

PETITION FOR DETERMINATION OF NON-REGULATED STATUS
FOR THE NEW PLANT VARIETY
HB4 SOYBEAN (IND-00410-5)
INTENDED FOR ENVIRONMENTAL RELEASE AND FOOD AND FEED USE

VERDECA, LLC

SUBMITTED TO THE UNITED STATES DEPARTMENT OF AGRICULTURE (USDA),
ANIMAL AND PLANT HEALTH INSPECTION SERVICE (APHIS)
BIOTECHNOLOGY REGULATORY SERVICES (BRS)

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AUGUST 7, 2017

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Dr. Keith Redenbaugh
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Date

SUMMARY

Verdeca, LLC is a United States-based joint venture between Bioceres and Arcadia Biosciences, which has developed a soybean line (IND-00410-5) using the *HaHB4* gene variant (*HaHB4v*) that provides the potential for increased yield in the natural range of soybean production areas. This line will be incorporated into traditional breeding programs to enhance the potential yield opportunity across a range of environmental conditions normally encountered by commercial soybeans. The selection of the IND-00410-5 event was based on data generated on multiple events of soybeans containing the *HaHB4v* gene over several years of field trials in Argentina and the US. These multi-event trials demonstrated improved yield across the normal range of yield variation in soybean production areas. The data presented here were generated on a single event (IND-00410-5, also designated as “HB4 soybean”) from two seasons of field trials, one in Argentina and the second in the US. The data support a conclusion that the *HaHB4v* gene provides the potential for increased yield across the current range of environments in which soybean is grown commercially. Argentina’s Ministry of Agriculture, Livestock and Fisheries has provided approvals for food and environmental safety, and international commerce pending China import approval.

Verdeca LLC completed safety assessments in Argentina on April 29, 2015 with the National Advisory Commission on Agricultural Biotechnology (CONABIA) and the Biotechnology Directorate of the Ministry of Agriculture, Livestock, & Fisheries. These Agencies completed their reviews and concluded that HB4 soybean is as safe for the environment as conventional soybeans. On October 6, 2015, Argentina’s Ministry of Agriculture, Livestock and Fisheries (2015, Resolución 397/2015) provided full approvals for food safety and international commerce for IND-00410-5. Verdeca LLC completed with FDA on August 2, 2017 a full safety consultation for HB4 soybeans (BNF No. 000155) and completed on August 7, 2015 an Early Food Safety Evaluation (EFSE) for the HAHB4 protein variant (HAHB4v) produced by HB4 soybeans (FDA 2015; NPC 000016; HAHB4).

The five major global soybean producers include the United States, Brazil, Argentina, China and India. Soybean is a self-pollinated species and does not have weediness characteristics in North and South America. The intended effect of the introduced trait in HB4 soybean to provide an increased yield opportunity under the normal growing conditions of commercial soybean production is unlikely to result in any negative or positive interactions with other organisms.

The HB4 soybean transgenic event IND-00410-5 was generated through *Agrobacterium*-mediated transformation. HB4 soybean IND-00410-5 contains the following introduced genes: 1) the *HaHB4* transcription factor gene variant (Appendix 7) from sunflower, 2) the *bar* gene from *Streptomyces hygroscopicus*, that confers resistance to glufosinate. The latter gene is only necessary for the plant transformation process and is not intended to provide field herbicide resistance. As discussed in detail in Appendix 7, changes were introduced into the *HaHB4* gene during the transformation process. As a result, the *HaHB4* gene present in IND-00410-5 is instead referred to as *HaHB4v* in this document and the resulting protein produced from the translation of *HaHB4v* is referred to as HAHB4v in this document. HB4 soybean is characterized by a single T-DNA locus comprised of a single copy of the selectable *bar* marker-gene, a single copy of the *HaHB4v* gene, and their

respective regulatory sequences. No unintended components from the binary vector DNA are present in IND-00410-5. The HAHB4 protein in IND-00410-5 belongs to the HD-Zip family of transcription factors, characterized by the presence of two functional domains: the homeodomain (HD), responsible for DNA binding, and a leucine zipper motif (LZ) involved in protein-protein interaction and dimerization.

Extensive analysis of the HAHB4v protein confirms its food, feed and environmental safety. This conclusion is based on a weight of evidence from multiple sources: gene source and history of use and exposure; bioinformatic comparisons of the amino acid sequence to known toxins, allergens and allergenic sequences; evaluation of the digestibility of HAHB4v protein using an *in vitro* assay; glycosylation status; level of HAHB4v protein in forage and grain of HB4 soybeans; and heat lability.

Compositional analysis of soybean event IND-00410-5 was conducted following the OECD consensus document for soybean (OECD 2012). A total of 43 components (nutrients, micronutrients, vitamins, minerals, and anti-nutrients) were analyzed in grain and forage from the transgenic event, the non-transgenic parental control Williams 82, and a set of commercial reference varieties all grown in the same field in different locations to represent a range of the natural variability across locations and commercial variety combinations. IND-00410-5 soybean was compositionally equivalent to its non-transgenic parental control Williams 82 and within the natural variability of conventional commercial reference varieties.

The agronomic performance of soybean transgenic event IND-00410-5 was evaluated in comparison with the conventional variety Williams 82 and with commercial comparators. The trials were conducted in several locations in Argentina (AR) and the United States (US). HB4 soybean characteristics measured included: 1) seed germination and dormancy; 2) pollen morphology and pollen fertility; 3) agronomic and phenotypic evaluations; and 4) ecological evaluations, including disease susceptibility, insect interactions, abiotic stress and plant-symbiont characteristics. The resulting data support the conclusion that soybean event IND-00410-5 is not fundamentally different than the Williams 82 soybean control and the conventional varieties, other than the intended effect of yield improvement. The results show the achievement of the desired trait of increased yield opportunity without introduction of adverse traits for this application, such as weediness or pest tolerance. Results demonstrated HB4 soybeans possess plant characteristics similar to those of conventional soybean varieties and do not pose an environmental risk compared to the conventional Williams 82 control.

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Abbreviations, Acronyms and Definitions

| | |
|-----------------------|---|
| <i>A. tumefaciens</i> | <i>Agrobacterium tumefaciens</i> |
| ACC | 1-aminocyclopropane-1-carboxylate oxygenase |
| ADF | Acid detergent fiber |
| AMD | Argentina Agriculture Market Directorate |
| AOS | Allene oxide synthase |
| AR | Argentina |
| ATDB | Animal toxins database |
| <i>bar</i> | Bialaphos resistance gene from <i>Streptomyces hygroscopicus</i> |
| C-t | Carboxy terminal region |
| CBI | Confidential Business Information |
| CONABIA | Argentina National Advisory Commission on Agricultural Biotechnology |
| DW | Dry weight |
| FDA | US Food and Drug Administration |
| <i>E. coli</i> | <i>Escherichia coli</i> |
| EFSE | US FDA Early Food Safety Evaluation |
| FW | Fresh weight |
| <i>G. max</i> | <i>Glycine max</i> |
| <i>HaHB4</i> | Transcription factor gene from sunflower (<i>Helianthus annuus</i>) characterized by a leucine zipper homeodomain |
| <i>HaHB4v</i> | Transcription factor gene from sunflower variant present in IND-00410-5 |
| HAHB4 | Protein encoded by the <i>HaHB4</i> gene from sunflower (<i>Helianthus annuus</i>) |
| HAHB4v | Protein encoded by the <i>HaHB4v</i> gene present in IND-00410-5 |
| HB4 | Soybean containing the <i>HaHB4v</i> gene |
| HD | Homeodomain |
| IND-00410-5 | OECD designation for HB4 soybean |
| LA | Lysogeny broth agar plates |
| LB | Lysogeny broth |
| LLOQ | Lower limit of quantification |
| LOD | Limit of detection |
| LOX | Lipoxygenase |
| LZ | Leucine Zipper |
| MMT | Million metric tonnes |
| NDF | Neutral detergent fiber |
| NGS | Next Generation Sequencing |
| OECD | Organization for Economic Cooperation and Development |
| ORF | Open reading frame |
| PAT | Phosphinothricin-N-acetyl transferase |
| PCR | Polymerase chain reaction |
| SENASA | Argentina Servicio Nacional de Sanidad y Calidad Agroalimentaria |
| T-DNA | Transfer deoxyribonucleic acid |
| US | United States of America |
| USDA APHIS | United States Department of Agriculture, Animal and Plant Health Inspection Service |
| Williams 82 | Parental variety for HB4 |

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RATIONALE FOR THE DEVELOPMENT OF HB4 SOYBEAN.**A. The name of the bioengineered food and the crop from which it is derived.**

The new plant variety is a soybean identified as “HB4 soybean” (OECD unique identifier IND-00410-5).

B. Basis for the Request for Approval

The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) has regulatory authority under the Plant Protection Act (7 U.S.C. § 7701-7772) and under the Plant Quarantine Act (7 U.S.C. § 151-167) to protect the United States from the introduction and spread of plant pests. Under APHIS regulation 7 CFR § 340.6, an organization may petition APHIS to evaluate and determine that a new plant variety does not represent a plant pest risk and therefore should no longer be regulated, allowing unrestricted introduction of the new variety.

Verdeca LLC requests the USDA APHIS review this safety assessment and issue a Determination of Non-Regulated Status for IND-00410-5 and any progeny generated from crosses between IND-00410-5 with conventional or other biotechnology-derived soybeans which have already been granted nonregulated status under 7 CFR Part 340.

C. Summary and Conclusions for the Development of HB4 Soybean.

Verdeca, LLC is a United States-based joint venture between Bioceres and Arcadia Biosciences, which has developed a soybean line (HB4 soybean) using the *HaHB4v* gene that provides the potential for increased yield in the range of soybean production areas. This line will be incorporated into traditional breeding programs to enhance the potential yield opportunity across a range of environmental conditions normally encountered by commercial soybeans. The selection of the IND-00410-5 event was based on data generated on multiple events of soybeans transformed with the *HaHB4v* gene over several years of field trials in Argentina and the US. These multi-event trials demonstrated improved yield across the normal range of yield variation in soybean production areas (data not shown). The data presented here were generated on a single event (HB4 soybean) from two seasons of field trials, one in Argentina and the second in the US. The data support a conclusion that the *HaHB4v* gene provides the potential for increased yield across the current range of environments in which soybean is grown commercially. Argentina’s Ministry of Agriculture, Livestock and Fisheries has provided approvals for food and environmental safety, and international commerce pending China import approval.

Verdeca LLC completed safety assessments in Argentina on April 29, 2015 with the National Advisory Commission on Agricultural Biotechnology (CONABIA) and the Biotechnology Directorate of the Ministry of Agriculture, Livestock, & Fisheries. These Agencies completed their reviews and concluded that soybean event IND-00410-5 is as safe for the environment as conventional soybeans. On October 6, 2015, Argentina’s Ministry of Agriculture, Livestock and Fisheries (2015, Resolución 397/2015) provided full approvals for food safety and international commerce for HB4. Verdeca LLC completed with FDA on August 2, 2017 a full safety consultation for HB4 soybeans (BNF No. 000155) and completed on August 7, 2015 an Early Food Safety Evaluation (EFSE) for the HAHB4 protein variant (HAHB4v) produced by HB4 soybeans (FDA 2015; NPC 000016; HAHB4).

The five major global soybean producers include the United States, Brazil, Argentina, China and India. Soybean is a self-pollinated species and does not have weediness characteristics in North and South America. The intended effect of the introduced trait in soybean event IND-00410-5 soybean to provide an increased yield opportunity under the normal growing conditions of commercial soybean production is unlikely to result in any negative or positive interactions with other organisms.

The HB4 soybean transgenic event HB4 was generated through *Agrobacterium*-mediated transformation. HB4 soybean IND-00410-5 contains the following introduced genes: 1) the *HaHB4* transcription factor gene variant from sunflower (Appendix 7), 2) the *bar* gene from *Streptomyces hygroscopicus*, that confers resistance to glufosinate. The latter gene is only necessary for the plant transformation process and is not intended to provide field herbicide resistance. As discussed in detail in Appendix 7, changes were introduced into the *HaHB4* gene during the transformation process. As a result, the *HaHB4* gene present in IND-00410-5 is instead referred to as *HaHB4v* in this document and the resulting protein produced from the translation of *HaHB4v* is referred to as HAHB4v in this document. HB4 soybean is characterized by a single T-DNA locus comprised of a single copy of the selectable *bar* marker-gene, a single copy of the *HaHB4v* gene, and their respective regulatory sequences. No unintended components from the binary vector DNA are present in HB4. The HAHB4v protein in HB4 belongs to the HD-Zip family of transcription factors, characterized by the presence of two functional domains: the homeodomain (HD), responsible for DNA binding, and a leucine zipper motif (LZ) involved in protein-protein interaction and dimerization.

Extensive analysis of the HAHB4v protein confirms its food, feed and environmental safety. This conclusion is based on a weight of evidence from multiple sources: gene source and history of use and exposure; bioinformatic comparisons of the amino acid sequence to known toxins, allergens and allergenic sequences; evaluation of the digestibility of HAHB4v protein using an *in vitro* assay; glycosylation status; level of HAHB4v protein in forage and grain of soybean event IND-00410-5; and heat lability.

Compositional analysis of soybean event IND-00410-5 was conducted following the OECD consensus document for soybean (OECD 2012). A total of 43 components (nutrients, micronutrients, vitamins, minerals, and anti-nutrients) were analyzed in grain and forage from the transgenic event, the non-transgenic parental control Williams 82, and a set of commercial reference varieties all grown together in different locations to represent a range of the natural variability across locations and commercial variety combinations. IND-00410-5 soybean was compositionally equivalent to its non-transgenic parental control Williams 82 and within the natural variability of conventional commercial reference varieties.

The agronomic performance of soybean event IND-00410-5 was evaluated in comparison with the conventional variety Williams 82 and with commercial comparators. The trials were conducted in several locations in Argentina (AR) and the United States (US). HB4 soybean characteristics measured included: 1) seed germination and dormancy; 2) pollen morphology and pollen fertility; 3) agronomic and phenotypic evaluations; and 4) ecological evaluations, including disease susceptibility, insect interactions, environmental conditions and plant-symbiont characteristics. The resulting data support the conclusion that soybean event HB4 is not fundamentally different than the Williams 82 soybean control and the conventional varieties, other than the intended effect of yield improvement. The results

show the achievement of the desired trait of increased yield opportunity without introduction of adverse traits for this application, such as weediness or pest tolerance. Results demonstrated soybean event IND-00410-5 possesses plant characteristics similar to those of conventional soybean varieties and does not pose an environmental risk compared to the conventional Williams 82 control.

D. Rationale and Benefits for the Development of HB4 Soybean.

Soybean is the number one source for protein in animal feed worldwide and the second largest source of vegetable oil worldwide. The United States remains the top producer of soybean. According to data from the USDA-NASS (2016b), approximately 83 million acres have been planted in the U.S. over the last five years, with a steady increase in yield to approximately 50 bushels per acre (USDA-ERS, 2016). The top 5 producing states are Illinois, Iowa, Minnesota, Indiana and Nebraska. Nearly 50% of the soybean crop from the US is exported to China, Europe and Mexico.

However, recently South America has emerged as a competitor market, particularly for export to China (USDA-NASS 2016a). As competition continues to increase from South America, soybean acreage could be limited in favor of corn to achieve the desired cost benefit. This in turn has led to an increase in planted soybean in areas traditionally used for small grains such as wheat. This has proven to be a benefit to wheat farmers because the rotation allows for interruption of wheat-based diseases (USDA-NASS 2016a). While soybeans are providing a timely benefit in those areas, this provides a wide variety of environments under which commercial soybean production occurs.

Soybean yield is impacted by a variety of factors including regional genetic adaptability, diseases, insects, nutrient deficiency, and normal environmental factors that can introduce stress to the plant. Typical environmental factors include but are not limited to soil crusting, frost damage, drought, flooding, hail damage, lightning damage, and sunburn or sunscald (Iowa State University 2011). Temperature, light and water availability also play key roles in soybean yield. These common environmental factors can be a major cause of yield reduction in soybean, affecting all production areas at one time or another, and, to varying degrees, during a growing season. One objective of soybean breeding programs is to develop varieties that maintain yield under the broad array of environmental conditions. Soybean varieties have for many years been developed using conventional plant breeding methods and, along with improved agronomic practices, have resulted in new varieties with enhanced yield maintenance and yield improvement. Given the multigenic components of yield in relation to adaption of soybean varieties to lower-yielding areas, as well as the need to develop regional soybean varieties adapted for specific environments, conventional plant breeding is limited in identifying yield improvement traits that can be applied across the entire soybean production environments. Discovery of specific genes that mitigate effects of broad environmental conditions offer the opportunity to develop, through genetic engineering, soybean varieties that will provide yield maintenance throughout the growing regions (USDA 2012).

HB4 soybean event IND-00410-5 was developed to provide an increased yield opportunity across the differing soybean environments. The *HaHB4v* gene encodes a DNA-binding protein that has demonstrated in the literature to be activated by various environmental stimuli normally encountered throughout a growing season. Data presented here generated

on soybean event IND-00410-5 over two years of field trials in Argentina and the US support a conclusion that the *HaHB4v* gene confers improved yield under a range of soybean growing conditions. Similar improvement has been observed in other crops containing the *HaHB4v* gene such as corn and wheat (internal, unpublished data). The *HaHB4v* transcription factor gene cloned from sunflower (*Helianthus annuus*) was inserted into soybean using *Agrobacterium*-mediated transformation. In sunflower, the gene expression is modified by biotic and abiotic stressors (Gago *et al.* 2002; Manavella *et al.* 2006 and 2008a). However in HB4 soybean, the gene is expressed at very low levels even under severe environmental stress (Verdeca 2015) and has been found to provide a yield benefit under conditions that might otherwise have reduced soybean yield. The sunflower *HaHB4v* gene expressed in soybean augments the plant's adaptability to the environment thereby potentially enabling a greater grain yield when integrated into commercial varieties.

E. Submission to the US Food and Drug Administration.

Verdeca LLC completed with FDA on August 2, 2017 a full safety consultation for HB4 soybeans (BNF No. 000155) and completed on August 7, 2015 an Early Food Safety Evaluation (EFSE) for the HAHB4 protein variant (HAHB4v) produced by HB4 soybeans (FDA 2015; NPC 000016; HAHB4).

F. Other Regulatory Agencies.

HB4 soybeans have been field trialed in two countries since 2011. Trials were conducted in Argentina in 2009, 2011, 2012, 2013, 2014 and 2015 under permit numbers 24817/2011 (extended permit), 95477/2012 and 531006/2013 from CONABIA. Trials were conducted in the United States in 2011, 2012, 2013, 2014 and 2015 under USDA notification numbers 11-122-110n, 12-097-106n, 12-121-104n, 12-131-102n, 13-018-102n, 13-106-105n, 13-130-103n and 14-101-108n. Additional trials for variety development have been conducted in both countries under appropriate permits/notifications.

In Argentina on April 29, 2015, CONABIA and the Biotechnology Directorate of the Ministry of Agriculture, Livestock, & Fisheries completed their reviews and concluded that soybean event IND-00410-5 is as safe for the environment as current commercial soybeans varieties. On October 6, 2015, Argentina's Ministry of Agriculture, Livestock and Fisheries (2015, Resolución 397/2015) provided approvals for food safety and environment international commerce pending China import approval.

The HB4 safety dossier was submitted in Uruguay Ministry of Livestock, Agriculture and Fisheries on February 9, 2015, requesting approval for environmental release for commercial production and for direct consumption and processing (Case no. 2015/7/1/1/378 - 02/09/15).

The HB4 soybean safety assessment for this new plant variety was submitted for consultation to the FDA Center for Food Safety and Applied Nutrition on May 12, 2016 (BNF 000155).

PRODUCTION AND BIOLOGY OF SOYBEAN.

A. Summary and Conclusions of the Production and Biology of Soybean.

Cultivated soybean, *Glycine max* (L.) Merr., is a diploidized tetraploid in the family Leguminosae with its center of origin in South-East Asia. The five major soybean producers are United States, Brazil, Argentina, China and India. Soybean is a self-pollinated species and does not have weediness characteristics in North and South America. The intended effect of the introduced trait in soybean event IND-00410-5 is to provide an increased yield opportunity under variable environmental conditions. This intended effect is unlikely to result in any negative or positive interactions with other organisms. The HB4 trait was engineered into Williams 82, a variety developed by the USDA and the Illinois Agriculture Experiment Station in 1981. Genetically engineered soybeans were first commercially introduced in the US in 1996. As of June 26, 2017 in the US, twenty genetically engineered soybean lines have been given non-regulated status by the USDA (USDA, 2017).

B. The Biology of Soybean

The biology of soybean is fully described by OECD (2000) “Consensus Document on the Biology of *Glycine max* (L.) Merr. (Soybean).” Language from the OECD document is incorporated in this dossier to provide the following details of soybean biology:

- a. General description including taxonomy and morphology and use as a crop plant.

“Cultivated soybean, *Glycine max* (L.) Merr., is a diploidized tetraploid ($2n=40$) in the family Leguminosae, the genus *Glycine* Willd. and the subgenus *Soja* (Moench). The primary leaves are unifoliolate, opposite and ovate, the secondary leaves are trifoliolate and alternate, and compound leaves with four or more leaflets are occasionally present” (OECD 2000).

Glycine max (L.) Merr. has the following taxonomy as described in the Integrated Taxonomic Information Systems (ITIS 2016).

Division Tracheophyta

Class Magnoliopsida

Superorder Rosidae

Order Fabales

Family Fabaceae - legumes

Genus *Glycine* Willd. - soybean

Species *Glycine max* (L.) Merr. – soybean

- i. Global Use.

Soybean is grown as a commercial crop in over 35 countries. The five major soybean producers in 2011/12 were the US, Brazil, Argentina, China and India, accounting for 90% of the total production. The US produced 83.2 million metric tonnes (MMT) of soybean grain and exported 4.7 MMT. Argentina produced 48 MMT of grain and exported 8.9 MMT (USDA 2013). Soybean is grown for the production of seeds and the derived products including oil and meal as food and animal feed. Soybean has a multitude of uses in the food and industrial sectors, and represents one of the major sources of edible vegetable oil and of proteins for livestock feed use. A bushel (27.2 kg) of soybeans yields about 21.8 kg of protein-rich meal and 5.0 kg of oil (OECD 2012).

ii. Production in Argentina.

Argentina produces an estimated 48 MMT soybeans a year (USDA 2013). About seventy percent of the beans are processed in Argentina into meal (75%), oil (18%) and biodiesel (5%) (Hilbert *et al.* 2012). Most of the unprocessed fraction is exported to China (70%) while the balance is further processed for domestic consumption (30%) in the food industry. As for the oil extracted in Argentina, about 70% is exported as crude oil, mainly to China and India (Hilbert *et al.* 2012). The remaining 30% is refined to edible quality for applications in the food and pharmaceutical industries or directed to the chemical industry for the preparation of cosmetics, cleaning products, biodiesel, fungicides and insecticides (Giancola *et al.* 2009). Only 2% of the soybean meal is consumed in Argentina as animal feed or food, or directed to other industrial uses. Most of the meal is exported to the European Union. Finally, about 60% of the biodiesel is exported to the European Union, the remainder being used domestically as fuel.

iii. Production in the United States.

Seventy-five million acres (M ac) (30.4 million hectares, M ha) of soybeans were grown in the United States in 2011, making it the second most planted crop in the US after corn. Average yield in the US in 2011 was 41.5 bushels/acre (2.79 MT/ha). 2013 soybean yield is projected at 43.0 bushels/acre (USDA 2013). Soybeans are widely adapted for US production, covering over 12 climatic zones stretching from Canada to the Gulf of Mexico. Soybeans are grown in 31 states, but more than 80% of the acreage is in the upper Midwest where soybean yields are highest. Soybeans are a seed crop planted primarily in the spring, with 6-9% of soybean acreage planted as a second crop, following rice, winter wheat or winter canola (Wilcox 2004). Soybean is frequently used as the rotational crop with corn or double cropped with wheat. The top five US soybean-producing states are Iowa (9.4 M ac), Illinois (8.9 M ac), Minnesota (7.1 M ac), Missouri (5.4 M ac) and Indiana (5.3 M ac). In the US, soybeans provided 66% of the edible consumption of fats and oils from 40% of the total soybean crop grown. The remaining 60% of the total soybean crop was exported as whole soybeans, soybean meal and soybean oil (Soystats 2012).

b. Agronomic practices.

“Soybean is a quantitative short day plant and hence flowers more quickly under short days. As a result, photoperiodism and temperature response is important in determining areas of variety adaptation. Soybean varieties are identified based on bands of adaptation that run east-west, determined by latitude and day length, including production in the US, Argentina and elsewhere” (OECD 2000).

c. Centers of origin of the species.

The genus *Glycine* Willd. is currently divided into two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The subgenus *Glycine* currently contains 23 wild perennial species and the subgenus *Soja* contains the cultigen *G. max* (L.) Merr and its wild annual purported ancestor *G. soja* Sieb and Zucc. The geographical origin of the genus *Glycine* was in South-East Asia (Hymowitz 2008). Hymowitz (2004, 2008) indicates that recent taxonomic, cytological and molecular systematic research and publications on the genus *Glycine* and related genera suggest the following: a putative ancestor of the current genus *Glycine* originated in South-East Asia with $2n = 2x = 20$. From this ancestral area Singh *et al.* (2001)

assume the northward migration to China of a wild perennial ($2n = 4x = 40$, unknown or extinct) with subsequent evolution to a wild annual ($2n = 4x = 40$; *G. soja*) and finally to the cultivated (domesticated) soybean ($2n = 4x = 40$; *G. max*, cultigen). Also, the wild perennial species found in Australia and Pacific islands today evolved from the putative ancestor in South-East Asia. The farmers of China domesticated the soybean.

d. Reproductive biology.

“Soybean is considered a self-pollinated species, propagated commercially by seed. Artificial hybridization is used for variety breeding” (OECD 2000).

e. Cultivated *Glycine max*.

“Cultivated soybean seed rarely displays any dormancy characteristics and only under certain environmental conditions grows as a volunteer in the year following cultivation. If this should occur, volunteers do not compete well with the succeeding crop and can easily be controlled mechanically or chemically. The soybean plant is not weedy in character. In North and South America, *Glycine max* is not found outside of cultivation” (OECD 2000).

“Soybean can only cross with other members of *Glycine* subgenus *Soja*. The potential for such gene flow is limited by geographic isolation. These species are not naturalized in North and South America” (OECD 2000).

“For a trait to become incorporated into a species genome, recurrent backcrossing of plants of that species by hybrid intermediaries, and survival and fertility of the resulting offspring, is necessary. The subgenus *Soja*, to which *G. max* belongs, also includes *G. soja* Sieb. and Zucc. ($2n=40$) and *G. gracilis* Skvortz. ($2n=40$), wild and semi-wild annual soybean relatives from Asia. Interspecific, fertile hybrids between *G. max* and *G. soja*, and between *G. max* and *G. gracilis* have been obtained. Hybrids between *G. max* and diploid wild species have been obtained using embryo rescue but the resulting hybrids were sterile” (OECD 2000).

f. Interactions with other organisms.

Cultivated soybeans have a long history of variety improvement through selection and plant breeding. Increasing yield opportunity under a range of environmental conditions typical for commercial soybean production is a basic and general objective of soybean breeding and has not resulted in any negative or positive interactions with other organisms.

g. Summary of ecology of *Glycine max*.

“*Glycine max* (L.) Merr., the cultivated soybean, is a summer annual herb that has never been found in the wild (Hymowitz 1970). This domesticate is in fact extremely variable, due primarily to the development of soybean “land races” in East Asia. The subgenus *Soja* contains, in addition to *G. max* and *G. soja*, the form known as *G. gracilis*, a form morphologically intermediate between the two. This is a semi-cultivated or weedy form, and is known only from Northeast China” (OECD 2000).

“*Glycine soja*, considered the ancestor of cultivated soybean, is an annual procumbent or slender twiner that is distributed throughout China, the adjacent areas of the former USSR, Korea, Japan and Taiwan. It grows in fields and hedgerows, along roadsides and riverbanks” (OECD 2000).

C. Characteristics of the Recipient Soybean Variety.

Williams 82 was developed by the USDA-ARS and the Illinois Agriculture Experiment Station and released in 1981. It is a late group III indeterminate variety (relative maturity 3.8). Williams 82 has white flowers, brown pubescence, tan pods and shiny yellow seed with a black to light black hilum. It is resistant to most *phytophthora* races and bacterial pustule. It is a tall (80 cm) variety that produces a large canopy and is well suited for row culture (Bernard and Cremeens 1988, MCI 2013, Wilcox and Christmas 1996). Williams 82 is an older variety and selected because of its ability to facilitate plant genetic transformation. Williams 82 was also the cultivar used for soybean whole-genome shotgun sequence of *Glycine max* (Schmutz *et al.* 2010). The IND-00410-5 soybean event will be crossed into elite soybean varieties and then backcrossed within the elite varieties to obtain the commercial product.

D. Selection of Comparators for HB4 Soybean.

The parent variety, Williams 82, was used as the conventional soybean comparator (control) in the safety assessment of IND-00410-5 soybean. Locally adapted, modern commercial varieties of soybean were also grown in randomized plots at each field test to establish ranges of typical measurements for soybean at each trial site representative of the site-specific growing conditions. These local reference varieties were chosen specifically for each site in both Argentina and the US and are described in Appendix 1. The local reference varieties were selected because each is adapted for optimal yield for that growing region; such varieties provide the appropriate comparison for HB4 soybean. The 2013 trial design as planned by Verdeca included the test line HB4, the parent Williams 82, two common soybean varieties (Dow32R280, Pioneer 93Y82) and three local commercial comparator varieties at each location.

E. Safe Use of Other Genetically Engineered (GE) Soybeans.

- a. Soybean is the world's leading genetically engineered crop and typically contains one or more herbicide tolerance traits. In 2012, 81 million hectares, or 81% of the world's soybean crop was genetically engineered. This accounts for 48% (81M ha/170.3 M ha) of the world's total GE crops (ISAAA 2013).
- b. Beginning in 1996 with the commercialization of the first genetically engineered soybean, there have been 12 different lines (transformation events) commercialized, with cultivation approvals in nine countries and food/feed approvals in seventeen countries plus the European Union (GMO Compass 2016). In the US, twenty soybean lines have been approved for environmental release at USDA (ISB 2016; USDA 2016). The commercialized soybean lines have various traits including herbicide tolerance, insect resistance, high yield and oil modification. Over the past 20 years since genetically engineered soybeans were first commercialized there have been no incidents of environmental hazards or adverse human or animal effects derived from their use.

DESCRIPTION OF THE TRANSFORMATION AND DEVELOPMENT OF HB4 SOYBEAN.

A. Summary and Description of the Transformation System.

The HB4 soybean transgenic event IND-00410-5 was generated through *Agrobacterium*-mediated transformation using a modified (described as follows) procedure of Paz *et al.* (2004). Mature soybean seeds (*Glycine max* cv. Williams 82) were pre-germinated on basal medium in the dark. Cotyledonary nodes derived from mature half seed explants were isolated and infected with *Agrobacterium tumefaciens* strain EHA101 (Hood *et al.* 1986) carrying the binary vector with *HaHB4v* (selected target) and *bar* (for selection) genes within the T-DNA region. Although some of the native *Agrobacterium* DNA is retained, the EHA101 strain does not contain tumorigenic DNA. The explants were co-cultured for 5 to 7 days in the dark with the *Agrobacterium* strain.

B. Description of Plant Regeneration and Event Selection.

After inoculation and co-culture, the explants were placed on selection medium supplemented with cefotaxime, timentin and vancomycin to inhibit *Agrobacterium* overgrowth. Glufosinate selection was employed to inhibit the growth and differentiation of non-transformed plant cells so that only the cells containing T-DNA could survive (shoot induction selective medium, SISM was used, Appendix 2). Regenerant explants were maintained at 24°C for two to three weeks under cool white fluorescent light and a 16:8 photoperiod. During this shoot induction step, explants were sub cultured several times on fresh SISM medium containing phytohormones, antibiotics and the selective agent. As soon as leaves were visible, leafy stems were excised and transferred to shoot elongation selective medium (SESM, Appendix 2). Elongated shoots (two nodes), were transferred to a semi-solid rooting medium (RM, Appendix 2). Rooted plants with normal phenotypic characteristics were transferred to soil mix for growth and further assessment.

DONOR GENES AND REGULATORY SEQUENCES.

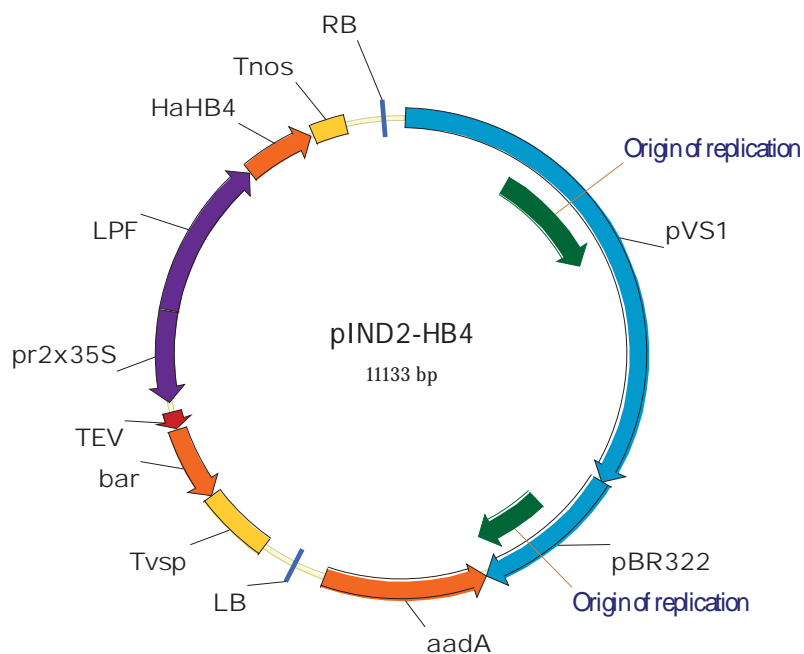
A. Summary and Conclusions of the Donor Genes and Regulatory Sequences

HB4 soybean IND-00410-5 contains the following introduced genes: 1) the *HaHB4v* transcription factor gene that is involved to improve yield, 2) the *bar* gene from *Streptomyces hygroscopicus*, that confers resistance to glufosinate. The latter gene is only necessary for the plant transformation process. None of the genes used to generate IND-00410-5 introduce plant pest interactions or influence plant health beyond the intended effects.

B. Vector (Map and Table with nt position).

HB4 soybean event IND-00410-5 was produced using *pIND2-HB4* vector (Figure IV.A). The *pIND2-HB4* vector, approximately 11.1 kb, derives from the *pPZP* family of binary vectors from *Agrobacterium* (Hajdukiewicz *et al.* 1994). It belongs to the series *pPZP202* (containing a spectinomycin resistance marker for bacterial selection) but includes a *2x35S-bar-Tvsp* cassette for *in vitro* glufosinate selection. In these vectors, the T-DNA borders derive from the *pTiT37* plasmid of *Agrobacterium*, the *bom* (basis of mobility) site from *pBR322* for *Escherichia coli* to *Agrobacterium* mobilization, and the origins of replication

from *ColE1* (from *E. coli*) and *pVS1* (from *Pseudomonas aeruginosa*) plasmids, for replication in *E. coli* and *A. tumefaciens*, respectively. The spectinomycin resistance gene, *aadA*, was cloned from the *pCN1* plasmid from *Shigella flexneri* serotype 2a (Chinault *et al.* 1986).

Figure IV.A. pIND2-HB4 Vector Map.**C. Identity and Source of Genetic Material.**

A summary of the IND-00410-5 genetic elements and their position on the source vector are provided in Table IV.A and discussed in detail in Appendix 3.

Table IV.A. IND-00410-5 Genetic Elements.

| Genetic element | Position (nt) | Description - Function | Donor | References |
|----------------------|---------------|---|-------------------------------|--|
| Intervening sequence | 01 - 08 | Sequence introduced for/during cloning | Sequence used in DNA cloning | |
| pVS1 | 09 - 3779 | Sequence derived from plasmid pVS1. The sequence between nt 948 and 1948 corresponds to the origin of replication pVS1ori, for replication and maintenance in <i>A. tumefaciens</i> . | <i>Pseudomonas aeruginosa</i> | Itoh <i>et al.</i> 1984; Itoh and Haas 1985; Hajdukiewicz <i>et al.</i> 1994 |
| pBR322 | 3780 - 4910 | Sequence derived from plasmid pBR322. The sequence between nt 4233 and 4852 corresponds to the origin of replication Col E1 for replication and maintenance in <i>E. coli</i> | <i>Escherichia coli</i> | Yanisch-Perron <i>et al.</i> 1985 |

| Genetic element | Position (nt) | Description - Function | Donor | References |
|----------------------|---------------|--|-----------------------------------|---|
| aadA | 4911 - 6153 | Aminoglycoside 3'-(O) adenylyltransferase gene confers resistance to spectinomycin/streptomycin. For selection in <i>E. coli</i> and <i>A. tumefaciens</i> (Complementary sequence). | <i>Shigella flexneri</i> Type 2a | Fling et al.1985; Chinault et al. 1986 |
| Intervening sequence | 6154 - 6396 | Sequence introduced for/during cloning | Sequence used in DNA cloning | |
| LB | 6397 - 6421 | The T-DNA left border sequence from the nopaline type pTi plasmid from <i>A. tumefaciens</i> . | <i>Agrobacterium tumefaciens</i> | Zambryski et al. 1982; Yadav et al. 1982 |
| Intervening sequence | 6422 - 6660 | Sequence introduced for/during cloning. | Sequence used in DNA cloning | |
| Tvsp | 6661 - 7210 | Sequence of the 3' terminator from a soybean vegetative storage protein gene. (Complementary sequence) | <i>Glycine max</i> | Rapp et al. 1990 |
| bar | 7211 - 7771 | L-Phosphinothricin (L-PPT) acetyltransferase gene that confers resistance to glufosinate herbicides by N-acetylation of L-PPT. (Complementary sequence). | <i>Streptomyces hygroscopicus</i> | Thompson et al. 1987; White et al. 1990; Becker et al. 1992 |
| Intervening sequence | 7772 - 7782 | Sequence introduced for/during cloning | Sequence used in DNA cloning | |
| TEV | 7783 - 7912 | Viral 5' leader sequence, acting as translational enhancer (Complementary sequence). | Tobacco Etch Virus | Carrington and Freed 1990 ; Gallie et al. 1995 |
| Intervening sequence | 7913 - 7984 | Sequence introduced for/during cloning. | Sequence used in DNA cloning | |
| pr2x35S | 7985 - 8671 | 2 x CaMV 35S promoter (duplicated CaMV 35S) (Complementary sequence). | Cauliflower Mosaic Virus | Odell et al. 1985, Haq et al. 1995 |
| Intervening sequence | 8672 - 8683 | Sequence introduced for/during cloning. | Sequence used in DNA cloning | |

| Genetic element | Position (nt) | Description - Function | Donor | References |
|-------------------------------|---------------|--|----------------------------------|--|
| LPF (large promoter fragment) | 8684 - 9892 | One of the allelic forms of the natural promoter of the <i>HaHB4v</i> gene (direct orientation) | <i>Helianthus annuus</i> | Dezar <i>et al.</i> 2005b |
| Intervening sequence | 9893 - 9903 | Sequence introduced for/during cloning. | Sequence used in DNA cloning | |
| HaHB4v | 9904 - 10434 | Gene coding for the transcription factor HAHB4v, involved to improve yield under varying environmental conditions (direct orientation). Translates to generate the HAHB4v protein. | <i>Helianthus annuus</i> | Dezar <i>et al.</i> 2005a, b; Manavella <i>et al.</i> 2008a; Gago <i>et al.</i> 2002 |
| Intervening sequence | 10435 - 10453 | Sequence introduced for/during cloning | Sequence used in DNA cloning | |
| Tnos | 10454 - 10706 | A 3' nontranslated region of the nopaline synthase gene from <i>A. tumefaciens</i> , which functions to terminate transcription. (Direct orientation). | <i>Agrobacterium tumefaciens</i> | Depicker <i>et al.</i> 1982 |
| Intervening sequence | 10707 - 10996 | Sequence introduced for/during cloning. | Sequence used in DNA cloning | |
| RB | 10995 - 11019 | The T-DNA right border sequence from the nopaline type pTi plasmid from <i>A. tumefaciens</i> | <i>Agrobacterium tumefaciens</i> | Zambryski <i>et al.</i> 1982; Yadav <i>et al.</i> 1982 |
| Intervening sequence | 11020 - 11133 | Sequence introduced for/during cloning | Sequence used in DNA cloning | |

GENETIC ANALYSIS OF THE INSERTION IN HB4 SOYBEAN.

A. Summary and Conclusions of the Genetic Analysis of HB4 Soybean

HB4 soybean is characterized by a single T-DNA locus comprised of a single copy of the selectable *bar* marker-gene, a single copy of the *HaHB4v* gene, and their respective regulatory sequences. No unintended components from the binary vector DNA are present in IND-00410-5. Results from both Southern blot analysis and Next Generation Sequencing (NGS) support these conclusions. The NGS approach allowed concurrent determination of 1) the number and full sequence of the T-DNA locus and 2) the T-DNA associated flanking sequences. In turn, the flanking sequences were used to locate the T-DNA precisely within chromosome 9. The locus of the T-DNA insertion was shown to be stable and its integrity

conserved both in six generations of self-pollinated progeny and in progeny of sexual crosses in F₂ plants (Appendix 4).

B. Organization of the Genetic Elements in the Insert of HB4 Soybean.

The organization of the genetic elements in the IND-00410-5 soybean event was the same as that in the binary vector T-DNA used in transformation to obtain this transgenic line. Data generated using both NGS and conventional technologies support the molecular characterization of event IND-00410-5 (Appendix 4, Figures 4-2 and 4-11).

C. The *bar* Gene and PAT Protein.

The *bar* gene was incorporated into HB4 soybeans for use only as a selectable marker and not for herbicide resistance. Consequently, determining the PAT expression level from the *bar* gene to ensure it is sufficient for herbicide resistance was not necessary. This protein was measured as part of the safety analysis (Appendices 3 and 6).

D. Genetic Elements of Plasmid Outside of the Transformation Fragment.

Results from Southern blot analysis, Next Generation Sequencing and PCR with appropriate probes and different primer combinations that amplify segments from the full plasmid DNA have shown the absence of plasmid backbone in the IND-00410-5 genomic sequence. Furthermore, this was also found when the IND-00410-5 genomic sequences were analyzed (Appendix 4).

E. Flanking Sequences.

The complete sequence of the T-DNA insert and its flanking soybean sequences (Appendix 4, Figure 4-6) were assembled *de novo* from the [CBI

CBI deleted

CBI]

F. Inheritance of the HB4 Trait in HB4 Soybean.

Segregation of the T-DNA was assessed over six generations and in F₂ progeny plants from crosses between IND-00410-5 and a commercial soybean cultivar. No changes in DNA sequence were detected, supporting the conclusion that the IND-00410-5 T-DNA resides at a single locus within the soybean genome and is inherited according to Mendelian inheritance principles (Appendix 4, Figure 4-11 and Table 4-4).

G. *HaHB4* Gene Mode of Action.

The sunflower transcription factor HAHB4 protein is a member of the sub-family I of HD-ZIP proteins. The HAHB4 protein homodimer complexes bind *in vitro* to a specific DNA sequence – CAAT(A/T)ATTG (Palena *et al.* 1999). The mRNA level of *HaHB4* has been shown to increase with the following stimuli: hyper-osmotic stress and abscisic acid (ABA) (Dezar *et al.* 2005b, Gago *et al.* 2002); ethylene and senescence (Manavella *et al.* 2006); jasmonate, wounding, and insect chewing (Manavella *et al.* 2008a); and darkness (Manavella *et al.* 2008b). These changes in *HaHB4* gene expression are consistent with reports in the literature for proteins of the HD-ZIP I and II sub-families, which have been shown to be responsive to various environmental stimuli (Dezar *et al.* 2005a 2011).

Comparisons of the native *HaHB4* promoter activity in sunflower to the activity of the native *HaHB4* promoter activity in transgenic *Arabidopsis* lines showed that responses of the promoter were the same. Exogenous ethylene caused an increase in *HaHB4* promoter activity in both transgenic *Arabidopsis* and sunflower (Manavella *et al.* 2006). Wounding and insect feeding also elicited similar responses in promoter activity in transgenic *Arabidopsis* and sunflower (Manavella *et al.* 2008a). The similarity in the responses of the *HaHB4* promoter in both *Arabidopsis* and sunflower suggests that the set of *cis*-acting elements in the *HaHB4* promoter (e.g., ABREs and DRE) are conserved (Dezar *et al.* 2005b; Gago *et al.* 2002).

Comparisons of the effects of transient *HaHB4* overexpression in sunflower leaf discs, *Arabidopsis*, and maize transgenic plants suggest that the set of downstream elements regulated by *HaHB4* is conserved. The wound-related genes, lipoxygenase (LOX) and allene oxide synthase (AOS), are upregulated in response to exogenous methyl jasmonate in sunflower leaves (Manavella *et al.* 2008a). Constitutive overexpression of *HaHB4* results in a high expression level of LOX and AOS in sunflower leaf discs, transgenic *Arabidopsis*, and transgenic maize (Manavella *et al.* 2008a).

The regulation of the expression of a set of target genes related to ethylene signaling and abiotic stress response was found to be similar in sunflower and transgenic *HaHB4 Arabidopsis* plants when expression is driven by a constitutive promoter. For example, expression of Cu/Zn superoxide dismutase (CSD1), the first enzyme in the cellular oxygen metabolism (Alscher *et al.* 2002, Gupta *et al.* 1993ab), was higher in both transient overexpressing *HaHB4* sunflower tissues and *HaHB4* transgenic *Arabidopsis* (Manavella *et al.* 2006) thereby protecting the plant from oxidative damage (Pan *et al.* 1981, Sakamoto and Murata 2000). Levels of betaine-aldehyde dehydrogenase (BADH), an important enzyme in the biosynthesis of plant osmoprotectant betaine-glycine, were higher in transient overexpressing *HaHB4* sunflower tissues and were induced to higher levels in *HaHB4* transgenic *Arabidopsis*. Manavella *et al.* (2006) concluded, "The expression of several ethylene-related regulatory genes is strongly transcriptionally repressed including, for example EIN3 and EIL1, which are involved in the ethylene signaling pathway ERF2 and ERF5, which are ethylene-responsive transcription factors and ACO and SAM, which participate in ethylene biosynthesis."

HAHB4 expression in *Arabidopsis* results in plants that survive a water deficit stress condition in which the untransformed control *Arabidopsis* plants die (Dezar *et al.* 2005a, Manavella *et al.* 2006, Cabello *et al.* 2007). The effect of *HaHB4* expression downregulating gene expression in the ethylene signaling pathway is consistent with the idea that *HaHB4* improves plant fitness by reducing the sensitivity to ethylene. Sharp and LeNoble (2002) and LeNoble *et al.* (2004) show that ABA exerts a growth maintenance effect on shoots by inhibiting the production of ethylene, and also by decreasing sensitivity to ethylene of the shoot tissues. ABA is a potent inducer of *HaHB4* expression (Dezar *et al.* 2005b), and *HaHB4* overexpression results in a decrease in the expression of genes leading to the synthesis of 1-aminocyclopropane-1-carboxylate oxygenase (ACC) and in a decrease in the expression of genes involved in the conversion of ACC to ethylene and in the response to this phytohormone (Manavella *et al.* 2006). These observations are consistent with a role for *HaHB4* in which plant metabolism is more strongly maintained because of the decrease in sensitivity to ethylene, which is a consequence of *HaHB4* overexpression.

Because plants are sessile organisms, the ability to adapt to the persistent changes in the environment improves the ability of the plant to tolerate those fluctuations. Therefore, because HAHB4 expression interrupts the ethylene pathway in the plant that would otherwise negatively impact growth, instead HB4 soybean is able to grow in the presence of average environmental stimuli with reduced negative impact on growth, development and yield.

H. ORF Analysis.

Bioinformatic analyses were performed to assess the potential toxicity or allergenicity of any putative peptides encoded by translation of the six reading frames within the insertion and the contiguous plant genomic DNA (Appendix 5).

Open reading frames (ORF) were identified by searching the encompassed nucleotide sequence for any initiation and stop codon producing a peptide of eight amino acids or greater in length.

Seventy-four peptides with up to 177 amino acids were found. The allergenic potential of each of these peptides was assessed by sequence comparison with known allergens in the FARRP database (FARRP 2016).

Using an 80 amino acids sliding window, no sequence identity greater than 35% was found with any allergen in the database. Additionally, alignments of eight contiguous amino acids along the whole sequence of each putative peptide with known allergens failed to find any significant match.

Bioinformatic analyses were also performed to assess the potential toxicity of any of the above putative peptides. The BLASTp algorithm (Altschul *et al.* 1990) was used to query the ATDB animal toxins database (He *et al.* 2008). The results of this search did not show any relevant homology (E score $< 1 \times 10^{-5}$) of the putative peptides with any of the toxins in the database.

From the above results, we conclude that none of the putative peptides generated by translation of the six reading frames arising from the insertion in IND-00410-5 matches with known toxic or allergenic proteins, from the ATDB and allergen online databases, respectively. We also conclude that these results obviate the need to perform further northern blot experiments for the search of putative transcripts.

CHARACTERIZATION OF PROTEINS EXPRESSED IN HB4 SOYBEAN.

A. Summary and Conclusions of HB4 and PAT Proteins in HB4 Soybean.

Gene expression can be regulated by transcription factors in response to a number of elements, and multiple classes of transcription factors exist. The HAHB4 protein belongs to the HD-Zip family of transcription factors, characterized by the presence of two functional domains: the homeodomain (HD), responsible for DNA binding, and a leucine zipper motif (LZ) involved in protein-protein interaction and dimerization.

In vitro selection for soybean event IND-00410-5 was developed using the herbicide bialaphos resistance (*bar*) gene. The *bar* gene, from *Streptomyces hygroscopicus*, encodes the enzyme phosphinothricin-N-acetyl transferase (PAT). PAT inactivates glufosinate ammonium herbicides providing tolerance (Block *et al.* 1987, Thompson *et al.* 1987, White

et al. 1990). Forage and seed were screened for PAT expression using an ELISA test. Both seed and forage were tested from the selected transgenic event HB4 soybean and the non-transgenic parent Williams 82. PAT protein was detected in HB4 soybean forage and seeds, but not in any of the Williams 82 samples, as expected. PAT expression in HB4 soybean was comparable to expression in other crops that have been approved by regulatory agencies and are currently being commercialized, thereby establishing a history of safe use for the PAT protein (Appendix 6). The use of the *bar* gene was only for selection processes and there is no intent for use in field herbicide resistance.

A number of experiments also were conducted to characterize the HAHB4v protein present in the soybean event IND-00410-5. HB4 soybean was shown to express an extremely low level of HAHB4v protein (Appendix 9). Consequently, recombinant HAHB4v protein was produced in *Escherichia coli* (Appendix 8) to generate material to conduct *in vitro* digestibility studies (Appendix 10). The studies described below demonstrate HAHB4v protein in HB4 soybean is present in low levels, even when the plant is placed under stress similar to what caused expression in the published literature, and is rapidly degraded under simulated digestion conditions. The protein safety data presented here support the conclusion that food and feed products generated from the soybean event IND-00410-5 should not pose a safety concern.

B. Biochemistry of the HB4 Protein.

The sunflower HAHB4 protein belongs to the HD-Zip family of transcription factors, characterized by the presence of two functional domains: the homeodomain (HD), responsible for DNA binding, and a leucine zipper motif (LZ) involved in protein-protein interaction and dimerization (Figure VI.A)(Elhiti and Stasolla 2009).

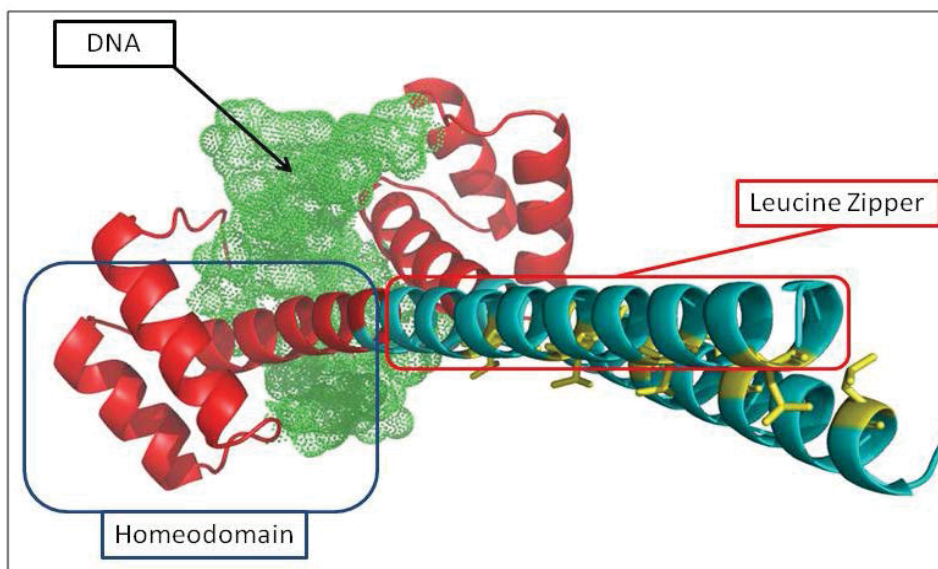
Figure VI.A. Representation of HAHB4 protein features. N-t: amino terminal region; HD: homeodomain; LZ: leucine zipper; C-t: carboxy terminal region.



The HD belongs to a designated class of transcription factors and is widely distributed among different kingdoms, genera and species including the crop plants canola, soybean, pea, rice, tomato, cotton and maize. This domain is typical of homeoboxes, DNA sequences involved in the regulation of morphogenesis, and encompasses a consensus sequence of 60-61 amino acids in length folding into three alpha helices connected by short loops.

HAHB4 functions as a dimer and is shown in Figure VI.B. The HD domain consists of a helix-turn-helix structure (shown in red) and is formed by the arrangement of the first two helices antiparallel to the third, which interacts directly with DNA (shown in green). The LZ domain (shown in aqua) consists of multiple leucine residues (shown in yellow) located at 7-amino acid intervals that form an amphipathic alpha helix that function as a dimerization domain.

Figure VI.B. Three dimensional representation of the HAHB4 protein. The diagram illustrates a DNA fragment (shown in green) interacting with an LZ-mediated HD-Zip dimer through the third helix of the HD domain (Ariel *et al.* 2007ab).



While both HD and LZ domains are commonly found in many proteins, their association in a single polypeptide chain is unique to plants (Schena and Davis 1992) and is the characteristic feature of the HD-ZIP family of transcription factors that is widely distributed among different plant species (Ariel *et al.* 2007ab).

The sunflower (*Helianthus annuus*) HAHB4 protein was identified by using a degenerate oligonucleotide derived from the conserved HD amino acid sequence WFQNRRA to screen a cDNA library generated from sunflower stem (Chan and Gonzalez 1994). HAHB4 was later shown to preferentially bind as a dimer to the dyad-symmetrical sequence CAAT(A/T)ATTG (Palena *et al.* 1999). Further research identified low-level expression in stem, roots, leaves and hypocotyls of sunflower plants (Gago *et al.* 2002). Additional experiments showed a significant increase in expression after exposure to water stress, abscisic acid (ABA), senescing tissues, darkness, and a number of other stressors (Gago *et al.* 2002; Manavella *et al.* 2006; Manavella *et al.* 2008b). Expression in these experiments has been observed in roots, stems and leaves.

In particular *HaHB4* belongs to a subclass of HD-Zip I genes that are primarily involved in abiotic stress responses in plants that help plants compensate with environmental changes. As discussed in Section V.F. concerning the mode of action, the native *HaHB4* gene in sunflower responds to a number of abiotic, as well as biotic, stress factors. The participation of HAHB4 transcription factor results in direct and indirect management of multiple environmental challenges including water deficit, saline exposure, abscisic acid and ethylene responses, photosynthesis, mechanical damage and herbivory. These environmental activities are part of a normal plant environment and the HD-Zip I proteins are expressed to help the plant compensate to such changes.

In the native sunflower plant, *HaHB4* expression is induced by water deficit in as short as an hour (Gago *et al.* 2002). When water deficit was introduced to transgenic *Arabidopsis* plants, plant survival after 5 days of water deficit was increased by more than 50% (Dezar *et al.* 2005b and Manavella *et al.* 2006). This can be akin to rain-fed crop management, when plants encounter prolonged periods of time without rain. Further, the native promoter driving expression of *HaHB4v* in IND-00410-5 soybean has shown a response to salt exposure, in addition to water stress (Dezar *et al.* 2006a). Saline conditions can often be encountered by soybean through irrigation systems or within the field soil.

The biochemistry of the HB4 protein was analyzed by comparison of the HAHB4 protein sequence translated from the original NCBI entry (GenBank Accession number AAA63768.2; Chan and Gonzalez 1994) with the sequence translated from the *HaHB4v* coding region actually inserted in soybean event IND-00410-5. While there are changes introduced to the *HaHB4v* coding sequence present in HB4 soybean, bioinformatic analysis suggests the changes are not located in the functional domains characteristic of HD-Zip proteins like HAHB4v and additionally are not predicted to impact the activity of the protein in the plant (Appendix 7). This is indicated by the protein being found in low levels when the plants are stimulated by environmental changes (Appendix 9).

C. Characterization of the HAHB4v Protein Produced in HB4 Soybean and Equivalence to the *E. coli*-Produced Protein Used in Safety Studies.

Recombinant HAHB4v protein was produced in *Escherichia coli* (Appendix 8). HB4 soybean was shown to express an extremely low level of HAHB4v protein (Appendix 9).

Based on collective data from LC-MS analysis, MALDI-TOF mass detection and N-terminal sequencing, *E. coli*-produced HAHB4v protein was shown to be equivalent to the protein present in HB4 soybean. These data support the conclusion that *E. coli*-produced HAHB4v protein is suitable for use in safety evaluations and to serve as a reliable standard for further studies, including the simulated gastric fluid digestion assay and as a positive control in quantifying HAHB4v in plant tissue samples.

D. Expression Levels of the Transgenic Proteins in HB4 Soybean Leaves, Seed And Roots.

HB4 soybean plants contain two transgenic proteins: HAHB4v and PAT. Genetically engineered crops that contain the *bar* or *pat* genes and express the PAT protein are as safe for use in food and feed as their conventional counterparts as determined in USDA approvals of engineered crops (Table VI.A). Using a standard assay, the measured values of PAT were well below previous reports of other crops expressing the transgenic protein (Appendix 6).

The levels of HAHB4v protein were determined in soybean seed and leaf tissue harvested from field trials in Argentina (2012-2013) and in the US (2013). A specific and sensitive LC-MS/MS method was developed and validated in order to detect the expected low levels of this transcription factor (Appendix 9). HAHB4v protein expression levels in the event IND-00410-5 were below the lower limit of quantification (LLOQ). Because the samples were collected from field trials grown across varying regions, the plants were subjected to various types of environments, as discussed in Appendix 12. Even under those conditions, the expression of HAHB4v being driven by the LPF promoter did not elicit expression at the time of harvest, as might be predicted from the literature. Consequently, an experiment was

conducted under abiotic stress conditions (growth chamber) like those described in the literature to elicit expression and HAHB4v protein was measured. The experiment in the growth chamber allowed for a controlled experiment to be conducted that introduced the stress repeatedly and then harvest the tissue, giving the best opportunity to capture protein expression in response to environmental stimuli. The highest amount of protein observed in soybean leaf tissue was 5 ng/g dry weight in a field trial leaf sample (Appendix 9). Even under severe stress conditions like those in the literature, the protein amount is extremely low (4 ng/g DW in seedling foliage and 5 ng/g DW in seedling root; Verdeca 2015) and barely above the limit of detection of an extremely sensitive and specific technique. This is likely due to the adjustment in expression of the native LPF sunflower promoter in soybean.

The HAHB4v protein was not detectable in any field samples and was barely detectable in a controlled growth stress experiment. This suggests the HB4 soybean event does not contain levels of transgenic protein that could negatively impact non-target organisms because the expression of the *HaHB4v* gene driven by the LPF promoter in the HB4 soybean event is extremely low.

E. Assessment of Potential Allergenicity and Toxicity of HAHB4v Protein in HB4 Soybean.

Assessment of the potential for allergenicity of the HAHB4v protein was based on Codex guidelines (2009) utilizing “an integrated, stepwise, case-by-case approach” employing “weight of evidence” including a bioinformatic search for homologies between the amino acids sequence of the introduced protein (HAHB4v) with known allergenic and toxic proteins and the assessment of certain physicochemical properties of the protein: digestibility in simulated gastric fluid and thermal stability.

The analyses and characteristics of HAHB4v protein supported the conclusion that the protein does not pose an allergenic or toxic risk to humans or toxic risk to animals (Appendix 10). A number of experiments were conducted, including a simulated digestibility assay. The amount of protein in the digestibility assay was more than 100,000X than what is the highest level detected in any IND-00410-5 plant (including severely stressed HB4 plants). Although the HAHB4v protein was heat stable, this was of no consequence due to the broad weight of evidence supporting its lack of allergenic or toxic potential, including:

1. HAHB4v was rapidly degraded *in vitro* with simulated gastric fluid,
2. HAHB4v protein is present in very low concentrations (generally below the level of detection) in HB4 soybean forage and grain,
3. HAHB4v protein sequence lacks homology with known allergens and toxins,
4. The source of the *HaHB4* gene is sunflower, which is not one of the eight major allergenic foods (Metcalf *et al.* 1996), and
5. HAHB4v protein sequence lacks homology with identified allergens from sunflower including 2S methionine-rich protein and sunflower profilin (Hel a 1 and Hel a 2) (Besler *et al.*, 2001).

F. Bioinformatics Search for Homologous Proteins, History of Safe Use.

The widespread distribution of proteins homologous to HAHB4 was confirmed by a BLASTP query of the HAHB4v sequence against the NCBI non-redundant protein

databases of higher plants (taxid:3193). Using the BLOSUM50 scoring matrix and an E-value threshold of 0.1, the top 100 significant protein matches against the higher plant database all had a bit score >100 and E-values of 1×10^{-30} or less (Pearson, 2013) (Table 7-2). The identified matching proteins were also homeobox-leucine zipper transcription factors and demonstrated their widespread distribution in commercial eudicots, monocots, seed and flowering plants; including spinach, asparagus, bean, potato, tomato, apple, pear, citrus, dates and walnuts. All of these matches had >50% coverage and > 50% identity to HAHB4v and contained the conserved homeobox DNA binding regions of the Homeodomain superfamily. These results, provided in Appendix 7, clearly support the case for a history of prior exposure to proteins that are significantly similar to HAHB4v protein and therefore a history of safe use.

G. Summary of the Food, Feed and Environmental Safety of the HB4 Protein.

Extensive analysis of the HAHB4v protein confirms its food, feed and environmental safety. This conclusion is based on a weight of evidence from multiple sources: gene source and history of use and exposure; bioinformatic comparisons of the amino acid sequence to known toxins, allergens and allergenic peptides; evaluation of the digestibility of HAHB4v protein using an *in vitro* assay; level of HAHB4v protein in forage and grain of HB4 soybeans.

The donor organism is *Helianthus annuus* (sunflower). The HAHB4 protein belongs to a large class of transcription factors, which are present in multiple plant species and are regulated by response to different environments. The levels detected in the plant leaf and seed samples harvested from multiple field trials were below the limit of detection and quantification. Growth chamber experiments designed to elicit HAHB4v protein production through exposure to severe and continuous stressors demonstrated expression levels ranging from below detection up to 5 ng/g DW in root and 4 ng/g DW in leaf from stressed plants (Verdeca 2015). Due to low expression in the selected event, *E. coli*-produced HAHB4v protein was purified to provide material for safety testing. The *in vitro* simulated gastric fluid assay revealed rapid degradation of the HAHB4v protein. No protein fragments were observed after the first 30 seconds of digestion. HAHB4v protein did not fragment during exposure to extended heat cycles, which allows the protein to be observed through SDS-PAGE experiments. There was slight change in electrophoretic mobility after a short period at 90°C, which was not significant. Multiple bioinformatics searches revealed no homology of HAHB4v to known allergenic or toxic proteins.

The results indicate the HAHB4v protein is unlikely to cause an allergic reaction in humans or be toxic to humans or animals, and therefore is safe for animal and human consumption (Verdeca 2015).

H. Characterization and Assessment of the Safe Use of *bar* Gene in Crops, Including Regulators' Conclusions.

Tolerance to the herbicide phosphinothricin (also known as glufosinate and bialaphos; trade names BASTA, Buster, Liberty) has been conferred to a variety of plant species by using recombinant DNA techniques to transfer one of two genes (*bar* or *pat*) from bacteria to enable the plant to produce an enzyme, phosphinothricin N-acetyltransferase (phosphinothricin acetyltransferase or PAT). The sources of the *bar* and *pat* gene,

respectively, are *Streptomyces hygrosopicus* and *S. viridochromogenes* (Thompson *et al.* 1987; Strauch *et al.* 1988; Wohlleben *et al.* 1988). Expression of PAT within the plant cell detoxifies the L-isomer of phosphinothricin (L-PPT), and thereby makes the plant tolerant to this herbicide. The *pat* and *bar* genes are very similar: they share 87 per cent homology at the nucleotide sequence level. Similarly, their expressed PAT proteins share 85 percent homology at the amino acid level (Wohlleben *et al.* 1988; Wehrmann *et al.* 1996). In some of the plants engineered with the *pat* or *bar* gene, the gene serves only as selectable marker, which is the case for HB4 soybean. Marker genes are routinely used in developing transgenic plants because they enable the selection of successful transformants in the laboratory. In addition, tolerance to L-PPT can be used as a selectable marker in the greenhouse and field.

In the US, phosphinothricin acetyltransferase and the genetic material necessary for its production in all plants are exempt from the requirement for food or feed tolerances in all crops (40 CFR 174.522; EPA 1997). The USDA has issued 26 Determinations of Nonregulated Status for crops containing either the *bar* or *pat* gene, including canola (rapeseed), chicory (radicchio), corn, cotton, rice, soybean and sugar beet (USDA 2016). The European Food Safety Authority (EFSA) GMO Panel has evaluated the safety of the PAT protein in the context of several applications for the placing on the EU market food and feed from GE crops expressing PAT (EFSA 2005a&b, 2006, 2007a,b&c, 2008, 2011, 2012, 2014), and no concerns were identified. Examples of global approvals of some of the crops containing either the *bar* or *pat* gene are listed in Table VI.A. These and other approvals of crops containing either the *bar* or *pat* gene demonstrate that the presence of the PAT protein in plants does not render them unsafe for consumption as food or feed.

Table VI.A. Examples of country approvals of crops containing either the *bar* or *pat* gene (USDA 2016; FDA 2013; ISAAA 2013).

| Company | Crop & Event | <i>pat</i> or <i>bar</i> | Food Use Approvals | Cultivation |
|-----------------------------------|---|--------------------------|--|----------------------|
| Dow AgroSciences | DAS-68416-4 glufosinate and 2,4- D tolerant soybean | <i>pat</i> gene | Australia, Canada, Mexico, New Zealand, USA (2011) | Canada USA |
| Dow AgroSciences (Mycogen) | DAS-06275-8 (TC6275) insect resistant, glufosinate tolerant corn | <i>bar</i> gene | Canada, Japan, USA (2004) | Canada USA (2004) |
| Bayer CropScience (Aventis) | LLCotton25 glufosinate tolerant cotton | <i>bar</i> gene | Australia, Brazil, Canada, EU, Japan, Mexico, New Zealand, South Africa, South Korea, USA (2000) | Brazil USA (2003) |
| Bayer CropScience (Aventis) | LLRICE06 and LLRICE062 glufosinate tolerant rice | <i>bar</i> gene | Australia, Canada, Colombia, Mexico, New Zealand, Russia, South Africa, USA (2000) | USA (1999) |

| Company | Crop & Event | pat or bar | Food Use Approvals | Cultivation |
|---|---|-----------------|---|--|
| Bayer CropScience (AgrEvo) | MS8/RF3 hybrid canola | <i>bar</i> gene | Australia, Canada, EU, Japan, New Zealand, USA (1998) | Australia Canada USA (1999) |
| Bayer CropScience (AgrEvo) | T120-7 glufosinate tolerant sugar beet | <i>pat</i> gene | Canada, Japan, USA (1998) | Canada USA (1998) |
| Bayer CropScience (AgrEvo) | A2704-12 and A5547-127 glufosinate tolerant soybean | <i>pat</i> gene | Argentina, Australia, Brazil, Canada, EU, Japan, Mexico New Zealand Philippines, Russia, South Korea, Taiwan, USA (1997), Uruguay | Argentina Brazil Canada USA (1998) Uruguay |
| Bayer CropScience (AgrEvo) | T45 (HCN28) glufosinate tolerant canola | | Australia, Canada, China, EU, Japan, Mexico, New Zealand, South Korea, USA (1997) | Australia Canada USA (1998) |
| Bejo Seeds | RM3-3, RM3-4 and RM3-6 male sterility chicory (radicchio) | <i>bar</i> gene | USA (1997) | USA (1997) |
| Bayer CropScience (Plant Genetic Systems) | MS3 male sterility corn | <i>bar</i> gene | Canada, USA (1996) | Canada USA (1996) |

According to available scientific evidence, PAT enzymes do not possess the characteristics associated with food toxins or allergens, i.e., they have no sequence homology with any known allergens or toxins, they have no N-glycosylation sites, they are rapidly degraded in gastric and intestinal fluids, and no adverse effects have been observed in several toxicology-related studies (Hérouet *et al.* 2005). Studies included acute oral single dose toxicity (mice) and 14-day repeated dose feeding (rats) studies with the isolated PAT protein and 42-day broiler chicken whole feed nutritional assessment, with no statistically significant adverse effects being observed (EFSA 2011).

Data collected from field studies, laboratory analyses, reports and literature references reviewed in support of all the corresponding regulatory approvals, as well as the experience of many years in agricultural environments, have demonstrated that plants containing the *bar* or *pat* genes (42 entries in the CERA Crop Database, 2011) are as environmentally safe as their non-engineered counterparts. These plants do not exhibit pathogenic properties, are

not more likely to become a weed than their non-modified counterparts, are unlikely to increase the weediness potential of any other cultivated or wild type relative, do not cause damage to derived agricultural commodities, and are unlikely to harm other organisms, including those that are beneficial to agriculture (CFIA 1995 a&b, 1996a&b; USDA 1996).

Data on the expression levels of PAT proteins, reports of the environmental risk assessment and compositional analysis of PAT-expressing plants have been thoroughly reviewed by several regulatory authorities (CERA 2011; ISAAA 2013). The environmental risk assessments included risk hypotheses, the establishment and persistence in the environment, adverse effects on the phenotype, weediness in agricultural and non-agricultural environments, movement of the *bar* or *pat* genes to wild relatives, and adverse impacts on other organisms in the receiving environment. These assessments show that the PAT protein expressed in these GE plants has negligible impact on their phenotype, beyond conferring tolerance to the herbicide glufosinate.

Environmental risk assessments associated with regulatory review of PAT-expressing plants show that expression of PAT does not alter the potential for persistence or spread of the GE plants in the environment, does not alter the reproductive biology or potential for gene flow, and does not increase the risks for adverse effects through interactions with other organisms. Although the introduction of PAT to GE plants has the potential to affect the management of herbicide-tolerant volunteers or weedy relatives becoming tolerant, the evidence does not indicate that expression of PAT has impacted the effectiveness or availability of alternative control measures such as other herbicides or mechanical weed control (CERA 2011).

In conclusion, there is a reasonable certainty of the safety of the inclusion of the PAT proteins in human food or in animal feed (H rouet *et al.* 2005). Therefore, the PAT enzymes, as individual proteins and within the whole GE crop-derived food/feed, have been proven to be safe, have not changed the levels of natural constituents of the whole food/feed, and have not shown potential toxicity, allergenicity or any nutritional quality concerns, and have not demonstrated any meaningful risk to the environment.

COMPOSITIONAL AND NUTRITIONAL ASSESSMENTS OF HB4 SOYBEAN.

A. Summary and Conclusions of the Composition and Nutrition of HB4 Soybean.

Compositional analysis of soybean event IND-00410-5 was conducted following the OECD consensus document for soybean (OECD 2012). A total of 43 components (nutrients, micronutrients, vitamins, minerals, and anti-nutrients) were analyzed in grain and forage from the transgenic event, the non-transgenic parental control Williams 82, and a set of commercial reference varieties all grown together in different locations to represent a range of the natural variability across locations and commercial variety combinations (Appendix 11).

Results were analyzed as a single group across all locations to determine whether there were significant nutritional differences between IND-00410-5 and Williams 82. Values of all components were analyzed for each field trial separately to account for any differences between location and genotype. For all of the locations, only two nutrient components—vitamin K1 and cysteine—showed a significant difference between the soybean event IND-00410-5 and Williams 82. While the content of cysteine was lower in the transgenic event when compared to Williams 82, the mean values fell within the range reported in the

literature and those for the commercial reference varieties. The value of vitamin K1 was lower in IND-00410-5 than Williams 82; however, Williams 82 was also lower than the commercial reference varieties, suggesting a genotypic effect of the Williams 82 parental variety (Appendix 11). Nevertheless, the overall vitamin K1 values for both IND-00410-5 and Williams 82 were within the ranges for soybean (Souci *et al.* 2008); however two sites, IA and KS in the US, were above and below, respectively, the range from the literature. The values are not consistent and were all below the commercial reference varieties for those locations (Table 11-18).

With regard to the levels of nutrients, the soybean event IND-00410-5 was compositionally equivalent to its non-transgenic parental control Williams 82 and within the natural variability of conventional commercial reference varieties combinations (Appendix 11).

Although some anti-nutrients showed significant differences between the HB4 soybean and the control line Williams 82, the levels were in all cases within the values obtained for commercial varieties and/or reported in the literature. Consequently, the anti-nutrients levels in event IND-00410-5 can be considered equivalent to those of the non-transgenic parental control Williams 82 and within the natural variability of conventional commercial reference varieties.

These results support the conclusion the transgenic event IND-00410-5 is compositionally equivalent to conventional soybean.

B. Components analyzed include key nutrients and anti-nutrients.

Key nutrients are those that have a substantial impact in the overall diet of humans (food) and animals (feed). The major constituents were fats, proteins, and structural and non-structural carbohydrates and the minor compounds were vitamins and minerals. Similarly, the levels of known anti-nutrients were determined. Key anti-nutrients and toxicants known in soybeans were measured (Appendix 11) (OECD 2012). Homology searches were conducted to verify that the new expression products are not toxicants and allergens (Appendices 5 and 10).

Analysis of grain samples included proximates (protein, fat, ash, and moisture), amino acids, fatty acids (C8-C22), acid detergent fiber (ADF), neutral detergent fiber (NDF), phytic acid, trypsin protease inhibitor, isoflavones, lectins, raffinose, stachyose, Vitamin E and carbohydrates by calculation. Analysis of forage samples included proximates (protein, fat, ash, and moisture), ADF, NDF and carbohydrates by calculation (Appendix 11).

- a. Anti-nutrients were measured in soybean grain and forage: raffinose, stachyose, trypsin protease inhibitors, lectins and phytic acid. Anti-nutrients of potential concern in soybean meal are trypsin inhibitors, lectins and phytic acid, some of which are generally controlled by heating during processing.
- b. Other compounds analyzed in soybean grain were isoflavones (daidzein, genistein and glycitein).

The nutrient levels in the soybean event IND-00410-5 were similar to those measured in the non-transgenic parental control Williams 82 or fell within the ranges observed for commercial varieties and/or as reported in the literature. The only differences found were not consistent overall and do not significantly vary from controls.

The anti-nutrients measured in seed did not show a consistent significant difference between the transgenic event IND-00410-5 and the parental control Williams 82 and were within the values obtained for commercial varieties and/or reported in the literature.

The nutrient composition of forage obtained from the soybean event IND-00410-5 was similar to that found in the non-transgenic counterpart and within the range of the commercial reference varieties, supporting the compositional equivalence of the transgenic event with Williams 82 and conventional reference varieties. Therefore the nutritional composition of seed and forage derived from IND-00410-5 is equivalent to current commercially available soybean varieties.

AGRONOMIC PERFORMANCE AND ECOLOGICAL OBSERVATIONS OF HB4 SOYBEAN.

A. Summary and Conclusions of Agronomic Performance and Ecological Observations.

The agronomic performance of soybean transgenic event IND-00410-5 was evaluated in comparison with the conventional variety Williams 82 and with commercial comparators. The results of these studies support the conclusion that the soybean IND-00410-5 event is comparable to commercial soybeans and does not pose a specific plant pest risk.

The studies included multiple laboratory, greenhouse and field experiments to observe a range of characteristics including seed germination, plant growth and development, plant disease and plant-pest interactions.

The trials were conducted in several locations in Argentina (AR) and the United States (US). Locations as planted at both countries have similar temperate growing seasons, environmental conditions and cultivation practices. In addition to the IND-00410-5 event and Williams 82 soybean entries, a number of commercial check varieties locally adapted in each country were included as references, providing a range of comparative values for assessment of phenotypic, agronomic and environmental interactions.

The resulting data support the conclusion that soybean event IND-00410-5 is not fundamentally different than the Williams 82 soybean control and the commercial varieties, other than the intended effect. The biological qualities were evaluated to demonstrate the similarity in germination, growth, pest susceptibility and disease response. The results showed the achievement of the desired trait of increased yield opportunity under conditions of environmental variations that might elicit HAHB4v protein expression without introduction of adverse traits for this application, such as weediness or pest tolerance. Results demonstrate IND-00410-5 possesses plant characteristics similar to those of conventional soybean varieties and does not pose an environmental risk compared to the conventional Williams 82 control (Appendix 12).

B. Characteristics Measured.

HB4 soybean characteristics measured included the following: 1) seed germination and dormancy; 2) pollen morphology and pollen fertility; 3) agronomic and phenotypic evaluations; and 4) ecological evaluations, including disease susceptibility, insect interactions, abiotic stress and plant-symbiont characteristics.

Seed Germination and Dormancy Characteristics.

Germination and seed dormancy are adaptive traits with a complex genetic basis (Koorneef *et al.* 2002) and generally are affected by the environment (Foley and Fennimore 1998). They constitute relevant aspects of the interactions of the crop with the environment and are related with the volunteer and pest potential of the plant. High levels of seed dormancy are associated with species that are weeds (Benech-Arnold *et al.* 2000). Conversely, soybean has low levels of dormancy (few hard seed, Mullin and Xu 2001).

In order to assess if these traits show changes in the soybean event IND-00410-5, an experiment was developed to test the response of the event as compared with the non-transgenic parental control line Williams 82. Laboratory experiments were conducted testing seeds from one site with standard seed germination and dormancy protocols. Temperature regimes were the recommended regime from the Association of Official Seed Analysts (AOSA 2013a) and five additional temperature regimes to assess the genotype responses. Statistical analysis showed no significant differences for seed germination characteristics, neither for the AOSA recommended temperature regime nor for the additional temperature regimes used in these experiments (AOSA 2013a and 2013b). Based on these results, it can be concluded that the introduction of the *HaHB4v* gene in the soybean genome has not changed the seed dormancy and germination characteristics between the transgenic event IND-00410-5 and the parental control (Appendix 12).

Pollen Morphology and Pollen Fertility Assessment.

The assessment of pollen characteristic serves as one component in evaluating the plant pest risk potential of HB4 soybean as compared to the parental control. Pollen grains from the HB4 soybean event and parental control Williams 82 were collected and assessed for pollen fertility using the iodine-potassium iodide (I2-KI) staining test. Microscope and digital images of pollen grains were examined to assess morphological differences. There were no statistically significant differences for pollen fertility and diameter between IND-00410-5 and Williams 82. In addition, pollen general morphology was similar between both genotypes (Appendix 12). In conclusion, these results show equivalence between HB4 soybean event and the parental control Williams 82 with regard to the above relevant pollen characteristics.

C. Agronomic and Phenotypic Evaluations.

The transgenic soybean event IND-00410-5 along with conventional control variety (Williams 82) and multiple commercial varieties specific for the growing region were field tested at 15 locations in Argentina in the single season 2012-2013 and at 10 locations in the United States (US) during the 2013 season. Both countries have similar temperate growing seasons, environmental conditions and cultivation practices, including maturity groups. The trial locations provided a range of environmental and agronomic conditions representative of the major soybean growing regions where HB4 soybean is expected to be grown commercially in Argentina and the US. Twenty-one agronomic and selected ecological interaction data were collected during the growing season for all entries at each site.

The wide variety of environmental conditions tested are represented by rainfall (Table 12-8), the temperature ranges (Table 12-9), and soil type and characteristics (Table 12-10). Additionally, the range in yield of the reference varieties (Table 12-20) indicates the range

of typical soybean performance at each of the tested locations. Specifically, the range in the US for the commercial varieties was as low as roughly 1,400 kg/ha to as high as nearly three times that amount; in Argentina, the variability in yield was even greater. This provides a broad range of potential yield across soybean production areas and an adequate environment under which to test the yield performance and plant characteristics of HB4 soybean. The differences in yield of IND-00410-5 soybean compared to Williams 82 were also found across that range at low-yielding (Argentina W1), mid-yielding (US IL2), and high yielding (Argentina D2). At those locations, IND-00410-5 soybean did not demonstrate unfavorable environmental interactions outside of the normal range of the reference and/or commercial varieties (Appendix 12).

Results from the Argentina and US multi-site, replicated field trials demonstrated that HB4 soybeans could provide an increased yield opportunity as compared with Williams 82. For many measured traits there were no significant differences between IND-00410-5 and Williams 82, including: plant height at V2-V3, days to 50% flowering, plant height at R6-R7, lodging score, shattering score, flower color, and grain moisture. Significant differences did exist for the following parameters: days to 50% emergence, early plant stand, seedling vigor, days to 50% maturity, plant stand at R8 and 1000 count seed weight. However, while these were significant differences between HB4 soybean and Williams 82, all values were within the range of the commercial reference varieties (Table 12-14). The only exception was the 1000 seed weight in Argentina was slightly out of range, but Williams 82 was even higher than HB4 soybean suggesting a genotypic effect in that location. Therefore the data support the conclusion that the *HaHB4v* gene does not confer agronomic or phenotypic characteristics resulting in a selective advantage for HB4 soybean over the parental control Williams 82.

D. Ecological Evaluations: Disease Susceptibility, Insect Interactions and Abiotic Stress.

IND-00410-5 and Williams 82 were observed for diseases and pest damage four times throughout development at Vn, R1/R2, R3/R4, and R5/R6 at all locations in Argentina and three times throughout development at R1/R2, R3/R4, and R5/R6 at all locations the US. At all locations in Argentina and all locations in the US, there were nearly no significant differences between genotypes throughout the developmental stages for arthropod counts, plant diseases and plant pests both at the individual site level as well as in the combined global analysis. These data demonstrated that soybean event IND-00410-5 has equivalent plant-environment interactions as compared to Williams 82 and did not demonstrate changes in pest or disease tolerance.

E. Ecological Evaluations: Plant-Symbiont Characteristics.

Symbiotic relationships with the rhizosphere-inhabiting bacteria from the *Rhizobiaceae* and *Bradyrhizobiaceae* families play a significant role in the environmental interactions of the soybean plant. These interactions, involving nitrogen fixation, greatly impact on agronomic practices, in particular, on the need to add nitrogen fertilizers to sustain soybean production. In order to assess if these symbiotic interactions in soybean event IND-00410-5 are unchanged as compared with the non-transgenic parental control line Williams 82, growth chamber experiments were conducted using *Bradyrhizobium japonicum* as the typical symbiont with standard seed inoculation protocols. Several variables indicative of the symbiotic performance of IND-00410-5 were compared with those of the parental cultivar.

Statistical analysis of the results showed no significant differences for nodule number, nodule dry weight, shoot dry weight and total biomass between the transgenic and the non-transgenic parental line. Based on this analysis, it can be concluded that the introduction of *HaHB4v* gene in the soybean genome has not changed the symbiotic interaction between the transgenic event and *B. Japonicum*, as compared with the parental control (Appendix 12).

ENVIRONMENTAL ASSESSMENT AND IMPACT ON AGRONOMIC PRACTICES OF HB4 SOYBEAN.

Information about the environmental assessment and impact of HB4 soybean as compared to conventional soybeans include the following: 1) characteristics and environmental assessment of the genetic insert and expressed proteins, 2) potential changes in the composition of the forage and grain, 3) phenotypic and agronomic interactions, 4) ecological interactions, 5) impact on the introduction of IND-00410-5 soybean on agronomic practices, and 6) weed resistance management. All characteristics support the conclusion that IND-00410-5 soybean does not significantly differ and has no additional impact on the environment than Williams 82. Based on the data and information generated, there will be no environmental impact on the commercialization of IND-00410-5 soybean as compared with other soybean varieties.

IND-00410-5 soybean does not present adverse environmental effects as compared with its parental variety Williams 82 or with commercial soybean varieties. The IND-00410-5 trait provides only an increased yield opportunity under a range of environmental conditions that are typically encountered by commercial soybean production areas. This trait is not unlike other varieties developed using more traditional soybean breeding methods to achieve incremental yield increase.

Data and results collected from 15 Argentina and 10 US trials as well as laboratory analyses demonstrated that IND-00410-5 soybean: 1) does not have plant pathogenic properties; 2) is no more likely to become a weed than non-transgenic soybeans; 3) is unlikely to increase the weediness potential of soybean; 4) does not cause damage to agricultural commodities; and 5) is unlikely to harm other organisms that are beneficial to agriculture.

Based on the overall assessment of IND-00410-5 soybean, there is no potential for it becoming a noxious weed nor a plant pest.

A. Characteristics and Environmental Assessment of the Genetic Insert and Expressed Proteins

Molecular characterization of IND-00410-5 by Southern blot analyses and NGS confirmed that a single copy of the T-DNA sequences from the transformation vector *pIND2-HB4* was integrated into the soybean genome at a single locus on chromosome 9. No additional sequences, including backbone elements, were introduced.

The function of the *HaHB4v* gene introduced in IND-00410-5 soybean should be considered as a timely expression of the normal physiological response of soybean to improve performance in its environment. It is expected that the biological activity derived from this yield phenotype will result in environmental interactions, which are the same as those of normal plant responses to the environmental. No metabolic processes involved in this response would indicate the possible expression of other effects that could in any way impact on agronomic practices. Therefore, there will not be an impact on agronomic practices derived from environmental interactions with IND-00410-5 soybean.

The levels of HAHB4v protein were determined in soybean seed and leaf tissue harvested from field trials in Argentina (2012-2013) and in the US (2013). Using a developed LC-MS/MS method, the expected low levels of this transcription factor were measured (Appendix 9) and found to be below the lower limit of quantification (LLOQ) in all samples

but above the method's limit of detection in two leaf samples. That is, even under the varied growing conditions including typical environments, HAHB4v protein was detectable in only two of the collected leaf samples and none of the seed samples. Because the promoter has been shown to be induced under severe abiotic stress (Manavella 2008a and 2008b), those experimental conditions were produced (growth chamber) and HAHB4v protein was measured, with the highest amount of protein observed in soybean leaf tissue at 0.0053 µg/g dry weight (Verdeca 2015). The amount of protein is extremely low, even when under direct severe stress, which would not be encountered in traditional soybean growing regions. Because the protein is not highly expressed and the HB4 plants did not demonstrate differences in disease susceptibility, insect interactions, and plant-symbiont characteristics, the results suggest the IND-00410-5 soybean event does not contain levels of transgenic protein that would have an impact on organisms different from other soybean varieties.

Results confirmed that HAHB4v protein does not have homology to known allergens or toxins. Although the HAHB4v protein was heat stable, it was rapidly degraded *in vitro* with simulated gastric fluid (Appendix 10).

In vitro selection for soybean event IND-00410-5 was developed using the herbicide bialaphos (*bar*) resistance gene. The *bar* gene, from *Streptomyces hygroscopicus*, encodes the enzyme phosphinothricin-N-acetyl transferase (PAT). Forage and seed were screened for PAT expression using an ELISA test. PAT protein was detected in IND-00410-5 soybean forage and seeds, but not in any of the Williams 82 samples as expected (Appendix 6). In other commercial genetically-engineered crops, the *bar* gene provides tolerance to glufosinate ammonium herbicides through a high expression of the PAT protein in the transformed plant. In IND-00410-5 soybean, the level of PAT is high enough for the screening of the desired soybean phenotype during the research and development process. However, this expression level is not high enough to provide field tolerance to glufosinate herbicides (data not shown). Comparatively, expression of PAT in HB4 soybean is 1.8-60 times lower than what has been expressed by glufosinate-tolerant plants (Appendix 6). Therefore, tolerance to glufosinate herbicides is not a phenotype expressed in the field by IND-00410-5 soybean.

Taken together, the low level of expression of the HAHB4v protein in IND-00410-5 soybean along with the lack of known allergenicity and rapid degradation of the protein supports the conclusion that the HAHB4v and PAT proteins in IND-00410-5 soybean will not pose an environmental risk.

B. Composition Observations in Forage and Grain

Compositional analyses were conducted on seed and forage material collected from all field trials in the US and Argentina. The results demonstrated that the nutrient levels in the seed of soybean event IND-00410-5 were similar to those measured in the non-transgenic parental control Williams 82 or fell within the ranges observed for commercial varieties or literature values. The anti-nutrient values measured in the same material of the transgenic event IND-00410-5 and the parental control Williams 82 were also within the commercial varieties and/or reported in the literature.

The nutrient composition of forage obtained from the soybean event IND-00410-5 was similar to that found in the non-transgenic counterpart and within the range of the

commercial reference varieties, supporting the compositional equivalence of the transgenic event with Williams 82 and conventional reference varieties.

Therefore the nutritional composition of seed and forage derived from IND-00410-5 is equivalent to current commercially available soybean varieties. These data support the conclusion that soybean event IND-00410-5 is comparable to other soybean varieties which are currently safely grown and consumed.

C. Phenotypic and Agronomic Interactions

The soybean event IND-00410-5 was further characterized to determine whether the presence of HAHB4v protein altered the plant pest potential as it relates to the phenotypic and agronomic characteristics compared to Williams 82. Multiple features were evaluated including pollen morphology and fertility; seed germination and dormancy; disease susceptibility, insect interactions and pest pressure response; and plant-symbiont interactions. Additionally, the propensity of IND-00410-5 for increased weediness or performance outside of the intended effect of improved yield was evaluated. Results from these studies demonstrated that soybean event IND-00410-5 is not fundamentally different than the Williams 82 soybean control. Therefore, it can be concluded that IND-00410-5 does not pose a plant pest risk nor will impact the environment any differently than other soybean varieties.

a. Pollen morphology and fertility.

General pollen morphology, pollen diameter and fertility were examined to compare potential differences between soybean event IND-00410-5 and the control Williams 82. Pollen grains from event IND-00410-5 and Williams 82 were assessed for fertility and size. There was no significant difference in pollen fertility or in mean diameter between IND-00410-5 and Williams 82 (Appendix 12).

The examination did not reveal any noticeable visual difference in pollen morphology in terms of appearance between grains from event IND-00410-5 and those from Williams 82. The results demonstrate that the introduction of *HaHB4v* gene into soybean did not alter the overall pollen morphology and pollen fertility of IND-00410-5 as compared to the parental control Williams 82 and therefore does not pose an environmental risk or change to conventional soybean.

b. Seed dormancy and germination.

Seed dormancy and germination results indicated that IND-00410-5 soybean seed had germination characteristics similar to the parental control Williams 82. Lack of hard seed (Table 12-2), associated with plants that are weeds, supported the conclusion that IND-00410-5 soybean did not have increased weediness potential when compared to Williams 82.

Evaluations of plant growth and development characteristics in the field, including potential weediness characteristics such as lodging and pod shattering, demonstrated no significant differences between IND-00410-5 soybeans and Williams 82. In regards to the other characteristics measured, one location showed a significant difference in seed germination at multiple temperatures, most likely due to the ideal growing conditions (Appendix 12). These differences were small in magnitude, and the mean values of IND-00410-5 soybeans at all

other locations were within the range of the control. Thus, these differences are unlikely to have biologically meaningful significance in terms of weediness potential of IND-00410-5 soybean.

c. Potential changes in susceptibility to pests and disease.

IND-00410-5 and Williams 82 were observed for diseases and pest damage four times throughout development at Vn, R1/R2, R3/R4, and R5/R6 at all locations in Argentina and three times throughout development at R1/R2, R3/R4, and R5/R6 at all locations the US. At all locations in Argentina and all locations in the US, there were nearly no significant differences between genotypes throughout the developmental stages for arthropod counts, plant diseases and plant pests both at the individual site level as well as in the combined global analysis (Appendix 12). These data demonstrated that soybean event IND-00410-5 has equivalent plant-environment interactions as compared to Williams 82 and did not demonstrate improved pest or disease tolerance.

Results from the studies on insect and disease presence as well as arthropod counts demonstrated no differences between IND-00410-5 soybean and the parental control. Additional results with commercial reference varieties also demonstrated no differences. No environmental impact beyond what is typical of conventional soybean farming is anticipated with IND-00410-5 soybean.

d. Consideration with regard to published report of herbivory.

Measurements of insect damage that includes damage from larval feeding made across all field trial sites in Argentina and the United States indicated no differences among the event, the non-transformed parent (Williams 82) and conventional comparators. The locations of the field trials were selected to represent the range of normal growing conditions encountered by soybean, and the targeted regions for IND-00410-5 soybean for yield improvement. Resistance to herbivory, as reported in Manavella *et al.* (2008a) under laboratory conditions in *Arabidopsis* and maize transgenic plants, was not detected in these field trials (Appendix 12). The plants in those tests in the literature contained constitutively high expression levels of HAHB4v and were tested in laboratory conditions, neither of which represent the plants and experiments presented in this document.

As discussed previously, the levels of HAHB4v protein were measured from all field trials, which experienced different growing conditions including various types of environments typically encountered by soybean. Only two of the samples collected from the field trials contained measurable levels of HAHB4v protein; both were leaf samples containing 4 and 5 ng/g DW (just above the limit of detection). To demonstrate the response of the LPF promoter in IND-00410-5 soybean, experiments were conducted to determine the levels of HAHB4v protein; the highest amount found was 0.0053 µg/g dry weight (Verdeca 2015). This demonstrates that under similar conditions as to those presented in Manavella *et al.* (2008a), those stressors barely elicited measureable levels of HAHB4v protein in IND-00410-5 soybean.

Appendix 12 summarizes the plant pest interactions measured in all of the field trials conducted in 2012-2013 in Argentina and the United States. For all of the plant pests and insects, there were no significant differences at any of the field locations individual or

combined. Samples were collected throughout development and the environments are representative of typical soybean growing conditions.

Taken collectively, the *HaHB4v* gene driven by the expression of the LPF promoter present in HB4 soybean does not display the characteristics of the constitutively expressed HAHB4 protein in the *Arabidopsis thaliana* and *Zea mays* plants presented in the Manavella *et al.* (2008a) publication. Therefore, IND-00410-5 soybean is not expected to impact herbivory or insect behavior any differently than commercially available soybean.

e. Plant symbiont characteristics.

Symbiotic relationships with the rhizosphere-inhabiting bacteria from the *Rhizobiaceae* and *Bradyrhizobiaceae* families play a significant role in the environmental interactions of the soybean plant. These interactions, involving nitrogen fixation, greatly impact on agronomic practices, in particular, on the need to add nitrogen fertilizers to sustain soybean production. In order to assess if these symbiotic interactions in soybean event IND-00410-5 are unchanged as compared with the non-transgenic parental control line Williams 82, growth chamber experiments were conducted using *Bradyrhizobium japonicum* as the typical symbiont with standard seed inoculation protocols. Several variables indicative of the symbiotic performance of IND-00410-5 were compared with those of the parental cultivar. Statistical analysis of the results showed no significant differences for nodule number, nodule dry weight, shoot dry weight and total biomass between the transgenic and the non-transgenic parental line. Based on this analysis, it can be concluded that the introduction of *HaHB4v* gene in the soybean genome has not changed the symbiotic interaction between the transgenic event and *B. japonicum*, as compared with the parental control (Appendix 12).

IND-00410-5 soybean was compared with the parental control Williams 82 to assess characteristics that could contribute to weediness potential, including increased seed dormancy, disease and pest susceptibility, pest preference and reproductive characteristics. Although there were some significant differences in several measured characteristics, these differences are minor, fall within range of other soybean varieties and do not alter the conclusion that IND-00410-5 soybeans do not represent an environmental risk as compared with the parental line Williams 82.

In the US, the US Fish and Wildlife Service manages the regulations concerning the Endangered Species Act (16 USC 1531 -1540). The Act requires federal agencies consult with the Fish and Wildlife Service when their activities may affect a listed species. The deregulation of IND-00410-5 soybean will increase the number of traits in soybean varieties available for a farmer to use. The gene products as expressed in IND-00410-5 soybean are not associated with any pesticide use or similar control of non-target organisms during cultivation as discussed in the previous section. The incremental increase of yield, under normal soybean growth conditions, has been a typical progression of plant breeding. Therefore, the expression of the gene products in soybean event IND-00410-5 will have no more impact on threatened, endangered or non-listed species than any other new improved yield soybean variety.

f. Weediness Potential of IND-00410-5 Soybean (Argentina and United States).

“Commercial soybean varieties in the U.S. and Argentina do not demonstrate weedy traits, have not been found moving into non-agricultural ecosystems, have not been included as a

weed in the major weed references (Holm *et al.* 1979), and are not listed as a noxious weed species (7 CFR Part 360). Furthermore, soybean does not possess any of the attributes generally associated with weeds (Baker 1974), such as seed dispersal and establishment as a dominant species in ecosystems. Nor do soybeans have the ability to compete well with native vegetation in North and South America. Because soybean seed lacks dormancy and germinates quickly under adequate temperature and moisture conditions, it is easily controlled in cultivated fields” (OECD 2000).

The HB4 trait in soybean provides an increased yield opportunity under typical environmental conditions of soybean production, compared with the Williams 82 control. This characteristic is not considered as weediness potential. Results from disease and insect damage, arthropods abundance, volunteer monitoring, symbiont interactions, dormancy and germination and pollen fertility demonstrated nearly no significant biological differences between IND-00410-5 soybean and the Williams 82 control that would indicate an overall selective advantage. Septoria Brown spot was measured to be significantly higher in HB4 soybean compared to Williams 82 at the R3/R4 growth stage. However, that trend was not repeated at other growth stages and does not confer a change in susceptibility different than traditional soybean varieties. In addition, any IND-00410-5 soybean volunteers would be controlled similarly to conventional soybeans, using herbicides and cultivation to remove the unwanted plants.

D. Gene Flow, Hybridization with Soybean and Other Plants

There are no sexually compatible relatives in North and South America; consequently, pollen-mediated gene flow can only occur between cultivated varieties. However, such gene flow would be minimal because of the biology of soybean.

Soybean is a self-pollinating crop that can only cross with other members of genus *Glycine*, that is subgenus *Soja*. The potential for gene flow in soybean is limited by 1) very low natural cross-pollination (less than 1%) with nearby soybean plants and 2) geographic isolation. “Wild soybean species are endemic in China, Korea, Japan, Taiwan and the former USSR. These species are not naturalized in North and South America, and although they could occasionally be grown in research plots, there are no reports of their escape from such plots to unmanaged habitats” (OECD 2000). Natural outcrossing in soybeans is so low that US Certified Seed Regulations (7CFR201.76) allows for seed production to be adjacent with only a separation “distance adequate to prevent mechanical mixture.” Consequently, the probability of gene transfer from IND-00410-5 soybean to other soybean plants is very low and inconsequential.

The risk of horizontal gene transfer (HGT) in plants is extremely low (Conner 2003). “In most cases the occurrence of HGT from GM crops to other organisms is expected to be lower than background rates. Therefore, HGT from GM plants poses negligible risks to human health or the environment” (Keese 2008). Rizzi et al. (2012) reported, “Animal feeding studies have demonstrated that a minor amount of fragmented dietary DNA may resist the digestive process...but stable integration and expression of internalized DNA has not been demonstrated.” Over the past 20 years, there have been many reports concerning HGT, but no evidence that such transfer has or could occur involving genetically engineered plants. Any sequence data suggestion that HGT may have occurred indicates that such transfer occurred over evolutionary time frames,” (Brown 2003). The Entransfood network

of the European Commission concluded that the probability of occurrence of HGT is extremely low (Van den Eede et al. 2004). In addition, the safety of the *HaHB4v* and *bar* transgenes shows there is little or no risk to the environment or human health from these genes or gene products. The risk from any horizontal gene transfer event concerning IND-00410-5 soybean is negligible.

E. Impact on the Introduction of IND-00410-5 Soybean on Agronomic Practices.

Agronomic practices used with the commercial cultivation of IND-00410-5 soybean will be identical to those used with other soybean varieties. IND-00410-5 soybean provides the opportunity for increased yields under typical growing conditions and therefore does not introduce a characteristic that requires specific or different cultivation activities. IND-00410-5 soybean will be planted with the recognition that most growing regions have varying conditions throughout the growing cycle and yields may be impacted accordingly.

Conventional soybeans have been bred for increased yield for many years. IND-00410-5 soybean will be an incremental increase, potentially replacing less robust varieties and increasing grain per acre in a range of soybean growing regions.

a. Cultivation of Soybean in Argentina.

Forty-nine million acres (M ac) (20 million hectares (M ha)) of soybean were grown in Argentina during the 2012-13 growing season. Average national yield was 38 bushels per acre (2.55 MT ha⁻¹). Soybean is widely adapted for Argentina climate, covering from latitude 23° to 39° South. Even though soybeans are grown across a wide range of agro-ecological zones, more than 90% of the cultivation area is concentrated in the Northern Pampas. The top five soybean-producing provinces are Buenos Aires (7 M ha), Córdoba (5 M ha), Santa Fe (3 M ha), Entre Ríos (1.5 M ha) and La Pampa (447,000 ha). For 2012-13, soybean production was estimated at 49 MMT (USDA 2013). These five provinces represent 90% of total soybean production, distributed as follows: Buenos Aires (17.8 MMT), Santa Fe (10.5 MMT), Córdoba (5.2 MMT), Entre Rios (3.5 MMT) and La Pampa (765,000 TN). More than 70% of soybean production (36.9 MMT) is exported. Exports products include soybean meal (23.5 MMT), oil (4.1 MMT), whole grain (7.8 MMT) (USDA 2013) and oil-derived biodiesel (2.3 MMT) (Hilbert *et al.* 2012).

b. Cultivation of Soybean in the U.S.

Soybeans are generally planted in the US as row crops, with planting in April/May and harvesting in October/November. With the advent of herbicide tolerant crops, soybeans are increasingly planted with no or reduced tillage systems, which has the advantages of decreased soil compaction, increased soil moisture and reduced soil erosion (Heatherly and Elmore 2004). Soybeans are typically grown in rotation with corn.

Glyphosate tolerant soybeans have been widely grown in the US since 1996, which has changed the way farmers manage weed control in soybeans. Currently, there are other herbicide tolerant soybean varieties reaching commercialization, including tolerance to glufosinate, dicamba and 2,4-D. Over 90% of US soybean acres are with varieties genetically engineered to contain herbicide tolerance.

Soybean cultivation relies on hot summers, with optimum growing temperatures of 20 to 30°C. In the US, the Midwest is the principle production region, extending from Louisiana

to North Dakota and from Nebraska to Ohio. Soybeans, like most legumes, fix nitrogen through a symbiotic relationship with rhizosphere-inhabiting bacteria from the *Rhizobiaceae* and *Bradyrhizobiaceae* families.

c. Comparison of the Cultivation of Soybean in Argentina and the US

Soybean farming practices are very similar in Argentina and the US, including comparable yield and greater adoption of no-till practices. Argentina farmers have almost 100% adoption of genetically engineered soybeans while in the US adoption is about 90%. Soybean regions in both countries are known for their rich, fertile soils (Lence 2010, Huerta 2002). Soybean growers in the US generally purchase seed each year because the genetically engineered varieties are protected by US patents. In Argentina, farmers can keep and produce their own seed, as per national law.

The introduction of soybean event IND-00410-5 will not substantially change or affect the management of soybean in Argentina or the US. The intended effect of improved yield does not require any adjustment in management of this soybean variety.

d. Potential Impact on volunteer management.

Determination of potential volunteers is important to consider in the context of weediness and persistence outside of cultivation. As discussed previously, soybean event IND-00410-5 does not possess weediness characteristics such as improved germination or changes in dormancy from the control variety Williams 82. These observations were made across all trials grown under various conditions and no changes in HB4 volunteers from Williams 82 were present. Therefore it is unlikely that any adjustments to traditional soybean cultivation and volunteer management will be required.

F. Weed Resistance Management.

Weed control programs are critical components for soybean production, specifically for crops engineered to contain a gene providing tolerance to a specific herbicide. IND-00410-5 soybeans were not developed to have field-level tolerance to glufosinate. The presence of the *bar* gene was only for the purpose of serving as a selectable marker.

It is likely that IND-00410-5 soybeans will be combined as a breeding stack with herbicide tolerant soybeans, thereby necessitating an integrated weed management program consistent with the herbicide. The presence of the PAT protein in IND-00410-5 soybean plants, however, is not intended and has not been shown to provide field tolerance to glufosinate (Appendix 6).

Creation of a full weed resistance management program will be done for each breeding stack incorporating a herbicide resistance trait that is consistent with the specific herbicide.

G. Environmental Impact Statement for Soybean Event IND-00410-5

The soybean event IND-00410-5 discussed herein does not possess commercial-level insect or herbicide tolerance. No genes have been introduced to achieve tolerance of any field application for a specific herbicide or insecticide and therefore does not require an Environmental Impact Assessment as the management of this soybean variety is similar to other soybean varieties achieved through traditional breeding.

ADVERSE CONSEQUENCES OF INTRODUCTION OF IND-00410-5 SOYBEAN.

Verdeca is not aware of any data or observations regarding IND-00410-5 soybean that would result in adverse environmental consequences from its introduction. As determined through field and laboratory studies, the only biologically relevant phenotypic difference between IND-00410-5 soybean and conventional varieties is the very low-level of expression of the transcription factor encoded by the *HaHB4v* gene and the resultant phenotype that provides an increased yield opportunity under a broad array of environmental conditions. Multiple lines of evidence support the conclusion that IND-00410-5 soybeans will not have adverse consequences: 1) molecular analysis, 2) protein expression analysis, 3) HAHB4 protein history of consumption, 4) compositional analysis, and 5) characterization of the plant phenotype.

Introduction of IND-00410-5 soybean will provide an increased yield opportunity under a variety of typical soybean growing conditions, which will be beneficial to farmers, help increase domestic and global supply of soybeans, and provide for food and feed uses of soybean products.

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APPENDICES.

- Appendix 1. Description of Commercial Soybean Varieties Used as Comparators.
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A. Appendix 1. Description of Commercial Soybean Varieties Used as Comparators.

The test line (IND-00410-5 soybean), parent (Williams 82) and common varieties (Dow 32R280 and Pioneer 93Y82) were provided to the cooperator without designating which were commercial and which were the test lines. Verdeca requested the cooperator choose three popular and adapted varieties in Maturity Group 3.4-3.9 for their area and provided the four blinded lines (IND-00410-5, Williams 82, Dow32R280 and Pioneer 93Y82). In three sites, a common variety selected by the cooperator was the same as one of the comparators, leading to double plot sets of Pioneer 93Y82. The double sets led to four comparator varieties instead of five varieties in these three trials but the duplicate 93Y82 plots acted as an internal control within the trial. As they were planted as commercial comparators in the three trials; they are listed in Appendix 1, Tables 1-1 and 1-2 as comparators.

Table 1-1. Commercial varieties used in 2013 US trials. Varieties selected for “Common varieties” were used at all US sites.

| Field Location | Variety | GE Technology ¹ | Maturity Zone | Company |
|------------------|---------------|----------------------------|---------------|---------------------------------|
| Common varieties | Dow 32R280 | RR2 Yield | 3.8 | BrodBeck (Dow) ² |
| | Pioneer 93Y82 | RR | 3.8 | Pioneer (DuPont) ³ |
| Hinton, OK | | | | |
| LC1 | Pioneer 93Y82 | RR | 3.8 | Pioneer (DuPont) ³ |
| LC2 | NK S38-S4 | RR/STS | 3.8 | NK (Syngenta) ⁴ |
| LC3 | NK S44-K7 | RR/STS | 4.4 | NK (Syngenta) ⁴ |
| York, NE | | | | |
| LC1 | Chanell 3806 | RR2/STS | 3.8 | Channel (Monsanto) ⁵ |
| LC2 | Big cob B38LL | LL | 3.7 | Big Cob Hybrids ⁶ |
| LC3 | Pioneer 93Y82 | RR | 3.8 | Pioneer (DuPont) ³ |
| Richland, IA | | | | |
| LC1 | Asgrow 3832 | Genuity/RR2 | 3.8 | Asgrow (Monsanto) ⁴ |
| LC2 | Stine 39LD02 | LL | 3.9 | Stine Seeds ⁷ |
| LC3 | Pioneer 93Y82 | RR | 3.8 | Pioneer (DuPont) ³ |
| Ladoga, IN | | | | |
| LC1 | Asgrow 3533 | Genuity/RR2 | 3.5 | Asgrow (Monsanto) ⁵ |
| LC2 | Asgrow 3731 | Genuity/RR2 | 3.7 | Asgrow (Monsanto) ⁵ |
| LC3 | Asgrow 3431 | Genuity/RR2 | 3.4 | Asgrow (Monsanto) ⁵ |
| Troy, OH | | | | |
| LC1 | Pioneer 93Y84 | | 3.8 | Pioneer (DuPont) ³ |

| Field Location | Variety | GE Technology ¹ | Maturity Zone | Company |
|----------------|----------------|----------------------------|---------------|--------------------------------|
| LC2 | DynaGro 36RY38 | Genuity/RR2 | 3.8 | DynaGro ⁸ |
| LC3 | Asgrow AG3832 | Genuity/RR2 | 3.8 | Asgrow (Monsanto) ⁵ |

Pemberton, OH

| | | | | |
|-----|----------------|-------------|-----|--------------------------------|
| LC1 | Pioneer 93Y84 | | 3.8 | Pioneer (DuPont) ³ |
| LC2 | DynaGro 36RY38 | Genuity/RR2 | 3.8 | DynaGro ⁸ |
| LC3 | Asgrow AG3832 | Genuity/RR2 | 3.8 | Asgrow (Monsanto) ⁵ |

Effingham, IL

| | | | | |
|-----|-------------------|-------------|-----|---------------------------------|
| LC1 | Asgrow AG3931 | Genuity/RR2 | 3.9 | Asgrow (Monsanto) ⁵ |
| LC2 | Hoffman H38-12CR2 | RR2 Yield | 3.8 | Hoffman Seed House ⁹ |
| LC3 | NK S39-U2 | Genuity/RR2 | 3.9 | Syngenta Seeds ⁴ |

Highland, IL

| | | | | |
|-----|-------------------|-------------|-----|---------------------------------|
| LC1 | Asgrow AG3931 | Genuity/RR2 | 3.9 | Asgrow (Monsanto) ⁵ |
| LC2 | Hoffman H38-12CR2 | RR2 Yield | 3.8 | Hoffman Seed House ⁹ |
| LC3 | NK S39-U2 | Genuity/RR2 | 3.9 | Syngenta Seeds ⁴ |

Carlyle, IL

| | | | | |
|-----|-------------------|-------------|-----|---------------------------------|
| LC1 | Asgrow AG3931 | Genuity/RR2 | 3.9 | Asgrow (Monsanto) ⁵ |
| LC2 | Hoffman H38-12CR2 | RR2 Yield | 3.8 | Hoffman Seed House ⁸ |
| LC3 | NK S39-U2 | Genuity/RR2 | 3.9 | Syngenta Seeds ⁴ |

Troy, KS

| | | | | |
|-----|---------------|-------------|-----|-------------------------------|
| LC1 | Pioneer 93Y84 | RR | 3.8 | Pioneer (DuPont) ³ |
| LC2 | Pioneer 93Y92 | Genuity/RR2 | 3.9 | Pioneer (DuPont) ³ |
| LC3 | Pioneer 93M94 | RR/STS | 3.9 | Pioneer (DuPont) ³ |

¹GE (Genetically Engineered) Technology. RR – Roundup Ready; RR2 – Roundup Ready 2; LL – Liberty Link; STS – Synchrony.

²BrodBeck (Dow), 15 Ringel Av, Wabash IN 46992.

³Pioneer (DuPont), 7100 NW 62nd Ave, Johnston, Polk County, IA 50131.

⁴Syngenta (NK), 11055 Wayzata Blvd, Minnetonka, MN 55305.

⁵Monsanto (Channel, Asgrow), 800 N. Lindbergh Blvd. St. Louis, MO 63167.

⁶Big Cob Hybrids, 990 Cottonwood St, Seward NE 68434.

⁷Stine Seeds, 360 Leiser Rd, New Columbia, PA 17856.

⁸DynaGro, 3005 Rocky Mountain Av. Loveland CO, 30538.

⁹Hoffman Seed House, 200 E 4th St, Hoffman, IL 62250.

Table 1-2. Commercial varieties used in 2012-2013 Argentina trials. Varieties selected for “Common varieties” were used at all Argentina sites. All reference varieties contain herbicide tolerance for glyphosate.

| Field Location | Variety | Maturity Zone | Company |
|--------------------------------|-------------|---------------|---------------------------------|
| Common variety | DM3810 | 3.8 | Don Mario Semillas ¹ |
| Aranguren, Entre Rios | | | |
| LC1 | Biosoja 4.6 | 4.6 | Bioceres Semillas ² |
| LC2 | DM 4210 | 4.2 | Don Mario Semillas ¹ |
| LC3 | DM 4670 | 4.6 | Don Mario Semillas ¹ |
| LC4 | SRM 3970 | 3.9 | Sursem ³ |
| San agustin (I2), Buenos Aires | | | |
| LC1 | FN 3.85 | 3.8 | FN semillas ⁴ |
| LC2 | A 3731 RG | 3.7 | Nidera Semillas ⁵ |
| LC3 | SRM 3410 | 3.4 | Sursem ³ |
| LC4 | SRM 3970 | 3.9 | Sursem ³ |
| Carmen de Areco, Buenos Aires | | | |
| LC1 | DM 4210 | 4.2 | Don Mario Semillas ¹ |
| LC2 | FN 3.85 | 3.8 | FN semillas ⁴ |
| LC3 | A 3731 RG | 3.7 | Nidera Semillas ⁵ |
| LC4 | SRM 3970 | 3.9 | Sursem ³ |
| Corral de bustos (D1), Cordoba | | | |
| LC1 | Biosoja 4.6 | 4.6 | Bioceres Semillas ² |
| LC2 | DM 4210 | 4.2 | Don Mario Semillas ¹ |
| LC3 | NS 4009 | 4.0 | Nidera Semillas ⁵ |
| LC4 | SPS 3900 | 3.9 | Syngenta ⁶ |
| LC5 | SRM 3970 | 3.9 | Sursem ³ |
| Corral de bustos (D2), Cordoba | | | |
| LC1 | DM4210 | 4.2 | Don Mario Semillas ¹ |
| LC2 | NS 4009 | 4.0 | Nidera Semillas ⁵ |
| LC3 | SPS 3900 | 3.9 | Syngenta ⁶ |
| LC4 | SRM3970 | 3.9 | Sursem ³ |
| Chilibroste, Cordoba | | | |
| LC1 | Biosoja 4.6 | 4.6 | Bioceres Semillas ² |
| LC2 | DM 4670 | 4.6 | Don Mario Semillas ¹ |
| LC3 | NS 4009 | 4.0 | Nidera Semillas ⁵ |
| LC4 | SPS 3900 | 3.9 | Syngenta ⁶ |
| LC5 | SRM 3970 | 3.9 | Sursem ³ |
| Daireaux, Buenos Aires | | | |
| LC1 | A 3731 RG | 3.7 | Nidera Semillas ⁵ |
| LC2 | SPS 3900 | 3.9 | Syngenta ⁶ |

| Field Location | Variety | Maturity Zone | Company |
|--------------------------------|-------------|---------------|---------------------------------|
| LC3 | SRM3410 | 3.4 | Sursem ³ |
| LC4 | SRM 3970 | 3.9 | Sursem ³ |
| Hughes, Santa Fe | | | |
| LC1 | DM 4210 | 4.2 | Don Mario Semillas ¹ |
| LC2 | NS 4009 | 4.0 | Nidera Semillas ⁵ |
| LC3 | SPS 3900 | 3.9 | Syngenta ⁶ |
| LC4 | SRM 3970 | 3.9 | Sursem ³ |
| Landeta, Santa Fe | | | |
| LC1 | Biosoja 4.6 | 4.6 | Bioceres Semillas ² |
| LC2 | DM 4210 | 4.2 | Don Mario Semillas ¹ |
| LC3 | DM 4670 | 4.6 | Don Mario Semillas ¹ |
| LC4 | NS 4009 | 4.0 | Nidera Semillas ⁵ |
| Monte buey, Cordoba | | | |
| LC1 | DM 4210 | 4.2 | Don Mario Semillas ¹ |
| LC2 | NS 4009 | 4.0 | Nidera Semillas ⁵ |
| LC3 | SPS 3900 | 3.9 | Syngenta ⁶ |
| LC4 | SRM 3970 | 3.9 | Sursem ³ |
| San agustin (II), Buenos Aires | | | |
| LC1 | FN 3.85 | 3.8 | FN semillas ⁴ |
| LC2 | A 3731 RG | 3.7 | Nidera Semillas ⁵ |
| LC3 | SPS 3900 | 3.9 | Syngenta ⁶ |
| LC4 | SRM 3410 | 3.4 | Sursem ³ |
| LC5 | SRM 3970 | 3.9 | Sursem ³ |
| Villa Saboya, Buenos Aires | | | |
| LC1 | DM 4210 | 4.2 | Don Mario Semillas ¹ |
| LC2 | FN 3.85 | 3.8 | FN semillas ⁴ |
| LC3 | A 3731 RG | 3.7 | Nidera Semillas ⁵ |
| LC4 | SRM 3970 | 3.9 | Sursem ³ |

¹Don Mario Semillas, Ruta 7 Km 208, Chacabuco, Buenos Aires.

²Bioceres Semillas, Ocampo 210 bis, Rosario, Santa Fe.

³Sursem, Ruta 32, Km 2, Pergamino, Buenos Aires.

⁴FN semillas, Ruta 31, Km 135, Salto, Buenos Aires.

⁵Nidera Semillas, Ruta 8, Km 376, Venado Tuerto, Santa Fe.

⁶Syngenta, Av. del Libertador 1855, Vicente Lopez, Buenos Aires.

B. Appendix 2. Plant Regeneration and Event Selection.

Following was the detailed protocol for transformation and regeneration of soybean shoots and for the differentiation of soybean transformed into plants.

1. Seed sterilization.

The seeds were washed in a dilute aqueous detergent solution for 5 minutes and washed for 6 times with sterile distilled water. The seeds were then alcohol washed (ethanol 70 %) for one to two minutes with occasional shaking. After decantation of the ethanol solution the seeds were placed for 10-12 hours into a bell jar desiccator within a fume hood for disinfection by gasification with chlorine gas. After gaseous disinfection seeds were soaked for twelve hours in sterile distilled water for softening of seed teguments.

2. Seed germination.

The seeds were peeled and placed on germination medium (GM) for a time period of about 72 to 96 hours in the dark until radical elongation. After the pre-germination period, the root and hypocotyledonous stem were removed. In order to increase the efficiency of infection by *Agrobacterium tumefaciens*, the adaxial epidermis of both cotyledons was mechanically partially removed to increase the intimate contact of the inoculums with the explants cells.

3. Transformation procedure.

i. Direct co-cultivation of explants with *A. tumefaciens* by infiltration.

Pre-germinated seeds were then placed in a vacuum infiltration infection medium (VIIM) containing a bacterial suspension (direct co-culture). Vacuum was drawn to 450 mm Hg. Explants stayed under vacuum for five to seven minutes. This procedure was repeated twice.

ii. Dissection of seeds and indirect co-culture thereof with transformed *A. tumefaciens*.

After infiltration procedure seeds were blotted on sterile filter paper and placed on top of a flat sterile surface (empty plate) for dissection. Cotyledons were ripped apart by using a sharp sterile scalpel blade and the apical shoot or plumule was also removed.

iii. Indirect co-culture of explants.

An extra treatment involving the dehydration/rehydration of the half seed explants was included to favor bacterial infection. Dissected half seed explants were allowed to lose their turgor pressure under the laminar flow hood for 30 min. Explants were then rehydrated by immersion in infection medium (IM) containing an *A. tumefaciens* suspension (OD 0.7) at 24°C for 30 min. Following this step explants were blotted on sterile paper and transferred immediately to co-cultivation medium (CCM) for 5-7 days in the dark at 24°C.

iv. Washing step.

To eliminate bacteria after co-cultivation, the explants were first dipped in sterile water for 10 minutes with gentle shaking and then blotted on sterile filter paper. Explants were then immersed in shoot induction washing medium (SIWM) (25 explants/flask). Washing was continued for 6 to 12 hours with continuous agitation (100 rpm) at 24°C, 16:8 photoperiod.

4. Selection and regeneration of transformed explants.

After the washing steps the explants were transferred to shoot induction selective medium (SISM) and maintained at 24°C for two weeks under cool white fluorescent light and a 16:8 photoperiod. The explants were laid on top of the selective media adaxial side up. Petri dishes (100 x 25 mm) were used for the selective and regeneration process. Every two weeks, explants were sub-cultured on fresh SISM medium containing phytohormones and antibiotic selection. As soon as leaves were visible, leafy stems were excised and transferred to dishes containing shoot elongation selective medium (SESM).

5. Rooting of transgenic shoots

When shoots were elongated (two nodes), they were transferred to a culture vials (1 plant/250 x 25 mm vial) containing semi-solid rooting medium (RM)(Figure 2-1). Transgenic plants were lately transferred to pot under growth chamber conditions for further selection and for phenotype and molecular characterization.

Figure 2-1. Shoot and root regeneration.



a, Soybean shoot regeneration on Shoot Induction Selective Medium; b, selection of rooted events

6. Media composition.

i. GM (Germination Medium).

Murashige & Skoog Basal Medium salts and vitamins, 30 g/L Sucrose, and 7 g/L Type A agar, pH 5.8.

ii. IM (Infection Medium).

1/10 X Gamborg B5 Basal Medium (Contains: 1/10X B5 major salts, 1/10X B5 minor salts, 1/10X B5 vitamins, 2.8 mg/L Ferrous, 3.8 mg/L NaEDTA), 30 g/L Sucrose, 3.9 g/L MES, pH 5.4. Filter sterilized GA3 (0.25 mg/L), BAP (2 mg/L), and 40 mg/L acetosyringone were added to this medium after autoclaving.

iii. VIIM (Vacuum Infiltration Infection Medium).

1/10 X Gamborg B5 Basal Medium (Contains: 1/10X B5 major salts, 1/10X B5 minor salts, 1/10X B5 vitamins, 2.8 mg/L Ferrous, 3.8 mg/L NaEDTA), 30 g/L Sucrose, 3.9 g/L MES, pH 5.4. Filter sterilized GA3 (0.25 mg/L), BAP (2 mg/L), Silwet L-77 (0.03 %) and 40 mg/L acetosyringone were added to this medium after autoclaving.

iv. CCM (Co-cultivation Medium).

1/10X Gamborg B5 Basal Medium (Contains: 1/10X B5 major salts, 1/10X B5 minor salts, 1/10 B5 vitamins, 2.8 mg/L Ferrous, 3.8 mg/L NaEDTA), 30 g/L Sucrose, 3.9 g/L MES, and 4.25 g/L Type A agar, pH 5.4. Filter sterilized GA3 (0.25 mg/L), BAP (2 mg/L), Cysteine (400 mg/L), Dithiothriitol (154.2 mg/L), and 40 mg/L acetosyringone were added to this medium after autoclaving. Medium was poured into sterile plates. When solidified the co-cultivation medium was overlaid with sterile filter paper to reduce bacterial overgrowth during co-cultivation.

v. Shoot Induction Washing Medium (SIWM).

1X Gamborg B5 Basal Medium (Contains: 1X B5 major salts, 1X B5 minor salts, 1X B5 vitamins, 28 mg/L Ferrous, 38 mg/L NaEDTA), 30 g/L Sucrose, and 0.59 g/L MES, pH 5.7. Filter sterilized BAP (1 mg/L), TDZ (0.1 mg/L), IBA (0.2 mg/L) Timentin (100 mg/L), Cefotaxime (100 mg/L), and Vancomycin (50 mg/L) were added to this medium after autoclaving.

vi. Shoot Induction Selective Medium (SISM).

1X Gamborg B5 Basal Medium (Contains: 1X B5 major salts, 1X B5 minor salts, 1X B5 Vitamins, 28 mg/L Ferrous, 38 mg/L NaEDTA), 30 g/L Sucrose, 0.59 g/L MES, and 7 g/L Type A agar, pH 5.7. Filter sterilized BAP (2 mg/L), IBA (0.2 mg/L) Timentin (50 mg/L), Cefotaxime (100 mg/L), Vancomycin (50 mg/L) and selective agent were added to this medium after autoclaving. Medium was poured into sterile plates.

vii. Shoot Elongation Selective Medium (SESM).

1X MS Modified Basal Medium with Gamborg Vitamins (Contains: 1X MS major salts, 1X MS minor salts, 1X B5 vitamins, 28 mg/L Ferrous, 38 mg/L NaEDTA), 30 g/L Sucrose, 0.59 g/L MES, and 7 g/L Type A agar, pH 5.7. Filter sterilized Asparagine (50 mg/L), L-Pyroglutamic Acid (100 mg/L), IAA (0.1 mg/L), GA3 (0.5 mg/L), Zeatin-R (1 mg/L), IBA (0.2 mg/L), Timentin (50 mg/L), Cefotaxime (100 mg/L), Vancomycin (50 mg/L) and selective agent were added to this medium after autoclaving. The medium was poured into sterile plates.

viii. Rooting Medium (RM).

½ X MS Modified Basal Medium with Gamborg Vitamins (Contains ½ X MS major salts, ½ X MS minor salts, ½ X B5 vitamins, 28 mg/L Ferrous, 38 mg/L NaEDTA), 20 g/L Sucrose, 0.59 g/L MES, and 7 g/L Type A agar, pH 5.6. Filter sterilized Indole-3-butyric acid (IBA, 2 mg/L), and selective agent were added to this medium after autoclaving. Medium was poured into sterile plates.

C. Appendix 3. Identity of Genes and Expressed Proteins.

The gene of interest is the *HaHB4v* Gene Coding Sequence, which expresses the HAHB4v protein.

The *HaHB4* (*Helianthus annuus homeobox 4*) gene codes for the sunflower transcription factor (TF) HAHB4, a member of the sub-family HD-Zip I of TFs. The DNA sequences present in HB4 soybean of the *HaHB4v* gene, promoter and terminator and the HAHB4v protein sequence are provided in Figures 3-1 to 3-4.

Figure 3-1. *HaHB4v* Gene cDNA (5'-3').

```
atgtctcttcaacaagtaacaaccaccaggaagaaccgaaacgaggggaggagacgatttaccgacaa
acaataagtttctagagtacatgtttgagacacagtcgagaccgagtttaaggatgaaacaccagt
tggcacataaactcgggcttcctcctcgtcaagtggcgatatggttccagaacaaacgcgcgcgatca
aagtcgaggcagattgagcaagagtataacgcgctaaagcataactacgagacgcttgcgtctaaatc
cgagtctctaaagaaagagaatcaggccctactcaatcaattggagggtgctgagaaatgtagccgaaa
agcatcaagagaaaactagtagtagtggcagcggggaagaatcggatgatcgggtttacgaactctccg
gacgttatgtttggcgaagaaatgaatgttccggttttgcgacgggttttgcgtaccttgaagaaggaaa
cagtttggtggagattgaagaacaactgccagaccttcaaaagtgggagggttctaa
```

Figure 3-2. *HaHB4v* Gene Promoter LPF (5' - 3').

```
acctggcacatcgatcttctcttttgcgtttccaacacaccacaacacacctacaaacgtgtca
attcacacttcaccaatttcatttccttttagtcaatcatattaaaagtagtagccccaccctt
tgttacctaccatttcccactttaataatcaccacgctatgtccacttgtacttttgtttgcacaca
actcttcccataaaatatacaaaccaatttttttagtggaacaaattccccaaatagaataactaa
cgaaattcatcgcatcagaatacactcatctctgaacagtgggcgaagcttgacgttttcgacgggggg
tcggaaaacgtatgtaccgaaatttctatagaatcggggggtcgaaaacgtatatacccaaaatttc
tatacgaaaactacatatataaactactgagcaaaaagttcgggggttcggggcgccctcccggccc
cttcaaagcttcgccaatgtctctgaaccgaagaaaaccctcactcgtctactagccaatgaatcctc
accagggaacccctcactcgtcttactggactattggcgcttccaaatggactacttgcgaaattca
ccacattgggatacactcgtctactcgggtgaggtaaaaccgcttgggtcaaggatcgaactagcga
ttgctgcctactcgcctaactctccatcatcaacaggtgcccgcgaaacaaaatgctggggcgggag
ttgaacctaggtccagtgacgcacccatgaatttttttagggatgcgaacgagtggtttaacct
acttttaagaggtgcgatcggaaattttacctataaaaatacactaaaaaagttccaaggggtccacca
cccctaacctaagtcgcctttgtctggatcacgtgaaacatcaggtctctcccttaccagtcacgc
tacgactcattgacaaaatatacaaacatatagatgttttgagttttatctcaaccgaaagtgcacatcat
gacagagaatcgacataaccaaaacgtgtaaacgtacaactcaccattgcggtgaaaaggacaaaaca
ggtaggattctgtcaaattcaacgcgtacacctgtgcttcatctaaacccatacttttaagaacct
ttataaagaccactcactatatacacatataatatacattatcaaacc
```

Figure 3-3. *HaHB4v* Gene Terminator Tnos (5' - 3').

```
gatcgttcaaacatttggcaataaagtttcttaagattgaatcctggtgcccgtcttgcatgattat
catataatttctggtgaattacgttaagcatgtaataattaacatgtaatgcatgacgttatttatga
gatgggtttttatgattagatcccgaattatacatttaatacgcgatagaaaacaaaatagcgcg
gcaactagataaattatcgcgcgcggtgtcatctatgttactagatc
```

Figure 3-4. HAHB4v Protein (NH3-COOH).

```
MSLQQVTTTRKNRNEGRRRF'FDKQISFLEYMFETQSRPELRMKHQLAHKLGHLHPRQVAIWFQNKRRS
KSRQIEQEYNALKHNYETLASKSESLKKNQALLNQLVLRNVAEKHQEKTSSSGSGEESDDRF'FTNSP
DVMFGQEMNVPFCDFAYLEEGNSLLEIEEQLPDLQKWWFEF
```

The selectable marker is the *bar* gene, which expresses the PAT protein.

HB4 soybean was developed using the *bar* gene for *in vitro* selection only. The *bar* gene (*bialaphos-resistance*), from *Streptomyces hygroscopicus*, codes for the enzyme

phosphinothricin-acetyl-transferase (PAT), which inactivates glufosinate ammonium herbicides providing tolerance to this herbicide (Thompson *et al.* 1987; White *et al.* 1990; Becker *et al.* 1992). Glufosinate selection was employed to inhibit the growth and differentiation of non-transformed plant cells so that only the cells containing the T-DNA could survive. Expression levels of this gene in HB4 soybean were, however, not sufficient for the plants to exhibit the glufosinate ammonium tolerance phenotype under field conditions. The DNA sequences of the *bar* gene, promoter, enhancer and terminator and the PAT protein sequence are provided in Figures 3-5 to 3-9.

Figure 3-5. *bar* Gene cDNA (5'-3').

```
atgagcccagaacgacgccccggccgacatccgcccgtgccaccgaggcggacatgccggcgggtctgcac
catcgtcaaccactacatcgagacaagcacgggtcaacttccgtaccgagccgcaggaaccgcaggagt
ggcaggacgacctcgtccgtctgcgggagcgtatccctggctcgtcggcagggtggcggcagggtc
gccggcatcgcctacgcgggccccctggaaggcacgcaacgcctacgactggacggccgagctgacccgt
gtacgtctccccccgcccaccagcggacgggactgggctccacgctctacaccacctgctgaagtccc
tggaggcacagggcttcaagagcgtggctcgtctcatcgggctgcccacgaccgagcgtgcgcatg
cacgaggcgtcggatattgccccccgcccgtgctgcccggcggcggcttcaagcacgggaactggca
tgacgtgggtttctggcagctggacttcagcctgccgggtaccgccccgtccgggtcctgccccgtcacccg
agatctgctcaacaatctag
```

Figure 3-6. *bar* Gene Promoter: pr2x35S (5'-3').

```
caacatgggtggagcacgacacacttgtctactccaaaaatatcaaagatacagtctcagaagacccaaa
gggcaattgagacttttcaacaaagggtaatatccggaaacctcctcggattccattgcccagctatc
tgtcactttattgtgaagatagtggaaggaaggtggctcctacaaatgccatcattgcgataaagg
aaaggccatcgttgaagatgcctctgccgacagtgggtcccaaagatggacccccaccacgaggagca
tcgtggaaaaagaagacgttccaaccacgtcttcaaagcaagtggattgatgtgataacatgggtggag
cacgacacacttgtctactccaaaaatatcaaagatacagtctcagaagaccaaagggcaattgagac
ttttcaacaaagggtaatatccggaaacctcctcggattccattgcccagctatctgtcactttattg
tgaagatagtggaaggaaggtggctcctacaaatgccatcattgcgataaaggaaagggccatcgtt
gaagatgcctctgccgacagtgggtcccaaagatggacccccaccacgaggagcatcgtggaaaaaga
agacgttccaaccacgtcttcaaagcaagtggattgatgtgatactcactgacgtaagggatgacg
cacaatcccactatccttcgcaagacccttctctatataaggaagttcatttcatttgggagaggac
```

Figure 3-7. *bar* Gene Enhancer: TEV (5'-3').

```
aattctcaacacaacatatacaaaaacaaacgaatctcaagcaatcaagcattctacttctattgcagc
aatttaaatcatttcttttaagcaaaagcaattttctgaaaattttcaccatttacgaagc
```

Figure 3-8. *bar* Gene Terminator: *Tvsp* (5'-3').

```
tagctagagtttgctcctatctatatgtaataaggtatgctgatatgcactattcaaataggagcatt
agctatgtttgtaatgtcactttatgttatgtgggtaagtcacctaagacactccacgtacctactt
gttgctctcttaccgcccgtttaaataaatcttctgcccttgttccatatttactaattatccctttctt
cactaaaagaaaattgttatcattaagtattagctctttagaacatagaggtctttaaattgggtagggt
ttacaaaattaactaataataaaatgtcataaaatccacgtgggttaaacaatgcagaaaatcgacgtc
gtctattggaccgacagttgctattaataataatgggccaccatagtagactgacaaaataaattacctg
acaacatcgtttcacaaaaaacacacaaaaagggagtgcattttccagggcatttttgaataaaa
aacagttaaaagggagtgcaatagaaaatagggggtgaggaaatagtgatttgagcacgtccttgaag
cgaatt
```

Figure 3-9. PAT Protein (NH3-COOH).

```
MSPERRPADIRRATEADMPAVCTIVNHYIETSTVNFRTPEPQEPQEWTDLVLRLRERYPWL
VAEVDGEVAGIAYAGPWKARNAYDWTAEESTVYVSPRHQRTGLGSTLYTHLLKSLEAQGFK
```

SVVAVIGLPNDPSVRMHEALGYAPRGMLRAAGFKHGNWHDVGFQWQLDFSLPVPVPPRPVLPV
TEICSTI

aadA gene and AAD protein.

The spectinomycin resistance gene, *aadA*, codes for the enzyme aminoglycoside 3'-
(O) adenylyltransferase (AAD), and was cloned from the *pCNI* plasmid from
Shigella flexneri serotype 2a (Chinault *et al.* 1986). Plasmids carrying spectinomycin
resistance genes are ubiquitous among gram-negative bacteria and fairly abundant in
natural bacterial populations (Fling *et al.* 1985; Shaw *et al.* 1993) and clinical
isolates (Heym *et al.* 1994). Consequently, *aadA* genes have been cloned from
several transposons. The *aadA* gene present in the vector used to produce HB4
soybean is located outside the T-DNA region and therefore has not been introduced
into the genome of HB4 soybean (Appendix 4). Furthermore, the spectinomycin
resistance gene has been accepted for such use for other genetically engineered crops
(EFSA 2009a and EFSA 2009b).

Regulatory sequences.

1. ori-pVS1

pVS1 is a 29 kb, non-conjugative plasmid from *Pseudomonas aeruginosa*. Its
replication origin, *ori-pVS1*, was used in the construction of the *pZPZ* series of
binary vectors (Itoh *et al.* 1984; Itoh and Haas 1985; Hajdukiewicz *et al.* 1994) for
replication in *Agrobacterium*. This regulatory sequence is outside the T-DNA region
in plasmid *pIND2-HB4*, and therefore is not present in the HB4 soybean genome.

2. ori-pBR322

pBR322 is a well-known, fully characterized plasmid from *E. coli*. Its replication
origin, *ori-pBR322*, has been widely utilized in the generation of genetically
engineered plants (Yanisch-Perron *et al.* 1985). This regulatory sequence is outside
the T-DNA region in plasmid *pIND2-HB4* and therefore is not present in the HB4
soybean genome.

3. Tvsp

Tvsp is the 3' untranslated region from soybean vegetative storage protein (VSPs)
genes, which functions to direct polyadenylation of the mRNA of the *bar* gene in
plasmid *pIND2-HB4*. The coding regions of VSPs have been characterized and
sequenced (Rapp *et al.* 1990) and show a typical organization of eukaryotic genes,
including the putative 3' untranslated polyadenylation signals (Rapp *et al.* 1990).

4. TEV 5' leader sequence

The genome of tobacco etch virus (TEV) lacks a 5' *cap* region but its 5' leader
sequence directs efficient translation of the viral genome (Carrington and Freed
1990; Gallie *et al.* 1995). In plasmid *pIND2-HB4*, this sequence directs translation of
the *bar* mRNA.

5. pr2x35S

The cauliflower mosaic virus (CaMV) 35S promoter is the most commonly used
viral-based promoter to drive transgene expression in plants (Odell *et al.* 1985; Haq

et al. 1995). In plasmid *pIND2-HB4*, the duplicated promoter enhancer, *pr2x35S*, was used for the expression of the *bar* gene.

6. LPF (large promoter fragment)

Two different allelic promoter regions (large and short promoter fragments, *LPF* and *SPF* respectively; Dezar *et al.* 2005b; Manavella *et al.* 2006) control the expression of *HaHB4* in sunflower. *LPF* is one of the two fragments of the natural promoter that may represent one of these different alleles. *LPF* directs the expression of *HaHB4* in specific plant tissues.

7. Tnos

Tnos is the 3' untranslated region of the nopaline synthase gene from *Agrobacterium tumefaciens*, which functions to direct polyadenylation of the mRNA of the *HaHB4* gene in plasmid *pIND2-HB4* (Depicker *et al.* 1982).

D. Appendix 4. Molecular Characterization of HB4 Soybean.

Introduction.

IND-00410-5 was analyzed to determine the number of loci and the sequence of the T-DNA insertion. Also determined were the stability of the T-DNA and the integrity of the sequence composition over six generations.

A detailed description of a transgenic event conventionally uses a combination of established techniques, including: 1) Southern blot analysis to probe for the number of T-DNA inserts and the possible presence of DNA elements from the carrier vector (Southern 1975), 2) the more time consuming “chromosome walking” needed to determine the native chromosomal T-DNA flanking sequences (Ochman *et al.* 1988; Singer and Burke 2003; Tan *et al.* 2005), and 3) Sanger sequencing technology and derived methods (Sanger 1977; Sengupta 2008) for determining the sequence of the T-DNA insertion(s).

The information above can also be determined with greater speed using Next Generation Sequencing (NGS) technology. The term NGS refers to several relatively new technologies developed to sequence and study whole genomes and transcriptomes (Metzker 2010; Xu *et al.* 2010). Illumina, Inc. (San Diego, CA) offers one of the most successful and most reliable of these technologies. There is now an abundant publication history covering NGS and the Illumina system in particular (Li *et al.* 2012; Lulin *et al.* 2012; Kovalic *et al.* 2012). Briefly, Illumina’s NGS combines high throughput sequencing and bioinformatics capable of handling and properly assembling the sequencing data. This process generates reliable, and practically error-free, sequences (Quail *et al.* 2012).

The NGS technology was used in parallel with conventional technologies to describe IND-00410-5. NGS was used to determine: the whole-genome sequence of IND-00410-5; the whole T-DNA sequence; and the junction sequences (JS) between the T-DNA and the soybean genome. The flanking sequences allowed further monitoring of the stability and the integrity of the T-DNA insertion across six self-fertilized generations, as well as in plants resulting from out-crossing with another soybean variety. Data generated using both technologies (NGS and conventional technologies) support the same molecular characterization of the event IND-00410-5.

Materials and Methods.

Plant Material

Leaf tissue from greenhouse- or field-grown homozygous IND-00410-5 or Williams 82 plants were used for DNA isolation for Southern blot analysis. For whole-genome sequencing and segregation analysis in F2 plants, DNA was extracted from embryo axes. A commercially available soybean cultivar, Bio 6.5 (Bioceres Semillas S.A., Ocampo 210 bis, Rosario, Argentina), was used for crossing with IND-00410-5. Bio 6.5 was developed by Roberto Wright a Cooperativa de Provisión de Servicios Agrícolas Criadero Santa Rosa Ltda., located in Rosario, Santa Fe province, Argentina. The relative maturity is 6.5, stems are indeterminate, the flowers are purple and the plants have gray pubescence, with brown pods at maturity. The

resulting seeds are yellow with dark brown hila and the line contains glyphosate tolerance.

DNA isolation

In general, prior to extraction, leaf tissue frozen in liquid nitrogen was processed to a fine powder in a mortar and pestle or in tubes in a 96-well format. The following techniques were used to extract plant DNA, depending on the amounts of DNA needed for the experimental purposes:

Genomic DNA was extracted following a hexadecyltrimethylammonium bromide (CTAB)-based method (Doyle and Doyle 1987). Briefly, 600 μ l of CTAB buffer (2% w/v CTAB, 100 mM Tris HCl, 20 mM EDTA, 1.4 M NaCl, and 0.14% β -mercaptoethanol) and 5 μ g RNaseA were added to approximately 100 mg of ground leaf tissue and incubated at 55-60°C for 15-20 minutes with intermittent mixing. 600 μ L of chloroform was added to the samples and mixed by hand for 2-3 minutes, then centrifuged at 10,000 rpm for 8 minutes. The upper aqueous phase was put into a clean microtube and the DNA was precipitated with 400 μ L of isopropanol. The sample was centrifuged at 12,500 rpm for 10 minutes to pellet the precipitated DNA. The DNA pellets were washed with 300 μ l of 70% ethanol by centrifuging the samples at 12,500 rpm for 5 minutes. The DNA pellets were air-dried, then resuspended in 100 μ l of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0). All extracted DNA was stored in a 4°C refrigerator or a -20°C freezer.

Genomic DNA was also extracted with DNeasy Plant Maxi Kit by Qiagen (Valencia, CA) for large preparations (Southern blot analyses) or with QIAprep® Miniprep (Qiagen Inc.) following the manufacturer's instructions.

For Illumina-based sequencing, and segregation studies of F2 progeny from (IND-00410-5 x Bio 6.5) crosses, the DNA was isolated from embryo axes. Prior to the extraction, seeds from IND-00410-5, Williams 82 and F2 were incubated in water at 37 °C to facilitate the disruption of the seeds. The embryo axes were then separated from the cotyledons and their DNA extracted following the CTAB method. The DNA was quantified either using a Quant-iT™ PicoGreen® (Invitrogen, Carlsbad, CA) or by QuBit fluorometer (Invitrogen) and the Quant-iT™ dsDNA BR Assay Kit by Invitrogen following the manufacturer's instructions. It was then stored in a 4°C refrigerator or a -20°C freezer.

Agarose gel electrophoresis of DNA

The DNA was resolved on 0.8% (w/v) agarose gels to assess the integrity of the samples. The gel was prepared with TAE 1x buffer (40mM Tris, 20mM acetic acid and 1mM EDTA) and run at 120v in the same buffer. The samples were loaded in a solution of 6x loading buffer (Glycerol 30% and bromophenol blue) and 200x GelRed™ Nucleic Acid Gel Stain (Biotium, Inc., Hayward, CA).

Agarose gels of 1.5% (w/v) were used for resolving amplicons with lengths between 200 bp and 1500 bp, while 1% (w/v) agarose gels were used for larger DNA fragments. The electrophoresis was performed in 1x TAE buffer and run at 120v. The samples were loaded using the loading buffer solution described above. Molecular size markers 100 bp (from 100 to 2080 bp) and/or Lambda *Bst*II (from

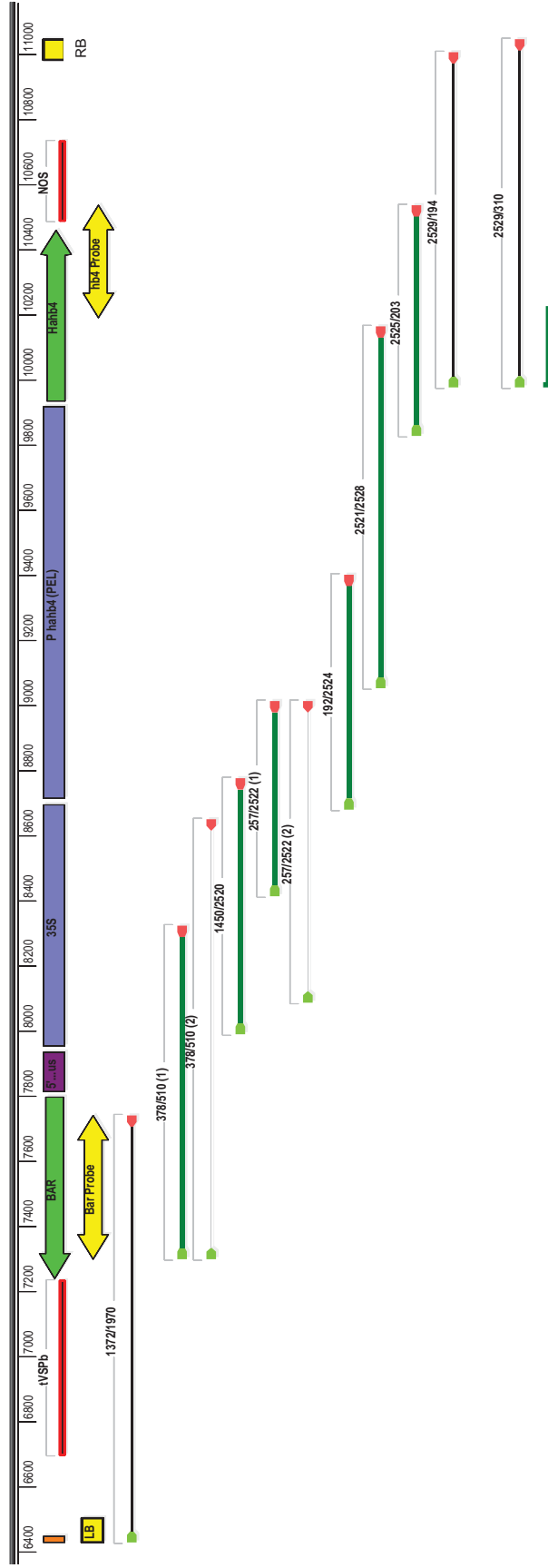
Table 4-2. List of primers used to determine the IND-00410-5 T-DNA sequence by conventional Sanger sequencing methods.

| Primer Set | Primer No. | Sequence | bp | T _m ¹ | Amplicon (bp) | PCR Extension (sec) | PCR T _m ¹ Range |
|-----------------------|------------|--------------------------------------|----|-----------------------------|---------------|---------------------|---------------------------------------|
| 1372/1970 | 1372 | TGGCAGGATATAT TGTGGTGTAACA | 26 | 56.3 | 1318 | 79 | 51-68 |
| | 1970 | GGCGGTCTGC ACCATCGTCA | 20 | 62.6 | | | |
| 378/510 ² | 378 | GAAGTCCAGC TGCCAGAAAC | 20 | 56.3 | 1031 | 62 | 51-65 |
| | 510 | AAGATAC AGTCTCAG AAGACCAAAGGGC | 28 | 59.8 | | | |
| 1450/2520 | 1450 | GGAAGGTCTTGCG AAGGATAGTGG | 24 | 59.2 | 793 | 48 | 54-65 |
| | 2520 | TGACACGTTTGTA GGTGTGTTGTGG | 25 | 59.4 | | | |
| 257/2522 ² | 257 | CTTCTTTTCCAC GATGCTCCTCG | 24 | 58 | 606 | 37 | 52-64 |
| | 2522 | AGAGATGAGTGTAT TCTGATGCGATGAA | 28 | 57.5 | | | |
| 192/2524 | 192 | AGTGTCTGTGCT CCACCATGTTG | 22 | 59.9 | 728 | 44 | 54-65 |
| | 2524 | GAGTAGGCAGCA ATCGCTAGTTCG | 24 | 59.5 | | | |
| 2521/2528 | 2521 | GGTCGGAAAAC GTATGTACCCG | 22 | 57.7 | 1118 | 68 | 52-66 |
| | 2528 | GCGTTATACTCTTG CTCAATCTGCCTCG | 28 | 60.8 | | | |
| 2525/203 | 2525 | CGTACACCTGTGC TTCATCTAAACCC | 26 | 59.3 | 715 | 43 | 51-65 |
| | 203 | CAAGACCGGC AACAGGATTC | 20 | 56.3 | | | |
| 2529/194 | 2529 | GAGGGGCGGA GACGATTTAC | 20 | 57.6 | 1036 | 62 | 50-63 |
| | 194 | TAAACTGAAG GCGGGAAACG | 20 | 55.4 | | | |
| 2529/310 | 2529 | GAGGGGCGGA GACGATTTAC | 20 | 57.6 | 1076 | 65 | 45-63 |
| | 310 | GGTTTACCCG CCAATATATC | 20 | 50.7 | | | |

¹ T_m = melting temperature.

² Primer pairs can bind twice due to a repeat sequence in the 35S promoter.

Figure 4-1. Conventional sequencing of the IND-00410-5 T-DNA and flanking sequences in chromosome 9. Overlapping amplicons are shown below the T-DNA map.



PCR product purification

All the PCR products to be sequenced were resolved by agarose gel electrophoresis as described above and further purified using Illustra GFX PCR DNA and Gel Band Purification Kit (GE, Piscataway, NJ) according to the manufacturer's instructions.

DNA Sequencing of PCR products

The sequencing of purified PCR products was outsourced to Macrogen Inc. (Seoul, Korea). For determination of the T-DNA sequence by Sanger sequencing (sequences presented in Figure 4-1), the amplicons were first cloned prior to Sanger sequencing (TOPO TA Cloning® kit by Invitrogen). These plasmid clones were then purified following the QIAprep Spin Miniprep Kit by Qiagen (Valencia, CA). Plasmid DNA was sent for sequencing to Davis Sequencing (Davis, CA). Sequences were analyzed using the software SeqMan Pro from DNASTAR (Madison, WI). At least three clones were sequenced for each amplicon.

Southern blot analysis

DNA extraction and concentration: One gram of leaf tissue from either IND-00410-5 or Williams 82 was flash frozen using liquid nitrogen and ground into fine powder using a pre-chilled pestle and mortar. DNA was extracted with Qiagen DNeasy Maxi Prep Kit following manufacturer's protocol. After elution, the DNA was precipitated by adding 0.1 volume of 3M sodium acetate and 2-3 volumes of 100% ethanol. The pellet was washed with 70% ethanol and resuspended in 80 µl of 1x TE buffer. The DNA was quantified using a QuBit fluorometer.

Restriction Digests: For each 50 µl digestion reaction, 5 µg genomic DNA was mixed with either *Hind*III enzyme or *Nde*I enzyme at concentrations of 10 U/µg of DNA. The samples were digested overnight (~16 hours) at 37°C. For digests of the plasmid control, 100-200 picograms of *pIND2-HB4* plasmid were used.

Gel Electrophoresis: The restriction digests of IND-00410-5 and Williams 82 genomic DNA were loaded into a 0.7% agarose gel along with DIG labeled Molecular Weight Marker VII (Cat No. 1669940910, Roche, Basel, Switzerland). The samples were run at 50 V overnight (approximately 16 hours). The gel was incubated in denaturing buffer twice for 30 minutes each time. The denatured gel was washed in transfer buffer for 15 minutes prior to alkaline transfer (outlined below).

Probe synthesis: Molecular probes for *HaHB4v* and *bar* genes (Table 4-3) were synthesized following the procedure outlined in the Roche PCR DIG Probe Synthesis Kit (Cat. No. 11636090910).

Table 4-3. List of primers used for the preparation of the probes used in Southern blot analyses.

| Probe | Size (bp) | Location on Vector | Hybridization (°C) | Primer Type | Primer Number | Sequence |
|-------|-----------|--------------------|--------------------|-------------|---------------|----------------------------------|
| HB4 | 226 | 10159..10510 | 45 | Forward | 2527 | CGCTTGCGTCTA AATCCGAGTCTC |
| | | | | Reverse | 203 | CAAGACCGGCA ACAGGATTC |
| Bar | 448 | 7267..7714 | 55 | Forward | 378 | ATATGGCGCTGA TCTCTGCT |
| | | | | Reverse | 1970 | GGCGGTCTGCAC CATCGTCA |
| STA | 357 | 1229..1585 | 54 | Forward | 1747 | AAGACGACCATC GCAACCCATCT A |
| | | | | Reverse | 1748 | TAGCCTTCCATC C GTGACCTCAAT |
| REP | 258 | 2979..3236 | 50 | Forward | 1745 | AGCTGATTGGAT G TACCGCGAGAT |
| | | | | Reverse | 1746 | TTCAAATCGTAC TC CGGCAGGTCA |
| aadA | 229 | 5935..6163 | 50 | Forward | 822 | ATCAAACATCGA C CCACGGCGTAA |
| | | | | Reverse | 1127 | GATCAATTCGGG C ACGAACCCAGT |

Alkaline transfer and DNA binding: Alkaline transfer of DNA from the agarose gel was performed using Turboblottter-Rapid downward transfer system (Whatman, Marlborough, MA). The DNA was transferred to a 12x21cm Nylon membrane (Nytran™ SuPerCharge, Sigma-Aldrich Co., St. Louis, MO) for 4 hours. The membrane was washed in neutralizing buffer (0.2M sodium phosphate, pH6.8). The DNA was permanently cross-linked to the membrane by Ultraviolet Cross linker (CL-1000, UVP, Upland, CA) with 2 exposures of 1500 mJ each.

Pre-Hybridization: The membrane was incubated in 50 mL of Roche DIG EasyHyb hybridization buffer (Cat. no. 11 603 558 001) at pre-calculated hybridization temperatures (45 °C and 55°C for the *bar* and *HaHB4v* gene probes respectively) under constant rotation.

Hybridization: Aliquots of 35 µL and 45 µL *bar* and *HaHB4v* probes, were diluted by adding 65 µL and 55 µL, respectively, of 1x TE buffer. The probe solutions were

incubated at 95°C for 10 min and cooled to 4°C for 2 min. They were added to 8.75 mL of Roche DIG EasyHyb hybridization buffer and poured to the bottom of the hybridization bottle. The membranes were incubated at the described hybridization temperatures for 16 hours in a hybridization oven (model 5420, VWR scientific products, Radnor, PA) under constant rotation.

Washing and Detection: After hybridization, the membranes were washed with washing buffer according to instructions provided with the Roche DIG Luminescent Detection Kit. After 1 hour blocking with 1x blocking reagent, each membrane was incubated for 30 min in a solution containing 50 mL of 1x blocking reagent and 5 µL of anti-digoxigenin-AP. The membranes were washed twice with washing buffer for 30 minutes each and treated with detection buffer for 5 minutes. Each membrane was then placed in a separate KPL Hybridization Bag (Cat. No. 60-00-51, KPL, Gaithersburg, MD). 5 mL of CSPD solution from the DIG Luminescent Detection Kit was applied evenly across the membrane and the bags were heat-sealed. The membranes incubated with CSPD solution for 5 minutes at room temperature (25°C) then 15 minutes at 37°C. The hybridization bag was placed in a cassette with Kodak Biomax Light Film (Cat. No. 178 8207, Rochester, NY) in a dark room and exposed for 20 minutes. The films were developed in the dark using a Konika QX-60A X-ray film processor. Subsequent exposures were made at 1 hour or 2 hours as necessary.

Illumina Junction Sequence Analysis (JSA) and IND-00410-5 TDNA sequence

DNA Samples: Seeds from 5 individuals were used to prepare embryo axes. DNA was isolated from embryo axes using a CTAB method (Doyle and Doyle 1987). The DNA integrity was checked by agarose gel electrophoresis. PCR confirmed the presence of the *HaHB4v* transgene. DNA samples were quantified using Quant-iT™ PicoGreen® dsDNA Reagent (Invitrogen) following the manufacturer's instructions.

Tru-Seq DNA Library preparation: DNA libraries were prepared using TruSeq DNA LT Sample Prep Kit (Illumina, San Diego). The total DNA (2µg) was fragmented by nebulization with nitrogen gas at 30 psi for 6 minutes. The resulting nebulized DNA was purified using QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions.

An end repair reaction was done using an End Repair Mix for 30 min at 30°C. A single 'A' nucleotide is added to the 3' ends of the blunt fragments using an A-Tailing Mix for 30 min at 37°C. Finally, DNA Adapter Indices were ligated.

A clean up step to remove small fragments was done using the Agencourt AM Pure XP system (Beckman Coulter) following the manufacturer's protocol. PCR was used to selectively enrich those DNA fragments that have adapter molecules on both ends and to amplify the amount of DNA in the library. After a final "clean up" step the libraries were eluted in 30 µl of resuspension buffer.

Library Quality control: TruSeq DNA libraries were quantified using Quant-iT™ PicoGreen® dsDNA Reagent (Invitrogen) and by qPCR using Library Quantification Kit - Illumina/LightCycler® 480 (KAPA) according to the manufacturer instructions.

Cluster generation: Cluster generation was performed on the cBOT (Illumina) using PE_Amp_Lin_Block_Hyb_v8.0 program. Libraries were included in the flow cell as follows:

Sequencing run. A high output run was performed on HiSeq1500 (Illumina) during 10 days to obtain paired ends reads PE 2x100bp. A total of 387.9Gb of high quality data were produced, 88.1% of bases with a quality score \geq Q30.

Standard operation procedure for junction sequence identification of plasmid elements on *Glycine max* genome. The SOP is based on the Junction Sequence Analysis work by Kovalic *et al.* (2012). The steps required to perform the analysis are described below.

The quality control (QC) of the library was performed using the Agilent 2100 Bioanalyzer. The average length of the library fragments obtained was around 600 bp, but a buffer amount was added and a value of 1000 bp was used for subsequent bioinformatics analyses.

Glycine max (soybean) genome was downloaded from the Illumina iGenomes webpage (Illumina 2014).

A well-known single copy locus, Lectin 1 (*le1*) was selected from the soybean genome to estimate the effective sequence coverage depth for each sample sequenced (NCBI 2014). This NCBI Reference Sequence was originally located at the following link:

<http://www.ncbi.nlm.nih.gov/nuccore/353336029/?from=1123511&to=1125662>

Both sequences (soybean genome in single FastA format file and the *le1* gene) were indexed for further analyses using BLAST (Altschul *et al.* 1990) and Bowtie2 (Langmead and Salzberg 2012).

pIND2-HB4 sequence (Figure IV.A.) was “circularized” by adding 200 bp of the sequence at its end. Also, two different annotation files were created for the plasmid, a GFF3 (Stein 2013) format file and a tab-separated value file (with the following fields: plasmid element, starting position, ending position).

Two lanes of the Illumina HiSeq 1500 sequencer were used to sequence the IND-00410-5 DNA, achieving a coverage between 30x and 70x. Reads were set in two gzipped FastQ formatted files, one per read type of the pair-ends (read 1 and read 2).

FastQ files were uncompressed (keeping the original files) and converted to FastA format for further analyses (ie., BLASTn or sequence extraction).

The effective sequence coverage depth and its standard deviation estimates were obtained by aligning the reads against *le1* with Bowtie2 and then running a local script for coverage and standard deviation calculation.

Sequence alignment (Bowtie2) against the plasmid was performed to identify plasmid elements present in IND-00410-5 DNA. The alignment was visualized with the Integrative Genomics Viewer (IGV) browser (Thorvaldsdóttir *et al.* 2012) and with the Tablet browser (Milne *et al.* 2013). To determine the effective sequence coverage, the values observed in this step were normalized with the values obtained in the previous step.

Reads for JS (junction sequences) analysis were selected on the basis of sequence similarity to the transformation plasmid using the local alignment software BLAST All. Reads with a match to the query sequence having an e-value of 1×10^{-5} or less and having a match length of at least 30 bases with at least 96.7% sequence identity were collected. The selection criteria have been established as providing the best possible sensitivity and specificity (Kovalic *et al.* 2012).

A modified version of the custom Perl script developed by Kovalic *et al.* (2012) was used to identify the junction points on the transformation plasmid and their supporting junction reads. For each junction position, all supporting junction reads were aligned at the 30 bases proximal to the junction position. The remaining bases of these reads were sorted to show the alignment and the consensus of the flanking junction sequences past the junction point.

Possible junction points were manually analyzed based on the output files obtained in the previous step. The files are described below:

a) JSC_ACCEPT.txt: contains a list of all possible junction points, reporting the position in the plasmid, the number of reads that support the possible joint and a message that suggests if each point should or should not be accepted. It should be noted that the protocol is tuned to identify a single insertion site (that is, two junction points). However, if there is more than one insertion site, each of the joints must be evaluated to see how they match to each other.

b) JSC_DETAILS.txt: contains a description of each of the junction points, informing the insertion position in the plasmid, the element that interrupts, the reads that support that joint and its alignment, showing the 30 bases proximal to the junction position plus the remaining bases past the junction point.

c) Tails vs. genome. BLASTn: Contains the result of running a BLAST between the junction sequence past the junction point against the soybean genome in order to identify the insertion site in the reference genome. The parameters to be observed here are: percent identity that should be 90% or higher; the alignment length that should be 60% or higher; and the subject id that shows the chromosome where the possible joint occurred.

d) Tails vs. plasmid BLASTn: contains the result of running a BLAST between the junction sequence before the junction point against the transformation plasmid thus confirming that the position within the plasmid is unique and the direction in which the insertion occurs.

T-DNA stability over sexual transfer

PCR reactions:

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Amplification reaction:

| | |
|-------|-----------------------------|
| 2µL | genomic DNA (200ng/µl) |
| 3.5µL | Dd (H ₂ O) |
| 10µL | qPCR SuperMix (2X) |
| 0.8µL | Native Probe 936 (10µM) |
| 0.8µL | Native Primer F 934 (10µM) |
| 0.8µL | Native Primer R 935 (10 µM) |

20µL

Amplification program:

| Temp. (°C) | Time (min) | Ramp rate (°C/s) | |
|------------|------------|------------------|------------------|
| 95 | 5 | 4.4 | Denaturation |
| Temp. (°C) | Time (s) | Ramp rate (°C/s) | |
| 95 | 10 | 4.4 | 35 cycles - qPCR |
| 55 | 30 | 2.2 | |
| 72 | 10 | 4.4 | |
| Temp. (°C) | Time (min) | Ramp rate (°C/s) | |
| 40 | 5 | 1.9 | Cooling |

PCR of Left Border Junction: oligonucleotides 868 and 752 amplify a chimeric fragment of 356bp (Figure 4-12) formed by right flanking sequences (soybean genome, “Gm chr9”) and part of the IND-00410-5 construct (“vector”).

Primer sequences:

868: 5' CCGCAATGTGTTATTAAGTTGTC 3'

752: 5' GGCTGCAAGTTTTGGTCAAT 3'

Amplification reaction:

| | |
|-------|--------------------------|
| 1µL | Genomic DNA (300ng) |
| 2µL | PCR Buffer (10X) |
| 1.6µL | MgCl ₂ (25mM) |
| 0.8µL | RBJ Primer F 868 (10mM) |

| | |
|---------|-------------------------|
| 0.8μL | RBJ Primer R 752 (10mM) |
| 0.4μL | dNTPs (10mM) |
| 0.16μL | Taq polymerase |
| 13.44μL | ultrapure water |
| <hr/> | |
| 20μL | |

Amplification program:

94°C
4min

94°C
30sec

60°C
30sec

35 cycles

72°C
30sec

4°C
2min

Statistical analysis

A Chi-square (χ^2) test was used to verify the F₂ 3:1 phenotype segregation (df=1, $\alpha=0.05$) and the 1:2:1 genotype segregation (df=2, $\alpha=0.05$).

Results and Discussion

Number of T-DNA inserts, chromosome allocation of T-DNA, and flanking sequences.

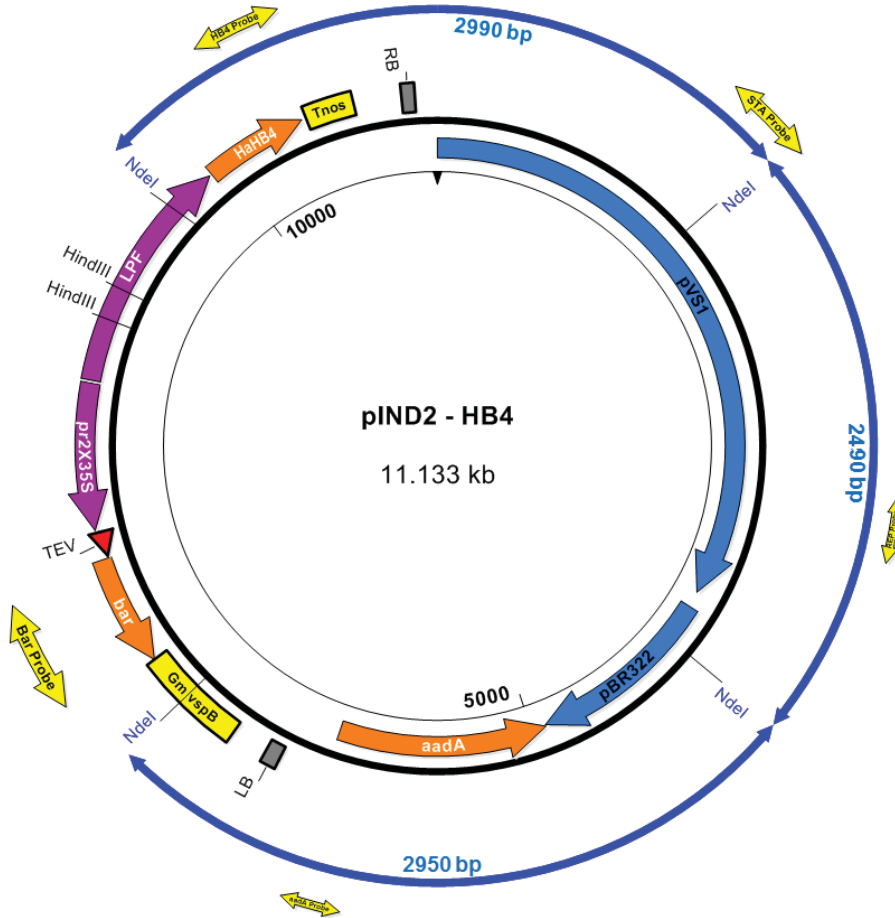
a) Southern blot analysis. The number of T-DNA inserts was determined in homozygous T5 IND-00410-5 plants by Southern blot analysis. The DNA from this event was digested with two restriction enzymes: *HindIII* and *NdeI*. There were two *HindIII* sites in the T-DNA, located near each other. No other *HindIII* site was present in the plasmid backbone (Figure 4-2). Assuming the occurrence of a single, intact T-DNA in the *HindIII* digested genome of IND-00410-5, the minimum fragment size detected by hybridization of the *HaHB4v* probe would be 1.85 kb. The probe for the selectable *bar* gene, on the other hand, would detect digests extending over the left border into the soy genome. These fragments should be longer than 2.6 kb (Figure 4-2, bottom). In the Southern blot presented in Figure 4-3 (blot a), the highlighted fragments showed the expected sizes and were consistent with a single T-DNA insert.

There are four *NdeI* restriction sites in the construct (Figure 4-2, top), two within the T-DNA and two in the binary vector. Complete *NdeI* digestion in the T-DNA releases a DNA segment of 2703 bp that contains the binding target for the *bar* probe. The *HaHB4v* probe detects a DNA fragment of a minimum size of 1.35 kb, assuming a single, intact T-DNA (as above). The hybridizing band in the *NdeI* digest

was longer than 8.6kb (Figure 4-3, blot a), which is consistent with the presence of a single, intact T-DNA insert. The hybridizing band corresponding to the bar probe was present in the HB4 soybean event but not Williams 82, as expected (Figure 4-3, blot b).

Figure 4-2. Map of the plasmid pIND2-HB4.

Binary vector plasmid with the T-DNA. Top figure: the fragments resulting from *NdeI* digestion are indicated as blue Left Right arrows along with their sizes. LB, left border; RB, right border. Probes are presented in yellow thick Left Right arrows. Bottom figure: detailed T-DNA portion of the pIND2-HB4 plasmid. *HindIII* digest fragments are presented as black arrows along with their minimum respective sizes to the LB and RB, respectively. *NdeI* digest fragments are presented in blue.



Detailed portion of pIND2-HB4 T-DNA.

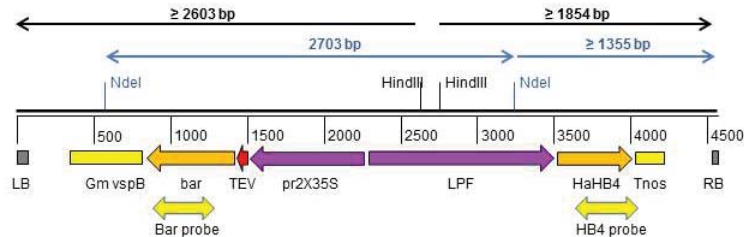
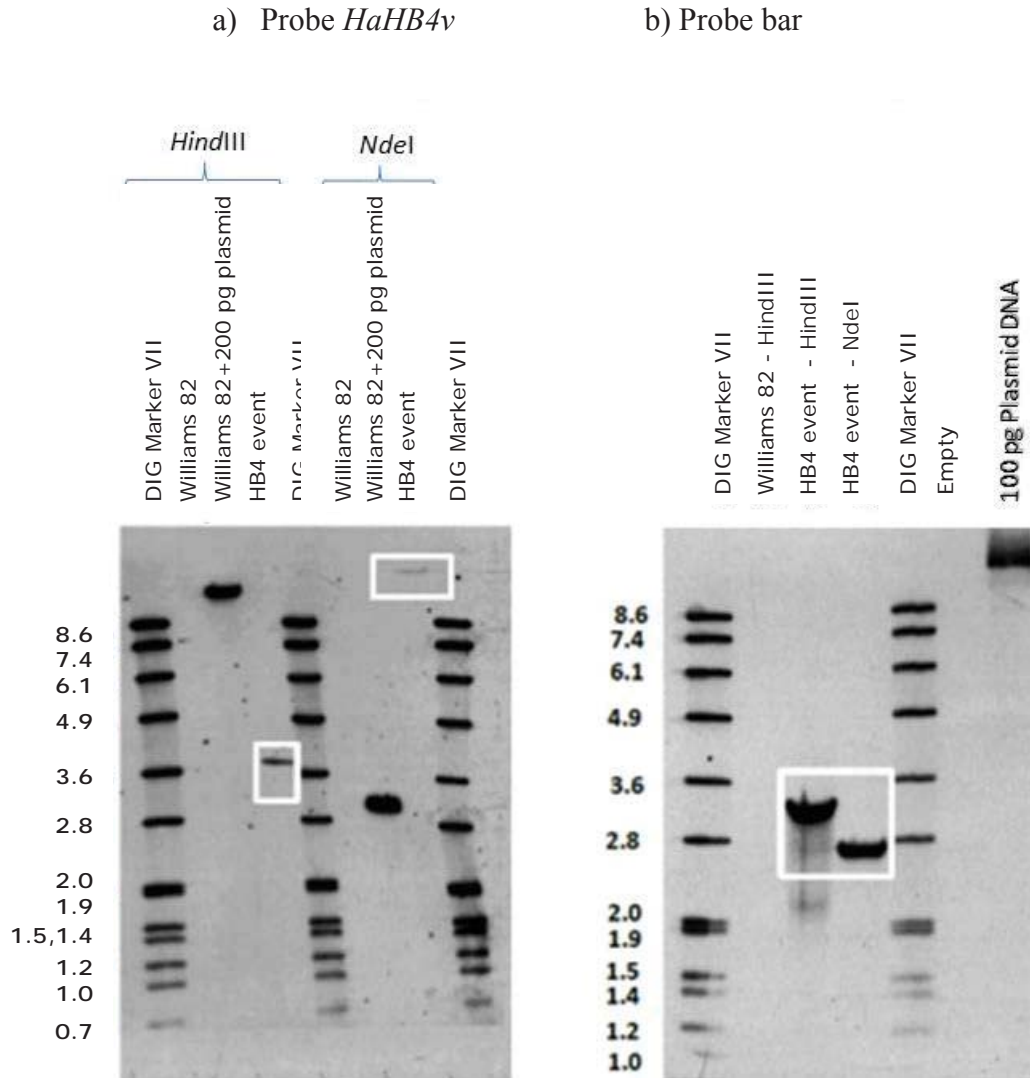


Figure 4-3. Southern blots of T5 IND-00410-5 plant DNA digested with *Hind*III and *Nde*I.

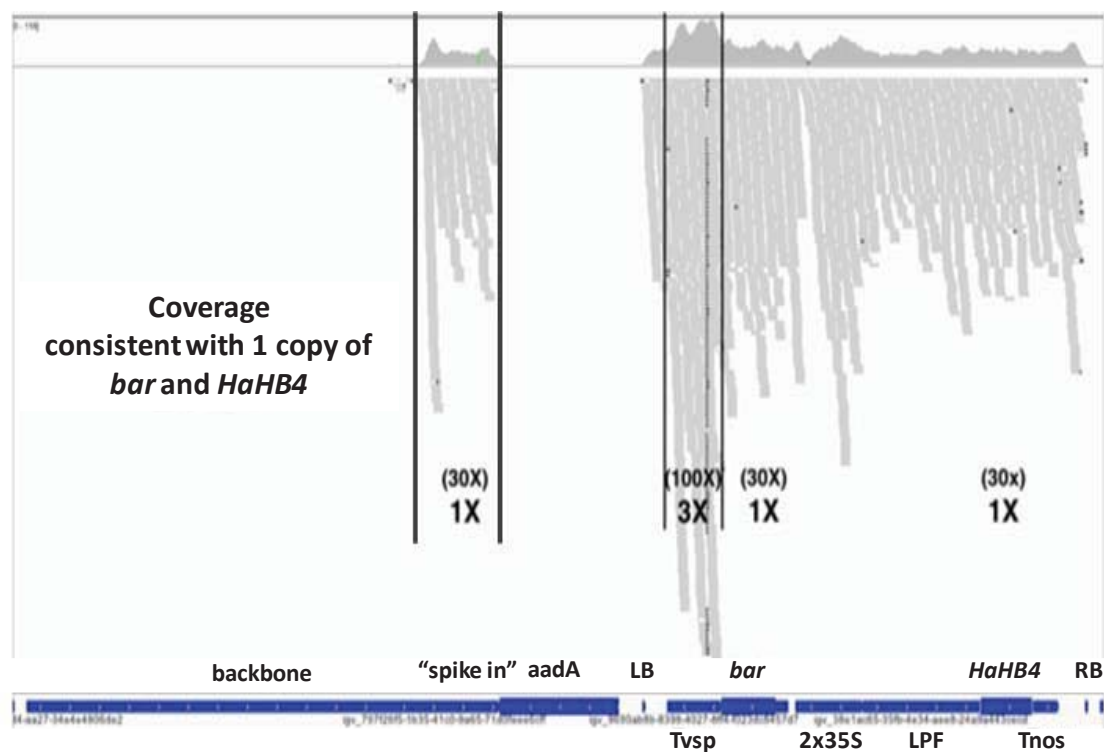
Blots were hybridized with DIG-labeled probes for a) *HaHB4v* and b) *bar* detection, respectively. DNA bands in IND-00410-5 digests hybridizing to the indicated probes are highlighted in white boxes. The HB4 *Nde*I band is faint as often occurs with large bands on a hybridized gel blot picture. Williams 82 + 200 pg plasmid DNA and 100 pg plasmid DNA were used as positive controls to demonstrate detection, not size. DIG-labeled Marker VII ladder band sizes are indicated on the left of the blots in kb.



b) Junction sequence analysis (JSA) and IND-00410-5 T-DNA sequence. The results from the Southern blot, concluding a single T-DNA insert, were confirmed by mapping the Illumina-generated sequence data against the whole sequence of the plasmid vector used for transformation. Results are presented in Figure 4-4.

Figure 4-4. Event IND-00410-5. DNA sequence reads were mapped against the complete plasmid vector sequence of pIND2-HB4 (shown in blue).

The total read coverage is presented in parentheses immediately above the normalized read coverage for each element in the plasmid vector. The normalized read coverage provided an estimate of the copy number of each element of the transformation vector present in the IND-00410-5 genome. Mapped sequence reads between the positions labeled “backbone” and “aadA” correspond to a sequence identical with control DNA included in the sequencing run to check for the sequencing error rate—this is labeled “spike in”. The resulting coverage of mapped reads supported the conclusion that no backbone elements from the vector were present in IND-00410-5. The 3X normalized coverage of the Tvsp element (soybean vegetative storage protein terminator) is due to the additional reads generated from at least two endogenous copies of the element in the soybean genome, with additional interference of other matching sequences found elsewhere in the genome. A BLAST search of the Tvsp element sequence on <http://soybase.org/> shows a single sequence and several other matching sequences which can generate multiple separate reads (indicating two or more copies in NGS).



The y-axis represents the sequencing coverage depth. The length of the gray bars in the x-axis represents the length of each read, and the length of the gray bar on the y-axis shows how many reads are mapping into a genomic position. The depth of coverage isn't uniform across a genomic region. For example in the Tvsp gene there are nucleotide positions with a coverage of 60x, others with a coverage of 102x and so on. On average the whole gene has a mean coverage of approximately 100x. The same happens with other genes, the mean coverage is around 30x, but some regions

have a higher coverage around 50x (and also others with less than 30x), which can be misleading in the above figure. However, the objective of demonstrating single copies of the *bar* and *HaHB4v* genes are present in HB4 was achieved.

The Junction Sequence Analysis (JSA) of IND-00410-5 using the Illumina-generated sequence data was consistent with the integration of a single T-DNA copy at a single locus and a single T-DNA. This result was supported by the finding of only two junction sequences in the whole-genome sequencing of IND-00410-5. The Junction Sequences (JS) were named after the chromosome in which the T-DNA is integrated. The positions in the soybean genome according to SoyBase data are JS-9L-32743826 and JS-9R-32743683. The JSA is presented in Figure 4-5.

Figure 4-5. Junction Sequence Analysis of event IND-00410-5.

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The complete sequence of the T-DNA insert and its flanking soybean sequences (Figure 4-6) were assembled *de novo* from the Illumina-generated DNA sequence reads.

Figure 4-6. Sequence of the T-DNA locus.

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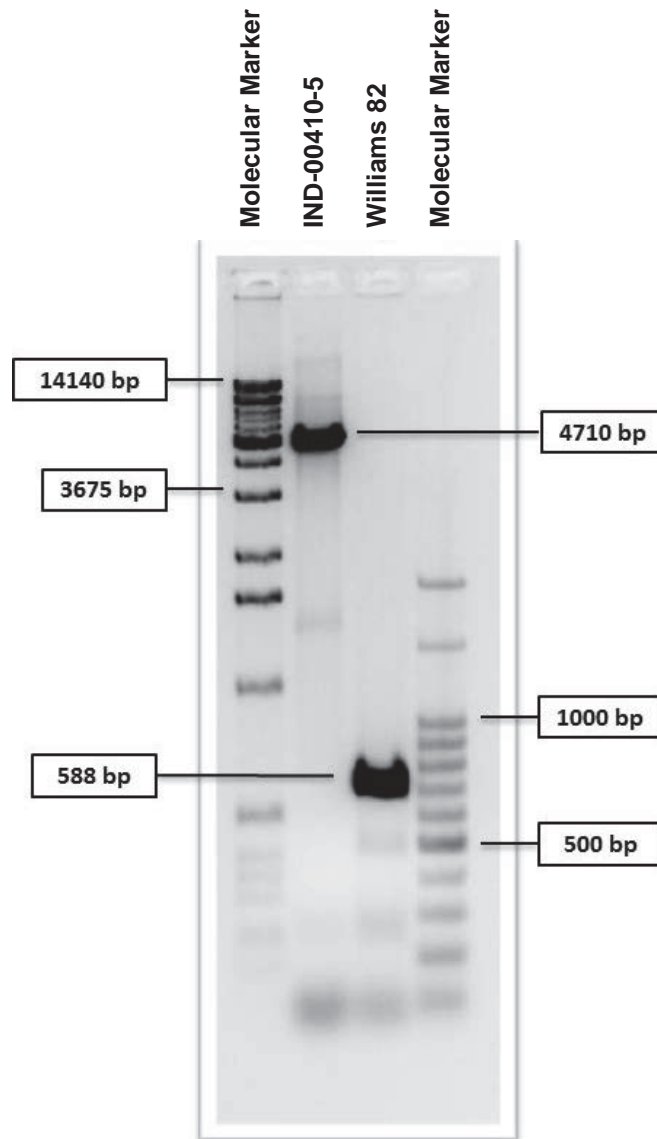
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The sequence of the T-DNA insert in event IND-00410-5 was identical to the sequence of T-DNA in the binary vector, with a single copy of each gene and each regulatory element (Figure 4-4; Figure 4-5, bottom figure). Conventional Sanger sequencing of multiple amplicons covering the whole insertion and its flanking sequences corroborated the JSA analysis of the Illumina-generated sequence. In addition, it was possible to generate a single amplicon of 4710 bp, using Expand Long Range PCR, with a set of primers complementary to the flanking sequences. The amplicons obtained using IND-00410-5 or Williams 82 DNA as templates were of the expected sizes (Figure 4-7) and the DNA sequence of this IND-00410-5 amplicon was identical to the T-DNA sequence derived from whole-genome sequencing.

Figure 4-7. Amplicons obtained from IND-00410-5 and Williams 82 DNA.

The primer pair 750-752 (see Material and Methods, Table 4-1), specific to the soybean sequences flanking the T-DNA insertion, generated amplicons of expected sizes with IND-00410-5 (4710 bp-lane 2) and Williams 82 (588 bp-lane 3) template DNA. The Williams 82 sample is overloaded with insufficient buffer concentration to create a thorough negative charge on the DNA and therefore is running slower than the expected size. Selected DNA band sizes are indicated as number of nucleotide base pairs (bp). The faint bands smaller than the labelled bands in each IND-00410-5 and Williams 82 are spurious bands and not related to the target.



c) Localization of IND-00410-5 T-DNA in the soybean genome. The flanking sequences were mapped to the soybean genome by homology search using BLASTn (Altschul *et al.* 1990). [CBI deleted

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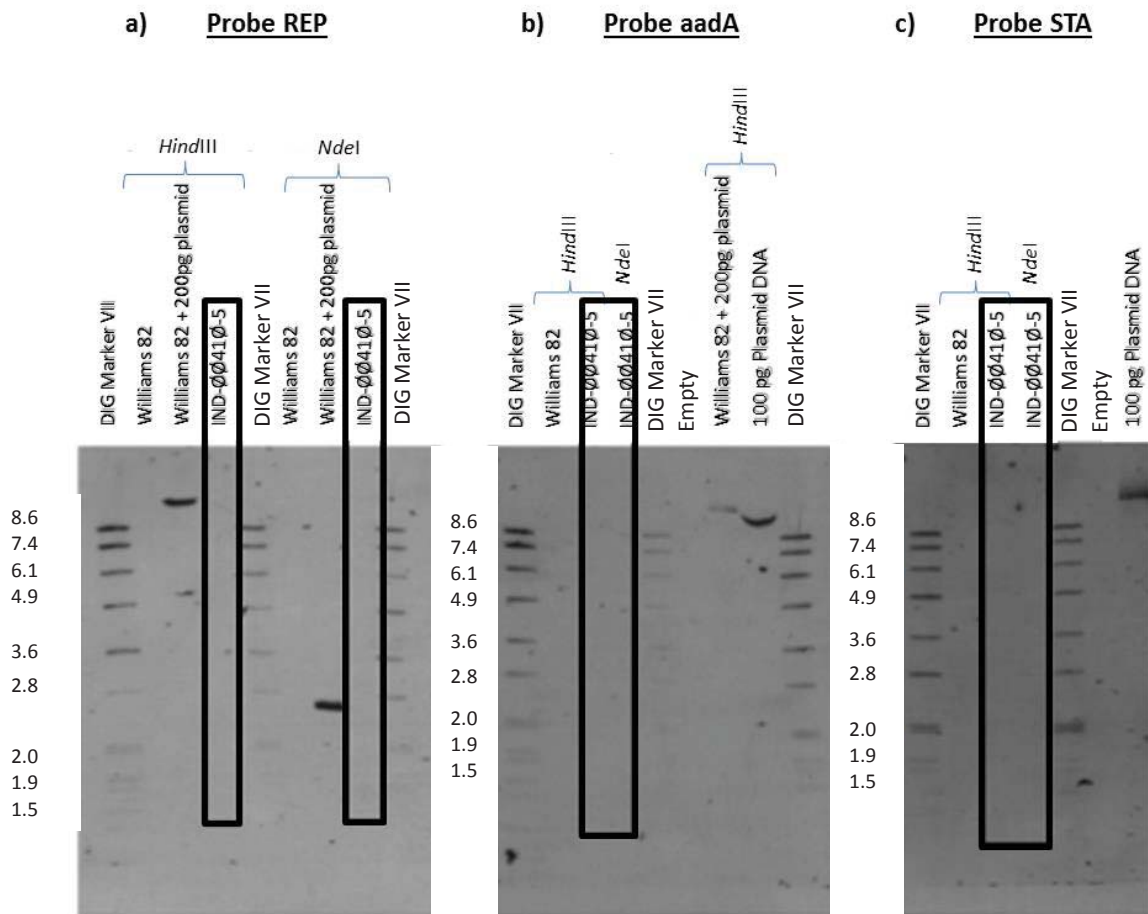
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d) Absence of binary vector elements. In principle, *Agrobacterium* should only transfer into the host cells its T-DNA the portion of the plasmid contained between the left border (LB) and right border (RB) sequences. However, it has been reported that *Agrobacterium* can also transfer a portion of or even the whole T-DNA binary vector, often referred to as backbone (De Buck *et al.* 2000). Assays for the presence of such unintended DNA in the HB4 soybean genome indicated that none was present.

The absence of vector backbone sequences in the IND-00410-5 event was determined unequivocally with the whole-genome sequence obtained with the Illumina NGS method. And while this set of data should be considered sufficient to support the conclusion, DNA blot analyses were employed to provide a second assay for vector backbone sequences. None of these probes for vector backbone sequence (aadA, STA, and REP; see Figure 4-2, top) hybridized to IND-00410-5 DNA (Figure 4-9).

Figure 4-9. DNA gel-blot analysis to test for the presence/absence of vector backbone DNA in event IND-00410-5.

Genomic DNA from leaves of either T5 generation event IND-00410-5 or non-transgenic control Williams 82 was digested with *Hind*III and *Nde*I and hybridized with DIG-labeled probes for a) REP, b) *aadA*, and c) STA sequence. Williams 82 + 200 pg plasmid DNA and 100 pg plasmid DNA were used as positive controls. DIG-labeled Marker VII ladder band sizes are indicated on the left of the blots in kb. Rectangles highlight lanes showing no probe hybridization signal in event IND-00410-5, any dark areas represent background from the process.



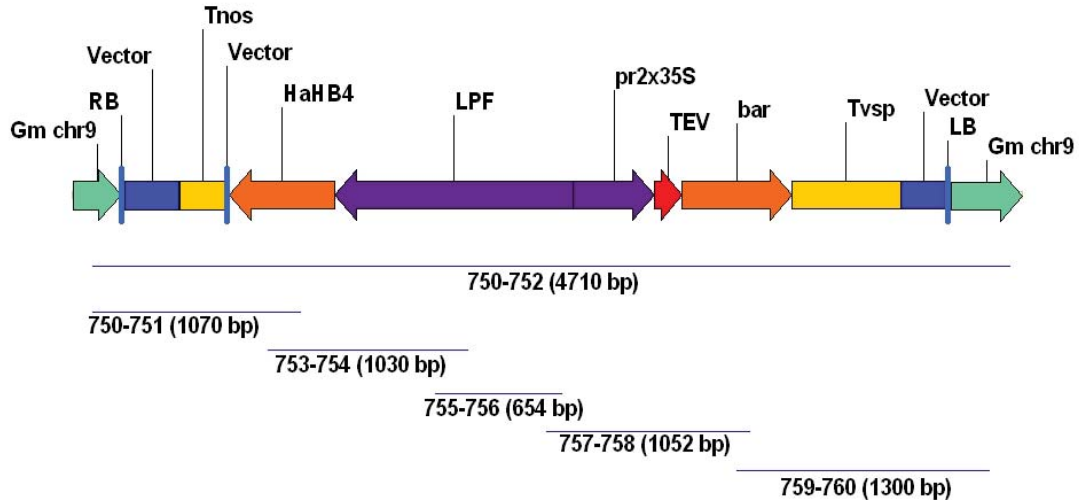
Stability and Integrity of the HB4 T-DNA

a) IND-00410-5 locus stability and T-DNA integrity. The position of the IND-00410-5 T-DNA across six generations was monitored. The scheme is summarized in Figure 4-10. A set of PCR primer pairs was selected to provide overlapping amplicons across the insertion locus of the IND-00410-5 T-DNA, inclusive of the soybean chromosome 9 flanking sequences. Sequences of the contigs assembled from these overlapping PCR products (Figure 4-10) suggested that the T-DNA was intact and stable. The organization of the genetic elements in the IND-00410-5 soybean event was the same as the one present in the binary vector T-DNA used in transformation to obtain this

transgenic line. No changes in DNA sequence were detected across six tested generations (Figure 4-11).

Figure 4-10. Strategy for conventional Sanger capillary sequencing of the IND-00410-5 T-DNA and flanking sequences in chromosome 9.

Blue lines indicate intended overlapping amplicons labeled with primer pair number (see Material and Methods, Tables 4-2 and 4-3) and the amplicon size, in parentheses. Gm: Glycine max. chr9: chromosome 9.



IND-00410-5

Figure 4-11. IND-00410-5 T-DNA with soybean chromosome flanking sequences.

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b) T-DNA segregation over sexual transfer. Segregation of the T-DNA was assessed in F₂ progeny plants from crosses between IND-00410-5 and a commercial soybean cultivar (Bio 6.5) using PCR. A set of PCR reactions, diagnostic of the T-DNA junction at the Left Border and of the native soybean allele, clearly showed that the T-DNA segregates as a single locus (Table 4-3, Figures 4-4 and 4-11).

A homozygous IND-00410-5 transgenic plant was crossed with Bio 6.5 to produce the F₁ progeny. Four F₁ plants were self-pollinated to produce F₂ seeds that were used for the segregation analysis. The DNA isolation and the corresponding experiments were conducted using 73 F₂ seeds. The summary of the data is shown in Table 4-4. F₂ plants were scored as homozygous for the IND-00410-5 T-DNA (I) when the amplicon for the Left Border Junction was present and the amplicon for the native allele was absent. F₂ plants were scored as hemizygous (H) when both amplicons described above were present. F₂ plants were scored as homozygous for the native Williams 82 cultivar allele (W) when the amplicon for the Left Border Junction was absent and the amplicon for the native allele was present.

Figure 4-12. Schematic representation of IND-00410-5 insertion locus and native allele.

Upper figure: scheme of the insertion in IND-00410-5 showing the elements present in T-DNA and primers used for analysis of segregation in F₂ plants. The labeled primers 868 and 752 were used to assay for the presence of the Left Border Junction. Lower figure: scheme of native allele showing the elements present in insertion region (without the T-DNA) and primers used for PCR test of segregation in F₂ plants. Primers 934 and 935 were used to assay for the presence of the native allele. Gm: Glycine max, Chr9: chromosome 9, UTR: untranslated region, CDS: coding sequence.

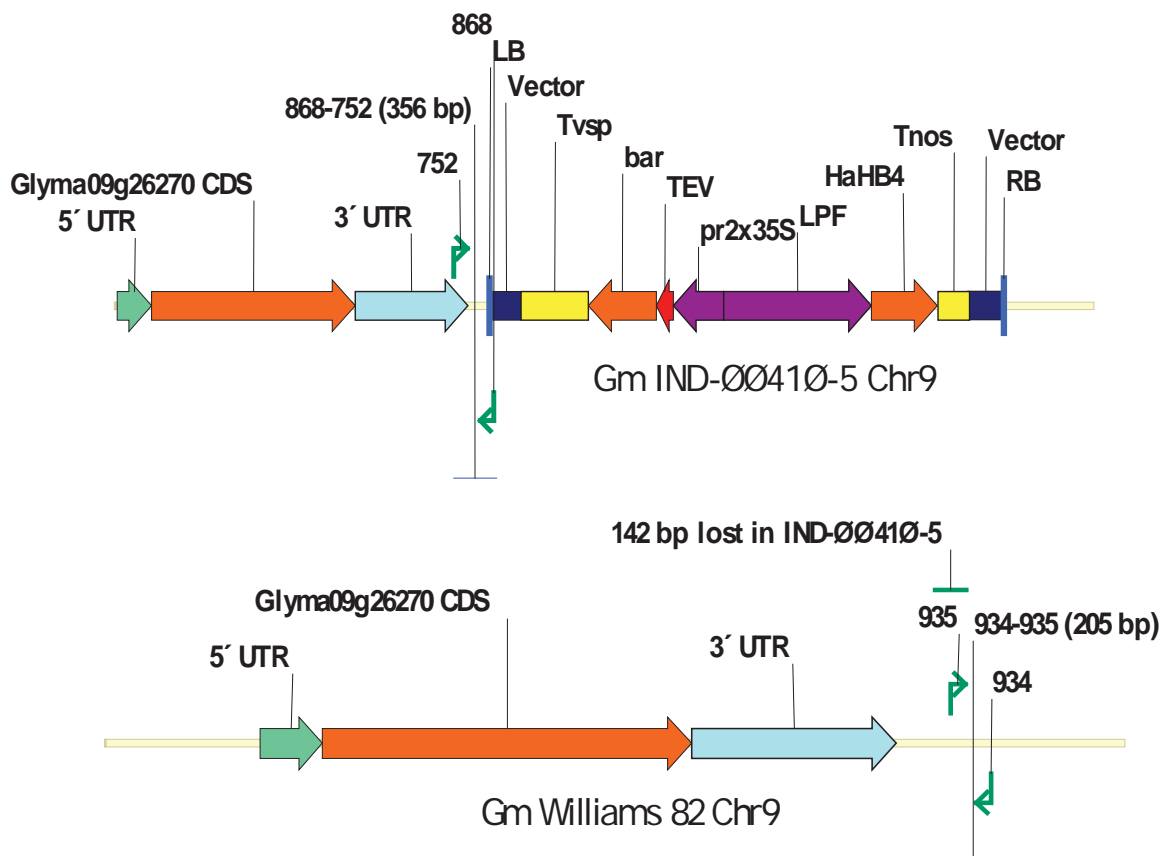


Table 4-4. Analysis of segregation of IND-00410-5 T-DNA in F₂ plants.

The χ^2 value (df=2, $\alpha=0.05$) indicated no statistically significant difference between the observed data and expected 1:2:1 (I:H:W) genotypic segregation. I: IND-00410-5 homozygous; H: hemizygous; W: Williams 82 homozygous.

| Expected Genotypes (Number of Plants) | | | Observed Genotypes (Number of Plants) | | | χ^2 | p-value |
|--|------|-------|--|----|----|----------|---------|
| I | H | W | I | H | W | | |
| 18.25 | 36.5 | 18.25 | 17 | 39 | 17 | 0.34246 | 0.8426 |

These results support the conclusion that the IND-00410-5 T-DNA resides at a single locus within the soybean genome and is inherited according to Mendelian inheritance principles.

Conclusions.

The selected transgenic event IND-00410-5 differs from the parental Williams 82 by a single T-DNA. This T-DNA carries a single copy of the selectable marker-gene *bar* and a single copy of the *HaHB4v* gene along with their regulatory sequences. The T-DNA was integrated in chromosome 9 between two native genes coding for an F-box At1g60400-like protein and an AtL6-like E3 ubiquitin ligase. The integration did not disrupt any known gene or annotated sequence but caused the loss of a 142 base pair sequence corresponding to an intergenic region. The locus of the insertion and the T-DNA structure were stable over six generations of self-pollination. The T-DNA insertion was shown to segregate in a Mendelian fashion following outcrossing. No sequences outside the T-DNA border were integrated into the IND-00410-5 event.

E. Appendix 5. Bioinformatic Analysis of HB4 Soybean Putative Peptides.

Introduction

Exposure to new allergenic or toxic substances is one of the major issues in the safety assessment of genetically-engineered new crops.

In addition to the new intended expression products, modifications introduced by the inserted sequences could shift existing open reading frames (ORFs) and/or create new ones. For this reason, the Codex Alimentarius (2009) extends the characterization of the genetic modification to include the “identification of any open reading frames (ORF) within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins” (Codex Alimentarius 2009).

In order to identify any putative expression products derived from these new ORFs, the insert and its 5′ and 3′ genomic DNA junctions are searched for start and stop codons along the six possible reading frames. The hypothetical translation products derived from this search are then analyzed to assess if any of these pose safety concerns involving allergenicity or toxicity potential.

Materials and Methods

Bioinformatic analyses were performed over a sequence encompassing the insert plus 200 bp of genomic DNA both 5′ and 3′ of the insert junctions.

To detect ORFs, the sequence was examined to find any start (ATG) and stop (TAA, TGA or TAG) codon using a local script developed using BioPython tools (<http://www.biopython.org>). All peptides of eight amino acids or greater in length were considered for analysis although only those with 100 residues or longer are included in Figure 5-1 and Table 5-1.

Homologies of putative peptides with any known protein were assessed using the BLASTp (Altschul *et al.* 1990) algorithm and the NCBI protein database (<http://www.ncbi.nlm.nih.gov>). Similarity was evaluated by the E score value, taking 1×10^{-5} as the cut off for genuine alignment (Doolittle 1990).

Allergenicity of each putative peptide was assessed by comparing its sequence with those of the allergens in the FARRP (FARRP 2016) database (AllergenOnline, <http://www.allergenonline.org>). Version number 16 of this database, updated in January 27 2016, includes the sequence of 1956 allergens. Sliding windows of both 80 and 8 contiguous amino acids from each putative peptide were assessed to detect homologies with allergens or identity with allergenic epitopes, respectively.

For toxicity analysis, the BLASTp algorithm (Altschul *et al.* 1990) was used to search for sequence homologies between each putative peptide and the 3844 toxins included in the ATDB (Animal Toxin Data Base, College of Life Sciences, Hunan Normal University) (He *et al.* 2008). Similarity was evaluated by the E score value, taking 1×10^{-5} as the cut-off for alignment significance.

Results and Discussion

The bioinformatic search for new ORFs created in a region encompassing the insert plus 200 bp of genomic DNA both 5' and 3' of each junction rendered seventy-four peptides having from 8 up to 177 amino acids in length. The complete description of each of these peptides is included in Table 5-2. All peptides were subjected to bioinformatic analysis for allergenicity and toxicity. For clarity, only seven of these, those having 100 amino acids or longer (Table 5-1) are described in Figure 5-1.

This search has shown that no new ORFs were created into the soybean genome as a consequence of the inserted DNA. However, two putative ORFs pre-existing in the soybean genome were modified by the insertion. Only one of them (peptide 15, 103 amino acids long) is displayed in Figure 5-1. The remaining putative peptides originate within the insert sequence.

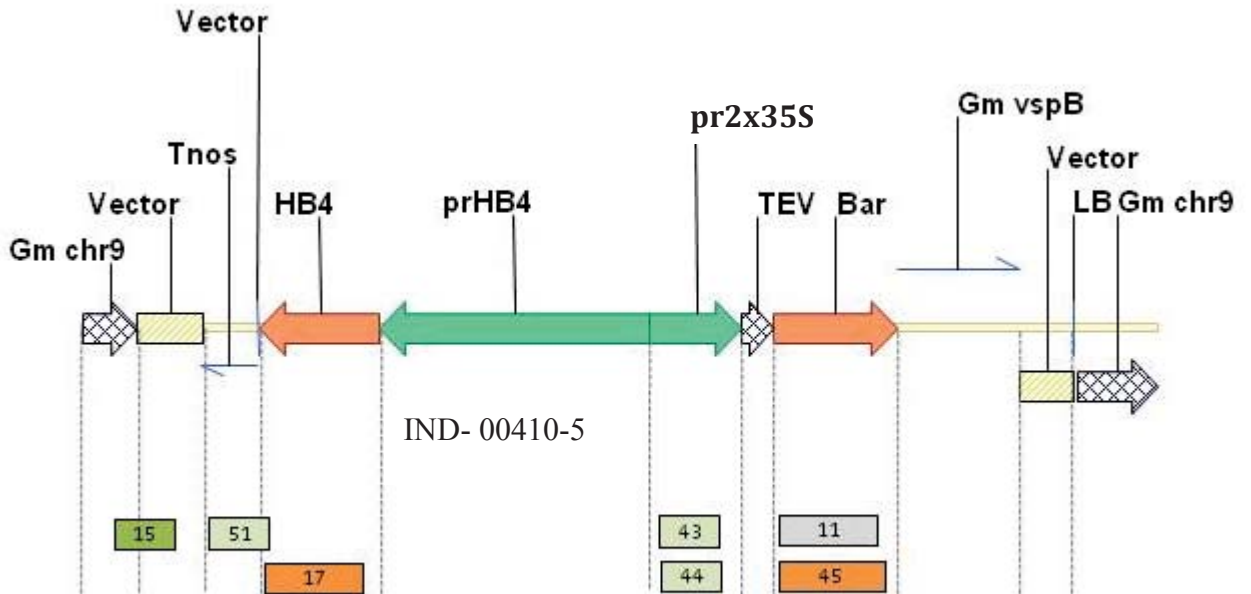
Homology of the putative peptides with any known protein was examined using the BLASTp algorithm (Altschul *et al.* 1990) and the NCBI protein database. Similarities were only found with hypothetical or putative proteins and a pair of cloning vectors (Table 5-1). Peptides 43 and 44 fall into the duplicated 35S promoter region and are identical to each other.

Bioinformatic analyses were performed to assess the potential toxicity or allergenicity of all the putative peptides.

No sequence identity greater than 35% was found with any allergen in the FARRP database when a sliding window of 80 contiguous amino acids from each putative peptide was analyzed. Similarly, no identity was found when a sliding window of 8 contiguous amino acids was aligned with allergenic epitopes in the database.

Figure 5-1. Location of putative peptides.

Boxes under the construct indicate the position of putative peptides with more than 100 amino acids along the insertion and flanking regions.



The assessment of the potential toxicity of the putative peptides by the BLASTp algorithm (Altschul *et al.* 1990) search for sequence homology with toxins included in the animal toxins database (ATDB, He *et al.* 2008) did not show any significant homology (E score $< 1 \times 10^{-5}$).

It must be emphasized that there is no experimental evidence indicating that transcription of the above putative ORFs indeed occurs. However, results included in this report suggest that in the highly unlikely event that any of the above sequences were to be transcribed and eventually translated, the resulting products would not be considered an allergenicity or toxicity risk based on a lack of sequence similarity to known allergens and toxins. Accordingly, we conclude that these results obviate the need to perform further northern blot experiments for the search of putative transcripts.

Table 5-1. Putative peptides from soybean IND-00410-5 event having 100 or more amino acids.

| Peptide ID | Frame | Start | Stop | AA | Amino acid Sequence | Best hit with NCBI database entries | Query cover | Identity |
|------------|-------|----------|----------|---------|--|--|-------------|----------|
| 11 | -3 | 339 4 | 389 7 | 16 7 | MPVPVLEAGRPPQHAAGGISERLIVHAH ARVVGQDDSDHLEALCLQLQVVG VERGAQSRPLVAGDVGRLGRPVVVG VACLPGARVGDAGDLAVHLGDEFGIA LPQTDEVVPRLLRFLRLGTEVDRACL DVVVDDGADRRRHVRLGGTADVGRASF WAHGRSPVRKW | None | - | - |
| 15 | -2 | 65 | 376 | 10 3 | MSELTHTINCVALTARFPVVGKPVVPA LMNRPTRGERRFAYWSLSLDQIVVSR LQFKLSVPSIISLHLLYFLLTSLLY YFKKIKSENLRKKNLSQVFLSISVV | Hypothetical proteins from Bacteroides dorei, | 47% | 90% |
| 43 | +1 | 252 7 | 284 4 | 10 5 | MVEHDTLVYSKNIKDVTSEDQRAIET FQQRVISGNLLGFHCPAICHFIVKIV EKEGGSYKCHHCDKGAIVEDASADS GPKDGPPTTRSIVEKEDVPTTSSKQV D | Inclusion body protein from cauliflower mosaic virus | 100% | 100% |
| 44 | +1 | 285 4 | 317 1 | 10 5 | MVEHDTLVYSKNIKDVTSEDQRAIET FQQRVISGNLLGFHCPAICHFIVKIV EKEGGSYKCHHCDKGAIVEDASADS GPKDGPPTTRSIVEKEDVPTTSSKQV D | Inclusion body protein from cauliflower mosaic virus | 100% | 100% |
| 51 | +2 | 668 | 988 | 10 6 | MIITARPATGFNLKLYCQMFERSGK FELLELPPLLKVWQLFFNLQQTVSFF KVRKTVAKRNHFLTKHNVRVRKPI IRFFTAATTSFLLMLFGYISQHLQL IE | Polymerase from rice ragged stunt virus | 27% | 100% |

Peptide ID: peptide identification number in the complete list considering all peptides with at least 8 amino acids; Frame: open reading frame; Start: nucleotide location of start codon; Stop: nucleotide location of the stop codon; AA: amino acid chain length.

Table 5-2. Complete list of putative peptides generated by the soybean IND-00410-5 insert.

| Peptide ID | Frame | Start | Stop | Length | |
|---|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| 1 | -3 | 463 | 498 | 36 | 11 |
| Coding sequence in Nucleotides: ATGTTACTAGATCGGGAATTCGTAATCATGTCATAG | | | | | |
| Coding sequence in Aminoacids: MLLDREFVIMS | | | | | |
| 2 | -3 | 562 | 618 | 57 | 18 |
| Coding sequence in Nucleotides: ATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAA | | | | | |
| Coding sequence in Aminoacids: MTLFMRWVFMIRVPQLYI | | | | | |
| 3 | -3 | 715 | 855 | 141 | 46 |
| Coding sequence in Nucleotides: ATGTTCCGTTTTGCGACGGTTTTGCGTACCTTGAAGAAGGAAACAGTTTTGTTGGAGATTG AAGAACAACCTGCCAGACCTTCAAAAGTGGTGGGAGTTCTAAGAGCTCGAATTTCCCCGAT CGTTCAAACATTTGGCAATAA | | | | | |
| Coding sequence in Aminoacids: MFRFATVLRRTLKKETVCRWLKNNCQTFKSGGSSKSSNFPDRSNIWQ | | | | | |
| 4 | -3 | 856 | 903 | 48 | 15 |
| Coding sequence in Nucleotides: ATGATCGGTTTACGAACCTCTCCGGACGTTATGTTTGGTCAAGAAATGA | | | | | |
| Coding sequence in Aminoacids: MIGLRTLRTLCLVKK | | | | | |
| 5 | -3 | 1657 | 1776 | 120 | 39 |
| Coding sequence in Nucleotides: ATGCTGGGGGCGGGAGTTGAACCTAGGTCCAGTGACGCACCCATGAATTTTTTTTTCTAGG GATGCGAACGAGTGGTTTAACCATACTTTTAAGAGGTGCGATCGGAAATTTTACCTATAA | | | | | |
| Coding sequence in Aminoacids: MLGAGVEPRSSDAPMNFSSRDANEWFNHTFKRCDRKFYL | | | | | |
| 6 | -3 | 1810 | 1974 | 165 | 54 |
| Coding sequence in Nucleotides: ATGAATCCTCACCAGGGAAAACCTCACTCGTCTTACTGGACTATTGGCGCTTCCAAATG GACTACTTGCGAAATTCACCACATTGGGATACACTCGTCTACTGCGGTGAGGTAAAACCC GCTTGGTTCAAGGATCGAACTAGCGATTGCTGCCTACTCGCCTAA | | | | | |
| Coding sequence in Aminoacids: MNPHQGKPSLVLLDYWRFQMDYLNRNSPHWDTLVYCGEVKPAWFKDRSDCCLLA | | | | | |
| 7 | -3 | 1978 | 2016 | 39 | 12 |
| Coding sequence in Nucleotides: ATGTCTCTGAACCGAAGAAAACCTCACTCGTCTACTAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|---|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| MSLNRRKPSLVY | | | | | |
| 8 | -3 | 2578 | 2715 | 138 | 45 |
| Coding sequence in Nucleotides: | | | | | |
| ATGATGGCATTGTAGGAGCCACCTTCCTTTTCCACTATCTTCACAATAAAGTGACAGAT | | | | | |
| AGCTGGGCAATGGAATCCGAGGAGGTTTCCGGATATTACCCTTTGTTGAAAAGTCTCAAT | | | | | |
| TGCCCTTTGGTCTTCTGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MMAFVGATFLFHYLHNKVTDSWAMESEEVSGYYPLLKSLNCPLVF | | | | | |
| 9 | -3 | 2905 | 3042 | 138 | 45 |
| Coding sequence in Nucleotides: | | | | | |
| ATGATGGCATTGTAGGAGCCACCTTCCTTTTCCACTATCTTCACAATAAAGTGACAGAT | | | | | |
| AGCTGGGCAATGGAATCCGAGGAGGTTTCCGGATATTACCCTTTGTTGAAAAGTCTCAAT | | | | | |
| TGCCCTTTGGTCTTCTGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MMAFVGATFLFHYLHNKVTDSWAMESEEVSGYYPLLKSLNCPLVF | | | | | |
| 10 | -3 | 3250 | 3330 | 81 | 26 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCTTGATTGCTTGAGATTGTTTTGTTTTGTATATGTTGTGTTGAGAATTAATTCTCGA | | | | | |
| GGTCTCTCCAAATGAAATGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MLDCLRFVCFVYVVLRIINSRGPLQMK | | | | | |
| 11 | -3 | 3394 | 3897 | 504 | 167 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCCAGTTCCCGTGCTTGAAGCCGGCCGCCCGCAGCATGCCGCGGGGGGCATATCCGAG | | | | | |
| CGCCTCGTGCATGCGCACGCTCGGGTCGTTGGGCAGCCCGATGACAGCGACCACGCTCTT | | | | | |
| GAAGCCCTGTGCCTCCAGGGACTTCAGCAGGTGGGTGTAGAGCGTGGAGCCCAGTCCCGT | | | | | |
| CCGCTGGTGGCGGGGGGAGACGTACACGGTTCGACTCGGCCGTCCAGTCGTAGGCGTTGCG | | | | | |
| TGCCTTCCAGGGGCCCCGCTAGGCGATGCCGGCGACCTCGCCGTCCACCTCGGCGACGAG | | | | | |
| CCAGGGATAGCGCTCCCGCAGACGGACGAGGTTCGTCCTCCACTCCTGCGGTTCTGCGG | | | | | |
| CTCGGTACGGAAGTTGACCGTGCTTGTCTCGATGTAGTGGTTGACGATGGTGCAGACCGC | | | | | |
| CGGCATGTCCGCTCGGTGGCACGGCGGATGTCCGGCCGGCGTTCGTTCTGGGCTCATGGT | | | | | |
| AGATCCCCCGTTTCGTAAATGGTGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MPVPVLEAGRPQHAAGGISERLVHAHARVVGQPDDSDHALEALCLQGLQQVVERGAQSR | | | | | |
| PLVAGGDVHGRLGRPVVGVACLPGARVGDAGDLAVHLGDEPGIALPQTDEVVRPLLRFLR | | | | | |
| LGTEVDRACLDVVVDDGADRRHVRLGGTADVGRASFWAHGRSPVRKW | | | | | |
| 12 | -3 | 4261 | 4395 | 135 | 44 |
| Coding sequence in Nucleotides: | | | | | |
| ATGTTGTCAGGTAATTTATTTGTCAGTCTACTATGGTGGCCATTATATTAATAGCAACT | | | | | |
| GTCGGTCCAATAGACGACGTTCGATTTTCTGCATTTGTTTAACCACGTGGATTTTATGACA | | | | | |
| TTTTATATTAGTTAA | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|---|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| Coding sequence in Aminoacids: | | | | | |
| MLSGNLFVSLLLWWPIILLIATVGPIDDVDFLHLFNHVDFMFTFYIS | | | | | |
| 13 | -3 | 4552 | 4611 | 60 | 19 |
| Coding sequence in Nucleotides: | | | | | |
| ATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MCKKAIKLGARVFPVTTL | | | | | |
| 14 | -3 | 4885 | 4989 | 105 | 34 |
| Coding sequence in Nucleotides: | | | | | |
| CCTCTCAGTTGGGTCAGCCTGAGTGATTTTTTTCTCAAATCAAGAACTTTATTTATAAA | | | | | |
| TCTAACATTATAATATTAATAAAAAACAAATATTAATAATATTCATGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| PLSWVSLSDFFLKSARNFIYKSNIIILKKQILKYS | | | | | |
| 15 | -2 | 65 | 376 | 312 | 103 |
| Coding sequence in Nucleotides: | | | | | |
| ATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAA | | | | | |
| CCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCATAT | | | | | |
| TGGAGCTTGAGCTTGATCAGATTGTCGTTTTCCCGCTTCAGTTTAAACTATCAGTACCC | | | | | |
| TCAATCATCTCACTTCATTATCTCCTATATTTTTTATTAACCTCTCTTTTATACTATTTT | | | | | |
| AAAAAATAAAAAGTGAGAATTTAAACAGAAAAACCTCTCTCAAGTCTTTCTCTCTATT | | | | | |
| TCAGTGGTCTGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MSELTHINCVALTARFPVGPVPAALMNRPTRGERRFAYWLSLSDQIVVSRLQFKLSVP | | | | | |
| SIISLHYLLYFLLTSLLYYFKKIKSENLRKNLSQVFLSISVV | | | | | |
| 16 | -2 | 584 | 622 | 39 | 12 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MHDVIYEMGFYD | | | | | |
| 17 | -2 | 755 | 1288 | 534 | 177 |
| Coding sequence in Nucleotides: | | | | | |
| ATGTCTCTTCAACAAGTAACAACCACCAGGAAGAACCAGAAACGAGGGGCGGAGACGATTT | | | | | |
| ACCGACAAAATAAAGTTTCTAGAGTACATGTTTGAGACACAGTCGAGACCCGAGTTA | | | | | |
| AGGATGAAACACCAGTTGGCACATAAACTCGGGCTTCATCCTCGTCAAGTGGCGATATGG | | | | | |
| TTCCAGAACAAACGCGCGGATCAAAGTTCGAGGCAGATTGAGCAAGAGTATAACGCGCTA | | | | | |
| AAGCATAACTACGAGACGCTTGCGTCTAAATCCGAGTCTCTAAAGAAAGAGAATCAGGCC | | | | | |
| CTACTCAATCAATTGGAGGTGCTGAGAAATGTAGCCGAAAAGCATCAAGAGAAAAGTAGT | | | | | |
| AGTAGTGGCAGCGGTGAAGAATCGGATGATCGGTTTACGAACTCTCCGGACGTTATGTTT | | | | | |
| GGTCAAGAAATGAATGTTCCGTTTTGCGACGGTTTTGCGTACCTTGAAGAAGGAAACAGT | | | | | |
| TTGTTGGAGATTGAAGAACAACCTGCCAGACCTTCAAAAAGTGGTGGGAGTTCTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|--|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| MSLQQVTTTRKRNREGRRRFTDKQISFLEYMFETQSRPELRMKHQLAHKLGHLHPRQVAIW FQNKRRARSKSRQIEQEYNALKHNYETLASKSESLKKNQALLNQLEVLNRNVAEKHQEKTS SSSGSEESDDRFTNSPDVMFQGEMNVPFCDFAYLEEGNSLLEIEEQLPDLQKWWEF | | | | | |
| 18 | -2 | 3365 | 3400 | 36 | 11 |
| Coding sequence in Nucleotides: ATGGTGAAAATTTTCAGAAAATTGCTTTTGTCTTTAA | | | | | |
| Coding sequence in Aminoacids: MVKIFRKL LLL | | | | | |
| 19 | -2 | 4136 | 4162 | 27 | 8 |
| Coding sequence in Nucleotides: ATGGAACAAGGGCAGAAGATTTATTAA | | | | | |
| Coding sequence in Aminoacids: MEQGQKIY | | | | | |
| 20 | -2 | 4295 | 4363 | 69 | 22 |
| Coding sequence in Nucleotides: ATGGTGGCCATTATATTAATAGCAACTGTCGGTCCAATAGACGACGTCGATTTTCTGCA TTTGTTTAA | | | | | |
| Coding sequence in Aminoacids: MVAHYINSNCRSNRRRRFSAFV | | | | | |
| 21 | -2 | 4400 | 4444 | 45 | 14 |
| Coding sequence in Nucleotides: ATGCCCTGGAAAATGCACTCCCTTTTTGTGTTTTGTTTTTTTTGTGA | | | | | |
| Coding sequence in Aminoacids: MPWKMHSLFVVFVFL | | | | | |
| 22 | -2 | 4850 | 4888 | 39 | 12 |
| Coding sequence in Nucleotides: ATGATATTTTTAAATCTAAATAATATTCTAAAAATTTGA | | | | | |
| Coding sequence in Aminoacids: MIFLNLNNILKI | | | | | |
| 23 | -2 | 4964 | 4990 | 27 | 8 |
| Coding sequence in Nucleotides: CCCTCTCAGTTGGGTCAGCCTGAGTGA | | | | | |
| Coding sequence in Aminoacids: PSQLGQPE | | | | | |
| 24 | -1 | 1068 | 1112 | 45 | 14 |
| Coding sequence in Nucleotides: ATGGTTCCAGAACAAACGCGCGATCAAAGTCGAGGCAGATTGA | | | | | |
| Coding sequence in Aminoacids: MVPEQTRAIKVEAD | | | | | |
| 25 | -1 | 1458 | 1526 | 69 | 22 |
| Coding sequence in Nucleotides: | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|---|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| ATGATTTTGGAGTTTTATCTCAACCGAAAGTGACATCATGACAGAGAATCGACATAACCAA AACGTGTAA Coding sequence in Aminoacids: MILSFISTESDIMTENRHNQNV | | | | | |
| 26 | -1 | 1647 | 1715 | 69 | 22 |
| Coding sequence in Nucleotides: ATGCGAACGAGTGGTTTAAACCATACTTTTAAAGAGGTGCGATCGGAAATTTTACCTATAAA ATACACTAA Coding sequence in Aminoacids: MRTSGLTILLRGAIGNFTYKIH | | | | | |
| 27 | -1 | 2292 | 2333 | 42 | 13 |
| Coding sequence in Nucleotides: ATGTCCACTTGTACTTTTGTGGCACACAACCTTTCCATAA Coding sequence in Aminoacids: MSTCTFVCTQLFP | | | | | |
| 28 | -1 | 2409 | 2528 | 120 | 39 |
| Coding sequence in Nucleotides: ATGTTGACCTGCAGGTGCACACCTGGCACATCGTATCTTATCTCTTTTGTGCGTTTCCAAC ACACCACAACACACCTACAAACGTGTCAATTCACACTTCACCAATTTTCATTTCTTTTAG Coding sequence in Aminoacids: MLTCRSTPGTSYLISFVVSNTQPQHTYKRVNSHFTNFISF | | | | | |
| 29 | -1 | 2712 | 2855 | 144 | 47 |
| Coding sequence in Nucleotides: ATGTTATCACATCAATCCACTTGGCTTTGAAGACGTGGTTGGAACGTCTTCTTTTTTCCACG ATGCTCCTCGTGGGTGGGGTCCATCTTTGGGACCACTGTGCGCAGAGGCATCTTCAACG ATGGCCTTTCTTTTATCGCAATGA Coding sequence in Aminoacids: MLSHQSTCFEDVVGTTSSFSSTMLLVGGGPSLGPLSAEASSTMAFPLSQ | | | | | |
| 30 | -1 | 3039 | 3122 | 84 | 27 |
| Coding sequence in Nucleotides: ATGCTCCTCGTGGGTGGGGTCCATCTTTGGGACCACTGTGCGCAGAGGCATCTTCAACG ATGGCCTTTCTTTTATCGCAATGA Coding sequence in Aminoacids: MLLVGGGPSLGPLSAEASSTMAFPLSQ | | | | | |
| 31 | -1 | 3351 | 3491 | 141 | 46 |
| Coding sequence in Nucleotides: ATGGTGCAGACCGCCGCATGTCCGCCTCGGTGGCACGGCGGATGTGCGCCGGGCGTCTGT TCTGGGCTCATGGTAGATCCCCCGTTTCGTAAATGGTGAAAATTTTTCAGAAAATTGCTTTT GCTTTAAAAGAAATGATTTAA Coding sequence in Aminoacids: MVQTAGMSASVARRMSAGRRSGLMVDPPFVNGENFQKIAFALKEMI | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|---|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| 32 | -1 | 3588 | 3632 | 45 | 14 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCCGGCGACCTCGCCGTCCACCTCGGCGACGAGCCAGGGATAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MPATSPSTSATSQG | | | | | |
| 33 | -1 | 3738 | 3860 | 123 | 40 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCCGCGGGGGGCATATCCGAGCGCCTCGTGCATGCGCACGCTCGGGTCGTTGGGCAGC CCGATGACAGCGACCACGCTCTTGAAGCCCTGTGCCTCCAGGGACTTCAGCAGGTGGGTG TAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MPRGAYPSASCMRTLGLSLGSPMTATTLKPCASRDFSRWV | | | | | |
| 34 | 1 | 22 | 81 | 60 | 19 |
| Coding sequence in Nucleotides: | | | | | |
| ATGATGTTTTTCCCAACCTAAAAGATTAAGAGACGCAACTGAACTCAGACCACTGAAATAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MMFSQPKRLRDATELRPLK | | | | | |
| 35 | 1 | 298 | 342 | 45 | 14 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCAGCTGGCAGCAGAGGTTTCCCGACTGGAAAGCGGGCAGTGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MQLARQVSRLESGQ | | | | | |
| 36 | 1 | 640 | 708 | 69 | 22 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCTTAACGTAATTCAACAGAAATTATATGATAATCATCGCAAGACCGGCAACAGGATT CAATCTTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MLNVIQQKLYDNHRKTGNRIQS | | | | | |
| 37 | 1 | 1489 | 1536 | 48 | 15 |
| Coding sequence in Nucleotides: | | | | | |
| ATGATGTCACTTTCGGTTGAGATAAAACTCAAAATCATATGGTTTTGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MMSLSVEIKLKIWF | | | | | |
| 38 | 1 | 1582 | 1620 | 39 | 12 |
| Coding sequence in Nucleotides: | | | | | |
| ATGTTTCACGTGATCCAGACAAAGGCGGACTTAGGTTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MFHVIQTKADLG | | | | | |
| 39 | 1 | 1693 | 1821 | 129 | 42 |
| Coding sequence in Nucleotides: | | | | | |
| ATGGTTAAACCACTCGTTTCGCATCCCTAGAAAAAAATTCATGGGTGCGTCACTGGACCT | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|---|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| AGGTTCAACTCCC GCCCCCAGCATTTTGTTCGGCGGCACCTGTTGATGATGGGAGATTA GGCGAGTAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MVKPLVRI PRKKI HGCVT GPRFNSRPQH FVSAAPVDDGRLGE | | | | | |
| 40 | 1 | 2227 | 2271 | 45 | 14 |
| Coding sequence in Nucleotides: | | | | | |
| ATGAATTT CGTTAGTATTCTATTTGGGGAATTTGTTTTCCACTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MNFVSILFGEFVFH | | | | | |
| 41 | 1 | 2374 | 2412 | 39 | 12 |
| Coding sequence in Nucleotides: | | | | | |
| ATGGGGGTGGGGGCTACTACTTTTAAATATGATTGACTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MGVGATTFNMID | | | | | |
| 42 | 1 | 2419 | 2454 | 36 | 11 |
| Coding sequence in Nucleotides: | | | | | |
| ATGAAATTGGTGAAGTGTGAATTGACACGTTTGTAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MKLVKCELTRL | | | | | |
| 43 | 1 | 2527 | 2844 | 318 | 105 |
| Coding sequence in Nucleotides: | | | | | |
| ATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGAC CAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCAT TGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAA TGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCC AAAGATGGACCCCCACCCACGAGGAGCATCGTGAAAAAGAAGACGTTCCAACCACGTCT TCAAAGCAAGTGGATTGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MVEHDTLVYSKNIKDTVSEDQRAIETFQQRVISGNLLGFHCPAICHFIVKIVEKEGGSYK CHHCCKGKAIVEDASADSGPKDGPPTRSIVEKEDVPTTSSKQVD | | | | | |
| 44 | 1 | 2854 | 3171 | 318 | 105 |
| Coding sequence in Nucleotides: | | | | | |
| ATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGAC CAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCAT TGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAA TGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCC AAAGATGGACCCCCACCCACGAGGAGCATCGTGAAAAAGAAGACGTTCCAACCACGTCT TCAAAGCAAGTGGATTGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MVEHDTLVYSKNIKDTVSEDQRAIETFQQRVISGNLLGFHCPAICHFIVKIVEKEGGSYK CHHCCKGKAIVEDASADSGPKDGPPTRSIVEKEDVPTTSSKQVD | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|---|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| 45 | 1 | 3421 | 3984 | 564 | 187 |
| Coding sequence in Nucleotides: | | | | | |
| ATGAGCCCAGAACGACGCCCGGCCGACATCCGCCGTGCCACCGAGGCGGACATGCCGGCG | | | | | |
| GTCTGCACCATCGTCAACCACTACATCGAGACAAGCACGGTCAACTTCCGTACCGAGCCG | | | | | |
| CAGGAACCGCAGGAGTGGACGGACGACCTCGTCCGTCTGCGGGAGCGCTATCCCTGGCTC | | | | | |
| GTCGCCGAGGTGGACGGCGAGGTCGCCGGCATCGCCTACGCGGGCCCCCTGGAAGGCACGC | | | | | |
| AACGCCTACGACTGGACGGCCGAGTCGACCGTGTACGTCTCCCCCGCCACCAGCGGACG | | | | | |
| GGACTGGGCTCCACGCTCTACACCCACCTGCTGAAGTCCCTGGAGGCACAGGGCTTCAAG | | | | | |
| AGCGTGGTGCCTGTCATCGGGCTGCCAACGACCCGAGCGTGCATGCACGAGGCGCTC | | | | | |
| GGATATGCCCCCGCGGCATGCTGCGGGCGGCCGGCTTCAAGCACGGGAAGTGGCATGAC | | | | | |
| GTGGGTTTCTGGCAGCTGGACTTCAGCCTGCCGGTACCGCCCCGTCCGGTCTGCCCGTC | | | | | |
| ACCGAGATCTGCTCAACAATCTAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MSPERRPADIRRATEADMPAVCTIVNHYIETSTVNFRTPEPQEPQEWTDLVLRLRERYPWL | | | | | |
| VAEVDGEVAGIAYAGPWKARNAYDWTAESTVYVSPRHQRTGLGSTLYTHLLKSLEAQGFK | | | | | |
| SVVAVIGLPNDPSVRMHEALGYAPRGLRAAGFKHGNWHDVGFWQLDFSLPVPVPRVLPV | | | | | |
| TEICSTI | | | | | |
| 46 | 1 | 4018 | 4224 | 207 | 68 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCTGATATGCACTATTCAAATAGGAGCATTAGCTATGTTTGTTAATGTCACTTTATGT | | | | | |
| TATGTGGGTAAGTCACCTAAGACACTCCACGTACCTACTTGTGTCTCTTACGCGGCTTT | | | | | |
| AATAAATCTTCTGCCCTTGTTCCATATTTACTAATTATCCCTTTCTTCACTAAAAGAAAA | | | | | |
| TTGTTATCATTAAAGTATTAGTCTTTAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MLICTIQIGALAMFVNVTLCYVGKSPKTLHVPTCCLLRGFNKSSALVPYLLIIPFFTKRK | | | | | |
| LLSLSISL | | | | | |
| 47 | 1 | 4303 | 4347 | 45 | 14 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCAGAAAATCGACGTCGTCTATTGGACCGACAGTTGCTATTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MQKIDVVYWTDSCY | | | | | |
| 48 | 2 | 401 | 442 | 42 | 13 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MLPARMLCGIVSG | | | | | |
| 49 | 2 | 470 | 529 | 60 | 19 |
| Coding sequence in Nucleotides: | | | | | |
| ATGATTACGAATTCCCGATCTAGTAACATAGATGACACCGCGCGGATAATTTATCCTAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MITNSRSSNIDDTARDNLS | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|---|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| 50 | 2 | 566 | 652 | 87 | 28 |
| Coding sequence in Nucleotides: | | | | | |
| ATGTATAATTGCGGGACTCTAATCATAAAAAACCCATCTCATAAATAACGTCATGCATTAC | | | | | |
| ATGTTAATTATTACATGCTTAACGTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MYNCGTLIIKTHLINNVMHYMLIITCLT | | | | | |
| 51 | 2 | 668 | 988 | 321 | 106 |
| Coding sequence in Nucleotides: | | | | | |
| ATGATAATCATCGCAAGACCGGCAACAGGATTCAATCTTAAGAACTTTATTGCCAAATG | | | | | |
| TTTGAACGATCGGGGAAATTCGAGCTCTTAGAACTCCCACCACTTTTGAAGGTCTGGCAG | | | | | |
| TTGTTCTTCAATCTCCAACAACTGTTTCCTTCTTCAAGGTACGAAAACCGTCGCAAAA | | | | | |
| CGGAACATTCATTTCTTGACCAAAACATAACGTCCGGAGAGTTCGTAAACCGATCATCCGA | | | | | |
| TTCTTCACCGCTGCCACTACTACTAGTTTTCTTCTTGATGCTTTTCGGCTACATTTCTCAG | | | | | |
| CACCTCCAATTGATTGAGTAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MIIIPATGFNLKLYCQMFERSGKFELLELPPLKLVWQLFFNLQQTVSFFKVRKTVAK | | | | | |
| RNIHFLTKHNVRVRKPIIRFFTAATTTSFLLMLFGYISQHLQLIE | | | | | |
| 52 | 2 | 1130 | 1171 | 42 | 13 |
| Coding sequence in Nucleotides: | | | | | |
| ATGAAGCCCAGATTTATGTGCCAACTGGTGTTCATCCTTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MKPEFMCQLVFHP | | | | | |
| 53 | 2 | 1442 | 1492 | 51 | 16 |
| Coding sequence in Nucleotides: | | | | | |
| ATGGTGAGTTGTACGTTTACACGTTTTGGTTATGTCGATTCTCTGTCATGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MVSCTFTRFGYVDSL | | | | | |
| 54 | 2 | 1547 | 1582 | 36 | 11 |
| Coding sequence in Nucleotides: | | | | | |
| ATGAGTCGTAGCTGGACTGGTAAGGGAGAGACCTGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MSRSWTGKGET | | | | | |
| 55 | 2 | 1733 | 1813 | 81 | 26 |
| Coding sequence in Nucleotides: | | | | | |
| ATGGGTGCGTCACTGGACCTAGGTTCAACTCCC GCCCCCAGCATTTTGTTCGGCGGCAC | | | | | |
| CTGTTGATGATGGGAGATTAG | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MGASLDLGSTPAPSILFRRHLLMMGD | | | | | |
| 56 | 2 | 2294 | 2335 | 42 | 13 |
| Coding sequence in Nucleotides: | | | | | |
| ATGGGAAGAGTTGTGTGCAAACAAAAGTACAAGTGGACATAG | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|---|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| Coding sequence in Aminoacids: | | | | | |
| MGRVVCKQKYKWT | | | | | |
| 57 | 2 | 2360 | 2404 | 45 | 14 |
| Coding sequence in Nucleotides: | | | | | |
| ATGGTAGGTAACAAATGGGGGTGGGGGCTACTACTTTTAAATATGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MVGNKWGWLLLLLI | | | | | |
| 58 | 2 | 2498 | 2620 | 123 | 40 |
| Coding sequence in Nucleotides: | | | | | |
| ATGTGCCAGGTGTCGACCTGCAGGTCAACATGGTGGAGCACGACACACTTGTCTACTCCA AAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGG TAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MCQVSTCRSTWWSTTHLSTPKISKIQSQKTKGQLRRLFNGK | | | | | |
| 59 | 2 | 2744 | 2947 | 204 | 67 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAA AAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGC ACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAA TTGAGACTTTTCAACAAAGGGTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MPLPTVVPKMDPHPRGASWKKKTFQPRLQSKWIDVITWWSTTHLSTPKISKIQSQKTKGQ LRLFNGK | | | | | |
| 60 | 2 | 3071 | 3193 | 123 | 40 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAA AAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACG TAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MPLPTVVPKMDPHPRGASWKKKTFQPRLQSKWIDVISPLT | | | | | |
| 61 | 2 | 3197 | 3424 | 228 | 75 |
| Coding sequence in Nucleotides: | | | | | |
| ATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTT ATTTGGAGAGGACCTCGAGAATTAATTCTCAACACAACATATACAAAACAAACGAATCTC AAGCAATCAAGCATTCTACTTCTATTGCAGCAATTTAAATCATTCTTTTAAAGCAAAAG CAATTTTCTGAAAATTTTACCATTACGAACGGGGGATCTACCATGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MTHNPTILRKTLPLYKEVHFIWRGPRELILNTTYTKQTNLQSSILLLLQFQKSFLLKQK QFSENFHHLRTGDLF | | | | | |
| 62 | 2 | 3845 | 3988 | 144 | 47 |
| Coding sequence in Nucleotides: | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|--|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| ATGCCCCCGCGGCATGCTGCGGGCGGCCGGCTTCAAGCACGGGAAGTGGCATGACGTGG GTTTCTGGCAGCTGGACTTCAGCCTGCCGGTACCGCCCCGTCCGGTCTGCCCCTCACCG AGATCTGCTCAACAATCTAGCTAG Coding sequence in Aminoacids: MPPAACCGRPASSTGTGMTWVSGSWTSACRYRPPVRSCPSRSAQQSS 63 2 4064 4171 108 35 Coding sequence in Nucleotides: ATGTCACCTTTATGTTATGTGGGTAAGTCACCTAAGACACTCCACGTACCTACTTGTGTC TCTTACGCGGCTTTAATAAATCTTCTGCCCTTGTTCATATTTACTAA Coding sequence in Aminoacids: MSLYVMWVSHLRHSTYLLVVSYAALINLLPLFHIY 64 2 4352 4489 138 45 Coding sequence in Nucleotides: ATGGGCCACCATAGTAGACTGACAAATAAATTACCTGACAACATCGTTTTACAAAAAAC AAACACAAAAAGGGAGTGCATTTTCCAGGGCATTTTTGTAAATAAAAAACAGTTAAAAGGG AGTGCAATAGAAATATAG Coding sequence in Aminoacids: MGHHSRLTNKLPDNIVSQKNKHKKGVHFPGHFCNKKQLKGSALIEI 65 2 4685 4714 30 9 Coding sequence in Nucleotides: ATGGCGAATGCTAGAGCAATTCGGCGTTAA Coding sequence in Aminoacids: MANARAIIR 66 3 3 41 39 12 Coding sequence in Nucleotides: GCCAATCTCCAAAAGAAGATGATGTTTTCCCAACCTAA Coding sequence in Aminoacids: ANLPKEDDVFPPT 67 3 189 218 30 9 Coding sequence in Nucleotides: ATGATTGAGGGTACTGATAGTTTAAACTGA Coding sequence in Aminoacids: MIEGTDSLN 68 3 501 587 87 28 Coding sequence in Nucleotides: ATGACACCGCGCGGATAATTTATCCTAGTTTGC GCGCTATATTTTGT TTTCTATCGCGT ATTAAATGTATAATTGCGGGACTCTAA Coding sequence in Aminoacids: MTPRAIIYPSLRAIFCFLSRIKCI IAGL 69 3 1197 1280 84 27 Coding sequence in Nucleotides: | | | | | |

| Peptide ID | Frame | Start | Stop | Length | |
|--|-------|-------|------|------------|-----------|
| | | | | Nucleotide | Aminoacid |
| ATGTACTCTAGGAAACTTATTTGTTTGTTCGGTAAATCGTCTCCGCCCTCGTTTCGGTTC | | | | | |
| TTCCTGGTGGTTGTTACTTGTGGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MYSRKLICLSVNRLRPSFRFFLVVVC | | | | | |
| 70 | 3 | 1323 | 1352 | 30 | 9 |
| Coding sequence in Nucleotides: | | | | | |
| ATGTGTATATATAGTGAGTGGTCTTTATAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MCIYSEWSL | | | | | |
| 71 | 3 | 1473 | 1508 | 36 | 11 |
| Coding sequence in Nucleotides: | | | | | |
| ATGTTCGATTCTCTGTCATGATGTCACTTTCGGTTGA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MSILCHDVTFG | | | | | |
| 72 | 3 | 4026 | 4064 | 39 | 12 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCACTATTCAAATAGGAGCATTAGCTATGTTTGTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MHYSNRSISYVC | | | | | |
| 73 | 3 | 4692 | 4727 | 36 | 11 |
| Coding sequence in Nucleotides: | | | | | |
| ATGCTAGAGCAATTCGGCGTTAATTCAGTACATTAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MLEQFGVNSVH | | | | | |
| 74 | 3 | 4887 | 4922 | 36 | 11 |
| Coding sequence in Nucleotides: | | | | | |
| ATGAATATTTTAATATTTGTTTTTTTTAATATTATAA | | | | | |
| Coding sequence in Aminoacids: | | | | | |
| MNILIFVFLIL | | | | | |

F. Appendix 6. Protein Expression of Phosphinothricin-N-Acetyl Transferase

Materials and Methods

Seed and forage samples were collected from the 2012-2013 growing season in Argentina and the 2013 season in the United States. Four plot samples from each of the locations were collected from the selected IND-00410-5 soybean event and the Williams 82 parental control. The field locations in Argentina were Monte Buey, Cordoba (A); Corral de Bustos, Cordoba (D2); Carmen de Areco, Buenos Aires (G1); Hughes, Santa Fe (Q1); Hughes, Santa Fe (Q2); and Aranguren, Entre Rios (W1). The five distinct locations in the United States include: Effingham, IL (IL3) Ladoga, IN (IN); Pemberton, OH (OH2); Richland, IA (IA); and Troy KS (KS). Forage samples were collected at the R3/R4 stage from three or more soybean plants in the interior rows of each plot. Leaf tissue samples were harvested and immediately frozen on dry ice and shipped to Bioceres Semillas (Rosario, Santa Fe, Argentina). The samples were stored in a -80 °C freezer until transferred to Arcadia Biosciences (Davis, CA, USA) on dry ice and then shipped to SGS Mid-West Seed Services (Brookings, South Dakota) on dry ice for analysis. Mature seed was collected and shipped at ambient temperature to the same location.

SGS Mid-West Seed Services was commissioned for detecting and quantifying PAT protein. Samples were homogenized through mechanical grinding under liquid nitrogen, weighed into individual 2 mL polypropylene tubes and 1.5 mL of 0.05% Tween-20 in phosphate buffered saline was added. The suspension was vortexed at maximum speed for 5 minutes with 2 tungsten beads, then at medium speed for an additional 10 minutes. The samples were spun at $10,000 \times g$ for 10 minutes and the supernatant was transferred to a fresh 2 mL polypropylene tube. The neat extract or a subsequently diluted extract was used in the assay. The dilution factors were 50, 100 and 200 for the leaf samples and 200, 400 and 800 for the seed samples. The Williams 82 control extracts were used undiluted. To yield quantitative results, a dilution series of purified PAT recombinant protein (phosphinothricin-N-acetyl transferase, >90% pure, MyBioSource, Inc., San Diego, CA; Cat# MBS1035286) was used as a standard for the quantification assay. Aliquots of varying concentrations—0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 10.0 ng/mL—of the PAT standard were used to generate a standard curve with quadratic curve regression. All values were reported on a fresh weight (FW) basis and the limit of detection for the assay was 0.025 $\mu\text{g/g}$ FW. The values for each plot were averaged and presented as $\mu\text{g/g}$ FW.

Data and Summary

PAT protein was detected in IND-00410-5 soybean forage and seeds (Table 6-1). There was no measurable PAT protein in any of the Williams 82 control samples as expected. The highest value measured in the IND-00410-5 seed samples was 71.17 $\mu\text{g/g}$ FW at Site A and 15.37 $\mu\text{g/g}$ FW in forage at Site OH2. These values are comparable to or less than previously reported values of PAT expression in other crops, such as 127 $\mu\text{g/g}$ FW (cotton seed) and 935 $\mu\text{g/g}$ FW (corn leaf) (CERA 2011). The reported values in other transgenic crops are 1.8 \times and 60 \times higher than the highest amount of PAT protein measured in IND-00410-5 seed and leaf, respectively. While the values vary across the different locations and do not share trends between forage and leaf samples, this can be due to slight differences in climate, harvest time and general trends of PAT behavior in crop plants (De Block *et al.*, 1987 and CERA, 2011).

Table 6-1. PAT Protein Levels in Forage and Seed.

| Site ¹ /Variety | HB4 ($\mu\text{g/g FW} \pm \text{SE}^2$) ³ | | Williams 82 ³ | |
|----------------------------|---|------------------|--------------------------|------|
| | Forage | Seed | Forage | Seed |
| A | 7.49 \pm 1.39 | 69.05 \pm 1.07 | 0 | 0 |
| D2 | 9.51 \pm 0.56 | 34.49 \pm 1.55 | 0 | 0 |
| G1 | 6.72 \pm 1.00 | 30.33 \pm 1.20 | 0 | 0 |
| Q1 | 5.44 \pm 0.74 | 65.57 \pm 1.54 | 0 | 0 |
| Q2 | 7.46 \pm 1.61 | 68.70 \pm 1.35 | 0 | 0 |
| W1 | 7.74 \pm 0.65 | 23.00 \pm 2.83 | 0 | 0 |
| IL3 | 10.19 \pm 0.39 | 48.46 \pm 1.82 | 0 | 0 |
| IN | 12.14 \pm 0.14 | 46.47 \pm 6.25 | 0 | 0 |
| OH2 | 12.68 \pm 0.93 | 58.68 \pm 2.69 | 0 | 0 |
| IA | 8.87 \pm 1.09 | 58.31 \pm 0.74 | 0 | 0 |
| KS | 4.94 \pm 3.24 | 50.80 \pm 6.03 | 0 | 0 |

¹The field locations in Argentina were Monte Buey, Cordoba (A); Corral de Bustos, Cordoba (D2); Carmen de Areco, Buenos Aires (G1); Hughes, Santa Fe (Q1); Hughes, Santa Fe (Q2); and Aranguren, Entre Rios (W1); and in the United States were Effingham, IL (IL3); Ladoga, IN (IN); Pemberton, OH (OH2); Richland, IA (IA); and Troy KS (KS).

²FW = fresh weight. SE = standard error.

³Each value is an average of four plots of each genotype from the specified location.

G. Appendix 7. Biochemistry of the HB4 Protein

Sequence analysis of the HAHB4v protein sequence in HB4 soybean

Comparison of the HAHB4v protein sequence translated from the original NCBI entry (GenBank Accession number AAA63768.2; Chan and Gonzalez 1994) with the sequence translated from the *HaHB4v* gene actually inserted in soybean event IND-00410-5 showed a few differences which are not predicted to affect the general properties of the protein expressed in the crop (Figure 7-1). The variant of the HAHB4 protein found in HB4 soybean is referred to as HAHB4 variant here for this discussion. At the amino acid level the differences include:

1. A four amino acid deletion (residue numbers 7 to 10), which changes residue numbering as indicated in the substitutions detailed below
2. A Lys to Arg substitution at position 22 (K22 → R18)
3. A Phe to Leu substitution at position 159 (F159 → L155)
4. A Pro to Leu substitution at position 175 (P175 → L171)

Figure 7-1. Alignment of HAHB4v protein molecules.

Alignment of the amino acid sequence translated from the nucleotide sequence registered in the GenBank (HAHB4) and the translated gene from IND-00410-5 soybean (HAHB4 variant). The numbers corresponding to amino acid positions are in frame with the GenBank HAHB4 accession.

| | | | |
|---------------|----------------------------|--------------------------------------|---------------------------------|
| HAHB4 | MSLQQVPTTETTTTRKKNRNEGRKRF | TDKQISFLEYMFETQSRPELRMKHQL | 50 |
| HAHB4 variant | MSLQQV----TTTRKKNRNEGRR | RF | TDKQISFLEYMFETQSRPELRMKHQL 46 |
| HAHB4 | AHKLGLHPRQVAIW | FQNKRRASKSRQIEQEYNALKHNYETLASKSESLKK | 100 |
| HAHB4 variant | AHKLGLHPRQVAIW | FQNKRRASKSRQIEQEYNALKHNYETLASKSESLKK | 96 |
| HAHB4 | ENQALLNQLEVL | RNVAEKHQEKTS | SSSGSGEESDDRFTNSPDVMFGQEMNV 150 |
| HAHB4 variant | ENQALLNQLEVL | RNVAEKHQEKTS | SSSGSGEESDDRFTNSPDVMFGQEMNV 146 |
| HAHB4 | PFCDGFAYFEEGNSLLEIEEQLPD | PQ | KWWEF 181 |
| HAHB4 variant | PFCDGFAYLEEGNSLLEIEEQLPD | L | QWWEF 177 |

The effects of these changes are considered minimal (as described in detail below) and are not expected to produce significant differences in the general properties of the protein based on bioinformatics analysis. The deletion (12 nucleotides) keeps the DNA in frame, and the amino acid substitutions are either conservative (positions 18 and 155) or a putative variant of the original sequence (position 171).

At the DNA level, these amino acid changes correspond to the following modifications (Figure 7-2):

1. Deletion of nucleotides 76 to 87, as numbered following the RNA sequence registered in the NCBI nucleotide database (original sequence, accession code original sequence, accession code AAA63768.2).
2. A22→G substitution, changing an AAA to an AGA codon producing the K22 → R18 change.
3. T432→C substitution, making a TTT into a CTT codon producing the F159 → L155 change.

4. Substitution C551→T, which changes a CCT for a CTT codon, leading to the P175 → L171 change.

In order to assess the possible effects of these changes on HAHB4v soy protein function, the Protein Variation Effect Analyzer algorithm (PROVEAN, <http://provean.jcvi.org>) was used (Choi 2012; Choi *et al.* 2012). This algorithm predicts damaging effects that amino acid changes could have on protein function based on the bioinformatic analysis of variants for more than 90,000 proteins (Choi *et al.* 2012). In this analysis, a score is assigned to each variant and a value below -2.5 would indicate a significant effect. As can be seen in Table 7-1, none of the changes found in the HAHB4v protein expressed in soybean event IND-00410-5 would have a significant effect on its functional properties.

Table 7-1. Prediction of the effects of HAHB4 variant amino acid changes on protein function.

| Modification | | PROVEAN score | Prediction |
|--------------|-----------|---------------|------------|
| Deletion | P7 to E10 | -1.025 | Neutral |
| Substitution | K22R | 0.911 | Neutral |
| | F159L | -0.494 | Neutral |
| | P175L | -1.811 | Neutral |

Changes in the sequence detailed above have been examined as to whether the conserved protein domains are affected. The deletion near the N-terminus and the K→R substitution are not located in the highly conserved homeobox domain. As mentioned earlier, this conserved region is characterized by participating in gene regulation through DNA binding and responds to similar stressors in multiple plant species (Chan and Gonzalez 1994; Hu *et al.* 2012; Ruberti *et al.* 1991; Zhao *et al.* 2011). Early studies of the expression of this transgene in plants demonstrated the anticipated response to various stressors (Manavella *et al.* 2006; Manavella *et al.* 2008a; Manavella *et al.* 2008b). The coding sequence changes toward the C-terminus are outside the predicted leucine zipper region and therefore are not anticipated to alter the behavior of the protein. Also, again the altered gene functions as the native sequence (Manavella *et al.* 2008b).

Some additional silent point mutations are also present; they do not change the amino acid residues: substitution G351→A, changing the CAG for an CAA codon, both coding for a glutamine residue (Q) and substitution A401→C, modifying the GCA for a GCC, both coding for Alanine (A).

Figure 7-2. Alignment of HAHB4 DNA molecules, the original from sunflower (*HaHB4* NCBI – AF339748) and the sequence in soybean IND-00410-5 (HAHB4 variant).

```

AF339748      ATGTCTCTTCAACAAGTACCCACAACAGAAACAACCACCAGGAAGAACCAGAAACGAGGGGCG 62
HAHB4variant ATGTCTCTTCAACAAGTA-----ACAACCACCAGGAAGAACCAGAAACGAGGGGCG 50

AF339748      GAAACGATTTACCGACAAACAAATAAGTTTCTTAGAGTACATGTTTGAGACACAGTTCGAGAC 124
HAHB4variant  GACACGATTTACCGACAAACAAATAAGTTTCTTAGAGTACATGTTTGAGACACAGTTCGAGAC 112

AF339748      CCGAGTTAAGGATGAAACACCAGTTGGCACATAAACTCGGGCTTCATCCTCGTCAAGTGGCG 186
HAHB4 variant CCGAGTTAAGGATGAAACACCAGTTGGCACATAAACTCGGGCTTCATCCTCGTCAAGTGGCG 174

AF339748      ATATGGTTCCAGAACAAACGCGCGCGATCAAAGTCGAGGCAGATTGAGCAAGAGTATAACGC 248
HAHB4 variant ATATGGTTCCAGAACAAACGCGCGCGATCAAAGTCGAGGCAGATTGAGCAAGAGTATAACGC 236

AF339748      GCTAAAGCATAACTACGAGACGCTTGCCTCTAAATCCGAGTCTCTAAAGAAAGAGAATCAGG 310
HAHB4 variant GCTAAAGCATAACTACGAGACGCTTGCCTCTAAATCCGAGTCTCTAAAGAAAGAGAATCAGG 298

AF339748      CCCTACTCAATCAGTTGGAGGTGCTGAGAAATGTAGCAAGAAAAGCATCAAGAGAAAACACTAGT 372
HAHB4 variant CCCTACTCAATCAATTGGAGGTGCTGAGAAATGTAGCCGAAAAGCATCAAGAGAAAACACTAGT 360

AF339748      AGTAGTGGCAGCGGTGAAGAATCGGATGATCGGTTTACGAACTCTCCGGACGTTATGTTTGG 434
HAHB4 variant AGTAGTGGCAGCGGTGAAGAATCGGATGATCGGTTTACGAACTCTCCGGACGTTATGTTTGG 422

AF339748      TCAAGAAATGAATGTTCCGTTTTTGCACGGTTTTTGCCTACTTTGAAGAAGGAAACAGTTTGT 496
HAHB4 variant TCAAGAAATGAATGTTCCGTTTTTGCACGGTTTTTGCCTACTTTGAAGAAGGAAACAGTTTGT 484

AF339748      TGGAGATTGAAGAACAACACTGCCAGACCTCAAAAAGTGGTGGGAGTTCTAA 546
HAHB4 variant TGGAGATTGAAGAACAACACTGCCAGACCTTCAAAAAGTGGTGGGAGTTCTAA 534

```

The bioinformatics results suggest that the changes found in the HAHB4v protein expressed in soybean event IND-00410-5 were neutral, i.e., would not introduce any significant difference in protein safety as compared with the original, sunflower-derived protein. Additionally, from a safety perspective, the HAHB4v protein is rapidly digested (Appendix 10) and does not confer increased pest resistance or negatively impact environmental interactions (Appendix 12).

HAHB4 is a small (21 kD) transcription factor, which is present in extremely low levels in sunflower as well as in the transgenic plant. Isolation of the protein from the selected event is not feasible because the levels are too low in the plant. To test for glycosylation, bioinformatics tools were used to search the putative acceptor sites.

The primary structure of HAHB4v was examined for the signal sequence required for transport to the endoplasmic reticulum (ER), a pre-requisite for glycosylation (Pattison and Amtmann 2009). Using two public algorithms (<http://www.cbs.dtu.dk/services/SignalP>; <http://urgi.versailles.inra.fr/Tools/Predotar>), no such signal peptide was found in HAHB4v (Nielsen *et al.* 1997; Small *et al.* 2004). Additionally, the presence of acceptor sites for oligosaccharides was analyzed (<http://turing.cs.iastate.edu/EnsembleGly/predict.html>, which moved to <http://ailab.ist.psu.edu/software.html>) (Caragea *et al.* 2007; Gomord *et al.* 2010) and no consensus sequences for glycosylation were found. The absence of both signal sequences for transport to ER and glycosylation acceptor sites suggests that glycosylation in HAHB4v is unlikely.

Bioinformatics Search for Homologous Proteins, History of Safe Use

To augment the case for prior exposure and a history of safe use for HAHB4v protein, a BLASTP search of the non-redundant NCBI higher plant database (taxid:3193) for proteins homologous to HAHB4v was performed (March 24, 2017). The BLOSUM50 scoring matrix was used with an E-value threshold of 0.1 to match the top 1,000 proteins. All 1,000 matches were significant with E-Values $\geq 1 \times 10^{-20}$, bit scores >85 and protein coverage of $> 50\%$.

The top 100 significant matches are listed in (Table 7-2) and include commercially grown eudicots, monocots, seed and flowering plants such as spinach, asparagus, bean, potato, tomato, apple, pear, citrus, dates and walnuts. The top 100 matches all had E-Values $\geq 1 \times 10^{-30}$ and bit scores > 100 . All the matches contained conserved homeobox DNA binding regions of the Homeodomain superfamily. To illustrate the widespread distribution of homologous proteins in the plant kingdom, a distance tree was created from this search using T pairwise alignments and a Max distance of 0.85 (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). These results clearly support prior exposure to proteins that are significantly similar to HAHB4v protein and therefore augment the case for a history of safe use of HAHB4v protein.

Table 7-2. Higher Plants (taxid:3193) Protein Database Matches for HAHB4.

| Identity | Description | E-Value | Bit Score | Coverage | % ¹ | Common Name |
|----------------|---|-----------|-----------|----------|----------------|-------------------|
| AAA63768.2 | homeobox-leucine zipper protein HAHB-4 (<i>Helianthus annuus</i>) | 1.00E-124 | 352 | 100% | 96% | sunflower |
| ALF46600.1 | HD-ZIP protein (<i>Chrysanthemum x morifolium</i>) | 1.00E-81 | 244 | 95% | 68% | Chrysanthemum |
| KVH95214.1 | Homeobox, conserved site-containing protein (<i>Cynara cardunculus</i> var. <i>scolymus</i>) | 1.00E-63 | 197 | 83% | 63% | artichoke thistle |
| XP_011070385.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Sesamum indicum</i>) | 2.00E-44 | 149 | 83% | 53% | sesame |
| XP_016500342.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Nicotiana tabacum</i>) | 2.00E-39 | 137 | 64% | 60% | tobacco |
| XP_009631116.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Nicotiana tomentosiformis</i>) | 2.00E-39 | 137 | 64% | 60% | wild tobacco |
| XP_011026784.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Populus euphratica</i>) | 2.00E-39 | 136 | 93% | 47% | desert poplar |
| XP_009796883.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Nicotiana sylvestris</i>) | 5.00E-39 | 136 | 84% | 48% | tobacco |
| XP_012087179.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Jatropha curcas</i>) | 1.00E-38 | 135 | 58% | 62% | Jatropha |
| XP_012842101.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Erythranthe guttata</i>) | 2.00E-38 | 134 | 69% | 58% | monkeyflower |
| XP_019226417.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Nicotiana attenuata</i>) | 3.00E-38 | 134 | 64% | 58% | wild tobacco |
| XP_002297947.1 | homeobox leucine zipper family protein (<i>Populus trichocarpa</i>) | 1.00E-37 | 132 | 93% | 45% | poplar |
| CDP12228.1 | unnamed protein product (<i>Coffea canephora</i>) | 4.00E-37 | 131 | 58% | 61% | coffee |
| XP_006343782.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Solanum tuberosum</i>) | 6.00E-37 | 130 | 68% | 59% | potato |
| KNA21035.1 | hypothetical protein SOVF_046880 (<i>Spinacia oleracea</i>) | 8.00E-37 | 130 | 72% | 48% | spinach |

| Identity | Description | E-Value | Bit Score | Coverage | % ¹ | Common Name |
|----------------|---|----------|-----------|----------|----------------|-----------------|
| XP_010559143.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Tarenaya hassleriana</i>) | 1.00E-36 | 130 | 67% | 55% | spider plant |
| XP_018831909.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Juglans regia</i>) | 5.00E-36 | 128 | 73% | 53% | english walnut |
| KZV45956.1 | homeobox-leucine zipper protein ATHB-7-like (<i>Dorcoeras hygrometricum</i>) | 6.00E-36 | 127 | 60% | 59% | |
| XP_004245456.2 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Solanum lycopersicum</i>) | 1.00E-35 | 127 | 62% | 59% | tomato |
| XP_015084842.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Solanum pennellii</i>) | 2.00E-35 | 126 | 63% | 59% | wild tomato |
| XP_017629690.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12 (<i>Gossypium arboreum</i>) | 3.00E-35 | 127 | 68% | 56% | tree cotton |
| XP_006476286.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like isoform X1 (<i>Citrus sinensis</i>) | 3.00E-35 | 126 | 67% | 53% | sweet orange |
| OAY48960.1 | hypothetical protein MANES_05G018700 (<i>Manihot esculenta</i>) | 5.00E-35 | 126 | 65% | 55% | cassava |
| EYU33472.1 | hypothetical protein MIMGU_mgv1a024502mg (<i>Erythranthe guttata</i>) | 9.00E-35 | 126 | 55% | 65% | monkeyflower |
| XP_002280048.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12 (<i>Vitis vinifera</i>) | 1.00E-34 | 124 | 91% | 47% | wine grape |
| XP_012490157.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Gossypium raimondii</i>) | 2.00E-34 | 125 | 68% | 56% | peruvian cotton |
| XP_016673458.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Gossypium hirsutum</i>) | 2.00E-34 | 125 | 66% | 55% | upland cotton |
| XP_017969913.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12 (<i>Theobroma cacao</i>) | 3.00E-34 | 124 | 68% | 56% | cocoa tree |
| XP_006439220.1 | hypothetical protein CICLE_v10022253mg (<i>Citrus clementina</i>) | 4.00E-34 | 123 | 53% | 61% | citrus |

| Identity | Description | E-Value | Bit Score | Coverage | %¹ | Common Name |
|-----------------|--|----------------|------------------|-----------------|----------------------|--------------------|
| XP_020241391.1 | homeobox-leucine zipper protein HOX6-like (<i>Asparagus officinalis</i>) | 5.00E-34 | 123 | 79% | 48% | Asparagus |
| XP_010054131.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12 (<i>Eucalyptus grandis</i>) | 6.00E-34 | 123 | 95% | 43% | Eucalyptus |
| XP_004133792.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12 (<i>Cucumis sativus</i>) | 9.00E-34 | 123 | 67% | 55% | cucumber |
| XP_019192855.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like isoform X2 (<i>Ipomoea nil</i>) | 1.00E-33 | 122 | 57% | 61% | morning glory |
| XP_008437846.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7 (<i>Cucumis melo</i>) | 2.00E-33 | 122 | 67% | 55% | muskmelon |
| XP_007039388.2 | PREDICTED: homeobox-leucine zipper protein ATHB-12 (<i>Theobroma cacao</i>) | 2.00E-33 | 122 | 63% | 57% | cocoa tree |
| AIA57938.1 | homeobox 12 (<i>Jatropha curcas</i>) | 2.00E-33 | 122 | 64% | 55% | |
| NP_182191.1 | homeobox 7 (<i>Arabidopsis thaliana</i>) | 2.00E-33 | 122 | 72% | 50% | Arabidopsis |
| XP_015896340.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Ziziphus jujuba</i>) | 3.00E-33 | 122 | 75% | 51% | Indian date |
| XP_018822320.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Juglans regia</i>) | 3.00E-33 | 121 | 88% | 47% | english walnut |
| EOY23889.1 | Homeobox 7, putative (<i>Theobroma cacao</i>) | 3.00E-33 | 122 | 63% | 57% | cocoa tree |
| XP_018805268.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Juglans regia</i>) | 3.00E-33 | 122 | 63% | 56% | english walnut |
| XP_010933776.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Elaeis guineensis</i>) | 3.00E-33 | 121 | 68% | 53% | oil palm |
| KHN06343.1 | Homeobox-leucine zipper protein ATHB-7 (<i>Glycine soja</i>) | 4.00E-33 | 121 | 63% | 58% | wild soybean |
| XP_016738107.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Gossypium hirsutum</i>) | 5.00E-33 | 120 | 62% | 56% | upland cotton |
| XP_017636812.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Gossypium arboreum</i>) | 5.00E-33 | 120 | 62% | 56% | tree cotton |
| XP_003528775.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Glycine max</i>) | 5.00E-33 | 121 | 60% | 60% | soybean |

| Identity | Description | E-Value | Bit Score | Coverage | % ¹ | Common Name |
|----------------|--|----------|-----------|----------|----------------|-----------------|
| XP_008376186.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Malus domestica</i>) | 6.00E-33 | 121 | 73% | 51% | apple |
| XP_002882084.1 | ATHB-7 (<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>) | 7.00E-33 | 121 | 64% | 54% | Arabidopsis |
| XP_016552550.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Capsicum annuum</i>) | 3.00E-32 | 118 | 89% | 47% | pepper |
| XP_019459554.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like isoform X1 (<i>Lupinus angustifolius</i>) | 3.00E-32 | 119 | 75% | 49% | lupin |
| XP_012437797.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Gossypium raimondii</i>) | 4.00E-32 | 119 | 62% | 55% | peruvian cotton |
| XP_006339757.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12 (<i>Solanum tuberosum</i>) | 4.00E-32 | 119 | 59% | 60% | potato |
| JAT56441.1 | Homeobox-leucine zipper protein HOX6 (<i>Anthurium amnicola</i>) | 5.00E-32 | 118 | 66% | 55% | tulip |
| XP_019420837.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Lupinus angustifolius</i>) | 6.00E-32 | 118 | 59% | 58% | lupin |
| XP_016538251.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Capsicum annuum</i>) | 6.00E-32 | 118 | 54% | 63% | pepper |
| XP_009361303.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Pyrus x bretschneideri</i>) | 7.00E-32 | 118 | 74% | 49% | pear |
| JAU69769.1 | Homeobox-leucine zipper protein ATHB-7 (<i>Nocca caerulea</i>) | 1.00E-31 | 118 | 57% | 58% | |
| KNA21535.1 | hypothetical protein SOVF_042290 (<i>Spinacia oleracea</i>) | 1.00E-31 | 117 | 67% | 51% | spinach |
| GAV72153.1 | Homeobox domain-containing protein/HALZ domain-containing protein (<i>Cephalotus follicularis</i>) | 1.00E-31 | 117 | 68% | 52% | pitcher plant |
| XP_003627511.1 | homeobox associated leucine zipper protein (<i>Medicago truncatula</i>) | 1.00E-31 | 117 | 61% | 59% | barrelclover |

| Identity | Description | E-Value | Bit Score | Coverage | %¹ | Common Name |
|-----------------|--|----------------|------------------|-----------------|----------------------|--------------------|
| XP_010506683.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Camelina sativa</i>) | 1.00E-31 | 118 | 61% | 56% | Camelina |
| XP_015947926.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Arachis duranensis</i>) | 1.00E-31 | 118 | 63% | 57% | |
| KDO76727.1 | hypothetical protein CISIN_1g027468mg (<i>Citrus sinensis</i>) | 2.00E-31 | 117 | 75% | 50% | sweet orange |
| AD150265.2 | homeodomain 20 transcription factor (<i>Nicotiana attenuata</i>) | 2.00E-31 | 117 | 66% | 55% | wild tobacco |
| XP_016181965.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Arachis ipaensis</i>) | 2.00E-31 | 118 | 63% | 57% | |
| JAU45030.1 | Homeobox-leucine zipper protein ATHB-7 (<i>Nocca caerulea</i>) | 2.00E-31 | 117 | 57% | 58% | |
| EOX95622.1 | Homeobox 7 (<i>Theobroma cacao</i>) | 2.00E-31 | 119 | 68% | 57% | cocoa tree |
| XP_009142615.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7 (<i>Brassica rapa</i>) | 3.00E-31 | 117 | 69% | 54% | turnip |
| XP_008810697.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Phoenix dactylifera</i>) | 3.00E-31 | 115 | 58% | 57% | date palm |
| NP_001031550.1 | homeobox 7 (<i>Arabidopsis thaliana</i>) | 3.00E-31 | 117 | 72% | 50% | |
| XP_006294845.1 | hypothetical protein CARUB_v10023896mg (<i>Capsella rubella</i>) | 3.00E-31 | 117 | 66% | 54% | |
| AFV30231.1 | homeobox-leucine zipper protein (<i>Medicago sativa</i>) | 4.00E-31 | 116 | 62% | 57% | alfalfa |
| OAY62110.1 | hypothetical protein MANES_01G242300 (<i>Manihot esculenta</i>) | 5.00E-31 | 116 | 58% | 59% | cassava |
| XP_018433939.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7 (<i>Raphanus sativus</i>) | 5.00E-31 | 116 | 66% | 55% | radish |
| JAU86530.1 | Homeobox-leucine zipper protein ATHB-7 (<i>Nocca caerulea</i>) | 7.00E-31 | 116 | 55% | 59% | |
| XP_018842791.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Juglans regia</i>) | 7.00E-31 | 115 | 68% | 53% | walnut |
| XP_004230017.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12 (<i>Solanum lycopersicum</i>) | 7.00E-31 | 115 | 53% | 62% | tomato |

| Identity | Description | E-Value | Bit Score | Coverage | % ¹ | Common Name |
|----------------|--|----------|-----------|----------|----------------|-----------------|
| AGV54280.1 | homeobox protein (<i>Phaseolus vulgaris</i>) | 8.00E-31 | 116 | 59% | 58% | bean |
| JAU22780.1 | Homeobox-leucine zipper protein ATHB-7 (<i>Nocca caerulea</i>) | 8.00E-31 | 116 | 54% | 60% | penny cress |
| EEF32403.1 | homeobox protein, putative (<i>Ricinus communis</i>) | 8.00E-31 | 116 | 55% | 61% | castor bean |
| XP_014524102.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12 (<i>Vigna radiata</i> var. <i>radiata</i>) | 8.00E-31 | 115 | 58% | 59% | mung bean |
| XP_017441838.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Vigna angularis</i>) | 1.00E-30 | 115 | 58% | 59% | adzuki bean |
| XP_017241533.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Daucus carota</i> subsp. <i>sativus</i>) | 1.00E-30 | 115 | 75% | 46% | wild carrot |
| XP_008791625.1 | PREDICTED: homeobox-leucine zipper protein HOX6-like (<i>Phoenix dactylifera</i>) | 1.00E-30 | 114 | 62% | 54% | date palm |
| AIN45171.1 | homeobox-leucine zipper protein 1 (<i>Rosa</i> hybrid cultivar) | 1.00E-30 | 115 | 61% | 56% | miniature rose |
| XP_004306630.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Fragaria vesca</i> subsp. <i>vesca</i>) | 1.00E-30 | 115 | 61% | 56% | wild strawberry |
| NP_001281285.1 | homeobox-leucine zipper protein ATHB-12-like (<i>Malus domestica</i>) | 1.00E-30 | 114 | 61% | 54% | apple |
| OEL19765.1 | Homeobox-leucine zipper protein HOX6 (<i>Dichanthelium oligosanthes</i>) | 2.00E-30 | 114 | 66% | 50% | perennial grass |
| XP_013631726.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7 (<i>Brassica oleracea</i> var. <i>oleracea</i>) | 2.00E-30 | 115 | 69% | 53% | cabbage |
| XP_015888788.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Ziziphus jujuba</i>) | 2.00E-30 | 114 | 62% | 55% | |
| XP_007135294.1 | hypothetical protein PHAVU_010G117200g (<i>Phaseolus vulgaris</i>) | 2.00E-30 | 116 | 59% | 58% | bean |

| Identity | Description | E-Value | Bit Score | Coverage | %¹ | Common Name |
|-----------------|---|----------------|------------------|-----------------|----------------------|--------------------|
| XP_007218369.1 | hypothetical protein PRUPE_ppa011221mg (<i>Prunus persica</i>) | 2.00E-30 | 114 | 62% | 52% | peach |
| XP_020223175.1 | homeobox-leucine zipper protein ATHB-12-like isoform X2 (<i>Cajanus cajan</i>) | 2.00E-30 | 114 | 56% | 61% | pigeon pea |
| XP_020114943.1 | homeobox-leucine zipper protein HOX6-like (<i>Ananas comosus</i>) | 2.00E-30 | 114 | 63% | 53% | pineapple |
| XP_004510630.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12 (<i>Cicer arietinum</i>) | 3.00E-30 | 115 | 61% | 59% | |
| XP_004307928.1 | PREDICTED: homeobox-leucine zipper protein ATHB-12-like (<i>Fragaria vesca</i> subsp. <i>vesca</i>) | 3.00E-30 | 113 | 72% | 48% | |
| XP_010273803.1 | PREDICTED: homeobox-leucine zipper protein ATHB-7-like (<i>Nelumbo nucifera</i>) | 3.00E-30 | 113 | 69% | 47% | |
| AAD37699.1 | homeodomain leucine zipper protein (<i>Oryza sativa</i> Indica Group) | 3.00E-30 | 113 | 90% | 46% | Indica rice |
| KNA09326.1 | hypothetical protein SOVF_154640 (<i>Spinacia oleracea</i>) | 3.00E-30 | 114 | 61% | 53% | spinach |
| AFP55588.1 | homeobox leucine zipper (<i>Rosa rugosa</i>) | 3.00E-30 | 113 | 63% | 53% | |

¹ % = Percent similarity

H. Appendix 8. HB4 Protein Production in *E. coli* and Equivalency to HAHB4v Protein in HB4 Soybean.

HAHB4v protein produced in *Escherichia coli* was shown to be equivalent to the protein present in HB4 soybean. These data support the conclusion that HAHB4v protein is suitable for use in safety evaluations and to serve as a reliable standard for further studies.

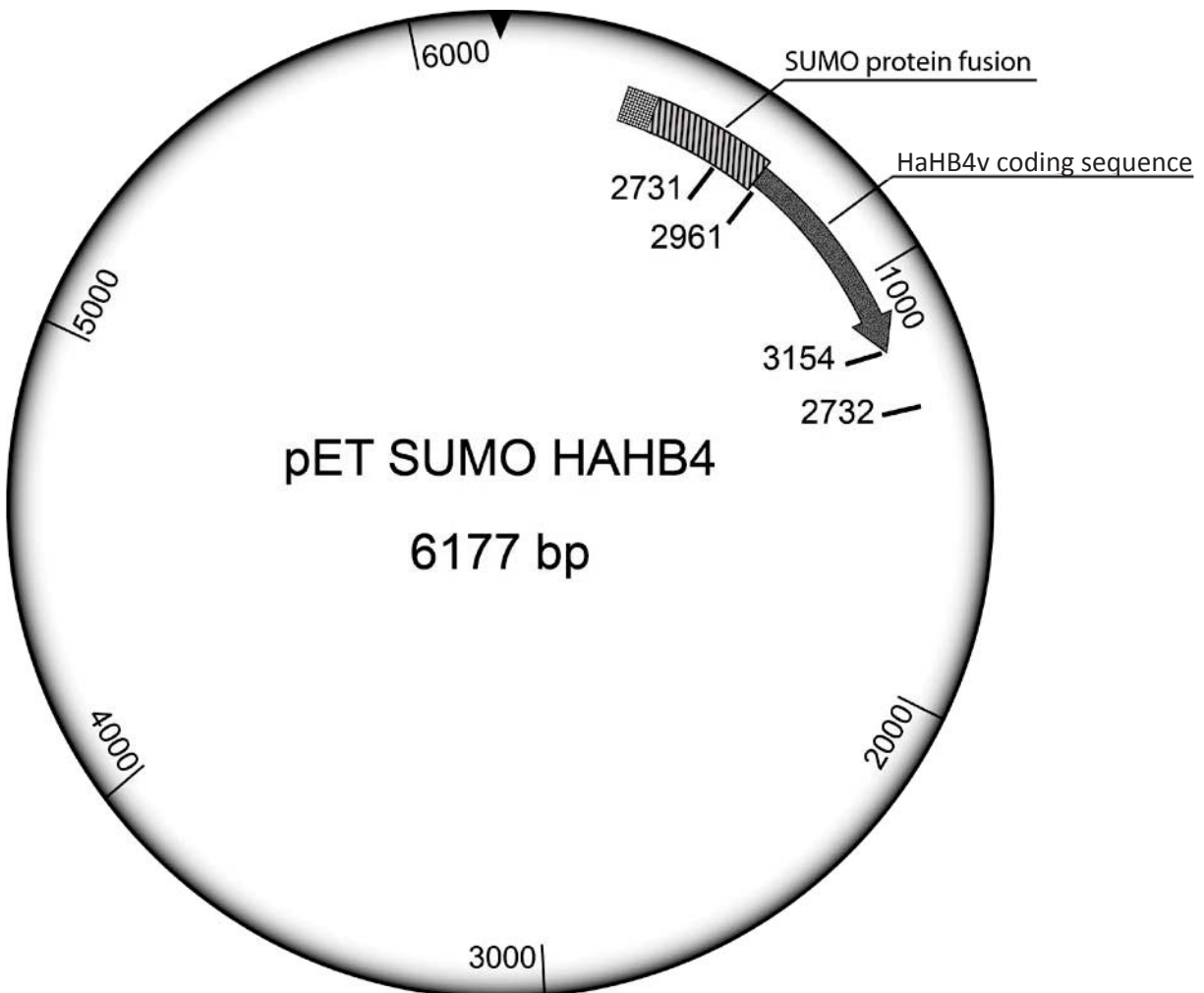
Materials & Methods

Cloning of the Protein Expression Vector

The *HaHB4v* coding sequence was cloned into a pET SUMO vector (Champion pET SUMO Protein Expression System, Invitrogen, Carlsbad, CA, Catalog no. K300-01) for expression and purification of HAHB4v protein (Figure 8-1). The pET SUMO expression system uses a polyhistidine tag to assist in purification from a crude extract. Afterward, the tag is removed and the target protein is isolated with no remaining amino acid residues as described below.

Figure 8-1. Plasmid Map for Protein Expression Vector pARC666.1 B8.

Primer 2731, Primer 2961, Primer 3154, Primer 2732 are indicated used to confirm the sequence and orientation of the target expression product.



The coding sequence is identical to that of the event IND-00410-5 (Figure 8-2). The HAHB4v coding sequence was cloned using primers 2961 and 3154 (Table 8-1 and Figure 8-1). PCR was performed using a proof-reading polymerase (Phusion® Hot Start Flex DNA Polymerase, New England Biolabs, Ipswich, MA; dNTPs, Thermo Scientific, Waltham, MA) to ensure sequence fidelity. PCR cycling conditions were 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds, 70°C for 35 seconds, and 68°C for 1 minute followed by 68°C for 10 minutes. Reactions were held at 4°C until used. All PCR reactions were carried out in an EP Gradient-S Eppendorf Mastercycler thermocycler. A sample of the PCR reaction was cleaned using Illustra GFX PCR and DNA Gel Band Purification Kit (GE Healthcare, Cleveland, OH) as described by the manufacturer's PCR product clean-up instructions. Following band purification, a sample was confirmed as matching the expected sequence present in the HB4 event by DNA sequencing (Davis Sequencing, INC., 1450 Drew Ave, Suite 100, Davis, CA 95618).

Figure 8-2. Coding Sequence Comparison

Sequence alignment showing the translated *E. coli*-produced HAHB4v sequence (ECOLI_HB4v) is identical to the protein sequence translated from the plasmid transformed into IND-00410-5 and used to clone the gene sequence present in the *E. coli* expression vector pARC666.1 B8. As reported in the Southern/sequencing data section, the T-DNA sequence found in IND-00410-5 is identical to the *pIND2-HB4* plasmid.

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ECOLI_HB4v  MSLQQVTTTRKRNNEGRRRFTDKQISFLEYMFETQSRPELRMKHQLAHKLGLHPRQVAIWFQNKRRSKSRQIEQEYNAL
Soy_HB4v    MSLQQVTTTRKRNNEGRRRFTDKQISFLEYMFETQSRPELRMKHQLAHKLGLHPRQVAIWFQNKRRSKSRQIEQEYNAL

ECOLI_HB4v  KHNYETLASKSESLKKNQALLNQLEVLRLNVAEKHQEKTSSSGSGEESDDRFTNSPDVMFQGEMNVPFCDGFAYLEEGNS
Soy_HB4v    KHNYETLASKSESLKKNQALLNQLEVLRLNVAEKHQEKTSSSGSGEESDDRFTNSPDVMFQGEMNVPFCDGFAYLEEGNS

ECOLI_HB4v  LLEIEEQLPDLQKWWEF
Soy_HB4v    LLEIEEQLPDLQKWWEF

```

Table 8-1. Cloning Primers

| Primer Number | Primer Name | Direction | Target Category | Target | Oligo Sequence (5' to 3') |
|---------------|---------------------|-----------|-----------------|----------------------------------|-----------------------------|
| 2731 | SUMO Forward primer | F | Vector | pET SUMO | AGATTCTTGTACGACGGTAT TAG |
| 2732 | T7 Reverse primer | R | Vector | T7 promoter | TAGTTATTGCTCAGCGGTGG |
| 2961 | cHaHahb4-F4 | F | Transgene | Homeobox leucine Zipper (Hahb4v) | ATGTCTCTTCAACAAGTAAC AACCAC |
| 3154 | cHaHahb4-R5 | R | Transgene | Homeobox leucine Zipper (Hahb4v) | TTAGAACTCCCACCACTTTT GAAG |

To clone the *HaHB4v* coding sequence into an expression vector (Champion pET SUMO Protein Expression Kit, Invitrogen, Carlsbad, CA), 3' A-overhangs were added to the

confirmed *HaHB4v* coding sequence PCR product by incubating 20 μ L of PCR product with 1 unit of standard Taq Polymerase (New England Biolabs) at 72°C for 8 minutes. Following the addition of 3' A-overhangs, the PCR product was used in a ligation reaction as described in the Champion pET SUMO Protein Expression Kit instructions with a 1:1 ratio of PCR product to pET SUMO linear vector and incubated overnight at 15°C. From the overnight ligation, 2 μ L was used for bacterial transformation into Mach1™ T1^R cells (Invitrogen) following the manufacturer's instructions provided with the Champion pET SUMO Protein Expression Kit. After transformation, cells were plated on lysogeny broth plus agar plates (LA = lysogeny broth agar plates, Bertani 1951, 2004) pre-warmed to 37°C (following instructions as described in the Champion pET Sumo Protein Kit) and containing 50 μ g/mL kanamycin (Gold Biotechnology, St. Louis, NJ). The plated cells were incubated overnight at 37°C. Individual colonies were selected and grown for 1 hour at 37°C in a 96-well microtiter plate with 150 μ L of lysogeny broth (LB = lysogeny broth, Bertani 1951) media with 50 μ g/mL kanamycin. The LB was prepared as described for LA, but omitting agar. Following incubation, colony PCR was performed on 2 μ L of each culture sample using primers 2731 and 2732 to verify insertion of the *HaHB4v* coding sequence in the proper orientation (Table 8-1) by PCR as described previously. Colonies producing PCR products of the correct size were selected for overnight cultures. For each selected sample, 10 μ L of culture was added to a fresh tube of 5 mL of LB media with 50 μ g/mL kanamycin and incubated overnight at 37°C with shaking at 250 rpm. Cultures were prepared for plasmid DNA using GeneJET Plasmid Mini-Prep Kit (Thermo Scientific) and plasmid DNA was quantified using the Qubit® dsDNA BR Assay Kit (Thermo Fisher Scientific). PCR was repeated on the purified plasmid DNA from the putative correct pET:SUMO:HAHB4v expression vectors as described previously. Clones verified through repeat PCR sequencing of the expression vector were sequenced from the plasmid DNA as a final verification of proper in-frame sequence for protein expression. The clone selected for HAHB4v protein expression work was named pARC666.1 B8.

Protein Extraction and Purification

To express the tagged HAHB4v protein, BL21(DE3) cells (New England BioLabs, Ipswich, MA) were transformed according to the manufacturer's instructions using 10 ng of pARC666.1 B8 DNA. After transformation, the entire cell culture was added to 10 mL of LB media with 50 μ g/mL kanamycin and incubated, with shaking, overnight at 28°C. The overnight culture was used to inoculate fresh LB media with 50 μ g/mL kanamycin at a proportion of 10 mL overnight culture to 500 mL of fresh media. Cells were grown at 28°C for approximately 3-4 hours to an OD600 of 0.4-0.6 before induction with a final concentration of 1 mM isopropyl β -D-1-thiogalactopyranoside (IPTG; Sigma-Aldrich, Buchs, Switzerland). Induced cells were grown at 28°C for 8-12 hours until an OD600 reached approximately 1.0. Cells were harvested in 500 mL bottles by centrifugation (4650 \times g, 4°C for 10 minutes). Pelleted cells were weighed and stored at -80°C until protein extractions were performed.

The *E. coli*-produced HAHB4v fusion protein was purified from both the soluble and insoluble protein fractions of the cell pellets using affinity tag column purification. To extract the soluble proteins, the CelLytic B Plus Kit (Sigma-Aldrich) was used according to manufacturer's instructions. The soluble fraction was processed immediately to minimize aggregation. The insoluble protein fraction pellet was stored at -80°C until needed. For processing, the insoluble fraction pellet was solubilized in phosphate buffered saline (PBS, 50 mM KH₂PO₄, 450 mM K₂HPO₄, 300 mM NaCl, Sigma-Aldrich) with the addition of 8 M urea.

The *E. coli*-produced HAHB4v was purified by affinity column purification using HisPur™ Cobalt Purification Kit with 3 mL resin/column (Thermo Scientific) following manufacturer's instructions. After column purification, the flow through, wash and elution fractions were quantified for protein content using the 660 nm Protein Assay (Thermo Scientific). The protein samples were visualized by SDS-PAGE gel electrophoresis followed by gel staining using Novex Colloidal Blue Staining Kit (Thermo Fisher Scientific). To determine protein sizes, a standard with known protein masses was included (Precision Plus Protein™ Kaleidoscope Standards, Bio-Rad). After verification of proper isolation of the HAHB4v fusion protein from the cell lysate, the fractions containing the HAHB4v fusion protein were combined for dialysis.

Dialysis of the *E. coli*-produced HAHB4v fusion protein was necessary prior to the following step, removing the 6xHis:SUMO tag by SUMO Protease digestion. The HAHB4v fusion protein fractions were quantified and combined with the appropriate amount of SUMO Protease (EnzyMax LLC, Lexington, KY) to achieve a concentration of 1 U protease per 25 µg of protein. The eluates plus protease was transferred into a dialysis cassette (Slide-A-Lyzer Dialysis Cassettes, Thermo Scientific) and placed into buffer G (100 mM Tris, 150 mM NaCl, 5% Glycerol, 1 mM DL-dithiothreitol) with 2 M urea for 4 hours at 4°C. Following the 4-hour incubation, two additional 2-hour dialysis steps were performed to raise the urea from 2 M to 8 M (which inactivates the enzyme) and replace Buffer G with PBS to prepare the cleaved protein sample for removal of the polyhistidine tag away from the HAHB4v protein. A second affinity column purification was performed as described previously to separate the HAHB4v protein from the cleaved 6xHis:SUMO tag and the His-tagged SUMO protease. Protein digestion and proper separation following the second purification was confirmed by SDS-PAGE. Purified HAHB4v protein eluates were stored at 4°C or -80°C until needed.

Protein Gel Electrophoresis for LC-MS detection

The purified *E. coli*-produced HAHB4v protein was denatured and reduced by adding 4× NuPAGE® LDS sample buffer (Thermo Fisher Scientific) and 10× NuPAGE® reducing agent (Thermo Fisher Scientific) to a final 1× concentration followed by heating at 70°C for 10 min. After treatment, solutions were centrifuged for 5 min to pellet any insoluble materials prior to loading clean supernatant on SDS-PAGE gel. Kaleidoscope Precision Plus MW Standards (Bio-Rad, Hercules, CA) were used and 70 µg of individual protein samples were loaded onto each lane of NuPAGE® Novex® 10% Bis-Tris Gel, 1.5-mm thick, 10-well (Thermo Fisher Scientific).

Protein gels were run using 1× MES-SDS buffer containing 0.5 mL of NuPAGE® antioxidant (both from Thermo Fisher Scientific). The protein bands were visualized using Colloidal Blue Staining Kit (Thermo Fisher Scientific). The excision of a gel band between 15 kDa and just above the 25 kDa protein standards containing the HAHB4v protein was guided by MW markers. Excised bands were diced into approximately 1.5 mm cubes and transferred to 1.5 mL Eppendorf Protein LoBind tubes (Eppendorf, Hamburg, Germany) for further processing and digestion.

In-Gel Protein Digestion

In-gel digestion of proteins after SDS-PAGE and subsequent analysis by mass spectrometry was performed as described by Lahm and Langen (2000). The excised and diced gel bands were destained three times with 50% acetonitrile (ACN) in 25 mM ammonium bicarbonate (AmBic) at 37 °C for 30 min, reduced with 50 mM tris(2-carboxyethyl)phosphine (TCEP) in 25 mM AmBic at 60 °C for 10 min, alkylated with 100 mM iodoacetamide in 25 mM AmBic (room temp for 60 min), washed twice with 50% ACN in 25 mM AmBic at 37 °C for 15 min, shrunk with ACN and finally allowed to air dry in a biological safety hood for ~ 20 min. Processed protein gel samples were digested with sequencing grade, modified porcine trypsin (Promega, Sunnyvale, CA). A 2.5 ng/μL trypsin solution was used to provide a protein to enzyme ratio of roughly 20:1.

After gel pieces were swollen with trypsin solution (30 min at room temperature), an additional 25 mM AmBic was added in order to completely cover gel pieces. Samples were incubated at 37°C for 16 hr before the supernatant was collected and the gel rinsed with 35 μL of 15% ACN and 1.5% formic acid in water. Digest and gel rinse were combined into 1.2 mL conical, reduced surface activity, glass autosampler vials (MicroSolv Technologies, Eatontown, NJ) for LC-MS/MS analysis.

LC-MS/MS Detection of HAHB4v

For characterization of *E. coli*-produced HAHB4v, data-dependent mass spectra were obtained on a LCQ Deca XP plus mass spectrometer (Thermo Scientific, San Jose, CA) as described (Appendix 9). Tryptic peptides were identified using one survey MS scan (350 to 2000 Da) followed by three data-dependent MS/MS scans of the three most abundant ions observed in the MS survey scan. Parameters for data-dependent MS/MS included a default charge state of 2, an isolation width of 2.0 Da and a collision energy of 35% for collision induced decay (CID) of ions having an abundance greater than 1×10^5 . The ionization conditions and lens voltages used for data-dependent MS were optimized using (Glu1)-Fibrinopeptide B standard (AnaSpec, Fremont, CA).

Mass spectral data were processed using the GPM manager application (GPM extreme edition, v. 2.2.1.0, Beavis Informatics Ltd, Manitoba, Canada) and Scaffold™ software (v. 3.00.03, Proteome Software Inc., Portland, OR) with comparison to proteins in the UniProtKB Soybean Glycine max database (downloaded on May 8, 2013) to which the sequence of HAHB4v as well as common contaminants were appended (e.g. human keratins, trypsin, BSA, and others in the cRAP database from GPM) prior to reverse concatenation using a Perl script (provided by Dr. Brett Phinney, UC Davis Genome Center Proteomics Core Facility). To assert whether a protein is present in a given sample, a minimum of two peptides with greater than 90% probability of being correctly identified is required as well as a minimum of 99% probability the protein is present. Probabilities were assigned by the Peptide Prophet and Protein Prophet algorithms within the Scaffold bioinformatics software (Keller *et al.* 2002; Nesvizhskii *et al.* 2003; Searle 2010; Searle *et al.* 2008). The peptides identified as exclusive to HAHB4v, in the soybean database, are shown in Table 8-2 and Figure 8-3.

Table 8-2. Peptides of *E. coli*-produced HAHB4 identified by data-dependent LC-MS.

| Position | Peptide Sequence | Number of Spectra | Peptide Probability |
|----------|--------------------|-------------------|---------------------|
| 1-10 | MSLQQVTTTR | 4 | 95% |
| 24-41 | QISFLEYMFETQSRPELR | 5 | 95% |
| 56-64 | QVAIWFQNK | 2 | 92% |
| 72-81 | QIEQEYNALK | 2 | 95% |
| 82-90 | HNYETLASK | 2 | 95% |
| 97-109 | ENQALLNQLEVLR | 8 | 95% |
| 159-173 | SLLEIEEQLPDLQK | 2 | 95% |

Figure 8-3. Protein coverage from data-dependent LC-MS of *E. coli*-produced HAHB4v.

The coding sequence of HAHB4v contains 177 amino acids. The seven peptide sequences identified by data-dependent LC-MS are underlined. Note there were two consecutive peptides (QIEQEYNALK and HNYETLASK) which have been separated by a blank space.

```

1   MSLQQVTTTRTKNRNEGRRRFTDKQISFLEYMFETQSRPELRMKHQLAHKLGLHPRQVAIW 60
61   FQNKRARSKSRQIEQEYNALK HNYETLASKSESLKKENQALLNQLEVLRNVAEKHQEKT 119
120  SSSGSGEESDDRFTNSPDVMFGQEMNVPFCDGFAYLEEGNSSLLEIEEQLPDLQKWWEF 177

```

HAHB4v Protein Characterization

To ensure the recombinant protein was produced in *E. coli* as expected, HAHB4v was characterized for N-terminal sequence and protein mass analysis. Both the soluble and insoluble *E. coli*-produced HAHB4v fractions were tested. For N-terminal sequencing, HAHB4v samples were run on a SDS-PAGE gel as described previously, followed by blotting of the separated proteins onto a PVDF membrane using the iBlot semi-dry transfer method (Thermo Fisher Scientific) following the manufacturer's instructions. The blot was stained with Coomassie Brilliant Blue. The bands of interest were marked on a photo of the blot and blot and photo were submitted for N-terminal sequencing analysis (Iowa State University, Protein Facility) of the first seven N-terminal amino acids using a 494 Procise Protein Sequencer/140C Analyzer (Applied Biosystems Inc).

For protein mass determination, a MALDI-TOF analysis was performed. Soluble and insoluble samples of 500 μ L each of HAHB4v were submitted to Rutgers Center for Advanced Proteomics Research. The samples were desalted, mixed with MALDI matrix and spotted on a MALDI plate. The protein mass was determined by a 4800 MALDI TOF/TOF MS instrument. The MS spectra were acquired in linear positive mode with apomyoglobin as an external calibration. The mass spectrometry data were obtained from an Orbitrap instrument funded in part by an NIH grant NS046593, for the support of the University of Medical and Dental School – New Jersey Neuroproteomics Core Facility.

Results and Discussion

To produce large amounts of protein for digestion studies and protein standards for LC-MS work a recombinant HAHB4v was produced in *E. coli*. The expression system chosen was the SUMO expression and purification system because it could yield highly purified protein without additional amino acid residues or modifications, such that the protein purified from *E. coli* had the same sequence as the protein present in the IND-00410-5 event (Figure 8-2). The production of *E. coli*-produced HAHB4v involved further extraction and purification of the HAHB4v fusion protein away from host proteins followed by cleavage of the polyhistidine tag using His-tagged SUMO protease. The cleaved tag and protease were then removed leaving highly purified and concentrated HAHB4v. Figures 8-4 and 8-5 represent an example of SDS-PAGE gels of HAHB4v during purification. The HAHB4v protein band migrates to approximately 20 kDa, which corresponds to the predicted size of 20.9 kDa.

Figure 8-4. SDS-PAGE Gel of Purified Insoluble *E. coli*-produced HAHB4v Protein.

Fractions from the second affinity purification of HAHB4v (insoluble) are shown run against the protein molecular weight marker (MW, 5 μ L). The combined eluates from the first purification were collected as described in the text and run here prior to purification (CE). The flow thru (FT) and wash fractions (W1, W2, W3) contain the targeted HAHB4v protein, free from the uncut HAHB4v fusion protein and 6 \times His:SUMO tag (E1, E2, E3). A total of 5 μ g of protein was loaded in each lane.

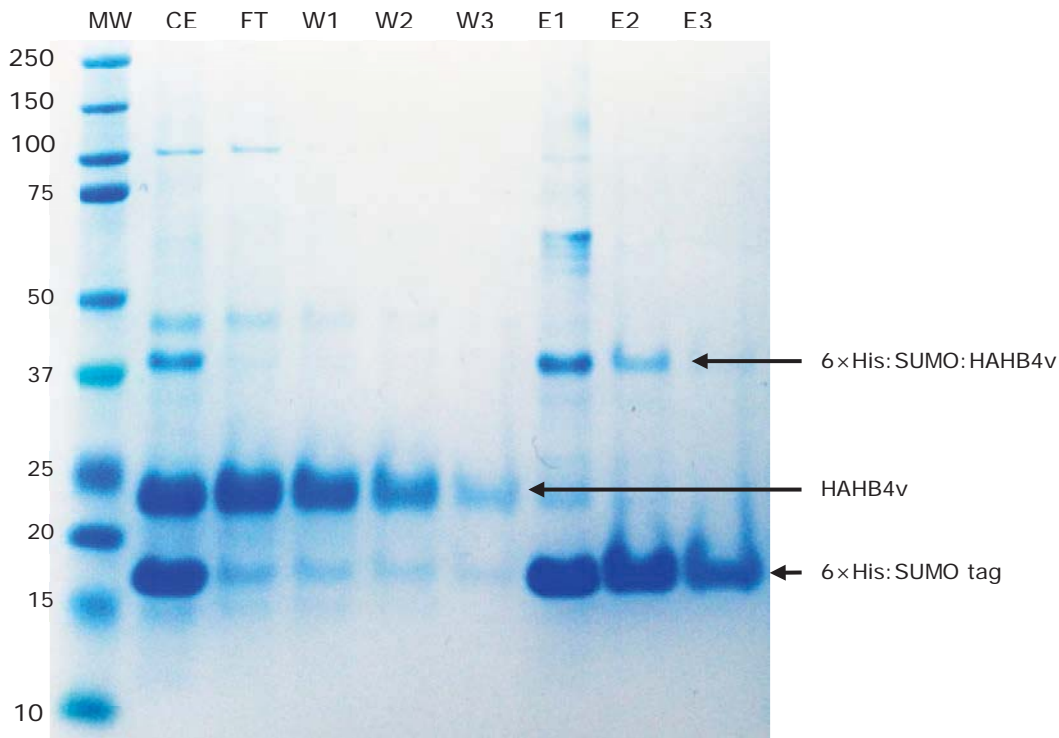
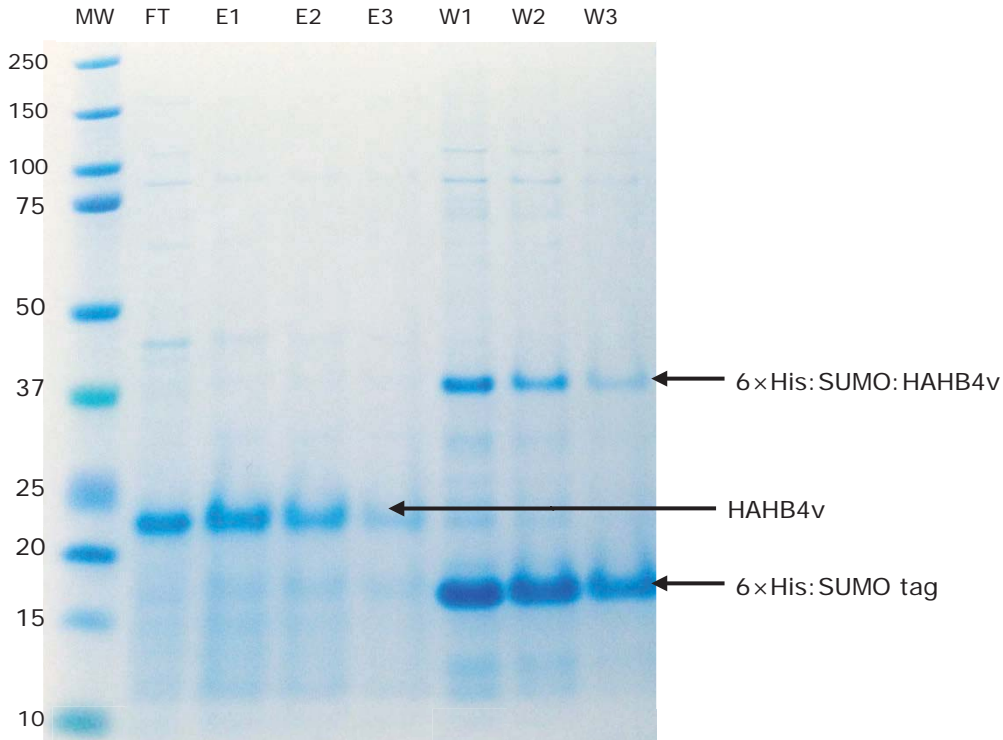


Figure 8-5. SDS-PAGE Gel of Purified Soluble HAHB4v Protein.

Fractions from the second affinity purification of *E. coli*-produced HAHB4v (soluble) are shown run against the protein molecular weight marker (MW, 5 μ L). Fractions were collected throughout the purification as described and visualized by SDS-PAGE. The flow thru (FT) and wash fractions (W1, W2, W3) contain the targeted HAHB4v protein, free from the uncut HAHB4v fusion protein and 6 \times His:SUMO tag (E1, E2, E3). A total of 5 μ g of protein was loaded in each lane (2-8). After purification, the protein was a purity of 78% according to UV-VIS analysis (data not shown).



To ensure the final HAHB4v protein no longer contained the 6xHis tag and was not modified during isolation from *E. coli*, data-dependent LC-MS/MS was used to detect peptides corresponding to HAHB4v. The application of peptide algorithm calculations to identify a protein in a mixture has been described and accepted in plant tissue matrices (Benschop *et al.* 2007; Obenland *et al.* 2008; Skinner *et al.* 2012; Slade *et al.* 2012) and other systems (Benson *et al.* 2009; Rice *et al.* 2012). A total of seven peptides were detected spanning the protein, including the N-terminal peptide (Figure 8-3). N-terminal sequencing of HAHB4v produced from the soluble and insoluble fractions confirmed no N-terminal modifications, correct N-terminal amino acid sequence for the first seven amino acids (MSLQQVT), and confirmed the polyhistidine tag had been removed. Detection of the peptides from both the soluble and insoluble fractions demonstrated 47% coverage of the HAHB4v protein with each peptide scoring a probability greater than 90% that the sequence had been correctly identified. Taken together, the algorithms in Protein Prophet assigned a 99% probability the protein was correctly identified in the samples. Further, no HAHB4v peptides were present in the UniProtKB Soybean *Glycine max* database ensuring the detected peptide sequences were only

derived from HAHB4v. MALDI-TOF analysis further showed the HAHB4v produced from the soluble and insoluble fractions were of the expected molecular mass. Based on the collective data from LC-MS analysis, MALDI-TOF mass detection, and N-terminal sequencing, HAHB4v protein produced in *E. coli* was shown to be equivalent to the protein present in HB4 soybean. These data support the conclusion that *E. coli*-produced HAHB4v protein is suitable for use in safety evaluations and to serve as a reliable standard for further studies.

I. Appendix 9. HAHB4v Protein Detection in Field-Grown Seed and Leaves.

Summary

Methods were developed to measure the levels of HAHB4v protein in soybean seed and leaf tissue harvested from field trials. HAHB4v protein expression in the selected transgenic event, IND-00410-5 was analyzed using a specific and sensitive LC-MS/MS method. However, the levels of the protein were below the lower limit of quantification (LLOQ). A separate report provides details and results of HAHB4v expression in plants exposed to abiotic stresses (Verdeca 2015).

Materials and Methods

Plant material samples

Soybean leaf and seed tissue samples from event IND-00410-5 and parental control, Williams 82, were harvested from six field sites in Argentina during the 2012-2013 growing season and from five field sites in the United States during the 2013 season. The field trials were grown across a range of soybean production areas to represent typical conditions encountered by the plants. Tissue samples were collected from four plots at each field site. The six geographically diverse field sites in Argentina include: Monte Buey, Cordoba (MB, Verdeca A); Corral de Bustos, Cordoba 2 (CB2FS, Verdeca D2); Carmen de Areco, Buenos Aires 1 (CA1FS, Verdeca G1); Hughes, Sante Fe 1 (HG1FS, Verdeca Q1); Hughes, Sante Fe 2 (HG2FS, Verdeca Q2); and Aranguren, Entre Rios 1 (AR1FS, Verdeca W1). The five distinct locations in the United States include: Effingham, IL (IL3); Ladoga, IN (IN); Pemberton, OH (OH2); Richland, IA (IA); and Troy KS (KS). These field sites were representative of soybean producing regions suitable for commercial production in each country. At each site, four replicated plots of event IND-00410-5 and the conventional control Williams 82 were planted using a randomized complete block field design. Leaf and seed tissue were collected from each replicated plot from all field sites at R3/R4 and maturity, respectively (Fehr and Caviness 1977). Prior to extraction, all samples were homogenized through mechanical grinding under liquid nitrogen.

Recombinant Protein Detection and Quantification

Recombinant HAHB4 variant (rHAHB4v) protein was used as an analytical reference standard for development and validation of the HAHB4v analytical measurement method. The rHAHB4v was produced as a 6×His-tagged, SUMO-fusion protein in *E. coli* as described in Appendix 8. The lot of rHAHB4v used for method validation was characterized by data-dependent LC-MS analysis using a LCQ Deca XP plus mass spectrometer (Thermo Scientific, San Jose, CA) equipped with the same HPLC and autosampler system described in the section LC-MS/MS Quantification of HAHB4v. The HPLC column and mobile phase program were also as described above. A gel band containing ~5 µg of rHAHB4v was digested in 25 mM AmBic containing 2.5 ng/µL trypsin solution (16 hr at 37°C) and the digest harvested and acidified before LC-MS characterization. Peptides were identified using one survey MS scan (350 to 2000 Da) followed by three data-dependent MS/MS scans of the three most abundant ions observed in the MS survey scan. Parameters for data-dependent MS/MS included a default charge state of 2, an isolation width of 2.0 Da and a collision energy of 35% for CID of ions having an abundance greater than 1×10^5 . The ionization conditions and lens voltages used for data-dependent MS were optimized using (Glu1)-Fibrinopeptide B standard (AnaSpec, Fremont, CA).

Mass spectral data were processed using the GPM manager application (GPM extreme edition, v. 2.2.1.0, Beavis Informatics Ltd, Manitoba, Canada) and Scaffold™ software (v. 3.00.03, Proteome Software Inc., Portland, OR) with comparison to proteins in the UniProtKB Soybean Glycine max database (downloaded from on May 8, 2013) to which the sequence of rHAHB4v as well as common contaminants were appended (e.g. human keratins, trypsin, BSA, and others in the cRAP database from GPM) prior to reverse concatenation using a Perl script (provided by Dr. Brett Phinney, UC Davis Genome Center Proteomics Core Facility). Protein identification required a minimum of 2 peptides of greater than 90% probability and a minimum of 99% protein probability. Probabilities were assigned by the Peptide Prophet and Protein Prophet algorithms within Scaffold bioinformatics software (Searle 2010). The peptides identified as exclusive to rHAHB4v, in the soybean database, are shown in Table 9-1.

Table 9-1. Peptides of rHAHB4v identified by data-dependent LC-MS and synthetic peptide chosen as quantitative LC-MS/MS targets.

| Position | Peptide Sequence | Number of Spectra | Peptide Probability | Synthetic Peptide ¹ |
|----------|--------------------|-------------------|---------------------|--------------------------------|
| 1-10 | MSLQQVTTTR | 4 | 95% | 3 |
| 24-41 | QISFLEYMFETQSRPELR | 5 | 95% | 5 |
| 56-64 | QVAIWFQNK | 2 | 92% | na ² |
| 72-81 | QIEQEYNALK | 2 | 95% | na |
| 82-90 | HNYETLASK | 2 | 95% | 2 |
| 97-109 | ENQALLNQLEVLR | 8 | 95% | 1 |
| 159-173 | SLLEIEEQLPDLQK | 2 | 95% | na |

¹ Synthetic peptide number corresponds to peptide number in Table 9-2.

² na: not available

Stable isotope labeled proteotypic peptides specific to HAHB4v were obtained from JPT Peptide Technologies GmbH (SpikeTides™ TQL, Berlin, Germany). The peptide reference standards were selected based on their detection upon data-dependent LC-MS of rHAHB4v and their uniqueness to HAHB4v in soybean, verified by BLAST searching against proteins in the UniProtKB Soybean Glycine max database (downloaded May 8, 2013).

LC-MS Reagents

LC-MS grade acetonitrile (ACN) and water were obtained from Honeywell Burdick and Jackson (Muskegon, MI). Formic acid (FA) and iodoacetamide were purchased from Sigma-Aldrich St. Louis, MO. Tris (2-carboxyethyl)phosphine hydrochloride (TCEP) was obtained from Thermo Fisher Scientific (Waltham, MA). Acetic acid was purchased from JT Baker (Center Valley, PA) and ammonium bicarbonate (AmBic) was from MP Biochemicals (Solon, OH). The Protease Inhibitor Cocktail was purchased from Sigma (St. Louis, MO).

Protein Extraction

Tissue samples were flash frozen on collection and stored at -80°C until used. Before extraction, tissues were ground to a fine powder while frozen in liquid nitrogen. Leaf tissue

was ground using a mortar and pestle and seeds were ground using a mechanical cryogenic mixer mill (Retsch, Haan, Germany) equipped with 50 mL stainless steel jar and ball. Tissues were extracted using the Plant Total Protein Extraction Kit (Sigma-Aldrich), which contains strong chaotropes and a detergent to solubilize HAHB4v protein and prevent its aggregation. The kit also contains a protease inhibitor cocktail that is a mixture of protease inhibitors with broad specificity for the inhibition of serine, cysteine, metalloproteases, aspartic, and aminopeptidases. It has been demonstrated to be highly effective in preventing protein degradation during the extraction process (Dubielka *et al.*, 2013 and Sigma Aldrich Plant Total Protein Extraction Kit PE0230). Before protein extraction, polyphenols, tannins and other interferences were removed from tissue by three successive extractions with -20°C methanol and one extraction with -20°C acetone. Each interference extraction employed centrifugation ($16,000 \times g$, 5 min, 4°C) to pellet the protein containing tissue while the interfering substances were decanted to waste. Pelleted tissue was dried on a Savant SpeedVac (Model SPD111V, Thermo Scientific, Asheville, NC) and weighed. For extraction of protein from dried plant tissue the kit uses the chaotropic Sigma Reagent Type 4 containing Trizma[®] buffered urea, thiourea, and detergent C7BzO. Sigma protease inhibitor cocktail was added at 1% v/v to the protein extraction reagent just prior to its addition to tissue pellets at 15 μ L per mg of dry tissue. Proteins were extracted by intermittent vortex at room temperature over 15 min and clarified by centrifugation ($18,000 \times g$ for 30 min at 23°C) before protein was harvested. The concentration of total protein in extracts was determined using a Qubit Model 2.0 Fluorometer and Qubit Protein Assay Kit (Life Technologies, Eugene, OR).

Protein Gel Electrophoresis

The extracted protein was denatured and reduced by adding 4 \times NuPAGE[®] LDS sample buffer (Thermo Fisher Scientific, Carlsbad, CA) and 10 \times NuPAGE[®] reducing agent (Thermo Fisher Scientific) to a final 1 \times concentration followed by heating at 70°C for 10 min. Samples were centrifuged for 5 min prior to loading on SDS-PAGE gel. The Kaleidoscope Precision Plus MW Standards (Bio-Rad, Hercules, CA) was used as a reference standard and 70 μ g of individual protein samples were loaded onto each lane of NuPAGE[®] Novex[®] 10% Bis-Tris Gel, 1.5-mm thick, 10-well and visualized using Colloidal Blue Stain (Thermo Fisher Scientific). In addition to analyzing protein extracted from tissues of each individual plot, a portion of the protein from the four test plots per field site was pooled, on a dry weight basis. The pooled protein samples from each field site were then separated by SDS-PAGE using six lanes of 70 μ g each (420 μ g total) and analyzed by LC-MS/MS, as described below. Each sample was run in duplicate.

Protein gels were developed using MES-SDS running buffer containing 0.5 mL of NuPAGE[®] antioxidant (both from Thermo Fisher Scientific) with electrophoresis conducted at 70V for 15 min then 100V for 1 hr more. The protein gels were fixed, stained and destained according to the manufacturer's instructions (Thermo Fisher Scientific). The excision of gel bands for analysis was guided by both MW markers and endogenous protein bands. Using a razor blade, bands were cut just above the 20 kDa and below the 25 kDa protein standards, a section that contains HAHB4v protein (based on known rHAHB4v migration). Recombinant HAHB4v protein (21 kDa) migrates with a slightly higher apparent MW on SDS-PAGE of approximately 23 kDa; this discrepancy between the predicted migration of a protein and its SDS-PAGE-displayed MW is not uncommon (Shi *et al.*, 2012; Guan *et al.*, 2015). Excised bands were placed on a microscope slide and diced into approximately 1.5 mm cubes and

transferred to 1.5 mL Eppendorf Protein LoBind tubes (Eppendorf, Hamburg, Germany) for further processing and digestion.

In-Gel Protein Digestion

In-gel digestion of proteins after SDS-PAGE and subsequent analysis by mass spectrometry has been described (Lahm and Langen 2000). The excised and diced gel bands were destained three times with 50% ACN in 25 mM AmBic at 37°C for 30 min, reduced with 50 mM TCEP in 25 mM AmBic at 60°C for 10 min, alkylated with 100 mM iodoacetamide in 25 mM AmBic (room temp for 60 min), washed twice with 50% ACN in 25 mM AmBic at 37°C for 15 min, shrunk with ACN and finally allowed to air dry in a biological safety hood for approximately 20 min. Protein gel samples were digested with sequencing grade, modified porcine trypsin (Promega, Sunnyvale, CA). A 2.5 ng/μL trypsin solution was used for leaf gel samples while a 5 ng/μL trypsin solution was used for the higher protein containing gel bands from seed samples, providing a protein to enzyme ratio of roughly 20:1.

Stable isotope-labeled heavy peptides were mixed with trypsin just prior to its addition to destained, reduced and alkylated gel pieces. Quantification of HAHB4v employed targeting four unique peptides, two of which were prepared with both non-oxidized and oxidized methionine residues, as shown in Table 9-2. The amount of each stable isotope (¹³C/¹⁵N) labeled peptide added to each gel sample at the start of digestion was 1,500 femtomoles (fmol). These synthetic peptides from JPT Peptide Technologies GmbH contain a small, proprietary C-terminal tag (Bronsema *et al.*, 2013 and JPT Peptide Technologies, Protocol) that is only removed by tryptic digestion.

Table 9-2. Peptides of HAHB4v Used for LC-MS/MS Quantification.

| Peptide | Sequence | MW |
|-----------|---|--------|
| 1 (Light) | ENQALLNQLEVLR | 1538.8 |
| 1 (Heavy) | ENQALLNQLEVLR (13C/15N - Arg) | 1548.8 |
| 2 (Light) | HNYETLASK | 1061.5 |
| 2 (Heavy) | HNYETLASK (13C/15N - Lys) | 1069.5 |
| 3 (Light) | MSLQQVTTTR | 1163.6 |
| 3 (Heavy) | MSLQQVTTTR (13C/15N - Arg) | 1173.6 |
| 4 (Light) | Met(O)SLQQVTTTR | 1179.6 |
| 4 (Heavy) | Met(O)SLQQVTTTR (13C/15N - Arg) | 1189.6 |
| 5 (Light) | QISFLEYMFETQSRPELR | 2273.1 |
| 5 (Heavy) | QISFLEYMFETQSRPELR (13C/15N - Arg) | 2283.1 |
| 6 (Light) | QISFLEYMet(O)FETQSRPELR | 2289.1 |
| 6 (Heavy) | QISFLEYMet(O)FETQSRPELR (13C/15N - Arg) | 2299.1 |

After the gel pieces were swollen with trypsin solution (30 min at RT), an additional 25 mM AmBic was added in order to completely cover gel pieces. Samples were incubated at 37°C for

16 hr before the digest was harvested and gel rinsed with 35 μL of 15% ACN in 1.5% FA in water. Digest and gel rinse were combined in 1.2 mL conical, reduced surface activity, glass autosampler vials (MicroSolv Technologies, Eatontown, NJ) for LC-MS/MS analysis.

LC-MS/MS Quantification of HAHB4v

HAHB4v was quantified by targeted LC-MS/MS detection using either an LTQ linear ion trap or a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA). LC-MS systems were equipped with a Michrom Captive Spray Source, Paradigm MDLC MS4™ liquid chromatography system (Bruker-Michrom, Auburn, CA) and CTC HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland). The HPLC was fitted with an ACE C18, 300Å, 0.3 x 150 mm capillary column (Advanced Chromatography Technologies, Aberdeen, Scotland) and a peptide cap-trap (Bruker-Michrom) that was loaded with 75 μL of digest and desalted with 25 μL of 2% ACN/0.1% FA, diverted to waste, before the trap was put in-line with the LC solvent by automated valve switching. Peptides were eluted from the trap and column using a 5 $\mu\text{L}/\text{min}$ flow rate and a gradient of ACN (solvent B) in 0.1% formic acid (solvent A) as follows: 5 to 40% B over 55 minutes, 40 to 80% B over 1 minute, hold at 80% B for 2 minutes, 80 to 5% B over 1 minute, and hold at 5% B for 14 minutes.

Heavy (isotope-labeled) internal standard and corresponding light (native) peptides were detected using scheduled segments of either full scan MS/MS on the ion-trap MS or by selected reaction monitoring (SRM) on the triple quadrupole MS. The ionization conditions, lens voltages, transfer line temperatures and MS/MS parameters were first optimized and tuned by infusion of individual heavy peptides in 20% ACN at a flow rate of 5 $\mu\text{L}/\text{min}$. During SRM on the triple quadrupole MS, quadrupole Q1 is used to selectively pass only the precursor ion of interest, at any given time, to the second quadrupole (Q2). Q2 is used for generating fragments from the ions allowed to pass into it from Q1, it is also referred to as the “collision cell” where fragmentation of the selected precursor ions occurs by collisional induced dissociation (CID) with a neutral gas (argon); quadrupole filter Q3 was set to allow only the fragment ions specific to the peptides of interest to pass through for subsequent quantification by the MS detector (e.g. electron multiplier).

Peptide, protein and mass spectral data

The mass filters, retention times and quantification ions used for LTQ linear ion trap MS are provided in Table 9-3. An MS isolation width of 1.5 Da was used except for peptides 1 and 2 where up to a 2.0 Da isolation width was needed for higher trapping efficiencies. Quantification measured MS/MS transitions that were found to be unique in soybean and specific for each heavy and light HAHB4v peptide. MS/MS transitions were chosen based on ion intensities and lack of interferences found upon LC-MS/MS of control matrix. The fragmentation pattern of peptide 2 by collision-induced dissociation (CID) yielded only two prominent ions in the full-scan MS/MS spectrum while the other peptides yielded three prominent ions that were used for quantification. A gel only control, excised from a region devoid of protein, was included with every sample batch to verify proper operation of the LC-MS/MS system, by detection of spiked heavy peptides and absence of light peptides.

Table 9-3. MS Parameters for Detection of HAHB4v Peptides on LTQ Ion-trap MS.

| Peptide Number ¹ | Retention Time (min) | MS/MS Filter Isolation Width (IW) | Quantification Ions (m/z) |
|-----------------------------|----------------------|---------------------------------------|---------------------------|
| 1 (Light) | 40 | 770.7 m/z, IW= 2.0, Scan 340-1400 m/z | 538.0, 871.5, 984.5 |
| 1 (Heavy) | 40 | 775.7 m/z, IW= 2.0, Scan 340-1400 m/z | 538.0, 881.5, 994.5 |
| 2 (Light) | 15 | 532.0 m/z, IW= 1.8, Scan 225-1000 m/z | 811.3, 925.3 |
| 2 (Heavy) | 15 | 536.0 m/z, IW= 1.8, Scan 225-1000 m/z | 819.3, 933.3 |
| 3 (Light) | 25 | 583.0 m/z, IW= 1.5, Scan 300-1050 m/z | 478.3, 705.3, 833.5 |
| 3 (Heavy) | 25 | 588.0 m/z, IW= 1.5, Scan 300-1050 m/z | 488.3, 715.3, 843.5 |
| 4 (Light) | 18 | 590.8 m/z, IW= 1.5, Scan 300-1000 m/z | 478.3, 581.5, 833.5 |
| 4 (Heavy) | 18 | 595.8 m/z, IW= 1.5, Scan 300-1000 m/z | 488.3, 586.5, 843.5 |
| 5 (Light) | 50 | 759.2 m/z, IW= 1.5; Scan 440-1050 m/z | 779.0, 843.6, 1017.3 |
| 5 (Heavy) | 50 | 762.6 m/z, IW= 1.5; Scan 440-1050 m/z | 784.0, 848.6, 1022.3 |
| 6 (Light) | 42 | 764.2 m/z, IW= 1.5; Scan 440-1050 m/z | 787.0, 851.6, 1025.2 |
| 6 (Heavy) | 42 | 767.7 m/z, IW= 1.5; Scan 440-1050 m/z | 792.0, 856.6, 1030.2 |

Table 9-4 lists the SRM transitions used for triple-quadrupole MS detection of digests from SDS-PAGE purification of 420 µg of leaf or seed protein, pooled from tissues collected at each field site. A resolution of 0.7 Da FWHM (full width at half maximum) was used for both Q1 and Q3. Collisional induced dissociations (CID) in Q2 were with argon at 1.3 mTorr and collision energies optimized by infusion of synthetic heavy peptides.

¹ The amino acid sequences of these numbered peptides are provided in Table 9-2.

Table 9-4. SRM Parameters for Detection of HAHB4v Peptides on TSQ Triple Quadrupole MS.

| Peptide Number ¹ | Retention Time (min) | Precursor Ion | Product Ion (collision energy voltage) |
|-----------------------------|----------------------|---------------|--|
| 1 (Light) | 37.5 | 770.5 m/z | 871.5 (27V), 984.5 (25V) |
| 1 (Heavy) | 37.5 | 775.5 m/z | 881.5 (27V), 994.5 (25V) |
| 2 (Light) | 9 | 531.9 m/z | 811.1 (19V), 915.5 (19V) |
| 2 (Heavy) | 9 | 535.9 m/z | 819.1 (19V), 925.5 (19V) |
| 3 (Light) | 20 | 582.9 m/z | 705.4 (21V), 833.5 (22V) |
| 3 (Heavy) | 20 | 587.9 m/z | 715.4 (21V), 843.5 (22V) |
| 4 (Light) | 13 | 590.8 m/z | 705.4 (16V), 833.5 (24V) |
| 4 (Heavy) | 13 | 595.8 m/z | 715.4 (17V), 843.5 (24V) |
| 5 (Light) | 50 | 759.0 m/z | 752.9 (14V), 1017.1 (17V) |
| 5 (Heavy) | 50 | 762.4 m/z | 756.2 (14V), 1022.1 (17V) |
| 6 (Light) | 38 | 764.2 m/z | 786.5 (16V), 819.5 (24V) |
| 6 (Heavy) | 38 | 767.7 m/z | 791.5 (16V), 824.5 (24V) |

¹ The amino acid sequences of these numbered peptides are provided in Table 9-2.

LC-MS data were processed with Xcalibur™ 2.2 software (Thermo Scientific). The criteria for detection of each native peptide were a distinct peak containing all quantification ions at the same retention time as the corresponding heavy peptide internal standard, and with the expected relative MS/MS ion intensities based on analogous transitions of heavy peptide standards. Detection of HAHB4v protein was affirmed only if quantification ions for each of the four target peptides: **1**, **2**, **3**, and **5** were observed, and at the expected relative intensities. The oxidized peptides, **4** and **6**, were not always detected, due to their variable and often low levels. The amount of HAHB4v protein was calculated based on the isotope dilution method (Kippen *et al.* 1997) using the equation:

$$\text{fmol protein} = \text{Average peak area ratio: L1/H1, L2/H2, (L3/H3 + L4/H4), (L5/H5 + L6/H6)} \\ \times \text{fmol heavy peptide added}$$

The calculations used in determining the method limit of detection (LOD) and lower limit of quantification (LLOQ) for 70 and 420 µg protein samples are provided in Tables 9-5, 9-6, 9-7 and 9-8. The LOD is generally defined as the lowest concentration at which 95% of positive samples are detected. The LOD is not necessarily within the linear range of an assay. By using the signal-to-noise method, the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to that noise level times 3 is taken as an estimate of the LOD. This method is commonly applied to analytical methods that exhibit baseline noise as suggested by the International Conference on Harmonisation (ICH, 1996). The LLOQ is the lowest level at which the concentration of an

analyte that can be accurately measured with a specified degree of confidence, in this case within $\pm 20\%$ of the amount added to parental control Williams 82. In this assay, it was found that 25 fmol was the LLOQ because the necessary criterion of $\pm 20\%$ of the amount of rHAHB4v added to Williams 82 was just barely quantifiable, eliminating the possibility of any lower limit being possible.

Table 9-5. Lower Limit of Quantification for 70 μg Protein Samples on LTQ ion-trap MS.

| LLOQ calculations for LTQ ion-trap MS following SDS-PAGE of 70 μg protein | | | | | | |
|--|---|---|---|---|---|---------------------------------------|
| Tissue | Amount rHAHB4v in 70 μg Protein (fmol) | Amount rHAHB4v in 70 μg Protein (μg) ¹ | Gram DW Equivalents for 70 μg of Seed Protein ² | Gram DW Equivalents for 70 μg of Leaf Protein ³ | Amount of rHAHB4v protein ($\mu\text{g}/\text{g}$ DW) ⁴ | Measured fmol of rHAHB4v ⁵ |
| Seed | 0 | 0.000000 | 0.0003825 | | - | ND ⁶ |
| | 0 | 0.000000 | 0.0003825 | | - | ND |
| | 5 | 0.000105 | 0.0003825 | | - | ND |
| | 5 | 0.000105 | 0.0003825 | | - | ND |
| | 10 | 0.000209 | 0.0003825 | | 0.55 | 6.5 |
| | 10 | 0.000209 | 0.0003825 | | 0.55 | 8.6 |
| | 25 | 0.000523 | 0.0003825 | | 1.37 (LLOQ ⁷) | 21 |
| | 25 | 0.000523 | 0.0003825 | | 1.37 (LLOQ ⁷) | 27 |
| Leaf | 0 | 0.000000 | | 0.0005109 | - | ND |
| | 0 | 0.000000 | | 0.0005109 | - | ND |
| | 5 | 0.000105 | | 0.0005109 | - | ND |
| | 5 | 0.000105 | | 0.0005109 | - | ND |
| | 10 | 0.000209 | | 0.0005109 | 0.41 | 11 |
| | 10 | 0.000209 | | 0.0005109 | 0.41 | 14 |
| | 25 | 0.000523 | | 0.0005109 | 1.02 (LLOQ ⁷) | 26 |
| | 25 | 0.000523 | | 0.0005109 | 1.02 (LLOQ ⁷) | 29 |

¹ MW of HAHB4v is 20,927 Da (20.9 kDa), and one fmol of HAHB4v corresponds to 0.000020927 μg .

² The average percentage of protein extracted from seed tissue was 18.3% of DW, and 0.000070 g protein/0.183 total protein represents 0.0003825 g protein/DW equivalents.

³ The average percentage of protein extracted from leaf tissue was 13.7%, and 0.000070 g protein/0.137 is equal to 0.0005109 g DW equivalents.

⁴ Amount of rHAHB4v added to 70 μg protein, expressed as $\mu\text{g}/\text{g}$ DW.

⁵ Amount of HAHB4v (fmol) measured in the 70 μg protein samples analyzed.

⁶ Not detected (ND).

⁷ LLOQ defined as level where the fmol of rHAHB4v measured were within $\pm 20\%$ of the amount added to Williams 82 protein sample prior to purification.

Table 9-6. Limit of Detection for 70 µg Protein Samples.

| Tissue | Highest Williams 82 Noise as fmol HAHB4v ¹ | Highest Williams 82 Noise as µg HAHB4v ² | Gram DW Equivalents for 70 µg of Seed Protein ³ | Gram DW Equivalents for 70 µg of Leaf Protein ⁴ | HAHB4v Noise as µg/g DW | LOD (µg/g DW) ⁵ |
|--------|---|---|--|--|-------------------------|----------------------------|
| Seed | 0.16 | 0.00000335 | 0.0003825 | | 0.0088 | 0.026 |
| Leaf | 0.22 | 0.00000460 | | 0.0005109 | 0.0090 | 0.027 |

¹ The amount of HAHB4v protein represented by the highest noise level detected in any Williams 82 control seed and leaf sample was calculated based on the isotope dilution method (Kippen *et al.* 1997) using the equation described above.

² Calculated from highest Williams 82 matrix noise in fmol x 0.000020927 (µg/fmol).

³ The average percentage of protein extracted from seed tissue was 18.3% of DW, and 0.000070 g protein/0.183 total protein represents 0.0003825 g protein/DW equivalents.

⁴ The average percentage of protein extracted from leaf tissue was 13.7%, and 0.000070 g protein/0.137 is equal to 0.0005109 g DW equivalents.

⁵ Three times the highest Williams 82 matrix noise found under HAHB4v peptides, expressed as µg/g DW.

Table 9-7. Lower Limit of Quantification for 420 µg Protein Samples on TSQ Vantage MS, in µg/g DW.

Lower Limit of quantification calculations for TSQ Vantage MS following SDS-PAGE of 420 µg protein

| Tissue | Amount rHAHB4v in 420 µg Williams 82 Protein (fmol) | Amount rHAHB4v in 420 µg Protein (µg) ¹ | Gram DW Equivalents for 420 µg of Seed Protein ² | Gram DW Equivalents for 420 µg of Leaf Protein ³ | Amount of HAHB4v protein (µg/g DW) ⁴ | Measured fmol of rHAHB4v ⁵ |
|--------|---|--|---|---|---|---------------------------------------|
| Seed | 0 | 0.0000000 | 0.002295 | | - | ND ⁶ |
| | 0 | 0.0000000 | 0.002295 | | - | ND |
| | 1.5 | 0.0000314 | 0.002295 | | - | ND |
| | 1.5 | 0.0000314 | 0.002295 | | - | ND |
| | 3 | 0.0000628 | 0.002295 | | 0.027 (LLOQ ⁷) | 3.18 |
| | 3 | 0.0000628 | 0.002295 | | 0.027 (LLOQ ⁷) | 3.28 |
| | 6 | 0.0001256 | 0.002295 | | 0.055 | 5.81 |
| | 6 | 0.0001256 | 0.002295 | | 0.055 | 6.30 |
| Leaf | 0 | 0.0000000 | | 0.003066 | - | ND |
| | 0 | 0.0000000 | | 0.003066 | - | ND |
| | 1.5 | 0.0000314 | | 0.003066 | - | ND |
| | 1.5 | 0.0000314 | | 0.003066 | 0.010 | 1.32 |
| | 3 | 0.0000628 | | 0.003066 | 0.020 | 4.16 |
| | 3 | 0.0000628 | | 0.003066 | 0.020 | 3.35 |
| | 6 | 0.0001256 | | 0.003066 | 0.041 (LLOQ ⁷) | 6.26 |
| | 6 | 0.0001256 | | 0.003066 | 0.041 (LLOQ ⁷) | 5.03 |

¹ MW of HAHB4v is 20,927 Da (20.9 kDa), and one fmol of HAHB4v corresponds to 0.000020927 µg.

² The average percentage of protein extracted from seed tissue was 18.3%, and 0.000420 g protein/0.183 is equal to 0.002295 g DW equivalents.

³ The average percentage of protein extracted from leaf tissue was 13.7%, and 0.000420 g protein/0.137 is equal to 0.003066 g DW equivalents.

⁴ Amount of rHAHB4v expressed as µg/g DW.

⁵ Measured amount of HAHB4v from 420 µg protein samples on TSQ Vantage MS system.

⁶ Not detected (ND).

⁷ LLOQ defined as level where the fmol of rHAHB4v measured were within ± 20% of the amount of rHAHB4v added to Williams 82 protein sample prior to purification.

Table 9-8. Limit of Detection for 420 µg Protein Samples.

| Tissue | Highest Williams 82 Noise as fmol HAHB4v ¹ | Highest Williams 82 Noise as µg HAHB4v ² | Gram DW Equivalents for 420 µg of Protein | | | HAHB4v Noise as µg/g DW | LOD (µg/g DW) ⁶ |
|-------------------|---|---|---|---------------------------|---------------------------|-------------------------|----------------------------|
| | | | Seed Protein ³ | Leaf Protein ⁴ | Root Protein ⁵ | | |
| Seed | 0.266 | 0.00000557 | 0.002295 | | | 0.0024 | 0.007 |
| Leaf | 0.166 | 0.00000349 | | 0.003066 | | 0.0011 | 0.003 |
| Root ⁷ | 0.070 | 0.00000146 | | | 0.008940 | 0.00016 | 0.0005 |

¹ The amount of HAHB4v protein represented by the highest noise level detected in any Williams 82 control seed and leaf sample was calculated based on the isotope dilution method (Kippen *et al.* 1997) using the equation described above.

² Calculated from highest Williams 82 matrix noise in fmol x 0.000020927 (µg/fmol).

³ The average percentage of protein extracted from seed tissue was 18.3% of DW, and 0.000420 g protein/0.183 total protein represents 0.0002295 g protein/DW equivalents.

⁴ The average percentage of protein extracted from leaf tissue was 13.7%, and 0.000420 g protein/0.137 is equal to 0.003066 g DW equivalents.

⁵ The average percentage of protein extracted from root tissue was 4.7%, and 0.000420 g protein/0.047 is equal to 0.00894 g DW equivalents.

⁶ Three times the highest Williams 82 matrix noise found under HAHB4v peptides, expressed as µg/g DW.

⁷ The LOD for root was calculated in support of the data discussed.

The sequence positions of targeted peptides of HAHB4v are shown in Figure 9-1. These peptides were selected based on results from analysis of rHAHB4v and their uniqueness to HAHB4v protein expected to be present in soybean event IND-00410-5. BLAST searching of individual targeted peptides and HAHB4v protein against the UniProtKB Soybean Glycine max database did not produce any soybean proteins with identical peptide sequences, indicating the selected peptides are unique to HAHB4v in soybean event IND-00410-5. It was also found from LC-MS/MS that the chosen proteotypic peptides and quantification ions lacked interfering MS/MS signals from endogenous soybean peptides at the relevant retention times. Targeted LC-MS/MS allows even a single amino acid difference in peptide sequence to be clearly distinguished, due to observed mass differences during the sequential fragmentation of the amino acid backbone upon MS/MS (Steen and Mann 2004). The sensitivity of the MS detector was maximized by measuring the most intense MS/MS transitions of the unique and well-resolved peptides, while minimizing any interference from endogenous matrix peptides.

Figure 9-1. Coding sequence of HAHB4v Protein and Peptides for MS/MS Quantification.

Locations of MS identified peptides are underlined and those selected for MS/MS targeting are numbered according to Table 9-2. Peptides 4 and 6 as listed in Tables 9-2 and 9-3 are oxidized versions of peptides 3 and 5, respectively.

| | | | | |
|-----|---|--|---|--|
| | 3 | | 5 | |
| 1 | <u>MSLQQVTTRK</u> NRNEGRRRFTDKQISFLEYMFETQSRPELRMKHQLAHKLG ² LHPRQVAIW ¹ 60 | | | |
| 61 | <u>FQNKRR</u> SKSRQIEQEYNALK HNYETLASKSESLK ² KENQALLN ¹ QLEVLRNVAEKHQEKTS 120 | | | |
| 121 | SSGSGEESDDRFTNSPDV ² MFQGEMNVPFC ¹ DGFAYLEEGNSLLEIEEQLPDLQK ² WWEF 177 | | | |

Method Validation

The extraction efficiency, linearity, limit of detection, precision and accuracy of the analytical method were investigated. Williams 82 soybean tissue and protein extracts thereof were fortified with known amounts of rHAHB4v reference material for method validation. The concentration of the rHAHB4v reference material in the working solution used for method validations was determined as described in the Protein Detection Report.

Method precision was established by fortifying three fresh ground seed tissue samples with rHAHB4v at 4.5 µg/g DW equivalents and four fresh ground leaf tissue samples at 3.9 µg/g DW equivalents, prior to extraction and analysis. The protein extracts (70 µg) were purified by SDS-PAGE and analyzed, in duplicate, on the LTQ-MS system. In order to determine the accuracy of the method, pooled protein extracts from Williams 82 soy seed and soy leaf were spiked at 4.5 and 4.8 µg/g DW equivalents, respectively (Table 9-9).

Table 9-9. Method Validation Results for Quantification of rHAHB4v in Soybean Seed and Leaf Extracts.

| Soybean Tissue | Linearity (R ²) ¹ | LLOQ (µg/g DW) ² | Extraction Efficiency (%) ³ | Precision (% RSD) ³ | Accuracy (% Recovery) ⁴ |
|----------------|--|-----------------------------|--|--------------------------------|------------------------------------|
| Seed | 0.996 | 1.37 (LTQ), 0.03 (TSQ) | 83 | 15 | 86 |
| Leaf | 0.995 | 1.02 (LTQ), 0.04 (TSQ) | 76 | 13 | 91 |

¹ R-squared value (R²) from linear regression analysis of duplicate Williams 82 protein samples fortified with rHAHB4v at 10, 25, 50, 150, 250 and 500 fmol; equivalent to 0.5 to 27 µg/g DW in seed and 0.4 to 20 µg/g DW in leaf tissue.

² LLOQ from duplicate Williams 82 protein samples fortified with 0, 5, 10 and 25 fmol of rHAHB4v for LTQ-MS of 70 µg protein samples; and 0, 1.5, 3 and 6 fmol for TSQ-MS of 420 µg protein samples.

³ Extraction efficiency and precision were established using ground Williams 82 seed and leaf tissue samples fortified at 4.5 and 3.9 µg/g DW, respectively. A total of 3 and 4 repeats of seed and leaf, respectively, were used to calculate each number.

⁴ Accuracy determined from Williams 82 seed and leaf pooled protein extracts fortified with rHAHB4v at 4.5 and 4.8 µg/g DW equivalents, respectively.

Method linearity was evaluated using duplicate protein samples containing 10, 25, 50, 150, 250 and 500 fmol rHAHB4v per 70 µg of seed and leaf protein. Linearity calibrators were analyzed on the LTQ MS system (except for leaf protein samples where the LTQ ion-trap was substituted with an LCQ Deca XP plus ion-trap MS). These rHAHB4v concentrations correspond to a range of 0.6 to 30 µg/g DW for seed tissue and 0.4 to 20 µg/g DW for leaf tissue. The lower limit of quantification (LLOQ) of the LTQ MS system was determined from the responses obtained for 70 µg of protein samples containing 0, 5, 10, and 25 fmol of rHAHB4v. The LLOQ represents the level of rHAHB4v in Williams 82 total protein that provided repeated measurements within ± 20% of the fortified level. The limit of detection was determined from evaluating noise in the Williams 82 matrix under the heavy peptide standards. Peaks containing quantification ions at the same retention time as the corresponding heavy peptide were integrated. A conservative estimate of the limit of detection was then obtained by multiplying the highest level of matrix noise from all Williams 82 leaf or seed samples by a factor of three. For the IND-00410-5 samples, all four peptides were required to be detected in the sample with matching peptide ratios to the heavy peptide standards. The calculations used in determining the LLOQ and LOD for analysis of 70 µg protein samples are shown in Tables 9-5 and 9-6.

A triple-quadrupole MS was used to increase the detectability of HAHB4v, and the LLOQ for these analyses was established by fortifying extracts of Williams 82 leaf and seed tissue at 1.5, 3 and 6 fmol of rHAHB4v per 420 µg of protein prior to SDS-PAGE, digestion and analysis. Calculations used in determining the LLOQ and LOD were identical to methods described above (Tables 9-7 and 9-8).

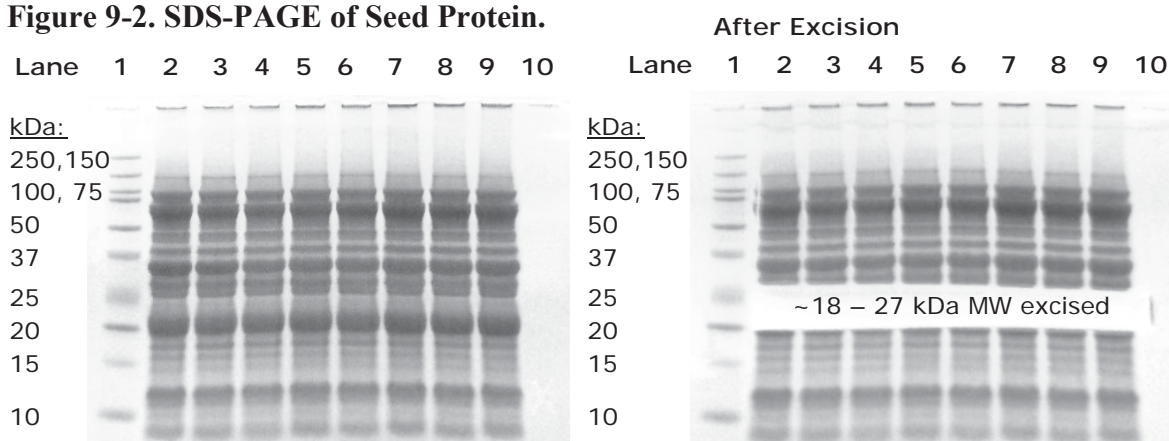
Results

Protein Extraction, Gel Purification and Protein Digestion

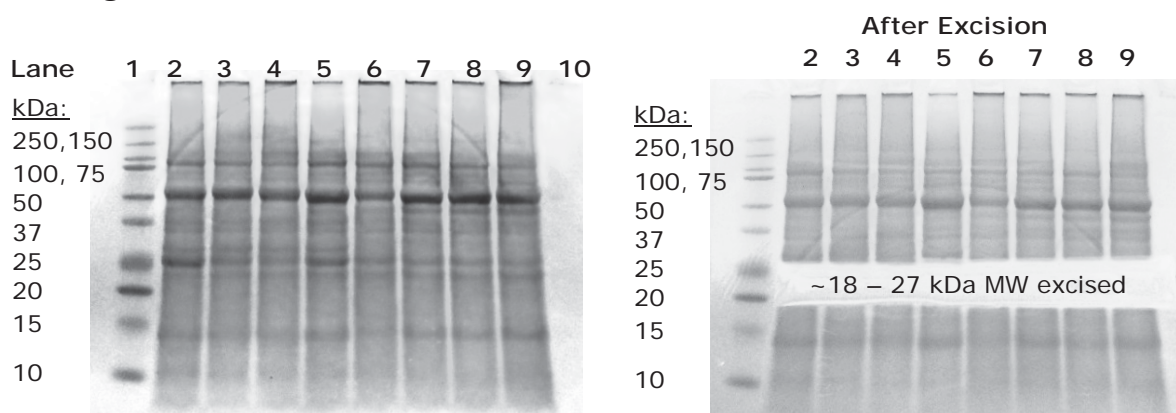
To maximize recovery of proteins present in the samples, extraction of total protein was carried out in the presence of a strong chaotropic reagent in order to break up protein-DNA and protein-protein interactions. The total protein yield, as a percentage of DW, was 18.3% for seeds and 13.7% for leaves. The effective extraction of HAHB4v was verified during method validation (Table 9-9) where 83 and 76% of rHAHB4v was subsequently recovered in the extracts of fortified Williams 82 seed and leaf tissue samples, respectively.

Protein gel electrophoresis provided well resolved protein bands in the expected MW region of HAHB4v (20.9 kDa). Protein bands and MW markers were visualized using Colloidal Blue stain and used to guide the excision of gel containing HAHB4v, examples of protein gels are provided in Figures 9-2 and 9-3.

Figure 9-2. SDS-PAGE of Seed Protein.



| Lane | Sample | Total Protein Load (μg) |
|------|------------------------------|--------------------------------------|
| 1 | Precision Plus MW Marker | N/A |
| 2 | IND-00410-5 Seed MB plot 131 | 70 |
| 3 | IND-00410-5 Seed MB plot 235 | 70 |
| 4 | IND-00410-5 Seed MB plot 334 | 70 |
| 5 | IND-00410-5 Seed MB plot 409 | 70 |
| 6 | Williams 82 Seed MB plot 151 | 70 |
| 7 | Williams 82 Seed MB plot 254 | 70 |
| 8 | Williams 82 Seed MB plot 307 | 70 |
| 9 | Williams 82 Seed MB plot 434 | 70 |
| 10 | Blank | 0 |

Figure 9-3. SDS-PAGE of Leaf Protein.

| Lane | Sample | Total Protein Load (μg) |
|------|------------------------------|--------------------------------------|
| 1 | Precision Plus MW Marker | N/A |
| 2 | IND-00410-5 Leaf AR plot 131 | 70 |
| 3 | IND-00410-5 Leaf AR plot 235 | 70 |
| 4 | IND-00410-5 Leaf AR plot 334 | 70 |
| 5 | IND-00410-5 Leaf AR plot 409 | 70 |
| 6 | Williams 82 Leaf MB plot 151 | 70 |
| 7 | Williams 82 Leaf MB plot 254 | 70 |
| 8 | Williams 82 Leaf MB plot 307 | 70 |
| 9 | Williams 82 Leaf MB plot 434 | 70 |
| 10 | Blank | 0 |

In-gel tryptic digestion of proteins employed highly purified and active porcine trypsin mixed with known amounts of stable isotope labeled peptides unique to HAHB4v in soybean. The tryptic digestion of proteins was monitored by data-dependent LC-MS and was found to deliver consistent peptide profiles. Also, the detection of synthetic heavy peptides, by targeted LC-MS/MS, provided an additional check of effective digestion as these peptide standards contained a C-terminal tag that is only removed by trypsin (Bronsema *et al.* 2013).

Expression Levels of HAHB4v Protein in Soybean Event IND-00410-5

The levels of HAHB4v in tissues of soybean event IND-00410-5 were determined by the absolute quantification (AQUA) method of protein quantification by targeted LC-MS/MS (Gerber *et al.* 2003). The transgenic proteotypic peptides were detected and quantified using stable isotope labeled peptide standards. Stable isotope labeled peptides were used as internal standards and spiked into the sample to accurately quantify the endogenous levels of transgenic protein. This workflow is similar to other targeted proteomic workflows for the identification

of biomarkers and low level endogenous proteins in complex matrices (Fortin *et al.* 2009; Yocum and Chinnaiyan 2009).

After SDS-PAGE, a region of gel expected to contain any HAHB4v protein based on molecular weight migration was excised and digested, in-gel, with trypsin. Stable-isotope labeled synthetic peptides unique to HAHB4v protein in soybean were added to the excised gel bands along with trypsin. The digests were analyzed by LC-MS/MS on a linear-ion trap mass spectrometer with integration of selected ion chromatograms of synthetic heavy peptide internal standards and co-eluting native (light) peptides. The levels of four unique HAHB4v peptides, plus two oxidized peptide forms, were determined using the isotope dilution method (Kippen *et al.* 1997). The arithmetic mean of the four peptides was taken as the level of HAHB4v protein and all four peptides were present in all samples used for method validation.

The analytical method used to quantify expression of transgenic HAHB4v protein was validated using rHAHB4v as an analytical reference standard to fortify the Williams 82 soybean tissue and protein samples prior to analysis. The validation results are summarized in Table 9-9 and representative chromatograms and spectra are shown in Figures 9-6, 9-7 and 9-8. The selected ion chromatograms show the MS/MS responses for the quantification ions of the heavy and light HAHB4v peptides **1**, **2**, **3**, and **5** (peptides **4** and **6** are oxidized forms of **3** and **5**; numbering as listed in Table 9-9). Each of the targeted peptides elute separately and are distinguishable from other potentially interfering peptide ions. All of the quantification ions were present in the full scan MS/MS spectra and in the correct relative ratios. The analytical method was found to provide precise and accurate measurements of HAHB4v, with relative standard deviations (RSD) of 15% or less, and recoveries of 86% and 91% for seed and leaf samples, respectively (Table 9-9). A linear response (R-squared of 0.996) was obtained for rHAHB4v in seed extract at six concentrations ranging from 0.5 to 27 µg/g DW tissue equivalents. Likewise, soybean leaf extract containing rHAHB4v at levels ranging from 0.4 to 20 µg/g DW equivalents provided a linear response (R-squared of 0.995). The response curves are shown in Figures 9-4 and 9-5. The absence of the expected MS/MS spectra in the Williams 82 samples indicates that the plants samples do not contain any measureable HAHB4v protein (Figure 9-6).

Figure 9-4. Linearity of HAHB4v Detection in Williams 82 (WILL) Soybean Seed Extract Fortified with Recombinant Protein.

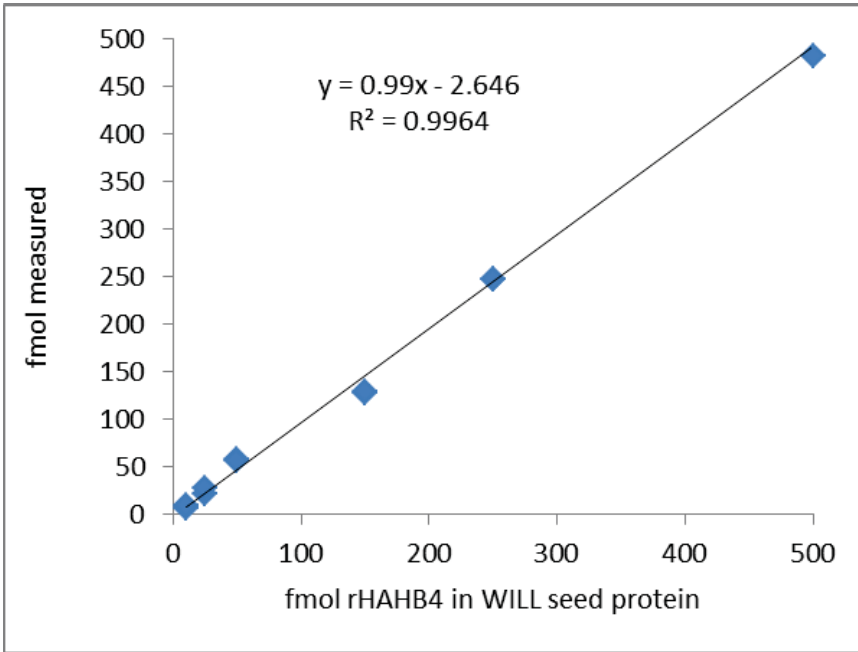


Figure 9-5. Linearity of HAHB4v Detection in Williams 82 (WILL) Soybean Leaf Extract Fortified with Recombinant Protein.

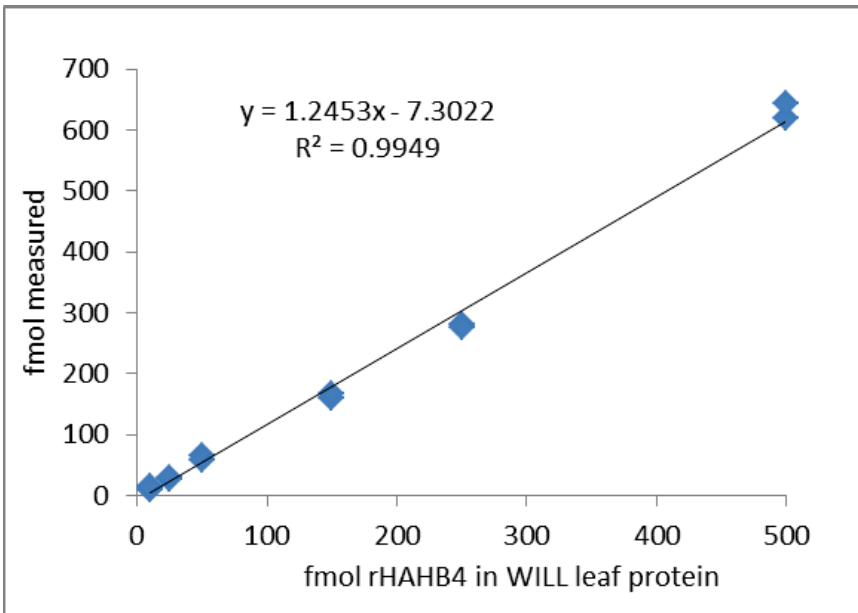


Figure 9-6. Detectability of HAHB4v Peptides in Williams 82. Chromatogram and full-scan MS/MS spectra of control (Williams 82) soybean leaf.

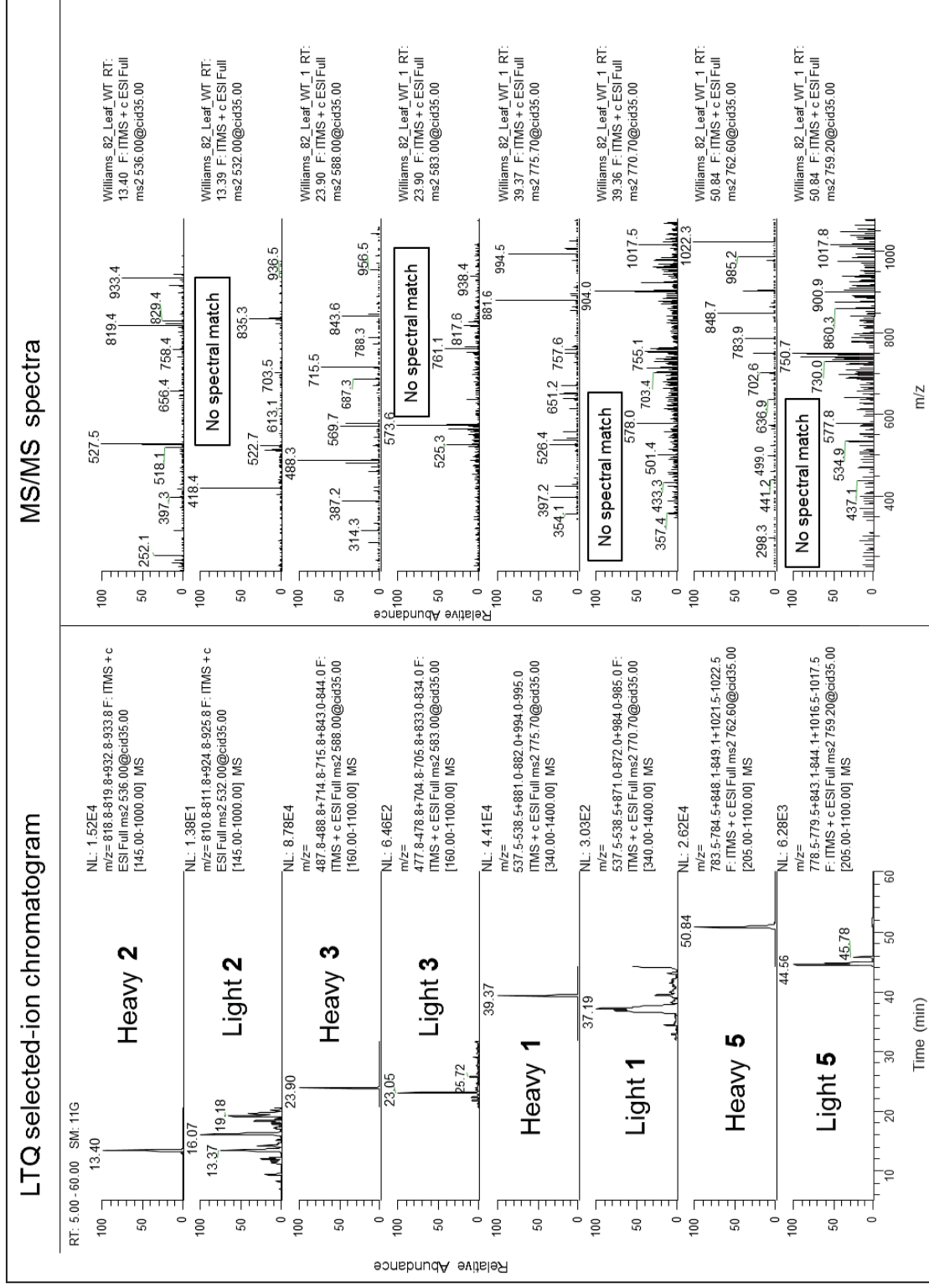


Figure 9-7. Detectability of HAHB4v Peptides in Control Leaf Extracts Fortified With rHAHB4v. Chromatogram and full-scan MS/MS spectra of Williams 82 soybean leaf tissue fortified with rHAHB4v at 3.9 µg/g DW.

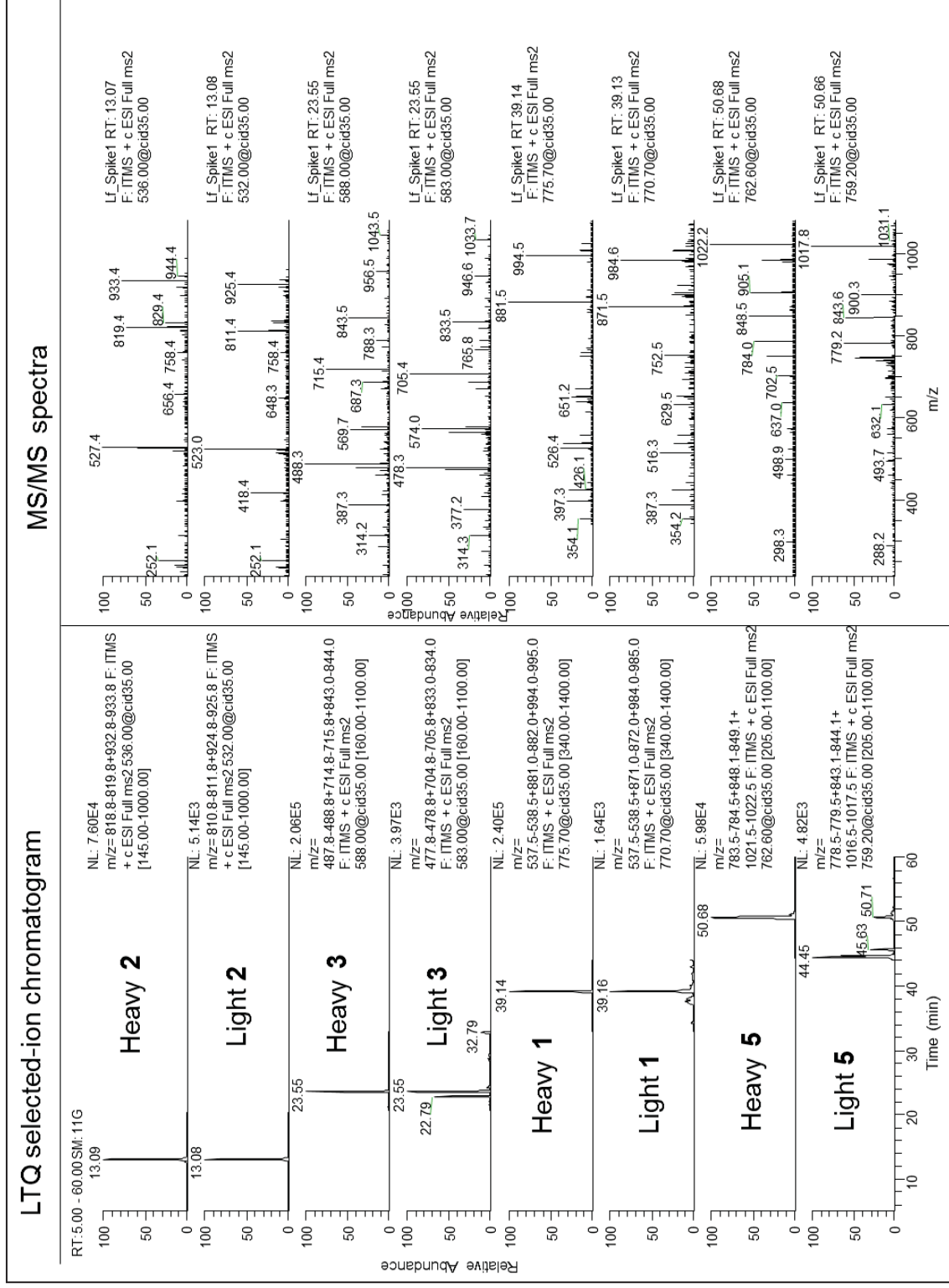
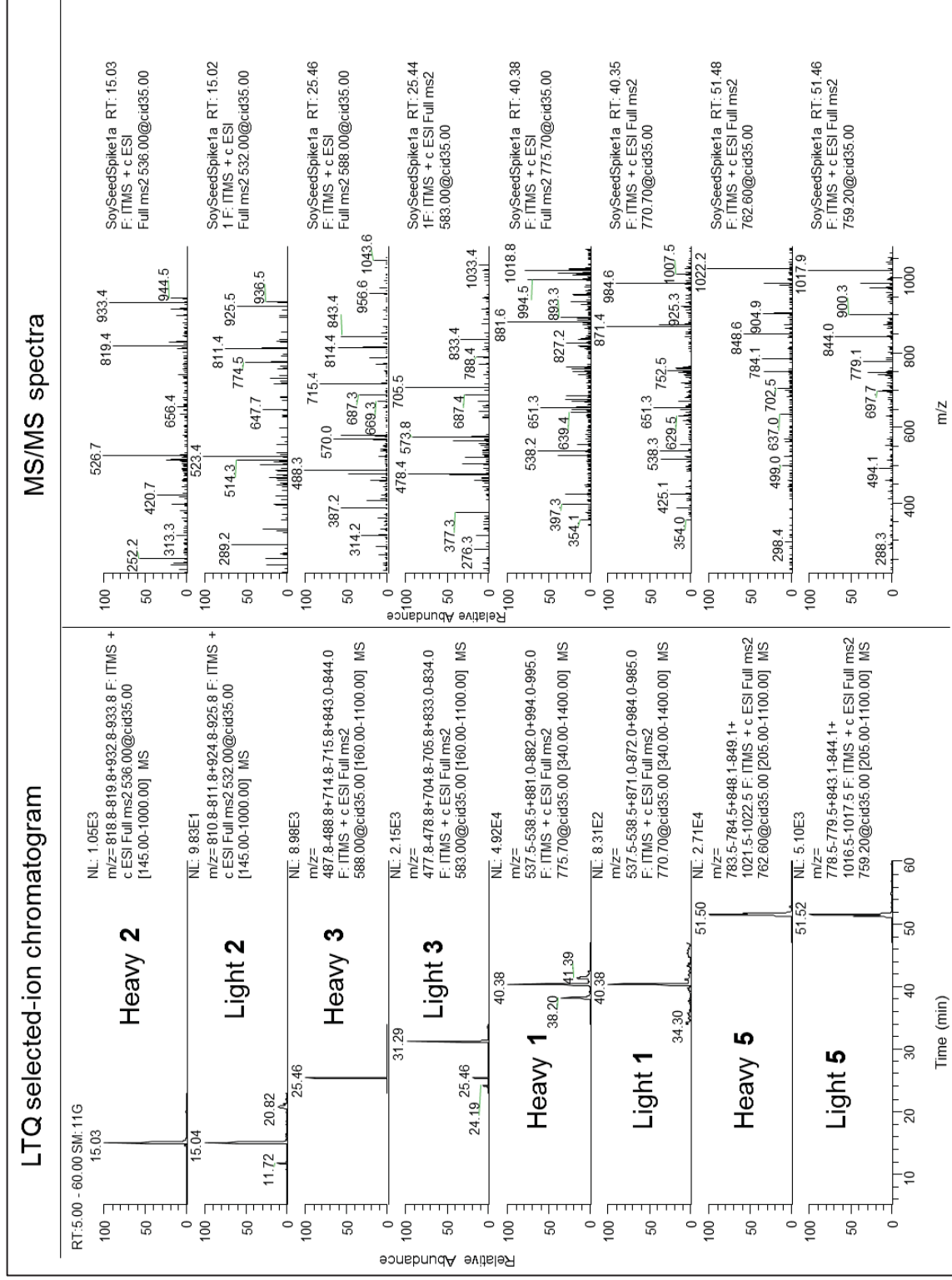


Figure 9-8. Detectability of HAHB4v Peptides in Control Seed Extracts Fortified with rHAHB4v. Chromatogram and full-scan MS/MS spectra of Williams 82 soybean seed tissue fortified with rHAHB4v at 4.5 µg/g DW.



LC-MS/MS analyses of HAHB4v protein levels from leaf and seed tissue samples collected from each of four individual plots from the six field sites in Argentina were carried out on 70 µg protein equivalents. No HAHB4v protein was detected in any of the Argentina or US samples. The results are summarized in Table 9-10. Examples of LC-MS/MS chromatograms from the analyses of individual plots are provided as Figures 9-9 and 9-10. The lower limit of quantification (LLOQ) for these LTQ-MS analyses was determined to be 25 fmol per 70 µg of both seed and leaf protein, equivalent to 1.37 µg/g DW seed and 1.02 µg/g DW leaf tissue (Table 9-5). The limit of detection was determined to be 0.026 µg/g DW and 0.027 for seed and leaf tissue, respectively (Table 9-6). In spite of high sensitivity of this LC-MS/MS method, no HAHB4v protein was detected in any of the analyzed samples. Therefore, a more sensitive method was developed using a triple-quadrupole MS and more samples were analyzed, as described below.

Figure 9-9. Analysis of HAHB4v Protein in IND-00410-5 Seed Extracts. Example of chromatogram and full-scan MS/MS spectra of IND-00410-5 seed extract (from plot 409 site Q1).

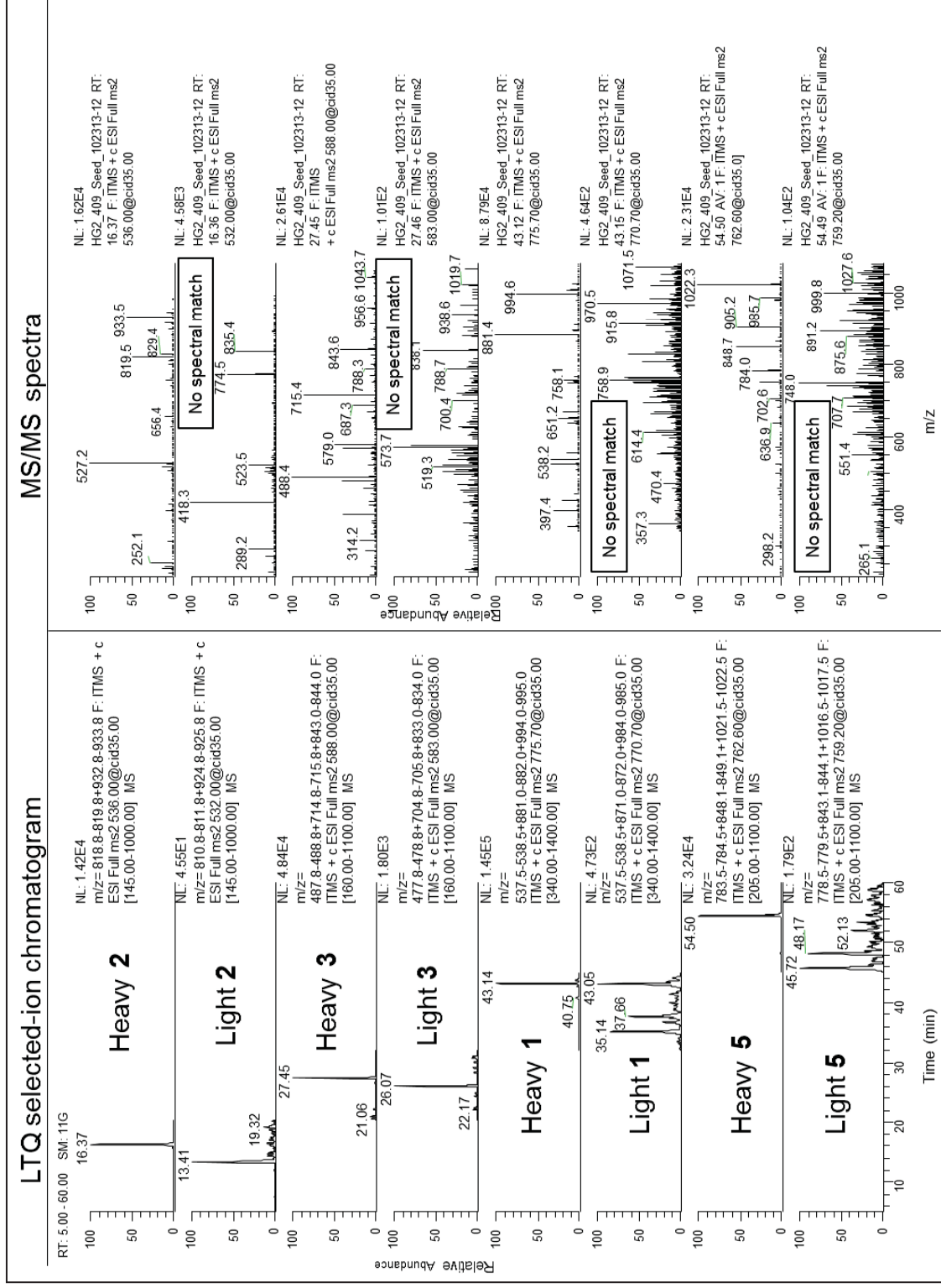
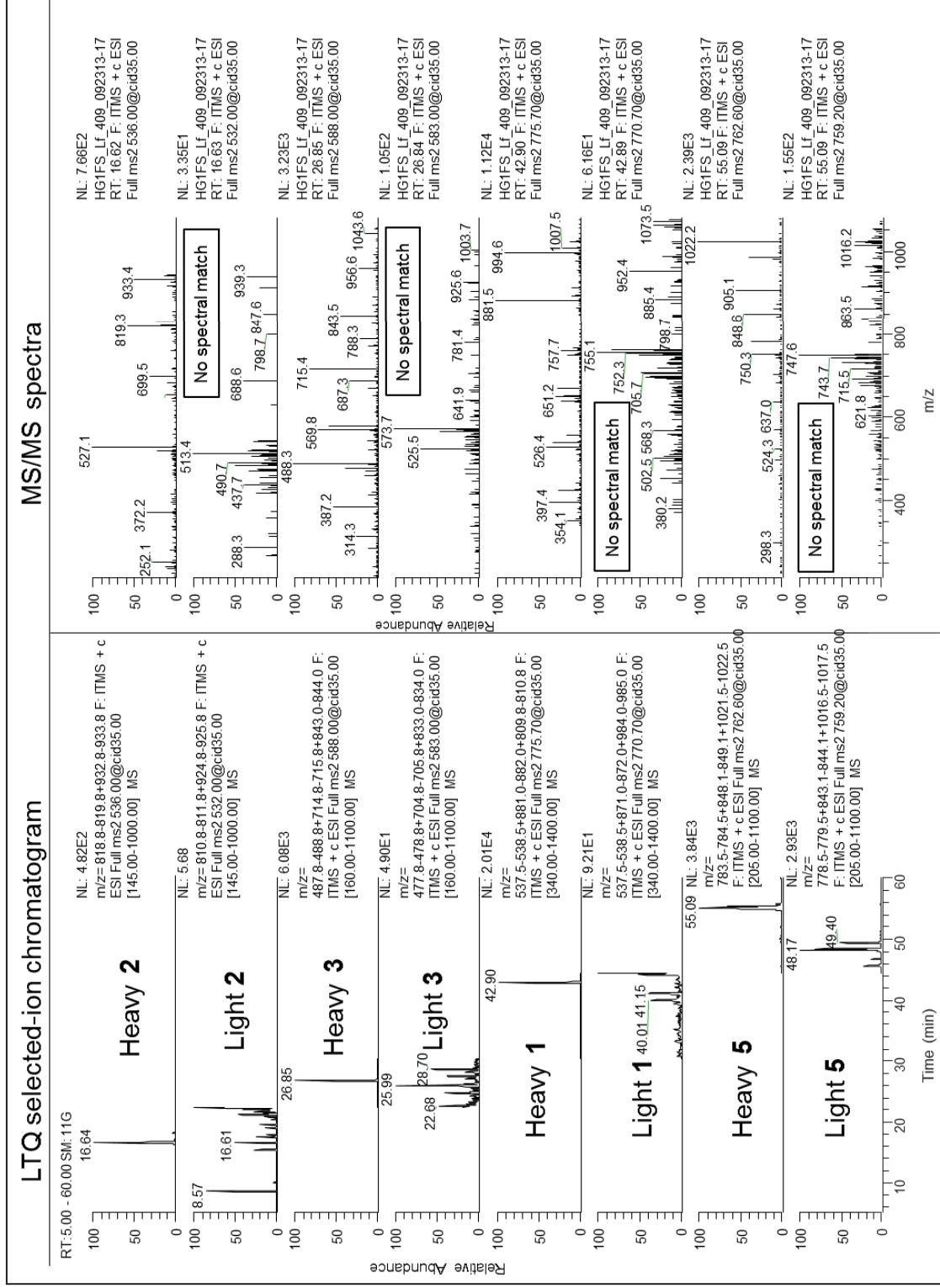


Figure 9-10. Analysis of HAHB4v Protein in IND-00410-5 Leaf Extracts. Example of chromatogram and full-scan MS/MS spectra of IND-00410-5 leaf extract (from plot 409 site Q1).



Due to the lack of detection of HAHB4v protein in the 70 µg protein samples, purification of leaf and seed tissue samples was scaled up 6-fold for all six of the field sites from Argentina and for all five of the field sites in the US. For these analyses, protein samples from each of the four plots for each field site were pooled before loading an SDS-PAGE gel with 420 µg of protein (six lanes of 70 µg each). To further increase sensitivity, these scaled-up samples were analyzed on a Thermo Scientific TSQ Vantage triple-quadrupole mass spectrometer. The LLOQ for these increased scale analyses on the TSQ MS was determined to be 3 fmol per 420 µg of both seed and leaf protein, equivalent to 0.027 µg/g DW seed and 0.041 µg/g DW leaf tissue (Table 9-7). The LOD for these samples was enhanced from the LTQ method to 0.007 µg/g DW and 0.003 µg/g DW for seed and leaf tissue, respectively (Table 9-8). The SRM chromatograms and spectra obtained at or below the LLOQ for seed and leaf protein are shown in Figures 9-11 and 9-12. In spite of the very high sensitivity of this TSQ MS method, HAHB4v protein was only detected in two of the field samples. The samples were leaf tissue from sites IN and OH2 which contained 5 and 4 ng/g DW, respectively (Table 9-10). The low expression of HAHB4v may be due to temporal, environmental, localized expression, and active regulatory control, as has been described for related HD Zip transcription factors (Appendix 9) (Chew *et al.* 2013; Henriksson *et al.* 2005).

Table 9-10. Summary of HAHB4v Protein Levels in Leaf and Seed from Soybean Field Trials with Event IND-00410-5 and Williams 82.

| Field | IND-00410-5 Seed ¹ | Williams 82 Seed ¹ | IND-00410-5 Leaf ² | Williams 82 Leaf ² |
|-------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| AR | <LOD | <LOD | <LOD | <LOD |
| CA | <LOD | <LOD | <LOD | <LOD |
| CB | <LOD | <LOD | <LOD | <LOD |
| HG-1 | <LOD | <LOD | <LOD | <LOD |
| HG-2 | <LOD | <LOD | <LOD | <LOD |
| MB | <LOD | <LOD | <LOD | <LOD |
| IL3 | <LOD | <LOD | <LOD | <LOD |
| IN | <LOD | <LOD | 0.005 µg/g DW | <LOD |
| OH2 | <LOD | <LOD | 0.004 µg/g DW | <LOD |
| IA | <LOD | <LOD | <LOD | <LOD |
| KS | <LOD | <LOD | <LOD | <LOD |

¹ Seed tissue from individual plots was extracted and analyzed for HAHB4v by targeted LC-MS/MS of 70 µg equivalents of protein, after SDS-PAGE. The limit of detection was 0.0026 µg/g DW for analysis of 70 µg protein samples from each individual field plot and 0.007 µg/g DW for analysis of 420 µg of pooled protein.

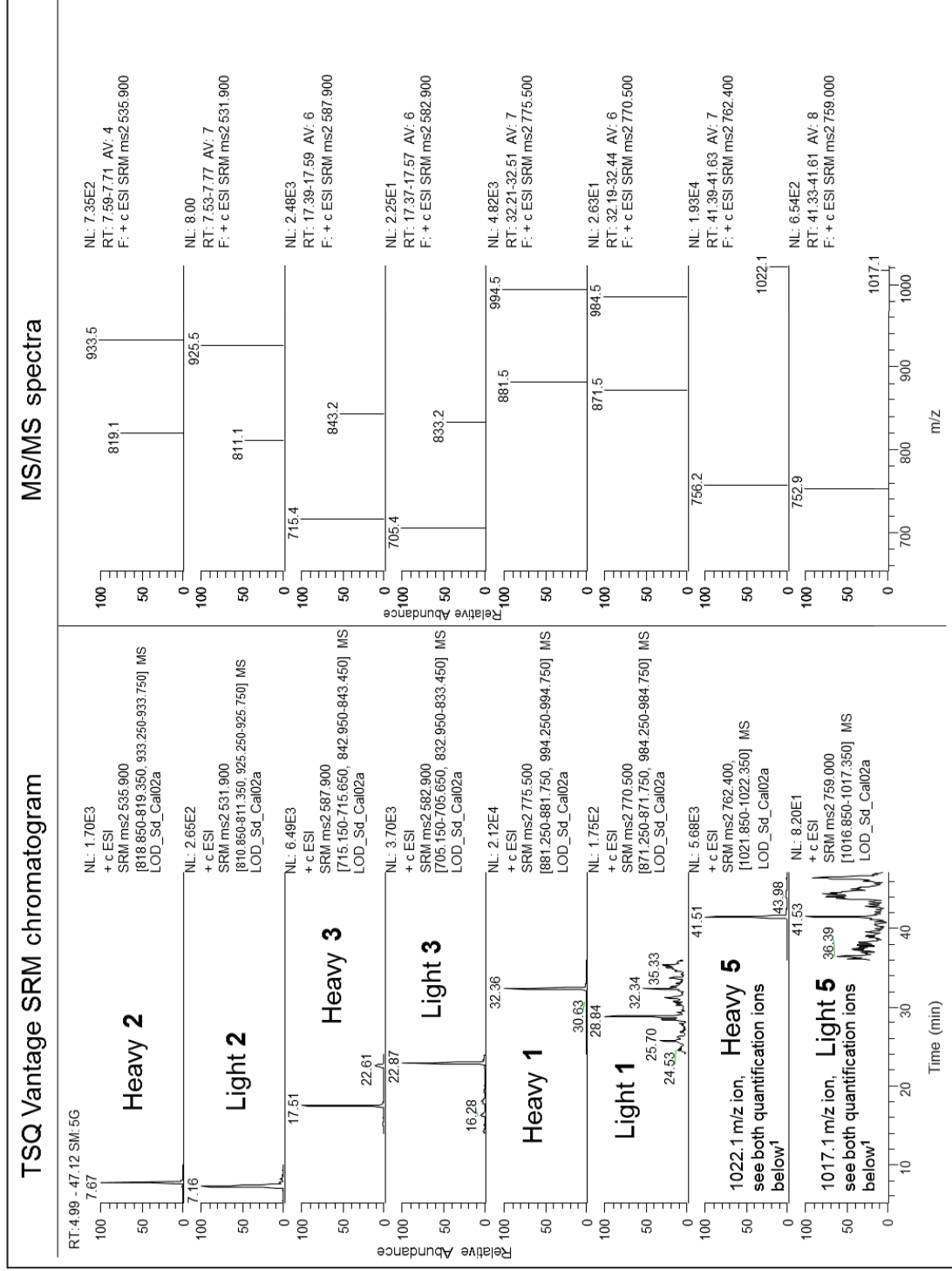
² Leaf tissue from individual plots was extracted and analyzed for HAHB4v by targeted LC-MS/MS of 70 µg equivalents of protein, after SDS-PAGE. The limit of detection was 0.027 µg/g DW for analysis of 70 µg protein samples from each individual field plot and 0.003 µg/g DW for analysis of 420 µg of pooled protein.

Figure 9-11. Detection of HAHB4v in Williams 82 Seed.

Selected reaction monitoring chromatograms and spectra of (A) individual peptides and (B) peptide 5 of pooled Williams 82 soybean seed protein fortified with 0.03 µg/g DW rHAHB4v (at LLOQ).

A. Individual

Peptides:



B. Peptide 5:

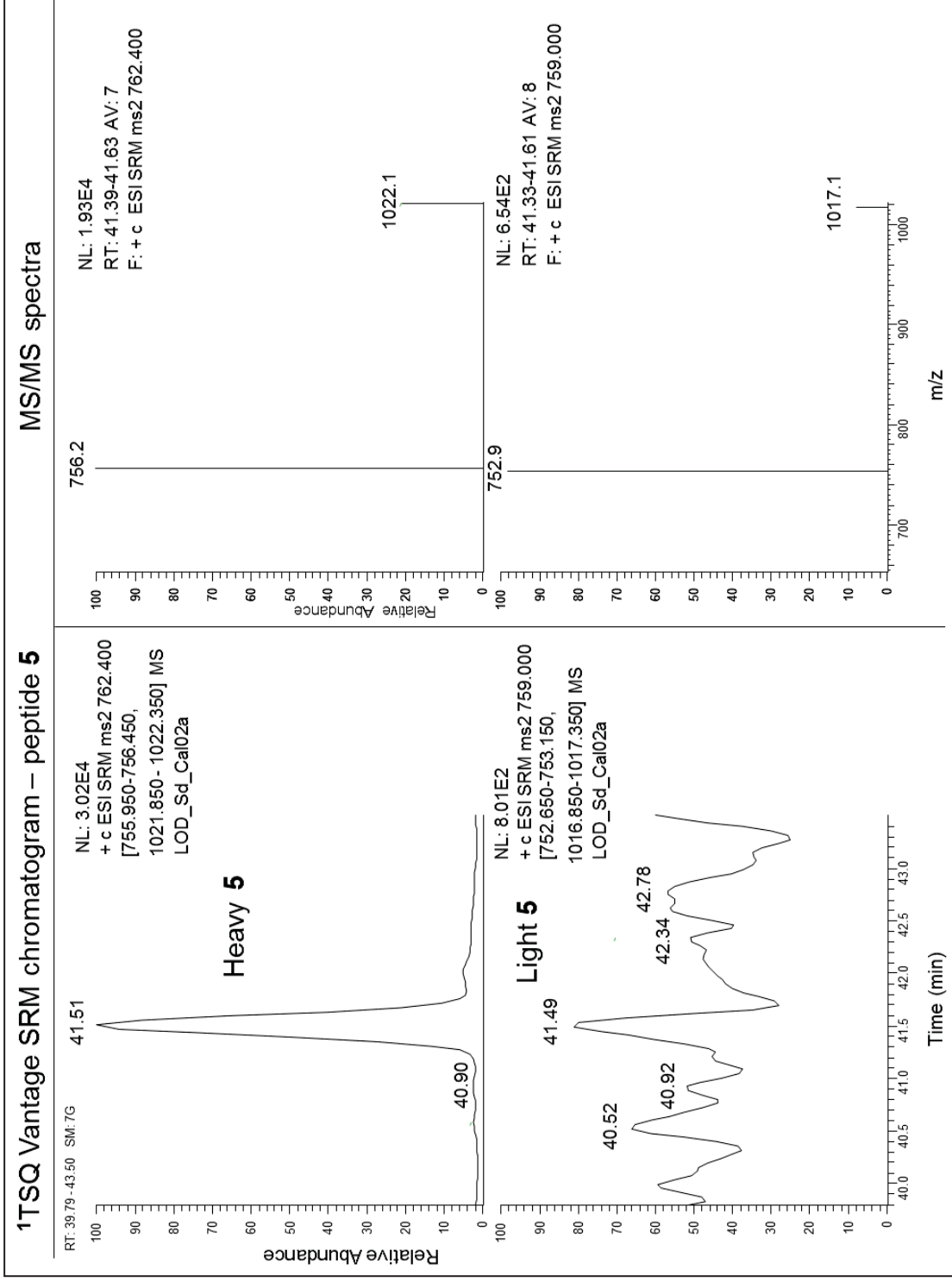
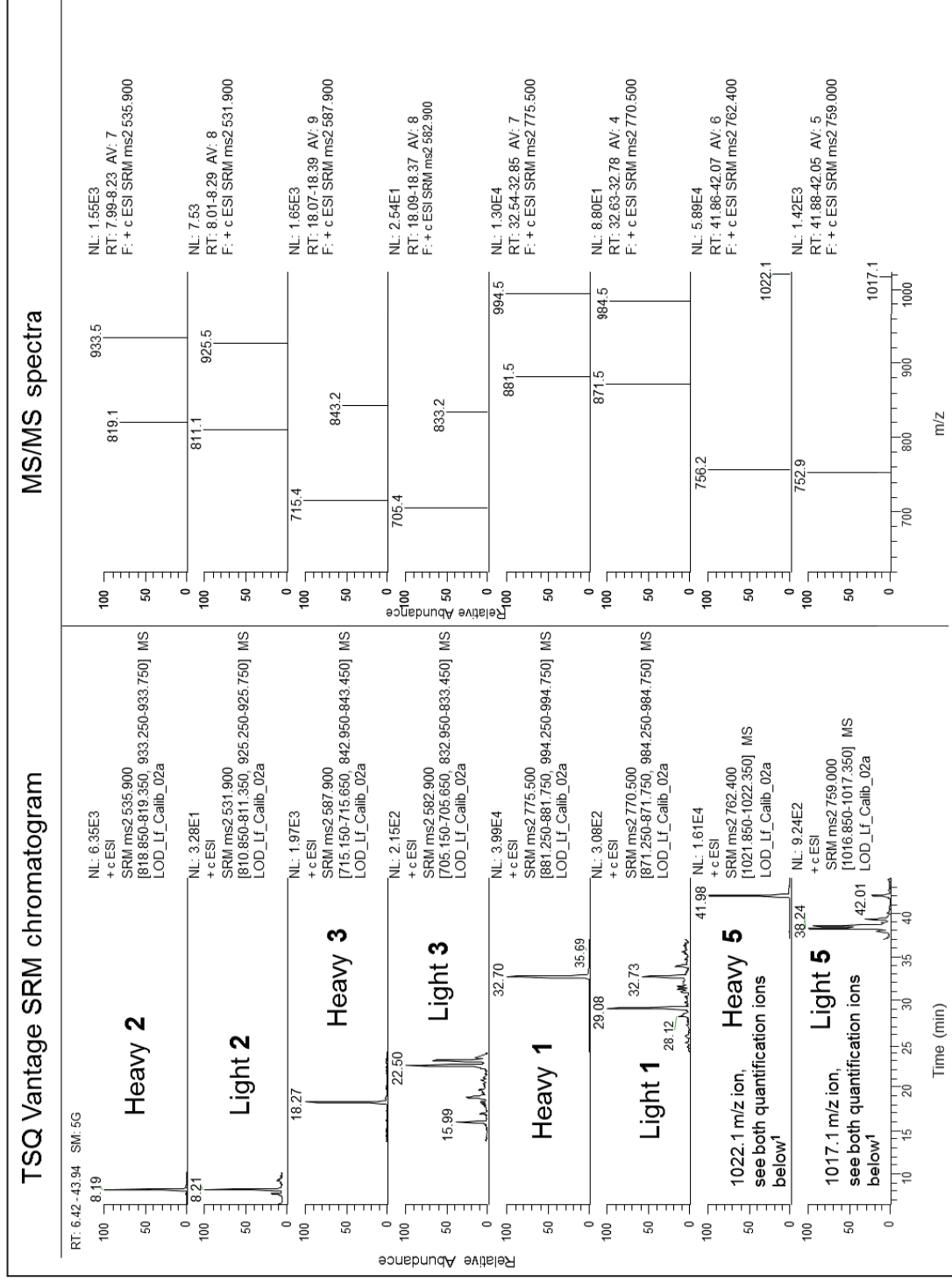
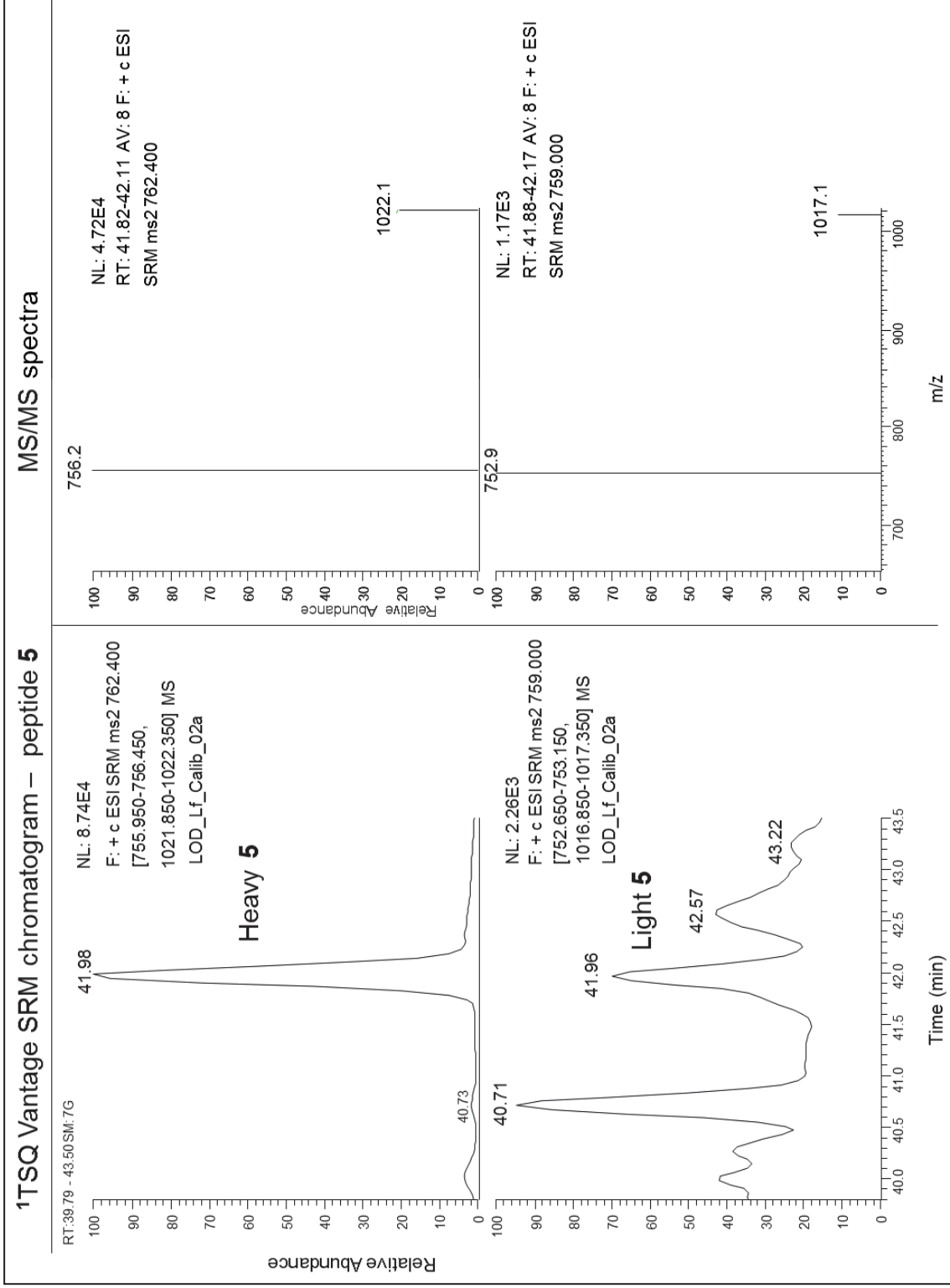


Figure 9-12. Detection of HAHB4v in Williams 82 Leaf. Selected reaction monitoring chromatograms and spectra of (A) individual peptides and (B) peptide 5 of Williams 82 soybean leaf protein fortified with rHAHB4v at 0.02 µg/g DW (below LLOQ).

A. Individual Peptides:



B. Peptide 5:



J. Appendix 10. Assessment of Potential Allergenicity and Toxicity of HAHB4v Protein in HB4 Soybean.

Analyses of HAHB4v protein were conducted to determine whether there was a potential for allergenicity or toxicity. Results confirmed that HAHB4v protein does not have homology to known allergens or toxins. Although the HAHB4v protein was heat stable, it was rapidly degraded *in vitro* with simulated gastric fluid.

Materials and methods

HAHB4v protein production

HAHB4v protein is a small (21 kDa) transcription factor present in extremely low concentration in its native sunflower as well as in the soybean event IND-00410-5. Isolation of the protein from the transgenic event was not feasible due to the low levels present in the plant (Appendix 9). Consequently, the protein safety analysis was carried out using *E. coli*-produced HAHB4v as described in Appendix 9.

HAHB4v antibodies

Two antibodies against HAHB4v were produced by GenScript (Piscataway, NJ). The first (α HB4), recognizing a HAHB4v specific C-terminal sequence (QEKTSSSGSGEESD) was raised by subcutaneous injection of this synthetic peptide into female New Zealand white rabbits and was used for the western blot and as the immobilized antibody in an enzyme-linked immunosorbent assay (ELISA). A second antibody was raised in mouse against the whole length *E. coli*-produced HAHB4v protein and used in the second layer of primary antibody in the sandwich ELISA.

Western Blot

An isolate of purified *E. coli*-produced HAHB4v was analyzed by SDS-PAGE followed by a Western blot to confirm the identity and molecular weight of HAHB4v. The protein aliquot was denatured and reduced by adding 4 \times Laemmli buffer (0.25 M Tris, 8% SDS, 20% 2-mercaptoethanol, 40% glycerol, pH6.8) to a final 1 \times concentration, followed by heating at 100°C for 5 min. Samples were centrifuged for 5 min prior to loading on a 15% acrylamide, 1.5-mm thick gel. HAHB4v protein (0.4 μ g/lane) was visualized using colloidal Coomassie Blue (Neuhoff 1988). Electrotransfer was performed at 50 V for 2 hours in 0.0125 M Tris, 0.19 M Glycine, 20% methanol buffer. The Precision Plus Protein Dual Xtra molecular weight standard (Bio-Rad, Hercules, CA) was used as a reference to verify protein size and transfer to nitrocellulose membranes.

For immunodetection, the membrane was incubated in blocking solution (BS): 2% w/v bovine serum albumin in phosphate saline buffer supplemented with 0.1% Tween 20 (PBS-T). The membrane was probed with a 1:1000 dilution in BS of affinity purified anti-C-terminal antibody followed by alkaline phosphatase conjugated anti-rabbit IgG (1:5000 in BS). All incubations were carried out for one hour at room temperature. After each incubation step, membranes were washed three times for 5 min with PBS-T. Immune complexes were visualized after colorimetric development with 0.3 mg/mL nitroblue tetrazolium, 0.15 mg/mL 5-bromo-4-chloro-3-indolyl-phosphate in 10% diethanolamine, 0.5 mM MgCl₂, pH 9.8).

HAHB4v Digestibility

E. coli-produced HAHB4v was subjected to simulated gastric fluid (SGF) assays developed following pre-established protocols (Thomas *et al.* 2004). Two control proteins (bovine serum albumin and soybean trypsin inhibitor) were also tested to demonstrate the efficacy of the assay under the conditions as described. A single tube for each protein (0.08 mL of a 5 mg/mL solution) was prepared with simulated gastric fluid (SGF: 4000 Pepsin units in 1.52 mL of 0.084 N HCl, 35 mM NaCl, pH 1.2) and warmed to 37°C. A ratio of 10 pepsin units per µg of protein was used for all assays. Sample aliquots (200 µl) were removed at timed intervals from 0.5 through 60 minutes. Separate control tubes, containing either only pepsin or the tested proteins, were incubated under the same conditions and included in the analysis. After collection, the aliquots were quenched by addition of 70 µL of 200 mM NaHCO₃, pH11. After all digestion samples had been collected, twenty µL aliquots were analyzed by SDS-PAGE on a 17% acrylamide Tricine gel and the proteins visualized by staining as indicated above. The molecular weight ladder used was Precision Plus Protein Dual Xtra molecular weight standard (Bio-Rad, Hercules, CA).

HAHB4v Thermal Stability

A sample of *E. coli*-produced HAHB4v was incubated at different temperatures (60, 75 or 90°C) for up to 60 minutes. Aliquots taken at 10, 30 and 60 min of incubation were analyzed by SDS-PAGE followed by protein staining (1.2 µg/lane) and ELISA. Control samples incubated at 4°C and at room temperature (25°C) were included as controls. In order to analyze a possible temperature-induced change in HAHB4v stability, protein reactivity was checked by a sandwich ELISA. The rabbit anti-C-terminal HAHB4v peptide (0.5µg/well) was immobilized in polystyrene wells by one-hour incubation at room temperature. After blocking remaining binding sites on the wells with BS, HAHB4v samples (40ng/well) were added. Bound HAHB4v was detected using mouse anti-HAHB4v antibody (1/100 in BS). After one more hour, biotin-conjugated anti-mouse IgG was added to each well, followed by avidin-alkaline phosphatase (both diluted 1/5,000 in BS). After each incubation step, wells were washed five times for 5 minutes each with PBS-T. The amount of HAHB4v bound to both antibodies could be inferred from the signal obtained at 495 nm after colorimetric development of the assay with 1 mg/mL p-nitro-phenyl-phosphate in 10% diethanolamine, 0.5 mM MgCl₂, pH 9.8.

Bioinformatic Assessment of HAHB4v Allergenicity and Toxicity

Potential allergenicity and toxicity of the HAHB4v protein was examined using five separate database searches. The FARRP database (Version 16, revised in January 27, 2016) contains 1956 allergens (Food Allergy Research and Resource Program, Department of Food Science and Technology, University of Nebraska). The AllergenOnLine (www.allergenonline.org) search was completed on April 1, 2016.

The search parameters for a positive allergen were either a sequence identity match $\geq 35\%$ over a stretch of 80 amino acids or a short peptide match of 8 contiguous amino acids. Identical parameters were used to search Allermatch (Fiers *et al.* 2004) and Allergen Online (www.allergenonline.org) with the added parameter of searching for any full FASTA sequences with more than 8 consecutive amino acids.

The possibility of structural homology between HAHB4v and allergens was examined using the SDAP database (<http://fermi.utmb.edu/SDAP/index.html>). The database contained 1526 allergen and isoallergens, 1312 protein sequences, 92 protein data base (PDB) structures, 458 3D models and 29 IgE epitopes, after its last update in February 2013 (Ivanciuc 2002, Ivanciuc 2003).

To analyze the putative toxicity of HAHB4v protein, the animal toxin database (ATDB), which contains 3844 toxins from 441 species (ATDB: Animal Toxin Data Base, College of Life Sciences, Hunan Normal University, He *et al.* 2008), was used.

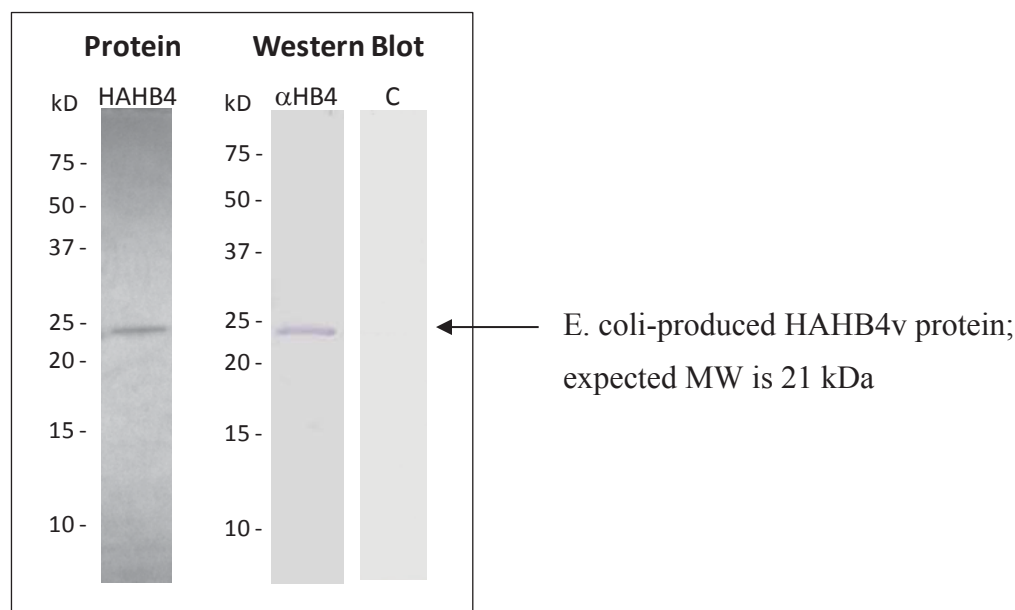
Results

Western Blot Analysis

The final preparation of HAHB4v protein showed a single protein band which migrated to the expected size (21 kDa, Figure 10-1, left panel). The immunoblots using the antibody raised against the C-terminal sequence of HAHB4v recognized the same band (Figure 10-1, right panel). Taken together, these results confirm the identity of *E. coli*-produced HAHB4v and its suitability for the studies here described.

Figure 10-1. Characterization of HAHB4v by SDS-PAGE and Western blot.

E. coli-produced HAHB4v was subjected to denaturing electrophoresis and detected by staining with Coomassie Blue (left panel) or with an anti-HAHB4v C-terminal antibody (α HB4) (right panel). C: control without primary antibody. The molecular weight marker is not pictured because it did not hybridize with the antibody for HB4 soybean. Instead, the band sizes were marked with a pencil on the blot prior to hybridization. Recombinant HAHB4v protein (21 kDa) migrates with a slightly higher apparent MW on SDS-PAGE of approximately 23 kDa; this discrepancy between the predicted migration of a protein and its SDS-PAGE-displayed MW is not uncommon (Shi *et al.*, 2012; Guan *et al.*, 2015).



HAHB4v Protein Digestibility

HAHB4v protein was tested for digestibility in simulated gastric fluid, as this characteristic is associated (together with other characteristics in a weight of evidence approach) with the allergenic potential. Controls were run in parallel to verify that assay conditions were able to distinguish a digestible (bovine serum albumin) from a non-digestible (soybean trypsin inhibitor) protein (Figure 10-2). The HAHB4v protein was rapidly degraded in the SGF assay conditions as revealed by the absence of the respective band and any protein fragments 0.5 min after initiation of the assay (Figure 10-3). These results suggest that the HAHB4v protein is rapidly digested by pepsin *in vitro* and does not generate fragments greater than 3.5kDa that could represent a safety concern (Codex Alimentarius 2009).

Figure 10-2. Digestibility of control proteins.

Soybean trypsin inhibitor (S, left panel) and bovine serum albumin (B, right panel) were incubated with pepsin (Pep) and analyzed by SDS-PAGE and protein staining. (<): indicates the location of the protein under analysis. *: indicates the location of pepsin band. St indicates the molecular weight standard lane.

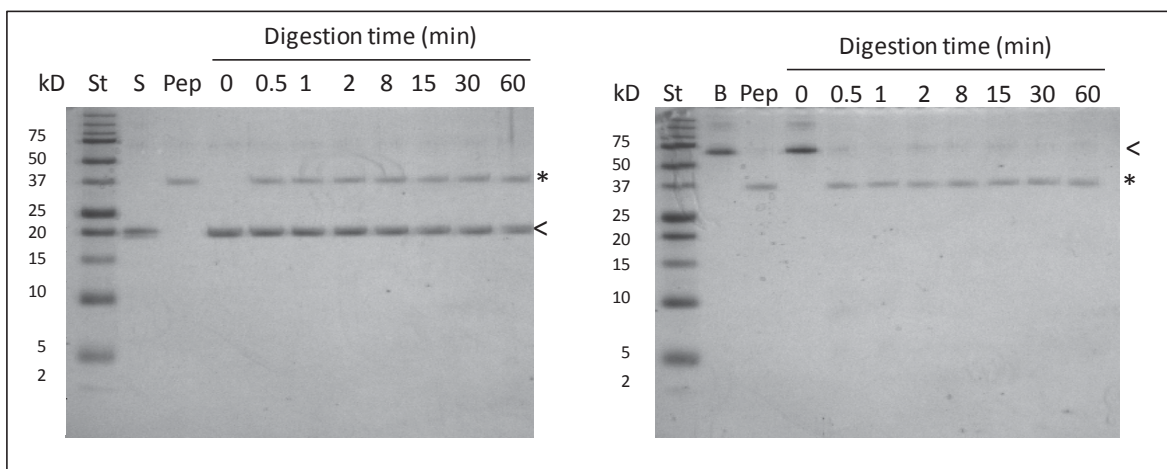
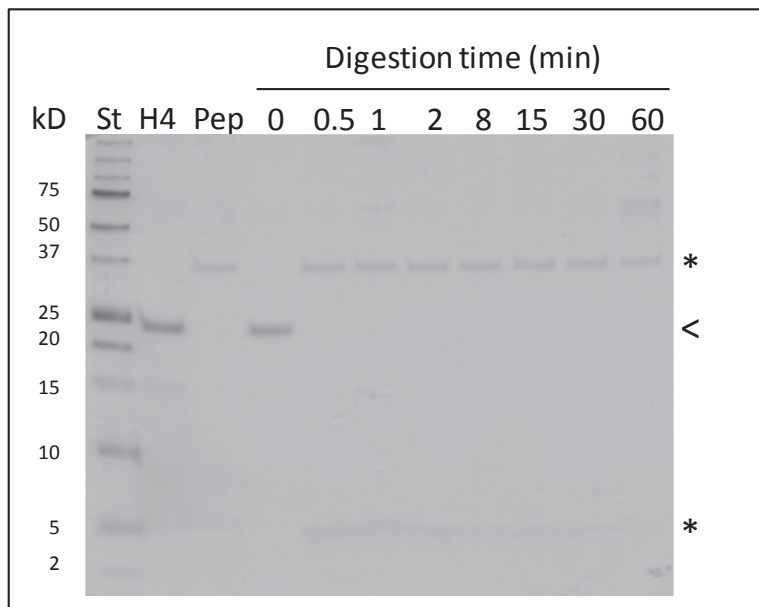


Figure 10-3. Digestibility of HAHB4v.

E. coli-produced HAHB4v (H4) incubated with pepsin (Pep) and analyzed by SDS-PAGE and protein staining. <: indicates the location of the *E. coli*-produced HAHB4v protein band. *: indicates the location of pepsin bands. St indicates the molecular weight standard lane.



Thermal Stability of HAHB4v Protein

HAHB4v protein was placed at multiple temperatures to test whether the protein is subject to thermal degradation. The possible presence of protein fragments was analyzed by SDS-PAGE. As shown in Figure 10-4, HAHB4v integrity is not affected by heating. Recombinant HAHB4v protein has a MW of 21 kD but migrates slightly higher than predicted on SDS-PAGE, as compared to the molecular weight standards, which has been shown to be a phenomenon associated with other proteins (Shi Y *et al.* 2012 and Guan Y *et al.* 2015). To further analyze if heating could affect other HAHB4v properties, antigenicity was analyzed by ELISA. Incubation at 90°C produced a slightly lower signal than the other tested temperatures even at short incubation times, but final absorbance values at 60 min did not show a significant difference from the control incubated at room temperature (Figure 10-5). These results suggest that the HAHB4v protein is not significantly degraded by high temperatures.

Figure 10-4. Effect of thermal treatment on *E. coli*-produced HAHB4v electrophoretic mobility.

HAHB4v protein was incubated at different temperatures for up to 60 minutes and analyzed by SDS-PAGE and protein staining. Samples kept at 4°C (C) or room temperature (RT) were included as controls. St indicates the molecular weight standard lane.

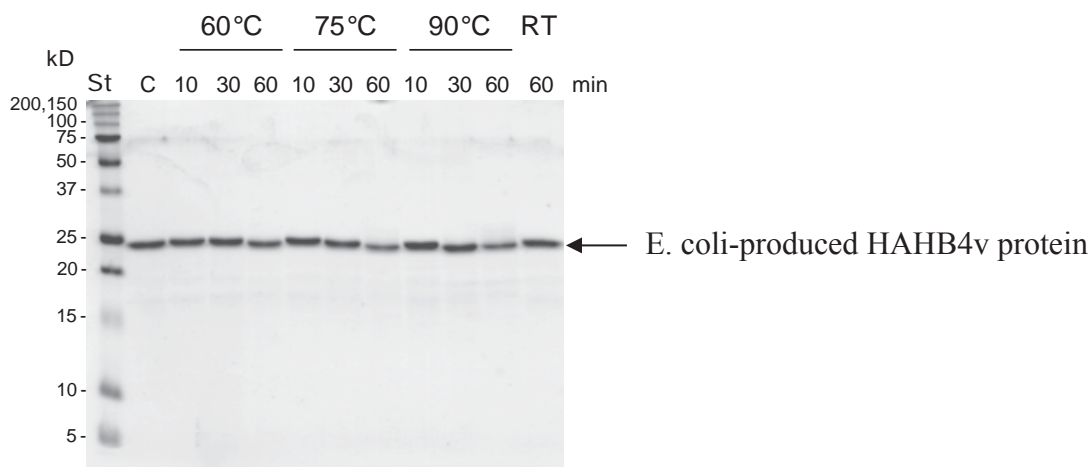
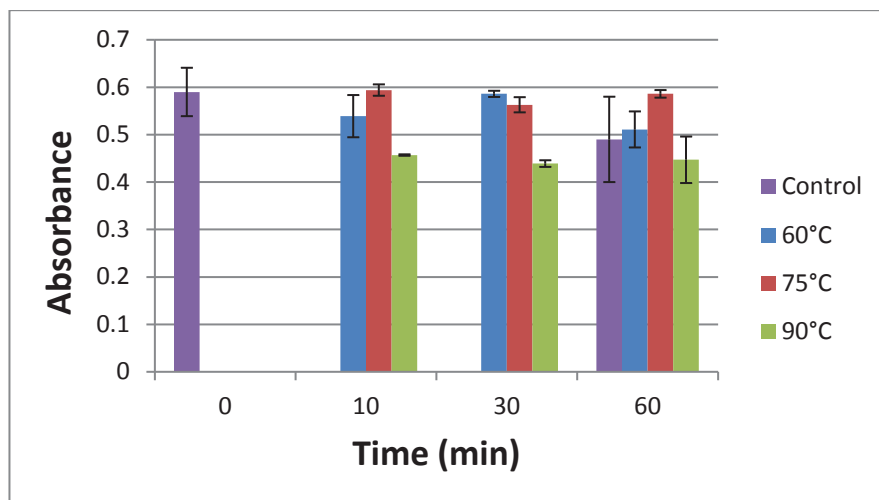


Figure 10-5. Effect of thermal treatment on HAHB4v antigenicity.

E. coli-produced HAHB4v protein was incubated at different temperatures for up to 60 minutes and analyzed by ELISA. Control = sample kept at room temperature. Bars indicate standard error.



HAHB4v Protein is Not Homologous to Known Allergens or Toxins

Bioinformatic analyses were used to assess the allergenic and toxic potential of HAHB4v. The sequence of HAHB4v protein was compared to known allergens in multiple databases and none met the criteria of shared homology to known allergens or allergen epitopes.

The possibility of structural homology between HAHB4v and allergens was examined using the SDAP database (<http://fermi.utmb.edu/SDAP/index.html>) (Ivanciuc *et. Al.* 2002 and 2003) and no structural homology with any member of this database was found.

To analyze the possible toxicity of HAHB4v, its amino acid sequence was compared to those registered in the animal toxin database (ATDB: Animal Toxin Data Base, College of Life Sciences, Hunan Normal University, He *et al.* 2008). The HAHB4v amino acid sequence was compared to those of the toxins in the database using the BLASTp algorithm and no relevant homology (E score $<1 \times 10^{-5}$) was found.

The results from the allergenicity and toxicity studies confirmed that HAHB4v protein does not have homology to known allergens or toxins. Although the HAHB4v protein was heat stable (Figures 10-4 and 10-5), it was rapidly degraded *in vitro* with simulated gastric fluid (Figures 10-2 and 10-3).

K. Appendix 11. Compositional Assessment of Event IND-00410-5.**Introduction**

Field trials were conducted at fifteen locations in Argentina during the 2012-2013 growing season and at ten locations in the United States during the 2013 growing season. Six locations in Argentina representing the environmental variation over the range of the 15 locations were selected for compositional analysis. Five of the ten locations in the US were similarly selected. Field trials were located within the major soybean production areas of both Argentina and the US thereby covering the diverse environmental conditions for soybean in these two countries. Four replicated plots of each seed line (IND-00410-5, Williams 82 and the commercial reference varieties) were planted using a randomized complete block field design. At each test site and planting date, harvested seed and forage materials were collected from each of the seed lines. Five commercial varieties were grown at the Argentina sites and five commercial varieties were grown at all the US locations except IA (4 commercial varieties were grown). The reference varieties selected were adapted for each site and represented the range of natural variability among commercial soybean varieties (see Tables 11-1 and 11-2). The six geographically diverse field sites in Argentina included: Monte Buey, Cordoba (A); Corral de Bustos, Cordoba (D2); Carmen de Areco, Buenos Aires (G1); Hughes, Sante Fe first planting date (Q1); Hughes, Sante Fe second planting date (Q2); and Aranguren, Entre Rios (W1). The five diverse locations in the United States include: Effingham, IL (IL3); Ladoga, IN (IN); Pemberton, OH (OH2); Richland, IA (IA); and Troy KS (KS). The commercial varieties included at each location are indicated in Tables 11-1 and 11-2.

Individual results from each plot were combined and used to calculate: mean values, standard error of the mean, the difference between mean values of IND-00410-5 and Williams 82 and the 95% confidence interval for each parameter. Therefore each value represents an average of 4 individual plot samples from each site. The occurrence of statistically significant differences between event IND-00410-5 and Williams 82 were analyzed using a two-way ANOVA and the Least Significant Difference (LSD) post-test using the SAS software (SAS Institute, Inc., Cary, NC). The differences between IND-00410-5 and Williams 82 were compared across all sites (combined-site analysis) and for each individual site. Statistical differences were established at a 5% level of significance ($\alpha = 0.05$).

Table 11-1. Commercial Reference Soybean Varieties Grown at the Field Sites in Argentina. Varieties Analyzed for Composition Are Indicated by a “+”.

| Variety/Site ¹ | A | D2 | G1 | Q1 | Q2 | W1 | Source ² |
|---------------------------|---|----|----|----|----|----|---------------------|
| A 3731 RG | | | + | | | | Nidera Semillas |
| FN 3.85 | | | + | | | | FN semillas |
| DM 3810 | + | + | + | + | + | + | Don Mario semillas |
| SRM 3970 | + | + | + | + | + | + | Sursem |
| SPS 3900 | + | + | | + | + | | Syngenta |
| NS 4009 | + | + | | + | + | | Nidera Semillas |
| DM 4210 | + | + | + | + | + | + | Don Mario semillas |
| DM 4670 | | | | | | + | Don Mario semillas |
| BIO 4.6 | | | | | | + | Bioceres Semillas |

1. Notation used to indicate site and planting date: A = Monte Buey, Córdoba; D2 = Corral de Bustos, Córdoba, second planting date; G1 = Carmen de Areco, Buenos Aires, first planting date; Q1 = Hughes, Santa Fe, first planting date; Q2 = Hughes, Santa Fe, second planting date; W1 = Aranguren, Entre Rios, first planting date.

2. Nidera Semillas: Ruta 8, Km 376, Venado Tuerto, Santa Fe; FN semillas: Ruta 31, Km 135, Salto, Buenos Aires; Don Mario Semillas: Ruta 7 Km 208, Chacabuco, Buenos Aires; Sursem: Ruta 32, Km 2, Pergamino, Buenos Aires; Syngenta: Av. del Libertador 1855, Vicente Lopez, Buenos Aires Bioceres Semillas: Ocampo 210 bis, Rosario, Santa Fe.

Table 11-2. Commercial Reference Soybean Varieties Grown at the Field Sites in the US. Varieties Analyzed for Composition Are Indicated by a “+”.

| Variety/Site ¹ | IL3 | IN | OH2 | IA | KS | Source ² |
|---------------------------|-----|----|-----|----|----|---------------------|
| Dow 32R280 | + | + | + | + | + | Dow AgroSciences |
| Pioneer 93Y82 | + | + | + | + | + | DuPont Pioneer |
| Asgrow AG3832 | | | + | + | | Monsanto |
| Pioneer 93Y84 | | | + | | + | DuPont Pioneer |
| DynaGro 36RY38 | | | + | | | Dyna-Gro Seed |
| Stine 39LD02 | | | | + | | Stine Seeds |
| Pioneer 93Y92 | | | | | + | DuPont Pioneer |
| Pioneer 93M94 | | | | | + | DuPont Pioneer |
| Asgrow 3533 | | + | | | | Monsanto |
| Asgrow 3731 | | + | | | | Monsanto |
| Asgrow 3431 | | + | | | | Monsanto |
| Asgrow AG3931 | + | | | | | Monsanto |
| Hoffman H38-12CR2 | + | | | | | Hoffman Seed House |
| NK S39-U2 | + | | | | | Syngenta Seeds |

1. Notation used to indicate site and planting date: (IL3) = Effingham, IL; IN = Ladoga, IN; OH2 = Pemberton, OH ; IA = Richland, IA; KS = Troy, KS.

2. Monsanto, St. Louis, MO; DuPont Pioneer, Johnston, IA; Dyna-Gro Seed, Richmond, CA; Stine Seeds, Adel, IA; Hoffman Seed House, Hoffman, IL; Syngenta Seeds, Minnetonka, MN.

Materials and Methods

Seed and forage material from transgenic event IND-00410-5, its parental control Williams 82 and several commercial reference varieties were collected from eleven locations in Argentina and the US (Tables 11-1 and 11-2). Compositional analyses were conducted according to the OECD consensus document for soybean (OECD 2012).

Nutrients measured included proximates (moisture, protein, fat, ash, and carbohydrates) and fiber (ADF and NDF) in forage, and proximates (moisture, protein, fat, ash, and carbohydrates), fiber (ADF, NDF and crude fiber), minerals (Ca and P), fatty acids, vitamins E and K1, and amino acids in seed. Anti-nutrients measured in seed included isoflavones (daidzein, genistein, and glycitein), stachyose, raffinose, phytic acid, lectin and trypsin inhibitors. All nutrients and anti-nutrients (except lectin) for locations A, D2, G1, Q1, Q2 and W1 were measured at Melacrom Laboratories (Mercedes, Buenos Aires, Argentina). Lectin for these sites was measured by Covance Laboratories (Madison, WI). All nutrients and anti-nutrients for locations IL3, IN, OH2, IA and KS were measured at Covance Laboratories (Madison, WI, United States). All of these components were measured in seed. In forage, all components were measured except fatty acids, amino acids and vitamins E and K1.

Acid Detergent Fiber

Sites A, D2, G1, Q1, Q2 and W1 (Melacrom Laboratories).

The samples were defatted by Soxhlet extraction and dried to remove excess moisture, placed into fritted glass crucibles and washed with an acidified quaternary detergent solution that dissolved the protein, carbohydrate and ash. After rinsing with acetone to remove fat and pigments, the remaining fiber was collected, dried and measured as a percent of the total weight of sample (AOAC Method 973.18). The limit of quantification is 0.5% dry weight.

Sites IL3, IN, OH2, IA and KS (Covance Laboratories).

Sample aliquots were weighed into pre-weighed filter bags. The fats and pigments were then removed by an acetone wash. The filter bags were placed in an ANKOM Fiber analyzer where the protein, carbohydrate, and ash content were dissolved by boiling acidic detergent solution. After drying, the bags were reweighed and the acid detergent fiber was determined gravimetrically (USDA 1970; Komarek *et al.* 1994). The limit of quantification was calculated as 0.100% on a fresh weight basis.

Amino Acid Composition

Sites A, D2, G1, Q1, Q2 and W1.

Ground samples were extracted with HCl to hydrolyze the amino acids (25 mL/g) and kept at 100°C in a water bath for 24 hours. After dilution with water, amino acids contained in the sample were derivatized in borate buffer pH 7 with fluorenylmethoxycarbonyl chloride (FMOC) for proline and OPA/ME (ortho-phthalaldehyde/mercaptoethanol) for other amino acids. Isolation was conducted by HPLC using a Hypersyl Gold column (4.6 mm ID x 250 mm long) (Thermo Scientific, San Jose, CA) using a fluorescent detector and acetonitrile/water as organic and aqueous phase, respectively. The amino acids were quantified using high-performance liquid chromatography (HPLC) with UV detection. Reference standards (Table 11-3) were used in triplicate to calibrate for quantification time (Herbert *et al.* 2000; Fabiani *et al.* 2002; Zhou *et al.* 2011). The limit of quantification was 0.1% on a fresh weight basis.

Table 11-3. Amino Acid Reference Standards for Argentina Samples.

| Component | Manufacturer | Lot Number | Purity (%) |
|---|---------------------|-------------------|-------------------|
| L-Alanine | Parafarm | 000996/008 | 98.5 |
| L-Arginine Monohydrochloride | Parafarm | 000930/009 | 98.5 |
| L-Aspartic Acid | Parafarm | 003271/009 | 99.1 |
| L-Cysteine | Parafarm | 000618/008 | 87.3 |
| L-Cystine | Parafarm | 002661/008 | 89.5 |
| L-Glutamic Acid | Parafarm | 001585/008 | 99.5 |
| Glycine | Parafarm | 000339/010 | 99.7 |
| L-Histidine Monohydrochloride Monohydrate | Parafarm | 004285/009 | 94.5 |
| L-Isoleucine | Parafarm | 001203/004 | 93.5 |
| L-Leucine | Parafarm | 001358/005 | 95 |
| L-Lysine Monohydrochloride | Parafarm | 001685/010 | 90.5 |
| L-Methionine | Parafarm | 008591/009 | 92.5 |
| L-Phenylalanine | Parafarm | 001478/010 | 92.5 |
| L-Proline | Parafarm | 002896/009 | 96 |
| L-Serine | Parafarm | 001414/008 | 99.5 |
| L-Threonine | Parafarm | 000829/008 | 96 |
| L-Tryptophan | Parafarm | 000843/010 | 96.3 |
| L-Tyrosine | Parafarm | 006244/008 | 94.4 |
| L-Valine | Parafarm | 002913/008 | 98.7 |

Sites IL3, IN, OH2, IA and KS.

The samples were hydrolyzed in 6N hydrochloric acid for approximately 24 hours at 106-118°C. Phenol was added to the 6N hydrochloric acid to prevent halogenation of tyrosine. Cystine and cysteine were converted to S-2-carboxyethylthiocysteine by the addition of dithiodipropionic acid. Tryptophan was hydrolyzed from proteins by heating at approximately 110°C in 4.2N sodium hydroxide for approximately 20 hours.

The samples were analyzed by high-performance liquid chromatography after preinjection derivatization. The primary amino acids were derivatized with o-phthalaldehyde (OPA) and the secondary amino acids were derivatized with fluorenylmethyl chloroformate (FMOC) before injection (Schuster 1988; Henderson *et al.* 2000; Henderson and Brooks 2010; Barkholt and Jensen 1989; AOAC Method 988.15). Reference samples are listed in Table 11-4. The limit of quantification for this study was 0.0100% on a fresh weight basis.

Table 11-4. Amino Acid Reference Standards for US Samples.

| Component | Manufacturer | Lot Number | Purity (%) |
|---|---------------------|-------------------|-------------------|
| L-Alanine | Sigma-Aldrich | 060M1776V | > 99 |
| L-Arginine Monohydrochloride | Sigma-Aldrich | SLBF3348V | 100 |
| L-Aspartic Acid | Sigma-Aldrich | 091M0201V | 100 |
| L-Cystine | Sigma-Aldrich | SLLB9524V | 100 |
| L-Glutamic Acid | Sigma-Aldrich | 060M01711V | 100 |
| Glycine | Sigma-Aldrich | 059K0040V | 100 |
| L-Histidine Monohydrochloride Monohydrate | Sigma-Aldrich | 110M00481V | 100 |
| L-Isoleucine | Sigma-Aldrich | 090M00842V | 100 |
| L-Leucine | Sigma-Aldrich | 110M00492V | 100 |
| L-Lysine Monohydrochloride | Sigma-Aldrich | 051M0016V | 100 |
| L-Methionine | Sigma-Aldrich | SLBF3077V | 100 |
| L-Phenylalanine | Sigma-Aldrich | SLBF2036V | 100 |
| L-Proline | Sigma-Aldrich | SLBF1872V | 100 |
| L-Serine | Sigma-Aldrich | 098K0161V | 99 |
| L-Threonine | Sigma-Aldrich | 081M01921V | 99 |
| L-Tryptophan | Sigma-Aldrich | SLBC5462V | 100 |
| L-Tyrosine | Sigma-Aldrich | BCBF4244V | 100 |
| L-Valine | Sigma-Aldrich | SLBF7406V | 100 |

Ash

Sites A, D2, G1, Q1, Q2, W1, IL3, IN, OH2, IA and KS.

An accurately weighed sample was incinerated in an oven at 585°C until a constant weight was achieved (AOAC Method 923.03). Ash content was calculated as the weight of the residue divided by the original weight of the sample, expressed as a percentage. The limit of quantification is 0.02% w/w dry weight.

Carbohydrates

Sites A, D2, G1, Q1, Q2, W1, IL3, IN, OH2, IA and KS.

Carbohydrates were calculated from the proximate analysis, using the difference in weight using the following equation (USDA 1973). The limit of quantification is 0.100% on a fresh weight basis.

$$\% \text{ carbohydrates} = 100\% - (\% \text{ protein} + \% \text{ fat} + \% \text{ moisture} + \% \text{ ash})$$

Crude Fiber

Sites A, D2, G1, Q1, Q2 and W1.

Samples were defatted by Soxhlet extraction and digested with sulfuric acid and sodium hydroxide to remove proteins, carbohydrates and lipid. The remaining residue, which contains cellulose and lignin was dried at 100°C for three hours, allowed to cool under desiccation, and weighed again. A percent was calculated from the loss in weight on ignition

minus the blank weight divided by the original sample weight (AACC Method 32-10.01). The limit of quantification is 0.5% on a fresh weight basis.

Sites IL3, IN, OH2, IA and KS.

Crude fiber was quantitated as the loss on ignition of dried residue remaining after digestion of the samples with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specific conditions (AOAC Method 962.09). The limit of quantification was calculated as 0.1% on a fresh weight basis.

Fat by Soxhlet Extraction

Sites A, D2, G1, Q1, Q2 and W1.

The ground sample was dried at 100°C until constant weight. A portion of dried sample was weighed and placed into a cellulose thimble and extracted with diethyl ether using soxhlet extraction for two hours. The extracted fat was dried at 100°C for 30 minutes and allowed to cool on a desiccator until constant weight. The fat content was calculated as a percentage of the total sample weight (AACC Method 30-20.01). The limit of quantification is 0.5% on a dry weight basis.

Sites IL3, IN, OH2, IA and KS.

The seed samples were weighed into a cellulose thimble containing anhydrous sodium sulfate and dried to remove excess moisture. Pentane was dripped through the samples to remove the fat using soxhlet extraction. The extract was then evaporated, dried, and weighed (AOAC Methods 960.39 and 948.22). The limit of quantification was calculated as 0.100% on a fresh weight basis.

Fat by Acid Hydrolysis

Sites IL3, IN, OH2, IA and KS.

Fat in forage samples was determined by acid hydrolysis with hydrochloric acid. The fat was extracted using ether and hexane. The extracts were dried down and filtered through an anhydrous sodium sulfate column. The remaining extracts were then evaporated, dried, and weighed (AOAC Methods 922.06 and 954.02). The limit of quantification was calculated as 0.100% on a fresh weight basis.

Fatty Acids

Sites A, D2, G1, Q1, Q2 and W1.

Fat obtained from samples as described above were dissolved in hexane and saponified with 2M KOH in methanol. The fatty acid methyl esters were removed from the upper phase and analyzed by gas chromatography on a Capilar Elite-WAX column (30 m x 0.32 mm x 0.25 um). External standards (Table 11-5) were used to calculate the amounts of the targeted fatty acids (AOAC Method 996.06). The limit of quantification was 0.05% on a fresh weight basis.

Table 11-5. Fatty Acids Reference Standards for Argentina Samples.

| Manufacturer | Lot Number | Component | Purity (%) |
|---------------------------------|------------|--------------------------|------------|
| SUPELCO | LC01538 | Cis-9-Oleic Methyl Ester | 99.9 |
| F.A.M.E. Mix, C8-C24/18918-1AMP | | Methyl Palmitate | 99.7 |
| | | Methyl Stearate | 99.9 |
| | | Methyl Linoleate | 99.9 |
| | | Methyl Linolenate | 99.9 |
| | | Methyl Arachidate | 99.9 |

Sites IL3, IN, OH2, IA and KS.

Lipids were extracted and saponified with 0.5N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride in methanol. The resulting methyl esters were extracted with heptane containing an internal standard (Table 11-6) to reference retention time. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation. The results are converted to their triglyceride equivalent (AOCS Methods Ce 2-66 and Ce 1i-07). The limit of quantitation was calculated as 0.0100% on a fresh weight basis.

Table 11-6. Methyl Esters Reference Standards for US Samples.

| Manufacturer | Lot No. | Component | JY10-W | | O1-X | |
|--|---------------------|-----------------------------|------------|------------|------------|------------|
| | | | Weight (%) | Purity (%) | Weight (%) | Purity (%) |
| Nu-Chek Prep GLC Reference Standard Covance 1 Covance 2 | JY10-W | Methyl Octanoate | 3.0 | 99.7 | 1.25 | 99.5 |
| | O1-X | Methyl Decanoate | 3.25 | 99.6 | 1.25 | 99.4 |
| | | Methyl Laurate | 3.25 | 99.8 | 1.25 | 99.7 |
| | | Methyl Myristate | 3.25 | 99.8 | 1.25 | 99.7 |
| | | Methyl Myristoleate | 1.0 | 99.5 | 1.25 | 99.4 |
| | | Methyl Pentadecanoate | 1.0 | 99.6 | 1.25 | 99.5 |
| | | Methyl Pentadecenoate | 1.0 | 99.4 | 1.25 | 99.4 |
| | | Methyl Palmitate | 10.0 | 99.8 | 15.75 | 99.7 |
| | | Methyl Palmitoleate | 3.0 | 99.7 | 1.25 | 99.7 |
| | | Methyl Heptadecanoate | 1.0 | 99.6 | 1.25 | 99.5 |
| | | Methyl 10-Heptadecenoate | 1.0 | 99.5 | 1.25 | 99.4 |
| | | Methyl Stearate | 7.0 | 99.8 | 14 | 99.5 |
| | | Methyl Oleate | 10.0 | 99.8 | 15.75 | 99.5 |
| | | Methyl Linoleate | 10.0 | 99.8 | 15.75 | 99.5 |
| | | Methyl GammaLinolenate | 1.0 | 99.4 | 1.25 | 99.5 |
| | | Methyl Linolenate | 3.0 | 99.5 | 1.25 | 99.4 |
| | | Methyl Arachidate | 2.0 | 99.8 | 1.25 | 99.5 |
| | | Methyl 11-Eicosenoate | 2.0 | 99.6 | 1.25 | 99.6 |
| | | Methyl 11-14 Eicosenoate | 1.0 | 99.5 | 1.25 | 99.5 |
| | | Methyl 11-14-17 Eicosenoate | 1.0 | 99.5 | 1.25 | 99.6 |
| | Methyl Arachidonate | 1.0 | 99.4 | 1.25 | 99.5 | |
| | Methyl Behenate | 1.0 | 99.8 | 1.25 | 99.5 | |

Isoflavones Analysis

Sites A, D2, G1, Q1, Q2 and W1.

Soybean grains were dry-milled in a coffee blender and mixed with 80% methanol (16 mL/g) for extraction. The mixture was heated in a water bath at 65°C for 2 hr with continuous shaking. After cooling to room temperature, the total volume was adjusted to 50 mL and the suspension centrifuged at 3000 rpm for 5 min. Concentrated HCl was added to a 4-mL aliquot of the supernatant, incubated at 80°C for 2 hr and cooled to room temperature. The solution was adjusted with ammonium hydroxide:acetic acid:DMSO (50:45:5) to pH 5 and diluted with 0.1% formic acid. Aglycone content was analyzed using an LC-ESI-MS/MS Acquity system (Waters Inc., Milford, MA) with a tandem quadrupole detector (TQD). The HPLC was fitted with a Hypersil Gold column (100 x 2.1 mm; 1.9 µm)(Thermo Scientific, San Jose, CA) and 0.1% formic acid in water or acetonitrile was used as aqueous and organic phase respectively. Targeted isoflavones were quantified by comparing to

external standards (AOAC 2001.10; Delmonte *et al.* 2006; Cesar *et al.* 2006; Seo and Morr 1984; Table 11-7). The limit of quantification was 5 mg/kg fresh weight.

Table 11-7. Isoflavones Reference Standards for Argentina Samples.

| Manufacturer | Component | Lot Number | Purity (%) |
|---------------|------------------------|------------|------------|
| Sigma Aldrich | Daidzein | 013M4027V | 99.5 |
| Sigma Aldrich | Glycitein | BCBK6136V | 99.4 |
| Sigma Aldrich | Genistein Synthetic | SLBC0762V | 98.7 |

Sites IL3, IN, OH2, IA and KS.

The samples were extracted at approximately 65°C with a 80:20 methanol:water solution and the extract was saponified with a dilute sodium hydroxide solution. The extract was then acidified, filtered, and diluted. The samples were analyzed on a HPLC system with ultraviolet spectrophotometric detection and were compared against an external standard curve (Table 11-8)(AOAC Method 2001.10). The limit of quantitation was calculated as 10 ppm ($\mu\text{g/g}$) for each component on a fresh weight basis.

Table 11-8. Isoflavones Reference Standards for US Samples.

| Manufacturer | Component | Lot Number | Purity (%) |
|------------------------------------|-----------|------------|------------|
| LC Laboratories | Daidzein | DA-121 | >99% |
| LC Laboratories | Glycitein | ARH-115 | >99% |
| LC Laboratories | Genistein | CH-148 | >99% |
| LC Laboratories | Daidzin | ARF-114 | >99% |
| Indofine Chemical Company, Inc. | Glycitin | 604029 | >99% |
| LC Laboratories | Genistin | ARE-109 | >99.4% |

Lectin

Sites A, D2, G1, Q1, Q2, W1, IL3, IN, OH2, IA and KS.

The binding properties of soybean agglutinin (SBA) to N-acetylgalactosamine (GalNAc) are utilized in an enzyme linked indirect sandwich assay. The polyacrylic acid form of GalNAc (α -GalNAc-PAA) is coated on a polystyrene microplate to act as a capturing agent. The SBA is extracted from the sample matrix in a phosphate-detergent buffer. The diluted extract is plated in the wells and SBA binds to the α -GalNAc-PAA. A biotinylated α -GalNAc-PAA is then added which also binds with the SBA and creates a SBA-sugar complex. Streptavidin horse radish peroxidase (HRP) is added. Streptavidin and biotin create a strong non-covalent bond which retains HRP in the wells proportional to the SBA concentration. The substrate 3,3',5,5'-tetramethylbenzidine (TMB) is added to the complex and reacts with the HRP enzyme, yielding a blue solution. The reaction is stopped with phosphoric acid producing a yellow color which is read at 450 nm and referenced to 620 nm to account for variations between the wells on the plate. The lectin is quantified using SBA as an external standard (Table 11-9) (Wang *et al.* 2009). The limit of quantitation was calculated as 0.375 mg/g on a fresh weight basis.

Table 11-9. Lectin Reference Standard.

| Manufacturer | Component | Lot Number | Purity (%) |
|---------------------|--------------------|------------|------------------------|
| Vector Laboratories | Soybean Agglutinin | X0820 | Homogenous by SDS-PAGE |

Minerals

Sites A, D2, G1, Q1, Q2 and W1 for Argentina Samples.

Following conversion of the material into ash in a 500°C oven, the samples were dissolved in nitric acid and analyzed by ICP emission spectroscopy. The concentration of each mineral was determined by reading at 3179 Å and 2149 Å for calcium and phosphorus, respectively (AOAC 985.01). The concentration values were reported as µg/mL. The limit of quantification for calcium and phosphorus was 0.001% and 0.005% on a dry weight basis, respectively. Reference standards are listed in Table 11-10).

Table 11-10. Minerals Reference Standards for US Samples.

| Mineral | Manufacturer | Lot Number | Concentration/Purity |
|------------|----------------------|--------------------------------|--------------------------|
| Calcium | Perkin Elmer Pure | Quality control standard 21 | 10-33YPY1 100 (ug/mL) |
| Phosphorus | Cicarelli | K2HPO4 | 57743 100 % |

Sites IL3, IN, OH2, IA and KS.

The samples were dried, precharred, and ashed overnight in a muffle furnace set to maintain 500°C. The ashed samples were re-ashed with nitric acid, treated with hydrochloric acid, taken to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown samples, measured on the inductively coupled plasma spectrometer, with the emission of the standard solutions (AOAC Methods 984.27 and 985.01). The limits of quantitation were calculated on a fresh weight basis (Table 11-11).

Table 11-11. Inorganic Ventures Reference Standards and Limits of Quantification.

| Mineral | Lot Number | Concentration (µg/mL) | Limit of Quantification (ppm) |
|------------|---------------------|-----------------------|-------------------------------|
| Calcium | G2- MEB500050MCA | 200 | 20 |
| Phosphorus | G2- MEB500050MCA | 200 | 20 |

Moisture

Sites A, D2, G1, Q1, Q2 and W1.

Seed was weighed into a pre-weighed vessel and heated for 72 hours at $130 \pm 1^\circ\text{C}$ in an oven, allowed to cool under desiccation, and weighed again. The resulting weight was used to calculate percent moisture (following AACC Method 44-15.02). The limit of quantification was 0.2% on a fresh weight basis.

Sites IL3, IN, OH2, IA and KS.

The samples were dried in a vacuum oven at approximately 100°C . The weight loss was determined and converted to percent moisture (AOAC Methods 926.08 and 925.09). The limit of quantitation was calculated as 0.100% on a fresh weight basis.

Neutral Detergent Fiber, Enzyme Method

Sites A, D2, G1, Q1, Q2 and W1.

The samples were defatted by soxhlet extraction and dried, placed into fritted glass crucibles and washed with a neutral detergent solution that dissolved proteins, sugars and lipids. The sample was rinsed with acetone to remove fat and pigments. The remaining residue containing cellulose, hemicellulose and lignin was collected, dried at 105°C for 12 hours and allowed to cool on a desiccator. Final weight was determined and the neutral fiber content calculated as a percent of the total weight of sample (AOAC 973.18). The reference standard is listed in Table 11-12.

Sites IL3, IN, OH2, IA and KS.

Sample aliquots were weighed into pre-weighed filter bags. The fats and pigments were then removed by an acetone wash. The filter bags were placed in an ANKOM Fiber analyzer where the protein, carbohydrate, and ash content were dissolved by a boiling detergent solution at a neutral pH. The starches were removed via an alpha amylase soak. Hemicellulose, cellulose, lignin and insoluble protein fraction were left in the filter bag and determined gravimetrically (AACC Method 32.20.01; USDA 1970; Komarek *et al.* 1994). The limit of quantitation was calculated as 0.1% on a fresh weight basis. The reference standard is listed in Table 11-12.

Table 11-12. NDF Reference Standard.

| Manufacturer | Component | Lot Number | Purity (%) |
|---------------------|---------------------------------|-------------------|-------------------|
| Sigma-Aldrich | Phytic Acid Sodium Salt Hydrate | BCBK8063V | Phosphorus 21.9% |

Phytic Acid

Sites A, D2, G1, Q1, Q2 and W1.

Homogenized samples were extracted in 3% trichloroacetic acid for 30 minutes. A 2 mg/mL ferric chloride solution was added to an aliquot of the supernatant and boiled for 1 hour. The phytate precipitate was dissolved in nitric acid, washed and diluted to volume. Potassium thiocyanate was added and the phytate ferric salt was measured colorimetrically at 480 nm. The phytate concentration was calculated using a 4:6 iron:phosphorus molecular ratio

(Wheeler and Ferrel 1971). The limit of quantification was 0.01% on a fresh weight basis. The phytic acid reference standard is listed in Table 11-13.

Sites IL3, IN, OH2, IA and KS.

The samples were extracted using hydrochloric acid and sonication, purified using a silica based anion exchange column, concentrated and injected onto a high-performance liquid chromatography system with a refractive index detector. The results were reported on a fresh weight basis (Lehrfeld 1989; Lehrfeld 1994). The limit of quantitation was calculated as 0.125% on a fresh weight basis. The phytic acid reference standard is listed in Table 11-13.

Table 11-13. Phytic Acid Reference Standard.

| Manufacturer | Component | Lot Number | Purity (%) |
|---------------|---------------------------------|------------|---|
| Sigma-Aldrich | Phytic Acid Sodium Salt Hydrate | BCBK8062V | Sodium 5 moles/moles; Phosphorus 21.8% (dried basis) |

Protein

Sites A, D2, G1, Q1, Q2 and W1.

Total nitrogen was determined through Kjeldahl analysis by digesting the sample in sulfuric acid in the presence of a copper catalyst. The resulting ammonia was distilled and titrated against a standardized acid. The percent nitrogen was determined and converted to protein using a factor of 6.25 (AACC Method 46-11.02). The limit of quantification is 0.5% on a fresh weight basis.

Sites IL3, IN, OH2, IA and KS.

The protein and other organic nitrogen in the samples were converted to ammonia by digesting the samples with sulfuric acid containing a catalyst mixture. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. Instrumentation was used to automate the digestion, distillation and titration processes. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25 (AOAC Method 979.09; AOCS Method Ac 4-91). The limit of quantitation was calculated as 0.100% on a fresh weight basis.

Raffinose and Stachyose

Sites A, D2, G1, Q1, Q2 and W1.

Ground material was extracted in 85% methanol for 30 minutes at 85°C, centrifuged, and the extracted sugars were collected. The procedure was repeated and the supernatants combined and dried at 40°C under vacuum. The product was dissolved in ACN:water 70:30 and analyzed by HPLC in a APS-2 column (Thermo Scientific, San Jose, CA). The oligosaccharide content was measured with a refractive index detector. Raffinose and stachyose were quantified by comparison with external standards (Table 11-14) (Muzquiz *et al.* 2004). The limit of quantification was 0.25% and 1% for raffinose and stachyose, respectively.

Table 11-14. Raffinose and Stachyose Reference Standards for Argentina Samples.

| Manufacturer | Component | Lot Number | Purity (%) |
|---------------|------------------------------|------------|------------|
| Sigma-Aldrich | D-(+)-Raffinose pentahydrate | SLBB1916V | 99.25 |
| Sigma-Aldrich | Stachyose hydrate | SLBD3565V | 98 |

Sites IL3, IN, OH2, IA and KS.

Sugars in the samples were extracted with a 50:50 water:methanol solution. Aliquots were taken, dried under inert gas, and then reconstituted with a hydroxylamine hydrochloride solution in pyridine containing phenyl- β -D-glucopyranoside as the internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoroacetic acid treatment, and then analyzed by gas chromatography using a flame ionization detector (Mason and Slover 1971; Brobst 1972). The limit of quantitation was calculated as 0.0500% on a fresh weight basis. The reference standards are listed in Table 11-15).

Table 11-15. Raffinose and Stachyose Reference Standards for US Samples.

| Manufacturer | Component | Lot Number | Purity (%) |
|---------------|------------------------------|------------|------------|
| Sigma-Aldrich | D-(+)-Raffinose pentahydrate | 019K1156 | 99.60% |
| Sigma-Aldrich | Stachyose hydrate | 049K3800 | 98% |

Trypsin Inhibitor

Sites A, D2, G1, Q1, Q2, W1, IL3, IN, OH2, IA and KS.

Ground samples were defatted with hexane. Defatted samples were weighed and extracted with 0.01 N sodium hydroxide. Duplicate samples were prepared at multiple dilutions and known amounts of trypsin and the substrate benzoyl-DL arginine-p-nitroanilide hydrochloride were added. The samples were incubated at 37°C for exactly 10 minutes then quenched with acetic acid. The samples were collected after filtration and the absorbance was measured at 410 nm. The activity was calculated by measuring the absorbance of the sample subtracted from the blank divided by the dilution factor for each assay (AOCS Method Ba 12-75; Kakade *et al* 1974). The limit of quantification was 1.00 TIU/mg.

Vitamin E

Sites A, D2, G1, Q1, Q2 and W1

Oil from soybean grains was extracted as stated in a previous section (Fat by Soxhlet Extraction) but using hexane supplemented with 0.1% BHT (butylated hydroxytoluene) as anti-oxidant. Alpha-tocopherol was quantified by HPLC using a Hypersyl Gold column (4.6 mm ID x 250 mm L, Thermo Scientific, San Jose, CA) and fluorescence detection (excitation 290 nm, emission 330 nm). The vitamin concentration was calculated by comparison of the peak area of vitamin E in the samples with those of standards (Table 11-16) (AACC Method 86-06). The quantification limit was 0.05 mg/100 g.

Sites IL3, IN, OH2, IA and KS.

The samples were saponified to break down fat and release vitamin E. The saponified mixtures were extracted with ethyl ether and then quantitated by high-performance liquid chromatography using a silica column (McMurray *et al.* 1980; Cort *et al.* 1983; Speek *et al.* 1985). The limit of quantitation was calculated as 5.00 mg/kg on a fresh weight basis.

Table 11-16. Vitamin E Reference Standards.

| Manufacturer | Component | Lot Number | Purity (%) |
|---------------------|----------------------|-------------------|-------------------|
| USP | Alpha Tocopherol | O0K291 | 98.5 |
| ACROS Organics | D-gamma-Tocopherol | A0083534 | 99.3 |
| Sigma-Aldrich | (+)-delta-Tocopherol | SLBG1716V | 93 |
| Matreya LLC | rac-beta-Tocopherol, | 23805 | >99% |

Vitamin K1

Sites IL3, IN, OH2, IA and KS.

Samples were prepared by the addition of dimethyl sulfoxide and extraction with multiple portions of hexane. The combined hexane extracts were concentrated and reconstituted in dichloromethane and methanol. Analysis was done by reverse phase high-performance liquid chromatography with a C30 column to separate the cis and trans isomers of Vitamin K. A post-column reduction assembly was used to produce a fluorescent derivative with detection at an excitation wavelength of 243 nm and emission at 430 nm (Woollard *et al.* 2002; USP 35-NF30 2012). The limit of quantitation was calculated as 0.0800 mg/kg on a fresh weight basis. Reference standard is listed in Table 11-17).

Table 11-17. Vitamin K1 Reference Standard.

| Manufacturer | Component | Lot Number | Purity (%) |
|---------------------|------------------|-------------------|-------------------|
| USP | Phytonadione | O0H310 | 99.7 |

Results

Nutrient Levels in Harvested Soybean Seed

The components in the nutrients group included: proximates (ash, moisture, fat, protein and carbohydrates), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, minerals (phosphorus and calcium), fatty acids (palmitic, stearic, oleic, linoleic, linolenic and arachidic), amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and tryptophan) and vitamins E and K1.

Results were analyzed as a single group across all locations to determine whether there were significant nutritional differences between IND-00410-5 and Williams 82. The generation of the plants tested was T6. Values of all components were analyzed for each field trial separately to account for any differences between location and genotype. For all of the locations, only two components—vitamin K1 and cysteine—showed a significant difference between the soybean event IND-00410-5 and Williams 82 (see Tables 11-18 and 11-19). While the content of cysteine was lower in the transgenic event when compared to Williams 82, the mean values fell within the range reported in the literature and those for the commercial reference varieties. The value of vitamin K1 was lower in IND-00410-5 than Williams 82; however, Williams 82 was also lower than the commercial reference varieties at those sites, suggesting a genotypic effect of the Williams 82 parental variety performance in that location (Table 11-18).

For some components, there were individual site significant differences between the mean values of event IND-00410-5 and Williams 82 (Tables 11-20 to 11-29). However, most of these values were either within the range of the commercial reference varieties or the literature values or both. There were only three exceptions outside of the referenced ranges and the commercial varieties. First, the ADF content at the D2 site (Table 11-18) was lower in event IND-00410-5 and below the commercial and literature ranges. Additionally, the vitamin K1 values at IA and KS were significantly lower in IND-00410-5 (Tables 11-29 and 11-30). However, the content of ADF and vitamin K1 in Williams 82 was also below the commercial range, suggesting a genotypic contribution coming from the parental control line (Tables 11-18, 11-21, 11-29 and 11-30). Also, the lysine levels at G1, Q1 and W1 (Tables 11-22, 11-23 and 11-25, respectively) were also statistically significantly lower and outside the range of the reference varieties. However, the majority of the values obtained for Williams 82 and the commercial reference variety values for all 11 locations were below the range reported in the literature. Consequently, the lower lysine content in event IND-00410-5 is likely affected by the growing region and not related to the transgenic event.

In summary, the other nutrient values in the soybean event IND-00410-5 not discussed above were similar to those measured in the non-transgenic parental control Williams 82 or fell within the ranges observed for commercial varieties and/or as reported in the literature.

In conclusion, with regard to the levels of nutrients, the soybean event IND-00410-5 was compositionally equivalent to its non-transgenic parental control line Williams 82 and within the natural variability of conventional commercial reference varieties.

Anti-Nutrient Levels in Soybean Seed

The anti-nutrient components measured included: phytic acid, lectins, the saccharides raffinose and stachyose, the trypsin inhibitor and the isoflavones daidzein, genistein and glycitein.

The analyses of the data of these components showed 5 site values (averaged from 4 samples from each site) significantly different between IND-00410-5 and Williams 82. These included phytic acid, stachyose, and the three isoflavones. The values for phytic acid and the isoflavones were higher in event IND-00410-5 than Williams 82; however, the values were within the range of the commercial reference varieties and literature ranges (Tables 11-18 and 11-31). The value for stachyose was higher in event IND-00410-5 than Williams 82. Again, the value fell within those obtained from the commercial reference varieties planted at the same locations (Tables 11-18 and 11-31).

The anti-nutrients were analyzed individually by site and most of these values were either within the range of the commercial reference varieties or the literature values or both (see Table 11-18 and Tables 11-32 to 11-42). There were few exceptions. In two of the field trials (D2 and W1), phytic acid levels of IND-00410-5 showed significantly higher values compared to Williams 82 and fell outside the range of commercial reference varieties and the literature values (Tables 11-18, 11-33 and 11-37). The stachyose values at two sites (D2 and G1) were significantly higher values compared to Williams 82 and fell outside the range of commercial varieties and literature values (Tables 11-18, 11-33 and 11-34). However, a significant difference was not observed in any of the other sites for both components. Raffinose levels were significantly different between event IND-00410-5 and Williams 82 and outside the reference ranges at two sites (D2 and Q1). At site D2, raffinose in event IND-00410-5 was higher (Tables 11-18 and 11-33) and at site Q1, the mean raffinose value was lower (Tables 11-18 and 11-35). The inconsistent changes in raffinose between these two sites, combined with the lack of a significant difference in the combined-site analysis, are not considered to be meaningful from a food and feed safety and nutritional perspective.

In summary, none of the anti-nutrients measured in seed showed a consistent between the transgenic event IND-00410-5 and the parental control Williams 82 at all planting sites and were within the values obtained for commercial varieties and/or reported in the literature. Consequently, the anti-nutrients levels in event IND-00410-5 were considered equivalent to those of the non-transgenic parental control Williams 82 and within the natural variability of conventional commercial reference varieties.

Nutrient Levels in Soybean Forage

The nutrients measured in forage samples from the individual plots for all the locations included: moisture, ash, protein, fat, carbohydrates, ADF, NDF, phosphorus and calcium.

No significant differences were found in the combined site analysis. There were four components for the individual site analysis which showed a significant difference between the event and Williams 82: carbohydrates, fat, ADF and NDF. The amount of total fat measured in IND-00410-5 at location IN was significantly higher than Williams 82 and carbohydrates in OH2 were calculated to be lower in HB4 than Williams 82. The values were both found to be within range observed for the commercial varieties grown in the same location (Tables 11-51 and 11-52). The analysis revealed greater levels of ADF for event

HB4 at location G1 and lower at location IA when compared with Williams 82. However, the values found were both within the range observed for the commercial varieties grown in the same location (Tables 11-46 and 11-53). Values of NDF at Site G1 and Q2 were found to be significantly different but were within range observed for the commercial varieties grown in the same locations (Tables 11-46 and 11-48).

Consequently, the nutrient composition of forage obtained from the soybean event HB4 was similar to that found in the non-transgenic counterpart and within the range of the commercial reference varieties, supporting the compositional equivalence of the transgenic event with Williams 82 and conventional reference varieties.

Summary

As discussed above, the nutrient and anti-nutrient levels measured in grain and forage from the transgenic event HB4 were found to be equivalent to those measured in the parental non-transgenic control Williams 82, and/or similar to the levels displayed by the commercial soybean reference varieties planted in the same locations, or comparable to the values reported in the literature. Only one value, Vitamin K1 at two sites were found to be outside of that range; however the values for both Williams 82 and HB4 were overall lower than the reference varieties, indicating a genotypic effect impacted at those two sites (IA and KS). These results support the conclusion the transgenic event HB4 is compositionally equivalent to conventional soybean.

Table 11-18. Summary of Differences for the Comparison of Soybean Components of HB4 vs. Parental Control Williams 82.

| Component (Units)¹ | HB4 Mean | Williams 82 Mean | Mean Difference (IND-00410-5 minus Williams 82) | Significance (p-Value)² | Commercial Reference Range | Literature Range (ILSI)³ |
|--|-----------------|-------------------------|--|---|-----------------------------------|--|
| Statistical Differences Observed in Combined-Site Analysis | | | | | | |
| Seed Amino Acids (% dwt) | | | | | | |
| Cysteine | 0.55 | 0.59 | -0.04 | 0.013 | 0.49 – 0.62 | 0.37 – 0.81 |
| Seed Vitamins (mg/kg) | | | | | | |
| Vitamin K1 | 0.38 | 0.43 | -0.05 | 0.014 | 0.44 – 0.85 | |
| Seed Anti-Nutrients | | | | | | |
| Phytic acid (% dwt) | 1.67 | 1.35 | 0.32 | 0.032 | 0.54 – 1.69 | 0.63 – 1.96 |
| Stachyose (% dwt) | 3.77 | 3.39 | 0.38 | 0.027 | 2.56 – 4.76 | 1.21 – 3.50 |
| Daidzein (mg/kg dwt) | 1240 | 1086 | 154 | 0.002 | 533 – 2150 | 60 - 2454 |
| Genistein (mg/kg dwt) | 1402 | 1282 | 120 | 0.009 | 671 – 2290 | 144 - 2837 |
| Glycitein (mg/kg dwt) | 276 | 239 | 37 | <0.001 | 126 – 344 | 15.3 – 310 |
| Statistical Differences Observed in More than One Individual Site | | | | | | |
| Seed Proximates (% dwt) | | | | | | |
| Carbohydrates Site G1 | 38.55 | 34.73 | 3.82 | 0.008 | 32.13 – 36.49 | 29.6 – 50.2 |
| Carbohydrates Site Q1 | 39.98 | 33.40 | 6.58 | 0.005 | 37.45 – 40.87 | |
| Carbohydrates Site KS | 34.38 | 33.63 | 0.75 | 0.04 | 33.40 – 36.30 | |
| Protein Site A | 39.07 | 39.12 | -0.05 | 0.038 | 37.95 – 40.11 | 33.2 – 45.5 |
| Protein Site D2 | 40.19 | 38.03 | 2.16 | <0.001 | 37.52 – 39.03 | |
| Protein Site G1 | 35.93 | 39.78 | -3.85 | 0.025 | 37.74 – 41.97 | |
| Protein Site Q1 | 36.52 | 41.24 | -4.72 | 0.036 | 37.10 – 38.90 | |
| Protein Site Q2 | 37.14 | 39.09 | -1.95 | 0.009 | 37.09 – 39.81 | |
| Protein Site IA | 38.23 | 39.03 | -0.80 | 0.011 | 36.60 – 39.80 | |
| Protein Site KS | 40.18 | 40.60 | -0.42 | 0.031 | 38.00 – 42.40 | |

¹ dwt = dry weight; fwt = fresh weight² The p value threshold is $p \leq 0.05$ ³ Reference range with values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014).

| Component (Units)¹ | HB4 Mean | Williams 82 Mean | Mean Difference (IND-00410-5 minus Williams 82) | Significance (p-Value)² | Commercial Reference Range | Literature Range (ILSI)³ |
|--------------------------------------|-----------------|-------------------------|--|---|-----------------------------------|--|
| Total Fat Site A | 18.30 | 20.48 | -2.18 | 0.003 | 19.40 – 22.10 | 8.1 – 23.6 |
| Total Fat Site D2 | 18.68 | 16.65 | 2.03 | 0.039 | 17.00 – 19.30 | |
| Total Fat Site Q2 | 20.95 | 16.10 | 4.85 | 0.003 | 17.46 – 18.10 | |
| Total Fat Site OH2 | 19.33 | 19.70 | -0.37 | 0.043 | 16.60 – 18.80 | |
| Seed Fiber (% dwt) | | | | | | |
| ADF Site A | 8.40 | 12.90 | -4.50 | 0.040 | 10.30 – 14.21 | 7.8 – 18.6 |
| ADF Site D2 | 7.08 | 11.60 | -4.52 | 0.003 | 12.89 – 16.19 | |
| ADF Site G1 | 13.83 | 9.65 | 4.18 | 0.004 | 11.34 – 15.73 | 7.8 – 18.6 |
| ADF Site W1 | 11.90 | 11.08 | 0.82 | 0.009 | 15.39 – 17.77 | |
| NDF Site A | 16.25 | 17.45 | -1.20 | 0.048 | 10.98 – 14.34 | 8.5 – 21.3 |
| NDF Site Q2 | 17.23 | 19.18 | -1.95 | 0.025 | 16.35 – 20.52 | |
| Seed Fatty Acids (% dwt) | | | | | | |
| Palmitic Site A | 2.04 | 2.26 | -0.22 | 0.002 | 2.14 – 2.73 | 0.67 – 2.78 |
| Palmitic Site D2 | 2.04 | 1.84 | 0.20 | 0.050 | 1.82 – 2.07 | |
| Palmitic Site Q2 | 2.54 | 1.84 | 0.70 | 0.026 | 1.86 – 2.05 | |
| Stearic Site Q2 | 0.86 | 0.67 | 0.19 | 0.004 | 0.78 – 0.93 | 0.28 – 1.13 |
| Stearic Site IA | 0.89 | 0.93 | -0.04 | 0.022 | 0.80 – 0.87 | |
| Oleic Site A | 3.65 | 4.63 | -0.98 | 0.005 | 3.47 – 4.54 | 1.36 – 6.56 |
| Oleic Site D2 | 3.87 | 3.55 | 0.32 | 0.048 | 3.44 – 4.45 | |
| Oleic Site OH2 | 4.52 | 4.80 | -0.28 | 0.013 | 3.81 – 4.64 | |
| Oleic Site IA | 4.87 | 5.23 | -0.36 | 0.008 | 4.15 – 4.87 | |
| Oleic Site KS | 4.30 | 4.67 | -0.37 | 0.037 | 4.01 – 4.71 | |
| Linoleic Site A | 10.44 | 11.27 | -0.83 | 0.015 | 10.89 – 13.34 | 3.46 – 13.36 |
| Linoleic Site D2 | 10.40 | 9.05 | 1.35 | 0.031 | 9.22 – 10.61 | |
| Linoleic Site Q2 | 11.65 | 8.92 | 2.73 | 0.002 | 9.32 – 10.26 | |
| Linoleic Site W1 | 10.74 | 9.47 | 1.27 | 0.020 | 10.70 – 11.72 | |
| Linoleic Site IA | 11.23 | 11.03 | 0.20 | <0.001 | 10.10 – 11.30 | |
| Linolenic Site A | 1.29 | 1.39 | -0.10 | 0.027 | 1.13 – 1.61 | 0.30 – 2.19 |
| Linolenic Site Q2 | 1.77 | 1.43 | 0.34 | 0.001 | 1.21 – 1.65 | |
| Linolenic Site W1 | 1.22 | 1.06 | 0.16 | 0.012 | 1.02 – 1.37 | |
| Linolenic Site IA | 1.36 | 1.33 | 0.03 | 0.032 | 1.25 – 1.49 | |

| Component (Units) ¹ | HB4 Mean | Williams 82 Mean | Mean Difference (IND-00410-5 minus Williams 82) | Significance (p-Value) ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---|----------|------------------|---|-------------------------------------|----------------------------|--------------------------------------|
| Arachidic Site IN | 0.06 | 0.07 | -0.01 | 0.029 | 0.06 – 0.07 | 0.02 – 0.11 |
| Arachidic Site IA | 0.07 | 0.07 | 0 | 0.033 | 0.06 – 0.07 | |
| Seed Vitamins | | | | | | |
| Vitamin E(mg/100 g dwt) Site Q2 | 2.16 | 1.32 | 0.84 | 0.004 | 1.26 – 1.98 | 0.19 – 6.17 |
| Vitamin E (mg/100 g dwt) Site IA | 2.66 | 2.82 | -0.16 | 0.014 | 1.42 – 3.13 | |
| Vitamin K1 ⁴ (mg/kg) Site IA | 0.50 | 0.57 | -0.07 | 0.029 | 0.71 – 0.85 | |
| Vitamin K1 (mg/kg) Site KS | 0.33 | 0.39 | -0.06 | <0.001 | 1.02 – 1.87 | |
| Seed Amino Acids (% dwt) | | | | | | |
| Arginine Site D2 | 2.58 | 2.97 | -0.39 | 0.013 | 2.71 – 3.07 | 2.28 – 3.4 |
| Arginine Site G1 | 2.54 | 2.88 | -0.34 | 0.036 | 2.58 – 3.07 | |
| Arginine Site Q2 | 2.47 | 2.98 | -0.51 | <0.001 | 2.70 – 3.07 | |
| Aspartic Acid Site A | 4.49 | 4.11 | 0.38 | 0.033 | 4.06 – 4.44 | 3.81 – 5.12 |
| Aspartic Acid Site Q1 | 4.27 | 4.94 | -0.67 | 0.004 | 3.98 – 4.47 | |
| Glycine Site D2 | 1.91 | 1.61 | 0.30 | 0.001 | 1.65 – 1.75 | 1.46 – 1.99 |
| Glycine Site Q1 | 1.76 | 1.66 | 0.11 | 0.026 | 1.67 – 1.75 | |
| Glycine Site W1 | 1.89 | 1.80 | 0.09 | 0.027 | 1.62 – 1.71 | |
| Isoleucine Site Q1 | 1.66 | 1.87 | -0.21 | 0.033 | 1.52 – 1.90 | 1.53 – 2.07 |
| Isoleucine Site Q2 | 1.58 | 1.84 | -0.26 | 0.002 | 1.51 – 1.93 | |
| Leucine Site A | 3.20 | 2.91 | 0.29 | 0.001 | 2.93 – 3.30 | 2.59 – 3.62 |
| Leucine Site D2 | 3.03 | 2.81 | 0.22 | 0.017 | 2.67 – 2.96 | |
| Leucine Site Q1 | 2.76 | 3.15 | -0.39 | 0.006 | 2.70 – 3.24 | |
| Leucine Site IA | 2.89 | 3.00 | -0.11 | 0.005 | 2.80 – 3.04 | 2.28 – 2.83 |
| Lysine Site G1 | 1.79 | 2.20 | -0.41 | 0.005 | 2.09 – 2.23 | |
| Lysine Site Q1 | 1.77 | 2.26 | -0.49 | 0.003 | 2.16 – 2.23 | |
| Lysine Site Q2 | 2.20 | 2.30 | -0.10 | 0.003 | 2.20 – 2.28 | |
| Lysine Site W1 | 1.92 | 2.27 | -0.35 | 0.003 | 2.06 – 2.23 | |
| Methionine Site Q1 | 0.50 | 0.55 | -0.05 | 0.043 | 0.48 – 0.58 | 0.43 – 0.68 |
| Methionine Site W1 | 0.57 | 0.49 | 0.08 | <0.001 | 0.50 – 0.57 | |

⁴ Measured only from locations IL3, IN, OH2, IA and KS.

| Component (Units)¹ | HB4 Mean | Williams 82 Mean | Mean Difference (IND-00410-5 minus Williams 82) | Significance (p-Value)² | Commercial Reference Range | Literature Range (ILSI)³ |
|--------------------------------------|-----------------|-------------------------|--|---|-----------------------------------|--|
| Methionine Site IA | 0.50 | 0.53 | -0.03 | 0.003 | 0.51 – 0.54 | |
| Serine Site D2 | 1.78 | 2.06 | -0.28 | 0.024 | 1.87 – 2.24 | 1.10 – 2.48 |
| Serine Site Q1 | 1.80 | 2.17 | -0.37 | 0.016 | 1.77 – 2.04 | |
| Threonine Site D2 | 1.64 | 1.41 | 0.24 | 0.001 | 1.34 – 1.55 | 1.14 – 1.86 |
| Threonine Site G1 | 1.48 | 1.40 | 0.08 | 0.049 | 1.36 – 1.49 | |
| Threonine Site W1 | 1.65 | 1.52 | 0.13 | 0.042 | 1.38 – 1.49 | |
| Tryptophan Site G1 | 0.39 | 0.45 | -0.06 | 0.008 | 0.42 – 0.52 | 0.36 – 0.50 |
| Tryptophan Site Q1 | 0.43 | 0.53 | -0.10 | 0.002 | 0.39 – 0.57 | |
| Seed Minerals (% dwt) | | | | | | |
| Calcium Site A | 0.26 | 0.29 | -0.03 | 0.002 | 0.19 – 0.22 | 0.12 – 0.31 |
| Calcium Site D2 | 0.21 | 0.20 | 0.01 | 0.014 | 0.17 – 0.22 | |
| Seed Anti-Nutrients (% dwt) | | | | | | |
| Phytic Acid Site D2 | 2.60 | 1.70 | 0.90 | 0.024 | 1.51 – 1.63 | 0.63 – 1.96 |
| Phytic Acid Site W1 | 2.78 | 1.63 | 1.15 | 0.003 | 1.47 – 2.02 | |
| Lectins Site A | 4.60 | 5.09 | -0.49 | 0.020 | 3.01 – 3.45 | 0.11 – 9.04 |
| Lectins Site D2 | 4.36 | 3.33 | 1.03 | 0.006 | 1.87 – 2.76 | |
| Raffinose Site D2 | 1.26 | 0.94 | 0.32 | 0.026 | 0.67 – 0.98 | 0.21 – 0.66 |
| Raffinose Site Q1 | 0.71 | 0.83 | -0.12 | 0.013 | 0.74 – 1.07 | |
| Raffinose Site IN | 0.84 | 0.76 | 0.08 | 0.046 | 0.80 – 0.90 | |
| Raffinose Site IA | 0.89 | 0.84 | 0.05 | 0.008 | 0.79 – 0.89 | |
| Stachyose Site D2 | 4.34 | 2.85 | 1.49 | 0.013 | 2.70 – 3.71 | 1.21 – 3.50 |
| Stachyose Site G1 | 3.64 | 2.70 | 0.94 | 0.013 | 2.56 – 3.17 | |
| Stachyose Site IA | 4.01 | 3.87 | 0.14 | 0.002 | 3.84 – 4.62 | |
| Seed Isoflavones (mg/kg dwt) | | | | | | |
| Daidzein Site A | 975 | 778 | 197 | 0.006 | 883 – 1159 | 60.0 – 2453.5 |
| Daidzein Site D2 | 1227 | 1025 | 202 | 0.010 | 1041 – 1323 | |
| Daidzein Site G1 | 1110 | 924 | 186 | 0.014 | 936 – 1244 | |
| Daidzein Site IL3 | 1385 | 1208 | 177 | 0.031 | 1280 – 2090 | |
| Daidzein Site IA | 1468 | 1062 | 406 | <0.001 | 1310 – 1720 | |
| Daidzein Site KS | 1780 | 1526 | 254 | 0.004 | 1260 – 2150 | 144.3 – 2837.2 |
| Genistein Site A | 1049 | 813 | 236 | 0.011 | 890 – 1478 | |

| Component (Units)¹ | HB4 Mean | Williams 82 Mean | Mean Difference (IND-00410-5 minus Williams 82) | Significance (p-Value)² | Commercial Reference Range | Literature Range (ILSI)³ |
|--|-----------------|-------------------------|--|---|-----------------------------------|--|
| Genistein Site IA | 1745 | 1348 | 397 | <0.001 | 1320 – 1740 | |
| Genistein Site KS | 1994 | 1801 | 194 | 0.008 | 1390 – 1940 | |
| Glycitein Site G1 | 259 | 219 | 40 | 0.007 | 163 – 201 | 15.3 – 310.4 |
| Glycitein Site IL3 | 350 | 297 | 53 | 0.042 | 172 – 409 | |
| Glycitein Site KS | 290 | 238 | 51.7 | 0.002 | 141 – 280 | |
| Statistical Differences Observed in One Individual Site | | | | | | |
| Seed Proximates (% fwt) | | | | | | |
| Moisture Site D2 | 9.49 | 8.12 | 1.37 | <0.001 | 7.50 – 8.05 | 4.7 – 34.4 |
| Seed Fiber (% dwt) | | | | | | |
| Crude Fiber Site Q1 | 4.84 | 6.48 | -1.64 | 0.014 | 3.39 – 4.97 | |
| Seed Amino Acids (% dwt) | | | | | | |
| Alanine Site Q2 | 1.66 | 1.86 | -0.20 | 0.012 | 2.04 – 2.16 | 1.51 – 2.10 |
| Cysteine Site IN | 0.56 | 0.58 | -0.02 | 0.029 | 0.56 – 0.61 | 0.37 – 0.81 |
| Histidine Site Q1 | 0.90 | 1.07 | -0.17 | 0.003 | 0.88 – 1.10 | 0.87 – 1.17 |
| Phenylalanine Site Q1 | 1.84 | 2.17 | -0.33 | 0.005 | 1.60 – 2.14 | 1.63 – 2.34 |
| Tyrosine Site Q1 | 1.22 | 1.50 | -0.28 | 0.038 | 1.20 – 1.40 | 1.01 – 1.61 |
| Valine Site IA | 1.78 | 1.85 | -0.07 | 0.034 | 1.72 – 1.87 | 1.59 – 2.20 |
| Seed Anti-Nutrients | | | | | | |
| Trypsin Inhibitor Site W1 (TIU/mg dwt) ⁵ | 44.24 | 35.05 | 9.19 | 0.006 | 50.60 – 62.90 | 19.59 – 118.68 |
| Forage Proximates (% dwt) | | | | | | |
| Carbohydrates Site OH2 | 67.9 | 68.5 | -0.55 | 0.049 | 65.0 – 68.8 | 29.6 – 50.2 |
| Total Fat Site IN | 3.27 | 2.46 | 0.81 | 0.001 | 3.02 – 3.54 | 1.30 – 5.13 |
| Forage Fiber (% dwt) | | | | | | |
| ADF Site G1 | 32.5 | 29.4 | 3.10 | 0.019 | 31.3 – 35.4 | |
| ADF Site IA | 27.0 | 32.6 | -5.62 | 0.023 | 25.6 – 31.2 | |
| NDF Site G1 | 47.88 | 45.03 | 2.85 | 0.042 | 46.80 – 57.20 | |
| NDF Site Q2 | 45.40 | 47.75 | -2.35 | 0.045 | 44.00 – 50.90 | |

⁵ TIU: trypsin inhibitor units

Table 11-19. Summary of Combined-Site Soybean Seed Nutrients for HB4 vs. Parental Control Williams 82.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 5.69 (0.05) (5.20 – 6.36) | 5.68 (0.05) (5.03 – 6.42) | 0.01 | -0.09 0.11 | NS | 4.83 – 6.35 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 35.84 (0.35) (32.27 – 41.52) | 35.19 (0.38) (32.16 – 40.97) | 0.65 | -1.14 2.44 | NS | 31.46 – 38.11 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 9.46 (0.18) (7.05 – 11.6) | 9.28 (0.18) (7.41 – 11.6) | 0.18 | -0.13 0.49 | NS | 7.78 – 11.82 | 4.7 – 34.4 |
| Protein | 39.03 (0.30) (34.58 – 42.74) | 39.78 (0.24) (36.49 – 43.93) | -0.75 | -1.99 0.49 | NS | 36.6 – 43.1 | 33.2 – 45.5 |
| Total Fat | 19.98 (0.19) (17.55 – 21.80) | 19.56 (0.28) (15.90 – 22.48) | 0.42 | -0.70 1.54 | NS | 16.6 – 21.64 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 12.51 (0.40) (6.69 – 16.0) | 12.99 (0.34) (9.18 – 18.3) | -0.48 | -2.13 1.17 | NS | 10.50 – 17.77 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 16.88 (0.27) (14.3 – 21.23) | 16.83 (0.23) (13.8 – 21.16) | 0.05 | -0.85 0.95 | NS | 14.10 – 18.07 | 8.5 – 21.3 |
| Crude Fiber | 7.35 (0.44) (3.21 – 12.50) | 7.74 (0.40) (4.66 – 13.20) | -0.39 | -0.96 0.18 | NS | 4.61 – 13.60 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.56 (0.01) (0.35 – 0.69) | 0.57 (0.01) (0.38 – 0.68) | -0.01 | -0.02 0.01 | NS | 0.36 – 0.61 | 0.50 – 0.94 |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSI)³ |
|---------------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Calcium | 0.26 (0.01) (0.20 – 0.37) | 0.25 (0.01) (0.18 – 0.35) | 0.01 | - 0.01 0.00 6 | NS | 0.20 – 0.31 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.17 (0.03) (1.82 – 2.88) | 2.12 (0.03) (1.74 – 2.61) | 0.05 | -0.10 0.20 | NS | 1.76 – 2.52 | 0.67 – 2.78 |
| 18:0 Stearic | 0.85 (0.01) (0.68 – 1.02) | 0.84 (0.01) (0.62 – 1.06) | 0.01 | -0.04 0.06 | NS | 0.61 – 1.15 | 0.28 – 1.13 |
| 18:1 Oleic | 4.31 (0.07) (3.35 – 5.25) | 4.46 (0.10) (3.03 – 5.37) | -0.15 | -0.44 0.14 | NS | 2.86 – 5.52 | 1.36 – 6.56 |
| 18:2 Linoleic | 10.85 (0.10) (9.51 – 12.40) | 10.43 (0.14) (8.62 – 12.31) | 0.42 | -0.20 1.04 | NS | 8.33 – 11.72 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.42 (0.02) (1.14 – 1.87) | 1.37 (0.02) (1.04 – 1.69) | 0.05 | -0.02 0.12 | NS | 1.20 – 1.66 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.06 (0) (0.04 – 0.09) | 0.06 (0) (0.03 – 0.11) | 0 | -0.01 0.01 | NS | 0.03 – 0.07 | 0.02 – 0.11 |
| Vitamins | | | | | | | |
| Vitamin E (mg/100 gr dwt) | 1.87 (0.06) (0.11 – 2.78) | 1.81 (0.07) (0.95 – 2.93) | 0.06 | -0.14 0.26 | NS | 1.37 – 3.13 | 0.19 – 6.17 |
| Vitamin K1 (mg/kg) ⁷ | 0.38 (0.02) (0.91 – 0.51) | 0.43 (0.02) (0.31 – 0.61) | -0.05 | -0.08 -0.02 | * | 0.44 – 0.85 | |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 1.85 (0.02) (1.57 – 2.14) | 1.86 (0.02) (1.65 – 2.17) | -0.01 | -0.06 0.04 | NS | 1.63 – 2.10 | 1.51 – 2.10 |
| Arginine | 2.83 (0.04) (2.43 – 3.22) | 2.96 (0.02) (2.57 – 3.19) | -0.13 | -0.29 0.03 | NS | 2.67 – 3.27 | 2.28 – 3.4 |
| Aspartic Acid | 4.51 (0.04) (4.04 – 5.07) | 4.56 (0.04) (4.04 – 5.08) | -0.05 | -0.26 0.16 | NS | 4.17 – 4.91 | 3.81 – 5.12 |

⁷Measured only from locations IL3, IN, OH2, IA and KS

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSI)³ |
|--------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Cysteine | 0.55 (0.01) (0.43 – 0.66) | 0.59 (0.01) (0.52 – 0.67) | -0.04 | -0.07 -0.01 | * | 0.49 – 0.62 | 0.37 – 0.81 |
| Glycine | 1.73 (0.01) (1.59 – 2.00) | 1.69 (0.01) (1.53 – 1.83) | 0.04 | -0.15 0.21 | NS | 6.28 – 7.41 | 1.46 – 1.99 |
| Glutamic Acid | 7.00 (0.05) (6.33 – 7.72) | 6.97 (0.05) (6.20 – 7.56) | 0.04 | -0.03 0.11 | NS | 1.58 – 1.79 | 5.84 – 8.20 |
| Histidine | 1.00 (0.01) (0.85 – 1.19) | 1.01 (0.01) (0.84 – 1.13) | -0.01 | -0.05 0.03 | NS | 0.90 – 1.10 | 0.87 – 1.17 |
| Isoleucine | 1.77 (0.02) (1.50 – 1.95) | 1.83 (0.01) (1.63 – 1.95) | -0.06 | -0.13 0.01 | NS | 1.60 – 1.87 | 1.53 – 2.07 |
| Leucine | 3.02 (0.02) (2.62 – 3.30) | 3.02 (0.02) (2.70 – 3.26) | 0 | -0.12 0.12 | NS | 2.80 – 3.15 | 2.59 – 3.62 |
| Lysine | 2.20 (0.05) (1.68 – 2.60) | 2.33 (0.02) (1.99 – 2.61) | -0.13 | -0.27 0.01 | NS | 2.14 – 2.59 | 2.28 – 2.83 |
| Methionine | 0.52 (0.01) (0.44 – 0.60) | 0.52 (0) (0.44 – 0.57) | 0 | -0.03 0.03 | NS | 0.45 – 0.55 | 0.43 – 0.68 |
| Phenylalanine | 1.96 (0.02) (1.56 – 2.18) | 1.99 (0.02) (1.67 – 2.19) | -0.03 | -0.11 0.05 | NS | 1.77 – 2.20 | 1.63 – 2.34 |
| Proline | 2 (0.02) (1.69 – 2.37) | 2.01 (0.02) (1.70 – 2.30) | -0.01 | -0.07 0.05 | NS | 1.85 – 2.29 | 1.68 – 2.28 |
| Serine | 1.94 (0.02) (1.64 – 2.33) | 2.03 (0.01) (1.78 – 2.24) | -0.09 | -0.19 0.01 | NS | 1.80 – 2.19 | 1.10 – 2.48 |
| Threonine | 1.54 (0.02) (1.30 – 1.69) | 1.50 (0.02) (1.34 – 1.71) | 0.04 | -0.01 0.09 | NS | 1.35 – 1.64 | 1.14 – 1.86 |
| Tryptophan | 0.50 (0.01) (0.34 – 0.61) | 0.52 (0.01) (0.40 – 0.62) | -0.02 | -0.05 0.01 | NS | 0.41 – 0.60 | 0.36 – 0.50 |
| Tyrosine | 1.38 (0.03) (1.04 – 1.63) | 1.39 (0.03) (1.03 – 1.62) | -0.01 | -0.07 0.05 | NS | 1.03 – 1.61 | 1.01 – 1.61 |
| Valine | 1.83 (0.02) (1.60 – 2.07) | 1.88 (0.01) (1.69 – 2.17) | -0.05 | -0.11 0.01 | NS | 1.71 – 2.10 | 1.59 – 2.20 |

Table 11-20. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site A.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 6.26 (0.05) (6.14 – 6.36) | 6.33 (0.04) (6.26 – 6.42) | -0.07 | -0.23 0.08 | NS | 5.87 – 6.35 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 36.78 (0.70) (34.79 – 37.85) | 36.15 (0.29) (35.42 – 36.75) | 0.63 | -1.22 2.47 | NS | 33.49 – 36.96 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 7.20 (0.05) (7.05 – 7.30) | 7.53 (0.06) (7.41 – 7.64) | -0.33 | -0.52 -0.13 | NS | 7.76 – 11.05 | 4.7 – 34.4 |
| Protein | 39.07 (0.43) (38.37 – 40.24) | 39.12 (0.55) (36.80 – 39.53) | -0.05 | -1.77 1.66 | * | 37.95 – 40.11 | 33.2 – 45.5 |
| Total Fat | 18.30 (0.20) (17.70 – 18.60) | 20.48 (0.28) (20.10 – 21.30) | -2.18 | -3.02 -1.33 | * | 19.40 – 22.10 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 8.40 (0.21) (7.91 – 8.81) | 12.90 (1.25) (10.24 – 15.50) | -4.50 | -7.61 -1.39 | * | 10.30 – 14.21 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 16.25 (0.43) (15.09 – 17.00) | 17.45 (0.31) (16.92 – 18.24) | -1.20 | -2.49 0.09 | * | 10.98 – 14.34 | 8.5 – 21.3 |
| Crude Fiber | 5.80 (0.27) (5.36 – 6.51) | 5.13 (0.20) (4.69 – 5.61) | 0.67 | -0.14 1.48 | NS | 5.13 – 6.10 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.65 (0.01) (0.61 – 0.67) | 0.66 (0.01) (0.63 – 0.67) | -0.01 | -0.05 0.03 | NS | 0.61 – 0.63 | 0.50 – 0.94 |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at p ≤ 0.05³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSD)³ |
|---------------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Calcium | 0.26 (0.01) (0.25 – 0.27) | 0.29 (0.004) (0.28 – 0.30) | -0.03 | -0.04 -0.01 | * | 0.19 – 0.22 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.04 (0.04) (1.98 – 2.16) | 2.26 (0.03) (2.19 – 2.34) | -0.22 | -0.35 -0.09 | * | 2.14 – 2.73 | 0.67 – 2.78 |
| 18:0 Stearic | 0.83 (0.02) (0.80 – 0.88) | 0.88 (0.01) (0.86 – 0.90) | -0.05 | -0.10 -0.01 | NS | 0.76 – 0.93 | 0.28 – 1.13 |
| 18:1 Oleic | 3.65 (0.09) (3.39 – 3.80) | 4.63 (0.09) (4.41 – 4.83) | -0.98 | -1.29 -0.67 | * | 3.47 – 4.54 | 1.36 – 6.56 |
| 18:2 Linoleic | 10.44 (0.16) (10.00 – 10.76) | 11.27 (0.21) (10.93 – 11.89) | -0.83 | -1.48 -0.18 | * | 10.89 – 13.34 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.29 (0.03) (1.21 – 1.35) | 1.39 (0.02) (1.36 – 1.45) | -0.10 | -0.19 0 | * | 1.13 – 1.61 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.05 (0.003) (0.05 – 0.06) | 0.05 (0.003) (0.05 – 0.06) | 0 | -0.01 0.01 | NS | 0.04 – 0.07 | 0.02 – 0.11 |
| Vitamins (mg/100 gr dwt) | | | | | | | |
| Vitamin E | 1.91 (0.16) (1.51 – 2.22) | 1.63 (0.20) (1.19 – 2.05) | 0.28 | -0.35 0.91 | NS | 1.37 – 2.16 | 0.19 – 6.17 |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 2.04 (0.03) (1.95 – 2.12) | 1.98 (0.07) (1.79 – 2.12) | 0.06 | -0.13 0.25 | NS | 1.67 – 2.11 | 1.51 – 2.10 |
| Arginine | 2.87 (0.07) (2.70 – 3.02) | 2.85 (0.08) (2.70 – 3.05) | 0.02 | -0.23 0.27 | NS | 2.48 – 3.05 | 2.28 – 3.4 |
| Aspartic Acid | 4.49 (0.10) (4.21 – 4.67) | 4.11 (0.03) (4.04 – 4.20) | 0.38 | 0.12 0.64 | * | 4.06 – 4.44 | 3.81 – 5.12 |
| Cysteine | 0.52 (0.04) (0.43 – 0.59) | 0.61 (0.02) (0.57 – 0.65) | -0.09 | -0.19 0.01 | NS | 0.57 – 0.64 | 0.37 – 0.81 |
| Glycine | 1.71 (0.01) (1.67 – 1.73) | 1.71 (0.04) (1.64 – 1.78) | -0.01 | -0.10 0.09 | NS | 1.77 – 1.84 | 1.46 – 1.99 |
| Glutamic Acid | 7.17 (0.05) (7.08 – 7.29) | 6.92 (0.08) (6.76 – 7.12) | 0.25 | 0.03 0.47 | NS | 6.68 – 7.15 | 5.84 – 8.20 |

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSD) ³ |
|-------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Histidine | 1.01 (0.06) (0.85 – 1.16) | 0.97 (0.03) (0.91 – 1.04) | 0.04 | -0.13 0.21 | NS | 0.93 – 1.09 | 0.87 – 1.17 |
| Isoleucine | 1.72 (0.07) (1.58 – 1.91) | 1.85 (0.06) (1.66 – 1.92) | -0.13 | -0.36 0.10 | NS | 1.49 – 1.86 | 1.53 – 2.07 |
| Leucine | 3.20 (0.05) (3.08 – 3.30) | 2.91 (0.04) (2.79 – 2.99) | 0.29 | 0.14 0.45 | * | 2.93 – 3.30 | 2.59 – 3.62 |
| Lysine | 1.84 (0.06) (1.71 – 1.97) | 2.07 (0.04) (1.99 – 2.16) | -0.23 | -0.40 -0.06 | NS | 2.17 – 2.29 | 2.28 – 2.83 |
| Methionine | 0.46 (0.01) (0.44 – 0.49) | 0.52 (0.01) (0.50 – 0.54) | -0.06 | -0.10 -0.03 | NS | 0.50 – 0.57 | 0.43 – 0.68 |
| Phenylalanine | 1.85 (0.11) (1.61 – 2.10) | 1.87 (0.06) (1.71 – 1.96) | -0.02 | -0.32 0.27 | NS | 1.71 – 1.95 | 1.63 – 2.34 |
| Proline | 2.07 (0.09) (1.85 – 2.24) | 2.05 (0.05) (1.93 – 2.15) | 0.02 | -0.24 0.28 | NS | 2.00 – 2.35 | 1.68 – 2.28 |
| Serine | 2.11 (0.07) (2.00 – 2.33) | 1.94 (0.06) (1.78 – 2.07) | 0.17 | -0.07 0.41 | NS | 1.89 – 2.11 | 1.10 – 2.48 |
| Threonine | 1.34 (0.01) (1.30 – 1.37) | 1.37 (0.01) (1.35 – 1.39) | -0.03 | -0.07 0.01 | NS | 1.34 – 1.38 | 1.14 – 1.86 |
| Tryptophan | 0.48 (0.04) (0.38 – 0.56) | 0.47 (0.03) (0.42 – 0.54) | 0.01 | -0.11 0.12 | NS | 0.48 – 0.56 | 0.36 – 0.50 |
| Tyrosine | 1.20 (0.03) (1.13 – 1.26) | 1.20 (0.04) (1.10 – 1.31) | 0 | -0.13 0.12 | NS | 1.37 – 1.52 | 1.01 – 1.61 |
| Valine | 1.82 (0.11) (1.60 – 2.07) | 1.81 (0.03) (1.75 – 1.88) | 0.01 | -0.28 0.29 | NS | 1.74 – 1.97 | 1.59 – 2.20 |

Table 11-21. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site D2.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 5.45 (0.01) (5.43 – 5.47) | 5.77 (0.10) (5.50 – 5.95) | -0.32 | -0.55 – 0.08 | NS | 5.65 – 5.80 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 37.20 (0.90) (35.64 – 39.03) | 39.45 (0.43) (38.38 – 40.44) | -2.25 | -4.69 – 0.19 | NS | 38.16 – 40.79 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 9.49 (0.07) (9.32 – 9.67) | 8.12 (0.04) (8.06 – 8.25) | 1.37 | 1.16 – 1.58 | * | 7.50 – 8.05 | 4.7 – 34.4 |
| Protein | 40.19 (0.41) (39.41 – 41.02) | 38.03 (0.34) (37.44 – 39.00) | 2.16 | 1.05 – 4.93 | * | 37.52 – 39.03 | 33.2 – 45.5 |
| Total Fat | 18.68 (0.48) (18.00 – 20.14) | 16.65 (0.38) (16.00 – 17.70) | 2.03 | 0.53 – 3.52 | * | 17.00 – 19.30 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 7.08 (0.25) (6.69 – 7.81) | 11.60 (0.49) (10.72 – 12.87) | -4.52 | -5.87 – -3.18 | * | 12.89 – 16.19 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 17.83 (0.56) (16.61 – 19.02) | 16.38 (0.46) (15.24 – 17.25) | 1.45 | -0.33 – 3.23 | NS | 14.93 – 15.72 | 8.5 – 21.3 |
| Crude Fiber | 3.85 (0.48) (3.21 – 5.29) | 5.81 (0.24) (5.12 – 6.15) | -1.96 | -3.28 – -0.64 | NS | 3.84 – 4.84 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.67 (0.01) (0.65 – 0.69) | 0.66 (0.01) (0.65 – 0.68) | 0.01 | -0.02 – 0.03 | NS | 0.62 – 0.66 | 0.50 – 0.94 |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation.⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSI)³ |
|---------------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Calcium | 0.21 (0.004) (0.20 – 0.22) | 0.20 (0.004) (0.18 – 0.20) | 0.01 | 0 | 0.03 | 0.17 – 0.22 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.04 (0.05) (1.93 – 2.15) | 1.84 (0.04) (1.76 – 1.96) | 0.20 | 0.05 | 0.36 | 1.82 – 2.07 | 0.67 – 2.78 |
| 18:0 Stearic | 0.83 (0.02) (0.78 – 0.89) | 0.83 (0.01) (0.80 – 0.85) | 0 | -0.07 | 0.06 | 0.83 – 0.94 | 0.28 – 1.13 |
| 18:1 Oleic | 3.87 (0.11) (3.63 – 4.17) | 3.55 (0.09) (3.39 – 3.75) | 0.32 | -0.03 | 0.67 | 3.44 – 4.45 | 1.36 – 6.56 |
| 18:2 Linoleic | 10.40 (0.26) (10.07 – 11.18) | 9.05 (0.22) (8.63 – 9.66) | 1.35 | 0.51 | 2.20 | 9.22 – 10.61 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.51 (0.06) (1.38 – 1.69) | 1.35 (0.05) (1.26 – 1.49) | 0.16 | -0.04 | 0.36 | 1.36 – 1.66 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.05 (0.005) (0.04 – 0.06) | 0.05 (0.003) (0.04 – 0.05) | 0 | -0.01 | 0.02 | 0.06 – 0.08 | 0.02 – 0.11 |
| Vitamins (mg/100 gr dwt) | | | | | | | |
| Vitamin E | 1.47 (0.16) (1.14 – 1.76) | 1.51 (0.10) (1.27 – 1.71) | -0.04 | -0.52 | 0.44 | 1.38 – 2.28 | 0.19 – 6.17 |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 1.88 (0.08) (1.71 – 2.05) | 1.85 (0.02) (1.80 – 1.91) | 0.03 | -0.17 | 0.22 | 1.82 – 1.99 | 1.51 – 2.10 |
| Arginine | 2.58 (0.07) (2.44 – 2.77) | 2.97 (0.04) (2.86 – 3.04) | -0.39 | -0.59 | -0.19 | 2.71 – 3.07 | 2.28 – 3.4 |
| Aspartic Acid | 4.91 (0.08) (4.68 – 5.06) | 4.56 (0.16) (4.23 – 4.91) | 0.35 | -0.11 | 0.79 | 4.01 – 4.37 | 3.81 – 5.12 |
| Cysteine | 0.51 (0.02) (0.48 – 0.58) | 0.62 (0.02) (0.57 – 0.67) | -0.11 | -0.19 | -0.04 | 0.51 – 0.64 | 0.37 – 0.81 |
| Glycine | 1.91 (0.03) (1.85 – 2.00) | 1.61 (0.04) (1.53 – 1.70) | 0.30 | 0.18 | 0.42 | 1.65 – 1.75 | 1.46 – 1.99 |
| Glutamic Acid | 7.30 (0.09) (7.09 – 7.49) | 6.94 (0.08) (6.73 – 7.09) | 0.36 | 0.06 | 0.65 | 6.64 – 7.48 | 5.84 – 8.20 |

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSI)³ |
|--------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Histidine | 1.00 (0.04) (0.91 – 1.08) | 0.97 (0.01) (0.95 – 1.00) | 0.03 | -0.08 0.14 | NS | 0.87 – 1.11 | 0.87 – 1.17 |
| Isoleucine | 1.87 (0.05) (1.71 – 1.95) | 1.78 (0.05) (1.71 – 1.92) | 0.09 | -0.09 0.27 | NS | 1.59 – 1.94 | 1.53 – 2.07 |
| Leucine | 3.03 (0.07) (2.90 – 3.17) | 2.81 (0.03) (2.74 – 2.87) | 0.22 | 0.04 0.39 | * | 2.67 – 2.96 | 2.59 – 3.62 |
| Lysine | 2.21 (0.02) (2.16 – 2.24) | 2.13 (0.05) (1.99 – 2.20) | 0.08 | -0.05 0.21 | NS | 2.13 – 2.27 | 2.28 – 2.83 |
| Methionine | 0.51 (0.03) (0.44 – 0.58) | 0.53 (0.01) (0.51 – 0.57) | -0.02 | -0.10 0.05 | NS | 0.45 – 0.54 | 0.43 – 0.68 |
| Phenylalanine | 1.95 (0.08) (1.75 – 2.13) | 1.91 (0.06) (1.75 – 2.00) | 0.04 | -0.19 0.28 | NS | 1.59 – 2.13 | 1.63 – 2.34 |
| Proline | 1.85 (0.07) (1.69 – 2.04) | 1.94 (0.04) (1.81 – 2.00) | -0.09 | -0.29 0.13 | NS | 1.87 – 2.06 | 1.68 – 2.28 |
| Serine | 1.78 (0.08) (1.64 – 1.98) | 2.06 (0.03) (2.00 – 2.12) | -0.28 | -0.48 -0.08 | * | 1.87 – 2.24 | 1.10 – 2.48 |
| Threonine | 1.64 (0.02) (1.60 – 1.67) | 1.41 (0.02) (1.34 – 1.67) | 0.24 | 0.16 0.31 | * | 1.34 – 1.55 | 1.14 – 1.86 |
| Tryptophan | 0.47 (0.04) (0.40 – 0.59) | 0.48 (0.02) (0.42 – 0.51) | -0.01 | -0.13 0.11 | NS | 0.40 – 0.57 | 0.36 – 0.50 |
| Tyrosine | 1.27 (0.08) (1.04 – 1.40) | 1.18 (0.06) (1.03 – 1.30) | 0.09 | -0.16 0.33 | NS | 0.95 – 1.25 | 1.01 – 1.61 |
| Valine | 1.88 (0.06) (1.76 – 2.04) | 1.85 (0.05) (1.75 – 1.96) | 0.03 | -0.16 0.22 | NS | 1.72 – 1.97 | 1.59 – 2.20 |

Table 11-22. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site G1.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 6.00 (0.09) (5.74 – 6.17) | 5.82 (0.09) (5.57 – 6.00) | 0.18 | -0.13 0.50 | NS | 5.81 – 6.61 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 38.55 (0.80) (36.8 – 40.1) | 34.73 (0.79) (32.60 – 36.43) | 3.82 | 1.08 6.58 | * | 32.13 – 36.49 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 8.17 (0.09) (7.97 – 8.34) | 7.99 (0.06) (7.91 – 8.15) | 0.18 | -0.08 0.43 | NS | 7.75 – 9.65 | 4.7 – 34.4 |
| Protein | 35.93 (0.58) (34.58 – 37.29) | 39.78 (1.20) (36.49 – 40.31) | -3.85 | -7.10 -0.59 | * | 37.74 – 41.97 | 33.2 – 45.5 |
| Total Fat | 21.08 (0.45) (19.80 – 21.80) | 21.50 (0.62) (19.66 – 22.48) | -0.42 | -2.29 1.44 | NS | 17.02 – 21.66 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 13.83 (0.70) (12.04 – 15.33) | 9.65 (0.22) (9.18 – 10.20) | 4.18 | 2.38 5.97 | * | 11.34 – 15.73 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 17.28 (0.27) (16.52 – 17.74) | 17.15 (0.33) (16.20 – 17.60) | 0.13 | -0.92 1.17 | NS | 14.81 – 18.77 | 8.5 – 21.3 |
| Crude Fiber | 4.41 (0.22) | 4.97 (0.11) | -0.56 | -1.16 0.04 | NS | 5.17 – 6.59 | |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation.⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (I.LSD)³ |
|---------------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|---|
| Minerals (% dwt) | (3.90 – 4.95) | (4.77 – 5.18) | | | | | |
| Phosphorus | 0.48 (0.02) (0.44 – 0.54) | 0.51 (0.01) (0.48 – 0.54) | -0.03 | -0.09 0.03 | NS | 0.45 – 0.53 | 0.50 – 0.94 |
| Calcium | 0.27 (0.02) (0.25 – 0.32) | 0.26 (0.01) (0.25 – 0.27) | 0.01 | -0.03 0.05 | NS | 0.18 – 0.25 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.54 (0.05) (2.48 – 2.70) | 2.43 (0.10) (2.15 – 2.61) | 0.12 | -0.17 0.40 | NS | 2.32 – 3.28 | 0.67 – 2.78 |
| 18:0 Stearic | 0.91 (0.03) (0.83 – 0.97) | 0.96 (0.04) (0.86 – 1.06) | -0.05 | -0.18 0.08 | NS | 0.61 – 0.81 | 0.28 – 1.13 |
| 18:1 Oleic | 4.35 (0.11) (4.12 – 4.64) | 4.83 (0.28) (4.01 – 5.32) | -0.48 | -1.21 0.27 | NS | 2.86 – 4.01 | 1.36 – 6.56 |
| 18:2 Linoleic | 11.76 (0.30) (10.96 – 12.40) | 11.76 (0.24) (11.15 – 12.31) | 0 | -0.95 0.95 | NS | 9.50 – 12.03 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.46 (0.05) (1.35 – 1.57) | 1.45 (0.04) (1.33 – 1.53) | 0.01 | -0.14 0.17 | NS | 1.30 – 1.60 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.06 (0.003) (0.05 – 0.06) | 0.07 (0.01) (0.05 – 0.11) | -0.01 | -0.05 0.02 | NS | 0.03 – 0.05 | 0.02 – 0.11 |
| Vitamins (mg/100 gr dwt) | | | | | | | |
| Vitamin E | 2.06 (0.15) (1.70 – 2.42) | 2.22 (0.21) (1.87 – 2.69) | -0.16 | -0.78 0.46 | NS | 1.14 – 2.06 | 0.19 – 6.17 |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 2.08 (0.03) (1.99 – 2.14) | 1.92 (0.08) (1.78 – 2.14) | 0.16 | -0.05 0.37 | NS | 1.79 – 1.95 | 1.51 – 2.10 |
| Arginine | 2.54 (0.05) (2.43 – 2.68) | 2.88 (0.11) (2.57 – 3.05) | -0.34 | -0.63 -0.05 | * | 2.58 – 3.07 | 2.28 – 3.4 |
| Aspartic Acid | 4.20 (0.07) (4.04 – 4.37) | 4.51 (0.13) (4.22 – 4.82) | -0.31 | -0.68 0.05 | NS | 4.29 – 4.85 | 3.81 – 5.12 |

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSD)³ |
|--------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Cysteine | 0.54 (0.04) (0.45 – 0.66) | 0.62 (0.01) (0.59 – 0.65) | -0.08 | -0.19 0.04 | NS | 0.50 – 0.63 | 0.37 – 0.81 |
| Glycine | 1.70 (0.02) (1.65 – 1.74) | 1.67 (0.03) (1.63 – 1.75) | 0.03 | -0.06 0.11 | NS | 1.65 – 1.79 | 1.46 – 1.99 |
| Glutamic Acid | 6.93 (0.13) (6.56 – 7.17) | 7.37 (0.03) (7.29 – 7.43) | -0.44 | -0.77 -0.11 | NS | 7.06 – 7.36 | 5.84 – 8.20 |
| Histidine | 0.93 (0.03) (0.88 – 1.01) | 0.97 (0.02) (0.93 – 1.02) | -0.04 | -0.13 0.04 | NS | 0.98 – 1.14 | 0.87 – 1.17 |
| Isoleucine | 1.62 (0.06) (1.50 – 1.77) | 1.73 (0.06) (1.63 – 1.90) | -0.11 | -0.32 0.10 | NS | 1.61 – 1.79 | 1.53 – 2.07 |
| Leucine | 2.90 (0.14) (2.62 – 3.16) | 2.90 (0.07) (2.70 – 3.01) | 0 | -0.38 0.37 | NS | 2.92 – 3.21 | 2.59 – 3.62 |
| Lysine | 1.79 (0.06) (1.68 – 1.96) | 2.20 (0.02) (2.15 – 2.26) | -0.41 | -0.57 -0.24 | * | 2.09 – 2.23 | 2.28 – 2.83 |
| Methionine | 0.52 (0.03) (0.46 – 0.57) | 0.47 (0.02) (0.44 – 0.51) | 0.05 | -0.02 0.14 | NS | 0.43 – 0.54 | 0.43 – 0.68 |
| Phenylalanine | 1.86 (0.11) (1.66 – 2.13) | 1.86 (0.07) (1.67 – 1.98) | 0 | -0.32 0.32 | NS | 1.81 – 2.20 | 1.63 – 2.34 |
| Proline | 1.85 (0.06)(1.72 – 2.01) | 2.08 (0.06) (1.92 – 2.19) | -0.23 | -0.44 -0.01 | NS | 1.85 – 2.35 | 1.68 – 2.28 |
| Serine | 1.77 (0.10) (1.65 – 2.05) | 1.97 (0.05) (1.85 – 2.09) | -0.20 | -0.47 0.07 | NS | 1.95 – 2.16 | 1.10 – 2.48 |
| Threonine | 1.48 (0.01) (1.46 – 1.50) | 1.40 (0.02) (1.35 – 1.45) | 0.08 | 0.02 0.14 | * | 1.36 – 1.49 | 1.14 – 1.86 |
| Tryptophan | 0.39 (0.02) (0.36 – 0.44) | 0.45 (0.02) (0.40 – 0.48) | -0.06 | -0.12 0.008 | * | 0.42 – 0.52 | 0.36 – 0.50 |
| Tyrosine | 1.20 (0.06) (1.09 – 1.35) | 1.19 (0.04) (1.12 – 1.28) | 0.01 | -0.17 0.19 | NS | 1.10 – 1.52 | 1.01 – 1.61 |
| Valine | 1.69 (0.01) (1.66 – 1.71) | 1.83 (0.07) (1.69 – 1.97) | -0.14 | -0.30 0.03 | NS | 1.74 – 2.10 | 1.59 – 2.20 |

Table 11-23. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site Q1.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 5.82 (0.10) (5.54 – 5.97) | 5.70 (0.09) (5.48 – 5.86) | 0.12 | -0.21 0.46 | NS | 5.72 – 6.21 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 39.98 (0.68) (38.18 – 41.52) | 33.40 (0.29) (32.65 – 34.14) | 6.58 | 4.77 8.38 | * | 37.45 – 40.87 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 8.81 (0.05) (8.71 – 8.93) | 9.07 (0.14) (8.81 – 9.45) | -0.26 | -0.61 0.10 | NS | 7.97 – 8.35 | 4.7 – 34.4 |
| Protein | 36.52 (0.91) (35.01 – 38.88) | 41.24 (0.41) (40.09 – 42.00) | -4.72 | -7.16 -2.29 | * | 37.10 – 38.90 | 33.2 – 45.5 |
| Total Fat | 18.63 (0.52) (17.55 – 19.87) | 18.88 (0.21) (18.47 – 19.36) | -0.25 | -1.61 1.11 | NS | 17.47 – 19.60 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 12.25 (0.88) (10.38 – 13.96) | 14.78 (0.74) (12.98 – 16.35) | -2.53 | -5.34 0.29 | NS | 10.27 – 14.76 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 19.55 (0.38) (18.73 – 20.38) | 18.60 (0.56) (17.03 – 19.44) | 0.95 | -0.69 2.59 | NS | 18.50 – 22.85 | 8.5 – 21.3 |
| Crude Fiber | 4.84 (0.23) (4.26 – 5.33) | 6.48 (0.09) (6.30 – 6.69) | -1.64 | -2.24 -1.04 | * | 3.39 – 4.97 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.56 (0.01) (0.53 – 0.59) | 0.52 (0.01) (0.51 – 0.54) | 0.04 | 0 0.07 | NS | 0.48 – 0.53 | 0.50 – 0.94 |

¹ SE = standard error of the mean

² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$

³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)

⁴ dwt = dry weight

⁵ Determined by calculation.

⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSD)³ |
|---------------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Calcium | 0.28 (0.01) (0.25 – 0.29) | 0.28 (0.01) (0.27 – 0.30) | 0 | -0.03 0.03 | NS | 0.22 – 0.26 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.19 (0.05) (2.10 – 2.32) | 2.31 (0.06) (2.16 – 2.46) | -0.12 | -0.31 0.07 | NS | 2.49 – 3.08 | 0.67 – 2.78 |
| 18:0 Stearic | 0.73 (0.02) (0.68 – 0.75) | 0.72 (0.01) (0.70 – 0.74) | 0.01 | -0.04 0.06 | NS | 0.61 – 0.77 | 0.28 – 1.13 |
| 18:1 Oleic | 3.95 (0.13) (3.64 – 4.25) | 3.90 (0.09) (3.65 – 4.06) | -0.05 | -0.34 0.45 | NS | 3.39 – 3.73 | 1.36 – 6.56 |
| 18:2 Linoleic | 10.31 (0.31) (9.79 – 11.05) | 10.50 (0.11) (10.21 – 10.70) | -0.19 | -0.99 0.60 | NS | 9.42 – 10.91 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.38 (0.03) (1.31 – 1.45) | 1.37 (0.01) (1.35 – 1.40) | 0.003 | -0.07 0.08 | NS | 1.22 – 1.65 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.05 (0.005) (0.04 – 0.06) | 0.04 (0.0003) (0.04 – 0.04) | 0.01 | 0 0.02 | NS | 0.03 – 0.04 | 0.02 – 0.11 |
| Vitamins (mg/100 gr dwt) | | | | | | | |
| Vitamin E | 1.75 (0.13) (1.39 – 2.03) | 1.87 (0.06) (1.77 – 2.02) | -0.12 | -0.48 0.23 | NS | 0.86 – 1.98 | 0.19 – 6.17 |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 2.06 (0.04) (1.96 – 2.13) | 2.11 (0.03) (2.04 – 2.17) | -0.05 | -0.16 0.07 | NS | 1.90 – 2.17 | 1.51 – 2.10 |
| Arginine | 2.68 (0.09) (2.48 – 2.91) | 3.01 (0.03) (2.94 – 3.09) | -0.33 | -0.57 -0.10 | NS | 2.46 – 3.09 | 2.28 – 3.4 |
| Aspartic Acid | 4.27 (0.07) (4.12 – 4.45) | 4.94 (0.07) (4.77 – 5.08) | -0.67 | -0.92 -0.42 | * | 3.98 – 4.47 | 3.81 – 5.12 |
| Cysteine | 0.61 (0.03) (0.53 – 0.65) | 0.63 (0.01) (0.59 – 0.66) | -0.02 | -0.09 0.05 | NS | 0.57-0.66 | 0.37 – 0.81 |
| Glycine | 1.76 (0.01) (1.74 – 1.80) | 1.66 (0.02) (1.62 – 1.71) | 0.10 | 0.05 0.17 | * | 1.67 – 1.75 | 1.46 – 1.99 |
| Glutamic Acid | 6.49 (0.07) (6.33 – 6.63) | 6.50 (0.11) (6.20 – 6.70) | -0.01 | -0.32 0.31 | NS | 6.91- 7.38 | 5.84 – 8.20 |

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSD)³ |
|--------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Histidine | 0.90 (0.02) (0.85 – 0.97) | 1.07 (0.02) (1.02 – 1.11) | -0.17 | -0.25 -0.08 | * | 0.88 – 1.10 | 0.87 – 1.17 |
| Isoleucine | 1.66 (0.04) (1.60 – 1.79) | 1.87 (0.04) (1.74 – 1.93) | -0.21 | -0.36 -0.06 | * | 1.52 – 1.90 | 1.53 – 2.07 |
| Leucine | 2.76 (0.04) (2.70 – 2.88) | 3.15 (0.02) (3.12 – 3.21) | -0.39 | -0.51 -0.28 | * | 2.70 – 3.24 | 2.59 – 3.62 |
| Lysine | 1.77 (0.07) (1.68 – 1.98) | 2.26 (0.03) (2.20 – 2.32) | -0.49 | -0.67 -0.30 | * | 2.16 – 2.23 | 2.28 – 2.83 |
| Methionine | 0.50 (0.01) (0.46 – 0.52) | 0.55 (0.01) (0.52 – 0.57) | -0.05 | -0.09 0 | * | 0.48 – 0.58 | 0.43 – 0.68 |
| Phenylalanine | 1.84 (0.07) (1.66 – 1.97) | 2.17 (0.01) (2.14 – 2.19) | -0.33 | -0.51 -0.14 | * | 1.60 – 2.14 | 1.63 – 2.34 |
| Proline | 1.88 (0.04) (1.78 – 1.94) | 1.76 (0.03) (1.70 – 1.82) | 0.12 | 0.01 0.23 | NS | 1.83 – 2.18 | 1.68 – 2.28 |
| Serine | 1.80 (0.09) (1.66 – 2.06) | 2.17 (0.03) (2.10 – 2.24) | -0.37 | -0.60 -0.14 | * | 1.77 – 2.04 | 1.10 – 2.48 |
| Threonine | 1.49 (0.01) (1.46 – 1.48) | 1.46 (0.06) (1.35 – 1.61) | 0.03 | -0.11 0.17 | NS | 1.35 – 1.43 | 1.14 – 1.86 |
| Tryptophan | 0.43 (0.01) (0.42 – 0.46) | 0.53 (0.02) (0.49 – 0.58) | -0.10 | -0.15 -0.05 | * | 0.39 – 0.57 | 0.36 – 0.50 |
| Tyrosine | 1.22 (0.07) (1.04 – 1.35) | 1.50 (0.04) (1.39 – 1.55) | -0.28 | -0.47 -0.10 | * | 1.20 – 1.40 | 1.01 – 1.61 |
| Valine | 1.79 (0.08) (1.68 – 2.04) | 1.94 (0.02) (1.90 – 1.97) | -0.15 | -0.36 0.06 | NS | 1.67 – 1.99 | 1.59 – 2.20 |

Table 11-24. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site Q2.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 5.65 (0.10) (5.48 – 5.90) | 5.65 (0.10) (5.37 – 5.82) | 0 | -0.35 0.35 | NS | 5.23 – 5.68 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 36.90 (0.56) (35.86 – 38.27) | 39.50 (0.50) (38.92 – 40.97) | -2.60 | -4.44 -0.76 | NS | 36.90 – 39.51 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 8.53 (0.07) (8.36 – 8.66) | 8.34 (0.09) (8.12 – 8.54) | 0.19 | -0.09 0.46 | NS | 7.91 – 8.31 | 4.7 – 34.4 |
| Protein | 37.14 (0.54) (36.44 – 38.75) | 39.09 (0.30) (38.31 – 39.80) | -1.95 | -3.46 -0.43 | * | 37.09 – 39.81 | 33.2 – 45.5 |
| Total Fat | 20.95 (0.37) (19.91 – 21.61) | 16.10 (0.17) (15.90 – 16.60) | 4.85 | 3.85 5.85 | * | 17.46 – 18.10 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 13.45 (0.51) (12.19 – 14.72) | 11.28 (0.77) (9.67 – 13.33) | 2.17 | -0.08 4.43 | NS | 10.87 – 16.82 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 17.23 (0.67) (15.98 – 19.15) | 19.18 (0.78) (17.80 – 21.16) | -1.95 | -4.46 0.56 | * | 16.35 – 20.52 | 8.5 – 21.3 |
| Crude Fiber | 4.94 (0.41) (4.28 – 6.02) | 5.25 (0.23) (4.66 – 5.79) | -0.31 | -1.46 0.83 | NS | 4.65 – 6.40 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.62 (0.01) (0.61 – 0.65) | 0.63 (0.004) (0.62 – 0.64) | 0.01 | -0.03 0.02 | NS | 0.56 – 0.63 | 0.50 – 0.94 |
| Calcium | 0.20 (0.003) | 0.20 (0.004) | 0 | 0 0.02 | NS | 0.18 – 0.22 | 0.12 – 0.31 |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation.⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| | (0.20 – 0.21) | (0.19 – 0.20) | | | | | |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.54 (0.12) (2.32 – 2.88) | 1.84 (0.07) (1.74 – 2.06) | 0.70 | 0.35 | 1.05 * | 1.86 – 2.05 | 0.67 – 2.78 |
| 18:0 Stearic | 0.86 (0.01) (0.83 – 0.90) | 0.67 (0.02) (0.62 – 0.70) | 0.19 | 0.14 | 0.25 * | 0.78 – 0.93 | 0.28 – 1.13 |
| 18:1 Oleic | 4.10 (0.32) (3.35 – 4.66) | 3.22 (0.07) (3.03 – 3.33) | 0.88 | 0.09 | 1.67 NS | 2.74 – 4.14 | 1.36 – 6.56 |
| 18:2 Linoleic | 11.65 (0.16) (11.41 – 12.12) | 8.92 (0.15) (8.62 – 9.34) | 2.73 | 2.18 | 3.27 * | 9.32 – 10.26 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.77 (0.04) (1.71 – 1.87) | 1.43 (0.02) (1.39 – 1.48) | 0.34 | 0.22 | 0.44 * | 1.21 – 1.65 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.05 (0.003) (0.04 – 0.05) | 0.04 (0.003) (0.03 – 0.04) | 0.01 | 0 | 0.02 NS | 0.05 – 0.07 | 0.02 – 0.11 |
| Vitamins (mg/100 gr dwt) | | | | | | | |
| Vitamin E | 2.16 (0.17) (1.78 – 2.57) | 1.32 (0.13) (0.95 – 1.53) | 0.84 | 0.32 | 1.37 * | 1.26 – 1.98 | 0.19 – 6.17 |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 1.66 (0.04) (1.57 – 1.74) | 1.86 (0.07) (1.68 – 2.03) | -0.20 | -0.40 | 0 * | 2.04 – 2.16 | 1.51 – 2.10 |
| Arginine | 2.47 (0.02) (2.43 – 2.54) | 2.98 (0.03) (2.94 – 3.06) | -0.51 | -0.60 | -0.42 * | 2.70 – 3.07 | 2.28 – 3.4 |
| Aspartic Acid | 4.57 (0.22) (4.14 – 4.98) | 4.46 (0.18) (4.08 – 4.95) | 0.11 | -0.60 | 0.82 NS | 3.91 – 4.36 | 3.81 – 5.12 |
| Cysteine | 0.61 (0.03) (0.52 – 0.66) | 0.65 (0.01) (0.62 – 0.66) | -0.04 | -0.12 | 0.04 NS | 0.55 – 0.69 | 0.37 – 0.81 |
| Glycine | 1.71 (0.02) (1.66 – 1.76) | 1.75 (0.02) (1.70 – 1.81) | -0.04 | -0.12 | 0.04 NS | 1.66 – 1.75 | 1.46 – 1.99 |
| Glutamic Acid | 6.99 (0.28) (6.47 – 7.72) | 6.61 (0.16) (6.21 – 6.89) | 0.38 | -0.40 | 1.16 NS | 7.03 – 7.36 | 5.84 – 8.20 |

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|-------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Histidine | 1.06 (0.04) (0.98 – 1.16) | 1.07 (0.03) (0.99 – 1.13) | -0.01 | -0.13 0.12 | NS | 0.94 – 1.07 | 0.87 – 1.17 |
| Isoleucine | 1.58 (0.02) (1.54 – 1.65) | 1.84 (0.03) (1.74 – 1.88) | -0.26 | -0.36 -0.16 | * | 1.51 – 1.93 | 1.53 – 2.07 |
| Leucine | 3.16 (0.03) (3.11 – 3.22) | 3.10 (0.07) (2.92 – 3.26) | 0.06 | -0.13 0.25 | NS | 2.88 – 3.22 | 2.59 – 3.62 |
| Lysine | 2.20 (0.02) (2.13 – 2.23) | 2.30 (0.03) (2.20 – 2.35) | -0.10 | -0.21 0 | * | 2.20 – 2.28 | 2.28 – 2.83 |
| Methionine | 0.51 (0.02) (0.47 – 0.55) | 0.54 (0.01) (0.52 – 0.57) | -0.03 | -0.09 0.01 | NS | 0.42-0.51 | 0.43 – 0.68 |
| Phenylalanine | 1.84 (0.11) (1.56 – 2.03) | 1.90 (0.05) (1.77 – 2.00) | -0.06 | -0.35 0.23 | NS | 1.61 – 2.14 | 1.63 – 2.34 |
| Proline | 1.96 (0.08) (1.82 – 2.15) | 2.02 (0.06) (1.87 – 2.14) | -0.06 | -0.31 0.18 | NS | 1.83 – 2.14 | 1.68 – 2.28 |
| Serine | 1.97 (0.08) (1.81 – 2.15) | 2.03 (0.06) (1.90 – 2.18) | -0.06 | -0.29 0.18 | NS | 1.74 – 2.06 | 1.10 – 2.48 |
| Threonine | 1.40 (0.02) (1.37 – 1.44) | 1.38 (0.01) (1.36 – 1.42) | 0.02 | -0.03 0.07 | NS | 1.26 – 1.38 | 1.14 – 1.86 |
| Tryptophan | 0.37 (0.02) (0.34 – 0.43) | 0.51 (0.02) (0.45 – 0.54) | -0.14 | -0.20 -0.06 | NS | 0.37 – 0.56 | 0.36 – 0.50 |
| Tyrosine | 1.31 (0.05) (1.20 – 1.41) | 1.27 (0.03) (1.21- 1.35) | 0.04 | -0.10 0.18 | NS | 1.08 – 1.21 | 1.01 – 1.61 |
| Valine | 1.79 (0.08) (1.68- 2.01) | 1.80 (0.03) (1.72- 1.87) | -0.01 | -0.22 0.19 | NS | 1.83 – 2.03 | 1.59 – 2.20 |

Table 11-25. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site W1.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 5.99 (0.14) (5.67 – 6.24) | 6.12 (0.02) (6.07 – 6.16) | -0.13 | -0.48 0.23 | NS | 5.75 – 6.56 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 33.75 (0.52) (32.27 – 34.50) | 35.10 (1.69) (32.16 – 39.60) | -1.35 | -5.69 2.99 | NS | 31.46 – 37.09 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 10.05 (0.15) (9.71 – 10.40) | 9.54 (0.18) (9.06 – 9.86) | 0.51 | -0.06 1.07 | NS | 10.52 – 11.82 | 4.7 – 34.4 |
| Protein | 41.16 (0.83) (39.43 – 42.74) | 42.81 (0.72) (37.23 – 43.93) | -1.65 | -4.33 1.04 | NS | 38.59 – 42.58 | 33.2 – 45.5 |
| Total Fat | 19.85 (0.56) (18.30 – 20.90) | 18.48 (0.15) (18.14 – 18.84) | 1.37 | -0.03 2.78 | NS | 19.32 – 21.64 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 11.90 (0.26) (11.38 – 12.62) | 11.08 (0.36) (10.32 – 11.89) | 0.82 | -0.25 1.90 | * | 15.39 – 17.77 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 19.58 (0.57) (18.70 – 21.23) | 17.43 (0.43) (16.77 – 18.62) | 2.15 | 0.41 3.89 | NS | 15.12 – 19.42 | 8.5 – 21.3 |
| Crude Fiber | 5.20 (0.29) (4.37 – 5.73) | 5.20 (0.17) (4.71 – 5.50) | 0 | -0.82 0.82 | NS | 4.23 – 4.93 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.58 (0.01) (0.57 – 0.61) | 0.59 (0.01) (0.57 – 0.61) | 0.01 | -0.02 0.04 | NS | 0.57 – 0.61 | 0.50 – 0.94 |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation.⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSD)³ |
|---------------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Calcium | 0.36 (0.01) (0.35 – 0.37) | 0.32 (0.02) (0.27 – 0.35) | 0.04 | -0.01 0.08 | NS | 0.24 – 0.29 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.15 (0.05) (2.01 – 2.22) | 2.18 (0.11) (1.96 – 2.49) | -0.03 | -0.33 0.26 | NS | 1.77 – 2.41 | 0.67 – 2.78 |
| 18:0 Stearic | 0.95 (0.02) (0.92 – 1.02) | 0.86 (0.02) (0.80 – 0.88) | 0.09 | 0.02 0.17 | NS | 0.85 – 1.15 | 0.28 – 1.13 |
| 18:1 Oleic | 4.72 (0.26) (4.14 – 5.25) | 4.87 (0.01) (4.83 – 4.90) | -0.15 | -0.78 0.48 | NS | 4.91 – 5.52 | 1.36 – 6.56 |
| 18:2 Linoleic | 10.74 (0.27) (10.02 – 11.32) | 9.47 (0.05) (9.32 – 9.56) | 1.27 | 0.59 1.95 | * | 10.70 – 11.72 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.22 (0.03) (1.14 – 1.30) | 1.06 (0.01) (1.04 – 1.08) | 0.16 | 0.08 0.24 | * | 1.02 – 1.37 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.07 (0.01) (0.06 – 0.09) | 0.06 (0.003) (0.05 – 0.06) | 0.01 | 0 0.03 | NS | 0.07 – 0.08 | 0.02 – 0.11 |
| Vitamins (mg/100 gr dwt) | | | | | | | |
| Vitamin E | 1.63 (0.16) (1.34 – 2.06) | 1.58 (0.14) (1.27 – 1.91) | 0.05 | -0.47 0.56 | NS | 1.02 – 1.87 | 0.19 – 6.17 |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 1.95 (0.06) (1.81 – 2.08) | 2.05 (0.05) (1.93 – 2.14) | -0.10 | -0.29 0.08 | NS | 1.86 – 2.16 | 1.51 – 2.10 |
| Arginine | 3.11 (0.03) (3.05 – 3.16) | 2.82 (0.11) (2.61 – 3.08) | 0.29 | 0.01 0.57 | NS | 2.59 – 3.07 | 2.28 – 3.4 |
| Aspartic Acid | 4.60 (0.21) (4.23 – 5.07) | 4.98 (0.07) (4.78 – 5.06) | -0.38 | -0.92 0.16 | NS | 4.54 – 5.04 | 3.81 – 5.12 |
| Cysteine | 0.51 (0.05) (0.43 – 0.64) | 0.60 (0.03) (0.52 – 0.66) | -0.09 | -0.23 0.05 | NS | 0.49 – 0.65 | 0.37 – 0.81 |
| Glycine | 1.89 (0.02) (1.84 – 1.94) | 1.80 (0.01) (1.78 – 1.83) | 0.09 | 0.03 0.14 | * | 1.62 – 1.71 | 1.46 – 1.99 |
| Glutamic Acid | 7.26 (0.06) (7.15 – 7.43) | 7.08 (0.08) (6.90 – 7.21) | 0.18 | -0.06 0.42 | NS | 6.93 – 7.28 | 5.84 – 8.20 |

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSD) ³ |
|-------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Histidine | 1.09 (0.04) (1.00 - 1.19) | 1.02 (0.06) (0.84 - 1.13) | 0.07 | -0.12 0.25 | NS | 0.90 - 1.12 | 0.87 - 1.17 |
| Isoleucine | 1.83 (0.05) (1.70 - 1.92) | 1.87 (0.04) (1.75 - 1.94) | -0.04 | -0.20 0.12 | NS | 1.64 - 1.90 | 1.53 - 2.07 |
| Leucine | 2.91 (0.07) (2.76 - 3.06) | 3.03 (0.07) (2.86 - 3.17) | -0.12 | -0.35 0.11 | NS | 2.89 - 3.03 | 2.59 - 3.62 |
| Lysine | 1.92 (0.03) (1.87 - 1.98) | 2.27 (0.02) (2.23 - 2.30) | -0.35 | -0.43 0.28 | * | 2.06 - 2.23 | 2.28 - 2.83 |
| Methionine | 0.57 (0.01) (0.56 - 0.60) | 0.49 (0.01) (0.47 - 0.52) | 0.08 | 0.05 0.12 | * | 0.50 - 0.57 | 0.43 - 0.68 |
| Phenylalanine | 2.06 (0.07) (1.89 - 2.18) | 2.00 (0.05) (1.88 - 2.11) | 0.06 | 0.08 -0.15 | NS | 1.77 - 2.15 | 1.63 - 2.34 |
| Proline | 2.24 (0.07) (2.05 - 2.37) | 2.16 (0.06) (2.04 - 2.30) | 0.08 | -0.14 0.29 | NS | 1.88 - 2.37 | 1.68 - 2.28 |
| Serine | 1.95 (0.11) (1.69 - 2.14) | 2.13 (0.05) (2.02 - 2.24) | -0.18 | -0.48 0.12 | NS | 1.80 - 2.19 | 1.10 - 2.48 |
| Threonine | 1.65 (0.02) (1.61 - 1.69) | 1.52 (0.03) (1.47 - 1.59) | 0.13 | 0.06 0.21 | * | 1.38 - 1.49 | 1.14 - 1.86 |
| Tryptophan | 0.46 (0.03) (0.38 - 0.49) | 0.45 (0.03) (0.40 - 0.52) | 0.01 | -0.08 0.10 | NS | 0.43 - 0.51 | 0.36 - 0.50 |
| Tyrosine | 1.23 (0.05) (1.09 - 1.32) | 1.25 (0.07) (1.07 - 1.41) | -0.02 | -0.25 0.21 | NS | 1.03 - 1.32 | 1.01 - 1.61 |
| Valine | 1.86 (0.04) (1.77 - 1.96) | 2.07 (0.05) (1.94 - 2.17) | -0.21 | -0.37 -0.05 | NS | 1.71 - 2.02 | 1.59 - 2.20 |

Table 11-26. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site IL3.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 5.47 (0.04) (5.41 – 5.57) | 5.39 (0.06) (5.23 – 5.51) | -0.07 | -0.27 0.13 | NS | 4.92 – 5.52 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 34.2 (0.31) (33.7 – 35.1) | 33.8 (0.19) (33.3 – 34.2) | 0.4 | -0.6 1.5 | NS | 32.8 – 37.2 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 9.3 (0.06) (9.2 – 9.4) | 9.2 (0.04) (9.1 – 9.3) | 0.1 | -0.1 0.4 | NS | 9.1 – 10.3 | 4.7 – 34.4 |
| Protein | 40.1 (0.16) (39.7 – 40.4) | 40.6 (0.20) (40.2 – 41.1) | -0.5 | -1.3 0.4 | NS | 38.1 – 41.0 | 33.2 – 45.5 |
| Total Fat | 20.2 (0.19) (19.8 – 20.6) | 20.3 (0.10) (20.1 – 20.5) | -0.1 | -0.9 0.7 | NS | 18.5 – 21.3 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 14.8 (0.8) (12.6 – 16.0) | 13.8 (1.2) (10.1 – 15.2) | 0.8 | -4.8 6.5 | NS | 12.8 – 15.4 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 16.9 (0.5) (15.9 – 18.2) | 15.7 (0.7) (13.8 – 16.6) | 1.2 | -1.7 4.0 | NS | 15.2 – 16.9 | 8.5 – 21.3 |
| Crude Fiber | 10.7 (0.6) (9.8 – 12.5) | 10.1 (0.4) (9.2 – 10.7) | 0.6 | -1.3 2.6 | NS | 9.7 – 11.8 | |
| Minerals (ppm) | | | | | | | |
| Phosphorus | 0.55 (0.01) (0.54 – 0.57) | 0.56 (0.01) (0.55 – 0.59) | -0.01 | -0.04 0.02 | NS | 0.52 – 0.61 | 0.50 – 0.94 |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation.⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSD) ³ |
|---------------------------------|------------------------------------|--------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Calcium | 0.20 (0.004) (0.20 – 0.22) | 0.21 (0.002) (0.20 – 0.21) | -0.01 | -0.01 0.08 | NS | 0.21 – 0.27 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.06 (0.02) (2.02 – 2.12) | 2.07 (0.01) (2.04 – 2.08) | -0.01 | -0.08 0.06 | NS | 1.99 – 2.39 | 0.67 – 2.78 |
| 18:0 Stearic | 0.86 (0.01) (0.84 – 0.88) | 0.87 (0.01) (0.86 – 0.89) | -0.01 | -0.04 0.02 | NS | 0.79 – 0.93 | 0.28 – 1.13 |
| 18:1 Oleic | 4.23 (0.05) (4.10 – 4.32) | 4.40 (0.02) (4.35 – 4.46) | -0.17 | -0.37 0.03 | NS | 3.74 – 4.95 | 1.36 – 6.56 |
| 18:2 Linoleic | 10.78 (0.18) (10.3 – 11.1) | 10.73 (0.06) (10.6 – 10.9) | 0.05 | -0.54 0.64 | NS | 9.53 – 11.40 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.54 (0.02) (1.49 – 1.61) | 1.51 (0.01) (1.48 – 1.53) | 0.03 | -0.07 0.13 | NS | 1.33 – 1.66 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.06 (0.001) (0.06 – 0.07) | 0.07 (0.0004) (0.65 – 0.66) | -0.01 | -0.013 0.01 | NS | 0.06 – 0.07 | 0.02 – 0.11 |
| Vitamins (mg/100 gr dwt) | | | | | | | |
| Vitamin E (mg/100 g dwt) | 1.62 (0.07) (1.49 – 1.76) | 1.73 (0.08) (1.52 – 1.91) | -0.11 | -0.25 0.04 | NS | 1.39 – 2.16 | 0.19 – 6.17 |
| Vitamin K1 ⁷ (mg/kg) | 0.38 (0.02) (0.35 – 0.43) | 0.43 (0.01) (0.41 – 0.45) | -0.05 | -0.12 0.02 | NS | 0.45 – 0.57 | |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 1.77 (0.02) (1.72 – 1.82) | 1.77 (0.01) (1.75 – 1.79) | 0 | -0.10 0.10 | NS | 1.63 – 1.82 | 1.51 – 2.10 |
| Arginine | 3.02 (0.03) (2.93 – 3.09) | 3.02 (0.02) (2.97 – 3.07) | 0 | -0.15 0.15 | NS | 2.76 – 3.07 | 2.28 – 3.4 |
| Aspartic Acid | 4.57 (0.03) (4.49 – 4.65) | 4.59 (0.03) (4.51 – 4.64) | -0.02 | -0.20 0.16 | NS | 4.18 – 4.68 | 3.81 – 5.12 |
| Cysteine | 0.58 (0.01) (0.55 – 0.60) | 0.58 (0.01) (0.56 – 0.59) | 0 | -0.05 0.05 | NS | 0.53 – 0.60 | 0.37 – 0.81 |

⁷ Measured only from locations IL3, IN, OH2, IA and KS.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSD) ³ |
|-------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Glycine | 1.71 (0.02) (1.66 – 1.76) | 1.71 (0.01) (1.68 – 1.73) | -0.12 | -0.61 0.37 | NS | 6.41 – 7.18 | 1.46 – 1.99 |
| Glutamic Acid | 7.07 (0.09) (6.87 – 7.30) | 7.19 (0.08) (6.97 – 7.32) | 0.00 | -0.10 0.10 | NS | 1.58 – 1.72 | 5.84 – 8.20 |
| Histidine | 1.02 (0.00) (1.01 – 1.03) | 1.03 (0.01) (1.00 – 1.03) | -0.01 | -0.03 0.01 | NS | 0.94 – 1.01 | 0.87 – 1.17 |
| Isoleucine | 1.84 (0.02) (1.81 – 1.89) | 1.87 (0.01) (1.85 – 1.88) | -0.03 | -0.09 0.03 | NS | 1.72 – 1.86 | 1.53 – 2.07 |
| Leucine | 3.09 (0.03) (3.04 – 3.14) | 3.09 (0.01) (3.06 – 3.11) | 0 | -0.11 0.11 | NS | 2.85 – 3.15 | 2.59 – 3.62 |
| Lysine | 2.56 (0.03) (2.47 – 2.60) | 2.53 (0.04) (2.44 – 2.61) | 0.03 | -0.11 0.17 | NS | 2.32 – 2.47 | 2.28 – 2.83 |
| Methionine | 0.54 (0.01) (0.52 – 0.55) | 0.54 (0.01) (0.53 – 0.55) | 0 | -0.03 0.03 | NS | 0.51 – 0.55 | 0.43 – 0.68 |
| Phenylalanine | 2.07 (0.03) (2.00 – 2.12) | 2.06 (0.01) (2.02 – 2.09) | 0.01 | -0.12 0.14 | NS | 1.87 – 2.05 | 1.63 – 2.34 |
| Proline | 2.07 (0.02) (2.03 – 2.11) | 2.06 (0.02) (2.01 – 2.10) | 0.01 | -0.09 0.11 | NS | 1.87 – 2.11 | 1.68 – 2.28 |
| Serine | 2.01 (0.03) (1.91 – 2.07) | 2.05 (0.01) (2.02 – 2.06) | -0.04 | -0.17 0.09 | NS | 1.86 – 2.10 | 1.10 – 2.48 |
| Threonine | 1.62 (0.01) (1.58 – 1.65) | 1.62 (0.00) (1.61 – 1.63) | 0 | -0.05 0.05 | NS | 1.51 – 1.64 | 1.14 – 1.86 |
| Tryptophan | 0.59 (0.00) (0.58 – 0.59) | 0.60 (0.01) (0.58 – 0.62) | -0.01 | -0.03 0.01 | NS | 0.55 – 0.60 | 0.36 – 0.50 |
| Tyrosine | 1.58 (0.01) (1.55 – 1.60) | 1.58 (0.01) (1.55 – 1.61) | 0 | -0.07 0.07 | NS | 1.43 – 1.61 | 1.01 – 1.61 |
| Valine | 1.90 (0.02) (1.86 – 1.93) | 1.91 (0.01) (1.88 – 1.93) | -0.01 | -0.08 0.06 | NS | 1.76 – 1.86 | 1.59 – 2.20 |

Table 11-27. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site IN.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 5.36 (0.12) (5.20 – 5.71) | 5.16 (0.05) (5.03 – 5.27) | 0.20 | -0.11 0.51 | NS | 4.83 – 5.10 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 32.9 (0.25) (32.4 – 33.6) | 33.7 (0.89) (32.4 – 36.3) | -0.8 | -4.0 2.4 | NS | 33.7 – 35.0 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 10.5 (0.09) (10.3 – 10.7) | 10.3 (0.37) (9.3 – 10.9) | 0.2 | -1.0 1.4 | NS | 9.9 – 10.5 | 4.7 – 34.4 |
| Protein | 40.4 (0.31) (39.7 – 41.2) | 40.0 (0.70) (37.9 – 41.0) | 0.4 | -2.1 3.0 | NS | 40.4 – 41.5 | 33.2 – 45.5 |
| Total Fat | 21.4 (0.09) (21.2 – 21.6) | 21.3 (0.26) (20.5 – 21.7) | 0.15 | -0.6 0.9 | NS | 18.5 – 20.0 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 14.7 (0.5) (13.6 – 15.8) | 15.3 (1.1) (12.9 – 18.3) | -0.7 | -3.6 2.3 | NS | 14.0 – 15.1 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 15.0 (0.3) (14.3 – 15.7) | 16.4 (0.9) (15.0 – 18.9) | 2.2 | -3.6 4.3 | NS | 14.1 – 16.2 | 8.5 – 21.3 |
| Crude Fiber | 10.3 (0.45) (9.4 – 11.5) | 11.1 (0.80) (9.4 – 13.2) | -0.8 | -2.3 0.7 | NS | 9.7 – 10.9 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.38 (0.01) (0.35 – 0.40) | 0.43 (0.04) (0.38 – 0.55) | -0.04 | -0.07 0 | NS | 0.36 – 0.40 | 0.50 – 0.94 |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation.⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSD) ³ |
|---------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Calcium | 0.26 (0.003) (0.25 – 0.26) | 0.25 (0.002) (0.25 – 0.26) | 0.01 | -0.01 0.08 | NS | 0.27 – 0.30 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.17 (0.01) (2.15 – 2.20) | 2.17 (0.05) (2.04 – 2.28) | 0 | -0.13 0.13 | NS | 1.84 – 2.23 | 0.67 – 2.78 |
| 18:0 Stearic | 0.86 (0.02) (0.82 – 0.89) | 0.88 (0.02) (0.82 – 0.91) | -0.02 | -0.04 0.00 | * | 0.75 – 0.82 | 0.28 – 1.13 |
| 18:1 Oleic | 4.90 (0.08) (4.70 – 5.03) | 4.97 (0.31) (4.04 – 5.37) | -0.07 | -0.84 0.70 | NS | 4.14 – 4.77 | 1.36 – 6.56 |
| 18:2 Linoleic | 11.25 (0.09) (11.0 – 11.4) | 11.35 (0.16) (11.1 – 11.8) | -0.10 | -0.60 0.40 | NS | 9.46 – 10.40 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.36 (0.01) (1.33 – 1.38) | 1.43 (0.09) (1.31 – 1.69) | -0.07 | -0.36 0.22 | NS | 1.14 – 1.33 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.07 (0.001) (0.06 – 0.07) | 0.07 (0.001) (0.06 – 0.07) | 0 | -0.001 0.001 | * | 0.06 – 0.07 | 0.02 – 0.11 |
| Vitamins (mg/100 gr dwt) | | | | | | | |
| Vitamin E (mg/100 g dwt) | 2.21 (0.04) (2.17 – 23.2) | 2.00 (0.17) (1.52 – 2.27) | 0.21 | -0.30 0.72 | NS | 1.97 – 2.66 | 0.19 – 6.17 |
| Vitamin K1 ⁷ (mg/kg) | 0.37 (0.01) (0.34 – 0.41) | 0.42 (0.04) (0.34 – 0.53) | -0.05 | -0.13 0.03 | NS | 0.50 – 0.72 | |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 1.76 (0.01) (1.74 – 1.79) | 1.76 (0.04) (1.65 – 1.82) | 0 | -0.12 0.12 | NS | 1.75 – 1.82 | 1.51 – 2.10 |
| Arginine | 3.03 (0.01) (2.99 – 3.05) | 2.94 (0.06) (2.76 – 3.05) | 0.09 | -0.13 0.31 | NS | 3.15 – 3.21 | 2.28 – 3.4 |
| Aspartic Acid | 4.61 (0.05) (4.47 – 4.70) | 4.55 (0.09) (4.29 – 4.73) | 0.06 | -0.32 0.44 | NS | 4.56 – 4.76 | 3.81 – 5.12 |
| Cysteine | 0.56 (0) (0.55 – 0.56) | 0.58 (0.01) (0.56 – 0.60) | -0.02 | -0.04 0.00 | * | 0.56 – 0.61 | 0.37 – 0.81 |

⁷ Measured only from locations IL3, IN, OH2, IA and KS.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSD) ³ |
|-------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Glycine | 1.72 (0.01) (1.69 – 1.73) | 1.68 (0.03) (1.60 – 1.74) | 0.02 | -0.64 0.68 | NS | 7.08 – 7.47 | 1.46 – 1.99 |
| Glutamic Acid | 7.05 (0.11) (6.80 – 7.26) | 7.03 (0.14) (6.62 – 7.26) | 0.04 | -0.07 0.15 | NS | 1.74 – 1.76 | 5.84 – 8.20 |
| Histidine | 1.02 (0.01) (0.99 – 1.05) | 0.99 (0.02) (0.94 – 1.04) | 0.03 | -0.07 0.13 | NS | 0.99 – 1.04 | 0.87 – 1.17 |
| Isoleucine | 1.90 (0.01) (1.86 – 1.92) | 1.83 (0.06) (1.68 – 1.95) | 0.07 | -0.13 0.27 | NS | 1.86 – 1.89 | 1.53 – 2.07 |
| Leucine | 3.13 (0.02) (3.08 – 3.14) | 3.03 (0.06) (2.86 – 3.14) | 0.10 | -0.13 0.33 | NS | 3.08 – 3.13 | 2.59 – 3.62 |
| Lysine | 2.55 (0.02) (2.52 – 2.59) | 2.44 (0.07) (2.28 – 2.60) | 0.11 | -0.14 0.36 | NS | 2.45 – 2.60 | 2.28 – 2.83 |
| Methionine | 0.53 (0) (0.52 – 0.54) | 0.53 (0.01) (0.50 – 0.56) | 0 | -0.05 0.05 | NS | 0.52 – 0.55 | 0.43 – 0.68 |
| Phenylalanine | 2.11 (0.01) (2.08 – 2.12) | 2.00 (0.04) (1.88 – 2.08) | 0.11 | -0.04 0.26 | NS | 2.06 – 2.12 | 1.63 – 2.34 |
| Proline | 2.06 (0.01) (2.03 – 2.09) | 2.02 (0.03) (1.92 – 2.07) | 0.04 | -0.06 0.14 | NS | 2.05 – 2.12 | 1.68 – 2.28 |
| Serine | 2.02 (0.04) (1.94 – 2.11) | 2.00 (0.02) (1.95 – 2.03) | 0.02 | -0.15 0.19 | NS | 2.01 – 2.10 | 1.10 – 2.48 |
| Threonine | 1.62 (0.01) (1.60 – 1.66) | 1.57 (0.01) (1.53 – 1.60) | 0.05 | -0.04 0.14 | NS | 1.60 – 1.62 | 1.14 – 1.86 |
| Tryptophan | 0.59 (0.01) (0.57 – 0.61) | 0.57 (0.01) (0.57 – 0.59) | 0.02 | -0.02 0.06 | NS | 0.55 – 0.59 | 0.36 – 0.50 |
| Tyrosine | 1.59 (0.01) (1.57 – 1.63) | 1.53 (0.03) (1.44 – 1.59) | 0.06 | -0.09 0.21 | NS | 1.55 – 1.60 | 1.01 – 1.61 |
| Valine | 1.91 (0.01) (1.57 – 1.63) | 1.85 (0.04) (1.74 – 1.95) | 0.06 | -0.09 0.21 | NS | 1.89 – 1.92 | 1.59 – 2.20 |

Table 11-28. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site OH2.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 5.69 (0.07) (5.56 – 5.86) | 5.58 (0.09) (5.47 – 5.87) | 0.11 | -0.01 | 0.24 | NS | 5.50 – 5.84 |
| Carbohydrates ⁵ | 34.6 (0.4) (33.8 – 35.5) | 33.8 (0.2) (33.4 – 34.4) | 0.8 | -0.3 | 1.9 | NS | 34.5 – 36.1 |
| Moisture (% fwt) ⁶ | 11.5 (0.09) (11.2 – 11.6) | 11.3 (0.15) (11.0 – 11.6) | 0.2 | -0.3 | 0.7 | NS | 10.7 – 11.7 |
| Protein | 40.5 (0.6) (39.3 – 41.9) | 41.0 (0.3) (39.3 – 41.9) | -0.5 | -2.0 | 0.9 | NS | 40.8 – 43.1 |
| Total Fat | 19.3 (0.2) (18.8 – 19.5) | 19.7 (0.1) (19.5 – 19.8) | -0.4 | -0.7 | -0.02 | * | 16.6 – 18.8 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 13.78 (0.7) (12.2 – 15.4) | 13.7 (0.5) (12.6 – 14.8) | 0.1 | -1.6 | 1.7 | NS | 10.5 – 14.3 |
| Neutral Detergent Fiber | 15.5 (0.5) (14.6 – 17.0) | 15.5 (0.5) (14.6 – 17.0) | 0.1 | -2.3 | 2.4 | NS | 14.1 – 15.4 |
| Crude Fiber | 10.8 (0.5) (9.7 – 12.2) | 10.1 (0.2) (9.6 – 10.4) | 0.6 | -1.0 | 2.2 | NS | 8.8 – 10.8 |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.59 (0.01) (0.57 – 0.63) | 0.59 (0.01) (0.57 – 0.61) | 0 | -0.03 | 0.03 | NS | 0.52 – 0.58 |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSD)³ |
|---------------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Calcium | 0.25 (0.003) (0.25 – 0.26) | 0.24 (0.001) (0.24 – 0.25) | 0.01 | -0.01 0.08 | NS | 0.24 – 0.27 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.89 (0.02) (1.82 – 1.92) | 1.93 (0.02) (1.89 – 1.97) | -0.04 | -0.09 0.01 | NS | 1.76 – 2.04 | 0.67 – 2.78 |
| 18:0 Stearic | 0.80 (0) (0.79 – 0.81) | 0.82 (0.01) (0.79 – 0.83) | -0.02 | -0.04 0.00 | NS | 0.66 – 0.77 | 0.28 – 1.13 |
| 18:1 Oleic | 4.52 (0.02) (4.48 – 4.56) | 4.80 (0.04) (4.69 – 4.87) | -0.28 | -0.45 0.11 | * | 3.81 – 4.64 | 1.36 – 6.56 |
| 18:2 Linoleic | 10.05 (0.18) (9.51 – 10.30) | 10.00 (0.11) (9.80 – 10.30) | 0.05 | -0.43 0.53 | NS | 10.70 – 11.72 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.36 (0.02) (1.29 – 1.40) | 1.36 (0.01) (1.34 – 1.38) | 0 | 0.07 0.07 | NS | 1.24 – 1.33 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.06 (0.0003) (0.06 – 0.06) | 0.06 (0.001) (0.06 – 0.06) | 0 | -0.003 0.003 | NS | 0.05 – 0.06 | 0.02 – 0.11 |
| Vitamins (mg/100 gr dwt) | | | | | | | |
| Vitamin E (mg/100 g dwt) | 1.58 (0.05) (1.46 – 1.69) | 1.55 (0.10) (1.36 – 1.73) | 0.03 | -0.32 0.38 | NS | 1.47 – 1.72 | 0.19 – 6.17 |
| Vitamin K1 ⁷ (mg/kg) | 0.32 (0.01) (0.31 – 0.34) | 0.33 (0.01) (0.31 – 0.35) | -0.01 | -0.05 0.03 | NS | 0.36 – 0.44 | |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 1.77 (0.01) (1.70 – 1.79) | 1.74 (0.02) (1.75 – 1.81) | 0.03 | -0.02 0.08 | NS | 1.69 – 1.84 | 1.51 – 2.10 |
| Arginine | 3.09 (0.05) (2.92 – 3.19) | 3.07 (0.06) (3.00 – 3.22) | 0.02 | -0.11 0.15 | NS | 2.91 – 3.28 | 2.28 – 3.4 |
| Aspartic Acid | 4.64 (0.04) (4.55 – 4.73) | 4.54 (0.07) (4.39 – 4.71) | 0.10 | -0.06 0.26 | NS | 4.44 – 4.75 | 3.81 – 5.12 |
| Cysteine | 0.54 (0.01) (0.51 – 0.57) | 0.53 (0.01) (0.52 – 0.55) | 0.01 | -0.04 0.06 | NS | 0.53 – 0.60 | 0.37 – 0.81 |

⁷ Measured only from locations IL3, IN, OH2, IA and KS.

| Component (Units) | HB4 Mean (SE)¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig² | Commercial Reference Range | Literature Range (ILSD)³ |
|--------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Glycine | 1.71 (0.02) (1.69 – 1.76) | 1.69 (0.02) (1.65 – 1.74) | 0.11 | -0.12 0.34 | NS | 6.91 – 7.61 | 1.46 – 1.99 |
| Glutamic Acid | 7.25 (0.11) (7.08 – 7.53) | 7.14 (0.16) (6.79 – 7.56) | 0.02 | -0.01 0.05 | NS | 1.66 – 1.73 | 5.84 – 8.20 |
| Histidine | 1.03 (0.01) (1.01 – 1.05) | 1.02 (0.02) (0.97 – 1.07) | 0.01 | -0.05 0.07 | NS | 0.98 – 1.03 | 0.87 – 1.17 |
| Isoleucine | 1.86 (0.02) (1.81 – 1.91) | 1.84 (0.02) (1.78 – 1.90) | 0.02 | -0.07 0.11 | NS | 1.80 – 1.89 | 1.53 – 2.07 |
| Leucine | 3.11 (0.03) (2.05 – 3.20) | 3.09 (0.05) (2.97 – 3.21) | 0.02 | -0.07 0.11 | NS | 2.99 – 3.18 | 2.59 – 3.62 |
| Lysine | 2.53 (0.03) (2.47 – 2.59) | 2.50 (0.04) (2.38 – 2.58) | 0.03 | -0.13 0.19 | NS | 2.41 – 2.55 | 2.28 – 2.83 |
| Methionine | 0.52 (0.01) (0.51 – 0.54) | 0.53 (0.01) (0.51 – 0.55) | -0.01 | -0.05 0.03 | NS | 0.53 – 0.55 | 0.43 – 0.68 |
| Phenylalanine | 2.07 (0.03) (2.02 – 2.14) | 2.07 (0.04) (1.97 – 2.16) | 0.00 | -0.09 0.09 | NS | 1.97 – 2.14 | 1.63 – 2.34 |
| Proline | 2.06 (0.02) (2.01 – 2.10) | 2.05 (0.03) (1.98 – 2.09) | 0.01 | -0.01 0.03 | NS | 2.01 – 2.16 | 1.68 – 2.28 |
| Serine | 2.04 (0.03) (1.97 – 2.12) | 2.01 (0.03) (1.94 – 2.07) | 0.03 | 0 0.06 | NS | 2.00 – 2.11 | 1.10 – 2.48 |
| Threonine | 1.62 (0.02) (1.60 – 1.67) | 1.61 (0.04) (1.55 – 1.71) | 0.01 | -0.05 0.07 | NS | 1.57 – 1.63 | 1.14 – 1.86 |
| Tryptophan | 0.56 (0) (0.55 – 0.57) | 0.57 (0.01) (0.55 – 0.58) | -0.01 | -0.03 0.01 | NS | 0.55 – 0.60 | 0.36 – 0.50 |
| Tyrosine | 1.57 (0.01) (1.54 – 1.60) | 1.56 (0.02) (1.52 – 1.62) | 0.01 | -0.03 0.05 | NS | 1.52 – 1.57 | 1.01 – 1.61 |
| Valine | 1.92 (0.02) (1.88 – 1.97) | 1.91 (0.04) (1.83 – 2.00) | 0.01 | -0.09 0.11 | NS | 1.83 – 1.94 | 1.59 – 2.20 |

Table 11-29. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site IA.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 5.47 (0.05) (5.37 – 5.61) | 5.54 (0.09) (5.29 – 5.71) | -0.07 | -0.47 | 0.33 | 5.34 – 5.64 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 35.0 (0.1) (34.8 – 35.4) | 34.0 (0.3) (33.2 – 34.8) | 1.1 | -0.1 | 2.2 | 34.9 – 36.4 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 9.6 (0.1) (9.3 – 9.9) | 9.9 (0.1) (9.6 – 10.1) | -0.2 | -0.8 | 0.4 | 9.7 – 10.3 | 4.7 – 34.4 |
| Protein | 38.2 (0.1) (38.0 – 38.5) | 39.0 (0.2) (38.6 – 39.5) | -0.8 | -1.3 | -0.4 | 36.6 – 39.8 | 33.2 – 45.5 |
| Total Fat | 21.3 (0.1) (21.2 – 21.4) | 21.5 (0.1) (21.3 – 21.6) | -0.2 | -0.5 | 0.1 | 19.3 – 21.5 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 13.8 (0.6) (12.3 – 15.4) | 14.5 (0.3) (14.0 – 15.3) | -0.8 | -2.6 | 1.0 | 14.30 – 17.50 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 15.5 (0.1) (15.3 – 15.8) | 16.1 (0.4) (14.9 – 16.8) | -0.6 | -1.8 | 0.8 | 15.9 – 18.1 | 8.5 – 21.3 |
| Crude Fiber | 10.3 (0.3) (9.6 – 10.5) | 10.8 (0.4) (9.7 – 11.5) | -0.5 | -1.5 | 0.5 | 10.3 – 12.3 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.50 (0.01) (0.48 – 0.52) | 0.53 (0.01) (0.51 – 0.56) | -0.03 | -0.06 | 0.01 | 0.50 – 0.53 | 0.50 – 0.94 |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSD) ³ |
|---------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Calcium | 0.27 (0.001) (0.27 – 0.28) | 0.27 (0.001) (0.27 – 0.28) | 0 | -0.01 0.08 | NS | 0.26 – 0.31 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.23 (0.01) (2.20 – 2.25) | 2.25 (0.01) (2.23 – 2.27) | -0.02 | -0.04 0.00 | NS | 1.99 – 2.39 | 0.67 – 2.78 |
| 18:0 Stearic | 0.89 (0) (0.89 – 0.90) | 0.93 (0.01) (0.91 – 0.94) | -0.04 | -0.07 -0.01 | * | 0.80 – 0.87 | 0.28 – 1.13 |
| 18:1 Oleic | 4.87 (0.03) (4.80 – 4.94) | 5.23 (0.05) (5.15 – 5.35) | -0.36 | -0.54 -0.18 | * | 4.15 – 4.87 | 1.36 – 6.56 |
| 18:2 Linoleic | 11.23 (0.03) (11.2 – 11.3) | 11.03 (0.02) (11.0 – 11.1) | 0.20 | 0.20 0.20 | * | 10.10 – 11.30 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.36 (0) (1.35 – 1.37) | 1.33 (0) (1.32 – 1.34) | 0.03 | 0 0.06 | * | 1.25 – 1.49 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.07 (0.0001) (0.07 – 0.07) | 0.07 (0.001) (0.07 – 0.07) | 0 | -0.003 0.003 | * | 0.06 – 0.07 | 0.02 – 0.11 |
| Vitamins (mg/100 gr dwt) | | | | | | | |
| Vitamin E (mg/100 g dwt) | 2.66 (0.05) (2.56 – 2.78) | 2.82 (0.05) (2.69 – 2.93) | -0.16 | -0.26 -0.06 | * | 1.42 – 3.13 | 0.19 – 6.17 |
| Vitamin K1 ⁷ (mg/kg) | 0.50 (0.01) (0.47 – 0.51) | 0.57 (0.01) (0.56 – 0.61) | -0.07 | -0.13 -0.01 | * | 0.71 – 0.85 | |
| Amino Acids (% dwt) | | | | | | | |
| Alanine | 1.69 (0.02) (1.64 – 1.72) | 1.72 (0.02) (1.68 – 1.77) | -0.03 | -0.15 0.09 | NS | 1.66 – 1.72 | 1.51 – 2.10 |
| Arginine | 2.78 (0.02) (2.72 – 2.83) | 2.96 (0.07) (2.87 – 3.17) | -0.18 | -0.38 0.02 | NS | 2.72 – 2.98 | 2.28 – 3.4 |
| Aspartic Acid | 4.28 (0.03) (4.23 – 4.36) | 4.43 (0.04) (4.38 – 4.55) | -0.15 | -0.35 0.05 | NS | 4.17 – 4.46 | 3.81 – 5.12 |
| Cysteine | 0.56 (0.01) (0.53 – 0.59) | 0.56 (0.01) (0.54 – 0.58) | 0.00 | -0.04 0.04 | NS | 0.55 – 0.60 | 0.37 – 0.81 |

⁷ Measured only from locations IL3, IN, OH2, IA and KS.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSD) ³ |
|-------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Glycine | 1.61 (0.01) (1.59 – 1.64) | 1.66 (0.01) (1.65 – 1.68) | -0.35 | -0.84 0.14 | NS | 6.28 – 6.93 | 1.46 – 1.99 |
| Glutamic Acid | 6.52 (0.07) (6.34 – 6.64) | 6.87 (0.09) (6.67 – 7.12) | -0.05 | -0.10 0.00 | * | 1.58 – 1.69 | 5.84 – 8.20 |
| Histidine | 0.96 (0.01) (0.94 – 0.98) | 1.00 (0.01) (1.73 – 1.79) | -0.04 | -0.09 0.01 | NS | 0.95 – 0.98 | 0.87 – 1.17 |
| Isoleucine | 1.76 (0.01) (1.73 – 1.79) | 1.81 (0.01) (1.79 – 1.82) | -0.05 | -0.11 0.01 | NS | 1.67 – 1.82 | 1.53 – 2.07 |
| Leucine | 2.89 (0.01) (2.88 – 2.92) | 3.00 (0.01) (2.99 – 3.04) | -0.11 | -0.16 -0.06 | * | 2.80 – 3.04 | 2.59 – 3.62 |
| Lysine | 2.34 (0.04) (2.27 – 2.41) | 2.44 (0.02) (2.38 – 2.48) | -0.10 | -0.28 0.08 | NS | 2.36 – 2.50 | 2.28 – 2.83 |
| Methionine | 0.50 (0.01) (0.48 – 0.52) | 0.53 (0.01) (0.51 – 0.54) | -0.03 | -0.04 -0.02 | * | 0.51 – 0.54 | 0.43 – 0.68 |
| Phenylalanine | 1.91 (0.01) (1.88 – 1.94) | 1.99 (0.02) (1.95 – 2.04) | -0.08 | -0.16 0.00 | NS | 1.85 – 2.07 | 1.63 – 2.34 |
| Proline | 1.93 (0.01) (1.92 – 1.94) | 1.98 (0.02) (1.94 – 2.05) | -0.05 | -0.14 0.04 | NS | 1.85 – 2.01 | 1.68 – 2.28 |
| Serine | 1.91 (0.01) (1.88 – 1.94) | 1.96 (0.01) (1.93 – 1.98) | -0.05 | -0.12 0.02 | NS | 1.84 – 2.03 | 1.10 – 2.48 |
| Threonine | 1.54 (0.01) (1.52 – 1.56) | 1.56 (0.01) (1.55 – 1.57) | -0.02 | -0.06 0.02 | NS | 1.51 – 1.58 | 1.14 – 1.86 |
| Tryptophan | 0.57 (0.00) (0.56 – 0.58) | 0.58 (0.00) (0.57 – 0.59) | -0.01 | -0.03 0.01 | NS | 0.55 – 0.57 | 0.36 – 0.50 |
| Tyrosine | 1.48 (0.02) (1.44 – 1.52) | 1.52 (0.01) (1.50 – 1.52) | -0.04 | -0.10 0.02 | NS | 1.43 – 1.56 | 1.01 – 1.61 |
| Valine | 1.78 (0.02) (1.74 – 1.82) | 1.85 (0.01) (1.84 – 1.87) | -0.07 | -0.13 -0.01 | * | 1.72 – 1.87 | 1.59 – 2.20 |

Table 11-30. Summary of Soybean Seed Nutrients for HB4 vs. Williams 82 in Site KS.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) ⁴ | | | | | | | |
| Ash | 5.38 (0.05) (5.27 – 5.48) | 5.44 (0.10) (5.22 – 5.67) | -0.06 | -0.27 0.15 | NS | 5.26 – 5.45 | 3.9 – 7.0 |
| Carbohydrates ⁵ | 34.4 (0.3) (33.8 – 35.0) | 33.6 (0.4) (32.6 – 34.0) | 0.8 | -0.1 1.4 | * | 33.4 – 36.3 | 29.6 – 50.2 |
| Moisture (% fwt) ⁶ | 11.0 (0.1) (10.6 – 11.2) | 10.9 (0.3) (10.1 – 11.3) | 0.1 | -0.4 0.5 | NS | 10.5 – 11.8 | 4.7 – 34.4 |
| Protein | 40.2 (0.5) (39.2 – 41.5) | 40.6 (0.5) (40.3 – 42.0) | -0.4 | -0.8 -0.1 | * | 38.0 – 42.4 | 33.2 – 45.5 |
| Total Fat | 20.1 (0.2) (19.5 – 20.4) | 20.3 (0.1) (20.0 – 20.5) | -0.3 | -0.5 0.03 | NS | 18.0 – 20.8 | 8.1 – 23.6 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 13.8 (0.7) (11.9 – 15.2) | 14.4 (0.7) (12.3 – 15.5) | -0.6 | -2.3 1.1 | NS | 13.0 – 14.9 | 7.8 – 18.6 |
| Neutral Detergent Fiber | 15.0 (0.3) (14.5 – 16.0) | 15.3 (0.4) (14.2 – 15.8) | -0.3 | -1.4 0.8 | NS | 14.3 – 16.6 | 8.5 – 21.3 |
| Crude Fiber | 9.8 (0.2) (9.5 – 10.3) | 10.3 (0.3) (9.6 – 11.1) | -0.5 | -1.0 0.0 | NS | 10.0 – 13.6 | |

¹ SE = standard error of the mean² Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ³ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁴ dwt = dry weight⁵ Determined by calculation.⁶ fwt = fresh weight

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|---------------------------------|------------------------------------|--------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.57 (0.01) (0.55 – 0.59) | 0.55 (0.01) (0.53 – 0.58) | 0.02 | -0.01 0.05 | NS | 0.52 – 0.59 | 0.50 – 0.94 |
| Calcium | 0.25 (0.002) (0.25 – 0.25) | 0.25 (0.003) (0.24 – 0.26) | 0 | -0.01 0.08 | NS | 0.28 – 0.32 | 0.12 – 0.31 |
| Fatty Acids (% dwt) | | | | | | | |
| 16:0 Palmitic | 2.06 (0.03) (1.98 – 2.09) | 2.10 (0.02) (2.07 – 2.14) | -0.04 | -0.12 0.04 | NS | 1.84 – 2.20 | 0.67 – 2.78 |
| 18:0 Stearic | 0.81 (0.01) (0.77 – 0.83) | 0.83 (0.00) (0.82 – 0.84) | -0.02 | -0.05 0.01 | NS | 0.76 – 0.85 | 0.28 – 1.13 |
| 18:1 Oleic | 4.30 (0.03) (4.22 – 4.38) | 4.67 (0.08) (4.56 – 4.82) | -0.37 | -0.69 -0.05 | NS | 4.01 – 4.71 | 1.36 – 6.56 |
| 18:2 Linoleic | 10.73 (0.14) (10.3 – 10.9) | 10.73 (0.09) (10.6 – 11.0) | 0 | -0.39 0.39 | NS | 9.13 – 10.80 | 3.46 – 13.36 |
| 18:3 Linolenic | 1.41 (0.01) (1.38 – 1.43) | 1.38 (0.01) (1.36 – 1.42) | 0.03 | -0.02 0.08 | NS | 1.30 – 1.46 | 0.30 – 2.19 |
| 20:0 Arachidic | 0.06 (0.0009) (0.06 – 0.06) | 0.06 (0.0008) (0.06 – 0.06) | 0 | -0.002 0.002 | NS | 0.06 – 0.07 | 0.02 – 0.11 |
| Vitamins | | | | | | | |
| Vitamin E (mg/100 g dwt) | 1.57 (0.06) (1.44 – 1.67) | 1.73 (0.10) (1.44 – 1.93) | -0.16 | -0.46 0.14 | NS | 1.27 – 2.20 | 0.19 – 6.17 |
| Vitamin K1 ⁷ (mg/kg) | 0.33 (0.01) (0.31 – 0.33) | 0.39 (0.01) (0.37 – 0.40) | -0.06 | -0.07 -0.05 | * | 1.02 – 1.87 | |
| Amino Acids (% dwt) | | | | | | | |

⁷ Measured only from locations IL3, IN, OH2, IA and KS.

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|-------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Alanine | 1.73 (0.02) (1.67 – 1.77) | 1.75 (0.02) (1.71 – 1.81) | -0.02 | -0.13 0.09 | NS | 1.63 – 1.82 | 1.51 – 2.10 |
| Arginine | 2.97 (0.04) (2.86 – 3.05) | 3.00 (0.04) (2.94 – 3.11) | -0.03 | -0.13 0.07 | NS | 2.79 – 3.25 | 2.28 – 3.4 |
| Aspartic Acid | 4.51 (0.05) (4.38 – 4.60) | 4.55 (0.06) (4.45 – 4.71) | -0.04 | -0.23 0.15 | NS | 4.26 – 4.71 | 3.81 – 5.12 |
| Cysteine | 0.57 (0.01) (0.53 – 0.59) | 0.57 (0.01) (0.54 – 0.59) | 0.00 | -0.02 0.02 | NS | 0.58 – 0.61 | 0.37 – 0.81 |
| Glycine | 1.65 (0.02) (1.65 – 1.69) | 1.69 (0.01) (1.66 – 1.71) | -0.08 | -0.33 0.17 | NS | 6.62 – 7.4 | 1.46 – 1.99 |
| Glutamic Acid | 6.98 (0.10) (6.80 – 7.24) | 7.06 (0.11) (6.83 – 7.35) | -0.04 | -0.09 0.01 | NS | 1.59 – 1.74 | 5.84 – 8.20 |
| Histidine | 0.99 (0.02) (0.95 – 1.03) | 1.03 (0.01) (1.00 – 1.06) | -0.04 | -0.09 0.01 | NS | 0.94 – 1.05 | 0.87 – 1.17 |
| Isoleucine | 1.80 (0.02) (1.73 – 1.84) | 1.85 (0.03) (1.78 – 1.92) | -0.05 | -0.16 0.06 | NS | 1.71 – 1.88 | 1.53 – 2.07 |
| Leucine | 3.03 (0.03) (2.93 – 3.09) | 3.10 (0.04) (3.00 – 3.18) | -0.07 | -0.22 0.08 | NS | 2.83 – 3.15 | 2.59 – 3.62 |
| Lysine | 2.46 (0.02) (2.40 – 2.51) | 2.47 (0.03) (2.39 – 2.50) | -0.01 | -0.11 0.08 | NS | 2.32 – 2.57 | 2.28 – 2.83 |
| Methionine | 0.53 (0.01) (0.50 – 0.54) | 0.52 (0.01) (0.50 – 0.54) | 0.01 | -0.03 0.05 | NS | 0.51 – 0.56 | 0.43 – 0.68 |
| Phenylalanine | 2.02 (0.03) (1.93 – 2.08) | 2.06 (0.02) (2.00 – 2.11) | -0.04 | -0.12 0.04 | NS | 1.86 – 2.13 | 1.63 – 2.34 |
| Proline | 2.04 (0.03) (1.99 – 2.11) | 2.03 (0.03) (1.99 – 2.11) | 0.01 | -0.05 0.07 | NS | 1.92 – 2.14 | 1.68 – 2.28 |

| Component (Units) | HB4 Mean (SE) ¹ (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ² | Commercial Reference Range | Literature Range (ILSI) ³ |
|-------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Serine | 1.99 (0.03) (1.92 – 2.04) | 2.00 (0.03) (1.93 – 2.07) | -0.01 | -0.09 0.07 | NS | 1.94 – 2.04 | 1.10 – 2.48 |
| Threonine | 1.59 (0.02) (1.54 – 1.61) | 1.61 (0.02) (1.56 – 1.67) | -0.02 | -0.08 0.04 | NS | 1.52 – 1.63 | 1.14 – 1.86 |
| Tryptophan | 0.57 (0.01) (0.56 – 0.59) | 0.58 (0.00) (0.58 – 0.59) | -0.01 | -0.03 0.01 | NS | 0.54 – 0.60 | 0.36 – 0.50 |
| Tyrosine | 1.54 (0.02) (1.49 – 1.59) | 1.56 (0.02) (1.53 – 1.60) | -0.02 | -0.06 0.02 | NS | 1.46 – 1.59 | 1.01 – 1.61 |
| Valine | 1.84 (0.03) (1.78 – 1.89) | 1.89 (0.02) (1.83 – 1.94) | -0.05 | -0.11 0.01 | NS | 1.74 – 1.93 | 1.59 – 2.20 |

Table 11-31. Summary of Combined-Site Soybean Seed Anti-Nutrients and Isoflavones for HB4 vs. Parental Control Line Williams 82.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 1.67 (0.10) (0.62 – 3.09) | 1.35 (0.04) (0.68 – 1.88) | 0.32 | 0.03 0.61 | * | 0.54 – 1.69 | 0.63 – 1.96 |
| Lectins (mg/g) | 4.78 (0.13) (2.43 – 6.34) | 4.73 (0.15) (3.02 – 7.03) | 0.05 | -0.27 0.37 | NS | 1.29 – 6.09 | 0.11 – 9.04 |
| Raffinose | 0.88 (0.03) (0.55 – 1.39) | 0.85 (0.02) (0.70 – 1.09) | 0.03 | -0.04 0.10 | NS | 0.64 – 1.2 | 0.21 – 0.66 |
| Stachyose | 3.77 (0.07) (2.50 – 4.85) | 3.39 (0.08) (2.27 – 4.32) | 0.38 | 0.06 0.70 | * | 2.56 – 4.76 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 35.04 (1.78) (18.60 – 60.30) | 33.46 (1.60) (19.30 – 56.10) | 1.58 | -1.06 4.22 | NS | 18.6 – 56.1 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 1240 (53) (497 – 1870) | 1086 (48) (462 – 1700) | 154 | 71 236 | * | 533 – 2150 | 60.0 – 2453.5 |
| Genistein | 1402 (64) (518 – 2130) | 1282 (61) (515 – 2060) | 120 | 41 199 | * | 671 – 2290 | 144.3 – 2837.2 |
| Glycitein | 276 (11) (133 – 412) | 239 (8) (123 – 344) | 37 | 22 53 | * | 126 – 344 | 15.3 – 310.4 |

¹ dwt = dry weight

² SE = standard error of the mean

³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$

⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)

⁵ TIU = trypsin inhibitor units

Table 11-32. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site A.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 1.63 (0.14) (1.29 – 2.05) | 1.23 (0.03) (1.19 – 1.30) | 0.40 | 0.04 | 0.76 | 1.57 – 2.14 | 0.63 – 1.96 |
| Lectins | 4.60 (0.33) (4.12 – 5.24) | 5.09 (0.31) (4.57 – 5.93) | -0.49 | -0.85 | -0.13 * | 3.01 – 3.45 | 0.11 – 9.04 |
| Raffinose | 0.99 (0.10) (0.70 – 1.17) | 0.99 (0.03) (0.91 – 1.09) | 0 | -0.26 | 0.26 | 0.85 – 1.23 | 0.21 – 0.66 |
| Stachyose | 3.51 (0.25) (2.80 – 3.93) | 3.36 (0.18) (2.94 – 3.76) | 0.15 | -0.59 | 0.90 | 3.24 – 3.78 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 44.08 (5.66) (31.40 – 56.70) | 41.15 (3.91) (30.80 – 49.70) | 2.93 | - | 19.7 | 31.70 – 59.40 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 975 (26) (899 - 1022) | 778 (39) (673 – 857) | 197 | 83 | 312 * | 883 - 1159 | 60.0 – 2453.5 |
| Genistein | 1049 (31) (1009 – 1139) | 813 (58) (652 – 916) | 236 | 75 | 395 * | 890 - 1478 | 144.3 – 2837.2 |
| Glycitein | 211 (20) (170 – 265) | 180 (5) (168 – 192) | 31 | -19 | 82 | 173 - 213 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-33. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site D2.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|--------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 2.60 (0.25) (1.99 – 3.09) | 1.70 (0.07) (1.63 – 1.85) | 0.90 | 0.27 | 1.53 * | 1.51 – 1.63 | 0.63 – 1.96 |
| Lectins | 4.36 (0.22) (3.86 – 4.82) | 3.33 (0.10) (3.02 – 3.45) | 1.03 | 0.56 | 1.50 * | 1.87 – 2.76 | 0.11 – 9.04 |
| Raffinose | 1.26 (0.06) (1.11 – 1.39) | 0.94 (0.05) (0.84 – 1.08) | 0.32 | 0.13 | 0.52 * | 0.67 – 0.98 | 0.21 – 0.66 |
| Stachyose | 4.34 (0.21) (3.90 – 4.85) | 2.85 (0.10) (2.56 – 3.01) | 1.49 | 0.93 | 2.05 * | 2.70 – 3.71 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 49.98 (4.31) (41.20 – 60.30) | 45.13 (4.60) (32.50 -53.60) | 4.85 | - | 20.28 NS | 33.30 – 45.80 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 1227 (46) (1130 – 1318) | 1025 (29) (964 – 1092) | 202 | 69 | 335 * | 1041 - 1323 | 60.0 – 2453.5 |
| Genistein | 1262 (34) (1164 – 1323) | 1159 (52) (1046 – 1253) | 103 | -49 | 255 NS | 963 - 1379 | 144.3 – 2837.2 |
| Glycitein | 225 (22) (188 – 284) | 209 (12) (185 – 241) | 16 | -46 | 78 NS | 170 - 210 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-34. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site G1.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 2.03 (0.17) (1.74 – 2.51) | 1.30 (0.07) (1.19 – 1.52) | 0.73 | 0.27 | 1.18 | 1.10 – 1.76 | 0.63 – 1.96 |
| Lectins | 5.50 (0.29) (5.05 – 6.33) | 5.39 (0.28) (4.61 – 5.79) | 0.11 | -1.64 | 1.86 | 2.90 – 4.09 | 0.11 – 9.04 |
| Raffinose | 0.80 (0.09) (0.67 – 1.05) | 0.80 (0.07) (0.70 – 0.99) | 0 | -0.27 | 0.27 | 0.86 – 1.01 | 0.21 – 0.66 |
| Stachyose | 3.64 (0.19) (3.31 – 4.19) | 2.70 (0.16) (2.27 – 2.98) | 0.94 | 0.32 | 1.55 | 2.56 – 3.17 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 41.48 (5.29) (29.80 – 55.10) | 42.20 (5.50) (30.00 – 54.40) | -0.62 | -19.29 | 18.04 | 28.70 – 50.80 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 1110 (50) (1013 – 1251) | 924 (20) (878 – 972) | 186 | 53 | 318 | 936 – 1244 | 60.0 – 2453.5 |
| Genistein | 1117 (79) (982 – 1033) | 1071 (28) (995 – 1124) | 46 | -160 | 252 | 898 – 1363 | 144.3 – 2837.2 |
| Glycitein | 259 (6) (244 – 273) | 219 (8) (204 – 240) | 40 | 16 | 0.73 | 163 – 201 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-35. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site Q1.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 1.95 (0.13) (1.64 – 2.19) | 1.73 (0.10) (1.54 – 1.88) | 0.22 | -0.17 | 0.62 | 1.10 - 1.53 | 0.63 – 1.96 |
| Lectins | 4.75 (0.26) (4.30 – 5.39) | 5.42 (0.27) (4.84 – 5.96) | -0.67 | -1.73 | 0.39 | 2.70 – 3.92 | 0.11 – 9.04 |
| Raffinose | 0.71 (0.07) (0.55 – 0.84) | 0.83 (0.05) (0.71 – 0.91) | -0.12 | -0.32 | 0.08 | 0.74 – 1.07 | 0.21 – 0.66 |
| Stachyose | 3.38 (0.48) (2.50 – 4.34) | 2.85 (0.13) (2.49 – 3.11) | 0.53 | -0.68 | 1.75 | 2.50 – 3.43 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 34.40 (3.99) (26.70 – 45.30) | 39.63 (5.50) (31.80 – 55.70) | -5.23 | -21.85 | 11.40 | 24.90 – 53.70 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 1005 (61) (846 – 1136) | 1028 (36) (942 – 1100) | -23 | -196 | 149 | 878 - 1132 | 60.0 – 2453.5 |
| Genistein | 1109 (56) (949 – 1213) | 1168 (20) (1131 – 1222) | -59 | -205 | 87 | 1014 - 1280 | 144.3 – 2837.2 |
| Glycitein | 241 (14) (221 – 280) | 232 (8) (215 – 255) | 9 | -31 | 49 | 161 - 233 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-36. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site Q2.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 1.75 (0.16) (1.42 – 2.08) | 1.30 (0.04) (1.20 – 1.42) | 0.45 | 0.06 | 0.84 | 1.42 – 1.96 | 0.63 – 1.96 |
| Lectins | 4.30 (0.30) (3.88 – 5.47) | 3.72 (0.12) (3.41 – 3.93) | 0.58 | -0.48 | 1.64 | 2.09 – 3.58 | 0.11 – 9.04 |
| Raffinose | 0.84 (0.01) (0.82 – 0.86) | 0.88 (0.06) (0.74 – 0.99) | -0.04 | -0.19 | 0.10 | 0.86 – 0.95 | 0.21 – 0.66 |
| Stachyose | 3.60 (0.12) (3.27 – 3.80) | 3.07 (0.12) (2.84 – 3.41) | 0.53 | 0.12 | 0.95 | 2.63 – 3.51 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 42.90 (6.91) (27.40 – 58.80) | 38.98 (6.74) (23.80 – 56.00) | 3.92 | - | 27.5 | 32.90 – 65.30 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 1048 (47) (959 – 1181) | 1030 (63) (903 – 1175) | 18 | -174 | 211 | 879 - 1508 | 60.0 – 2453.5 |
| Genistein | 1208 (39) (1130 – 1317) | 1119 (64) (949 – 1256) | 89 | -96 | 273 | 756 - 1217 | 144.3 – 2837.2 |
| Glycitein | 293 (30) (239 – 374) | 236 (15) (198 – 271) | 57 | -24 | 137 | 157 - 255 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-37. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site W1.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 2.78 (0.12) (2.45 – 3.01) | 1.63 (0.03) (1.54 – 1.66) | 1.15 | 0.85 1.45 | * | 1.47 – 2.02 | 0.63 – 1.96 |
| Lectins | 3.32 (0.40) (2.43 – 4.19) | 3.57 (0.11) (3.29 – 3.75) | -0.25 | -1.73 1.23 | NS | 1.29 – 2.45 | 0.11 – 9.04 |
| Raffinose | (0.09) (0.83 – 1.25) | 0.97 (0.04) (0.87 – 1.06) | 0.04 | -0.20 0.29 | NS | 0.90 – 1.21 | 0.21 – 0.66 |
| Stachyose | 3.90 (0.29) (3.23 – 4.66) | 3.52 (0.27) (2.86 – 4.06) | 0.38 | -0.59 1.35 | NS | 3.41 – 3.85 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 44.24 (4.58) (31.70 – 53.70) | 35.05 (3.85) (25.80 – 44.50) | 9.19 | -5.46 23.81 | * | 50.60 – 62.90 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 547 (19) (497 - 588) | 509 (21) (462 – 554) | 38 | -31 108 | NS | 472 - 574 | 60.0 – 2453.5 |
| Genistein | 629 (42) (518 – 707) | 569 (23) (515-624) | 60 | -57 176 | NS | 559 - 757 | 144.3 – 2837.2 |
| Glycitein | 149 (7) (134 – 165) | 145 (11) (123 – 177) | 4 | -28 36 | NS | 116 - 160 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-38. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site IL3.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 1.24 (0.04) (1.16 – 1.34) | 1.22 (0.02) (1.18 – 1.29) | 0.02 | -0.12 0.16 | NS | 0.74 – 1.4 | 0.63 – 1.96 |
| Lectins | 4.70 (0.16) (4.46 – 5.16) | 4.72 (0.19) (4.21 – 5.04) | -0.02 | -0.72 0.68 | NS | 2.95 – 6.09 | 0.11 – 9.04 |
| Raffinose | 0.80 (0.01) (0.76 – 0.83) | 0.79 (0.01) (0.76 – 0.82) | 0.01 | -0.06 0.08 | NS | 0.70 – 0.74 | 0.21 – 0.66 |
| Stachyose | 3.85 (0.09) (3.58 – 3.94) | 3.85 (0.03) (3.80 – 3.90) | 0 | -0.27 0.27 | NS | 3.79 – 4.85 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 26.0 (0.9) (24.0 – 28.4) | 25.8 (2.2) (19.3 – 27.7) | 0.2 | -9.5 10.0 | NS | 25.2 – 31.3 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 1385 (59) (1220 – 1500) | 1208 (41) (1130 – 1300) | 177 | 30 324 | * | 1280 – 2090 | 60.0 – 2453.5 |
| Genistein | 1688 (50) (1541 – 1770) | 1576 (50) (1480 – 1662) | 112 | -8 233 | NS | 1530 – 2290 | 144.3 – 2837.2 |
| Glycitein | 350 (18) (315 – 344) | 297 (4) (288 – 304) | 53 | 3 103 | * | 172 – 409 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-39. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site IN.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 0.71 (0.06) (0.62 – 0.85) | 0.87 (0.13) (0.68 – 1.25) | -0.16 | -0.66 0.34 | NS | 0.54 – 0.74 | 0.63 – 1.96 |
| Lectins | 5.99 (0.20) (5.59 – 6.34) | 5.65 (0.77) (3.68 – 7.03) | 0.34 | -2.29 2.97 | NS | 3.42 – 4.58 | 0.11 – 9.04 |
| Raffinose | 0.84 (0.02) (0.80 – 0.88) | 0.76 (0.02) (0.70 – 0.81) | 0.08 | 0.01 0.15 | * | 0.80 – 0.90 | 0.21 – 0.66 |
| Stachyose | 3.72 (0.04) (3.65 – 3.84) | 3.68 (0.25) (3.10 – 4.32) | 0.04 | -0.64 0.72 | NS | 3.99 – 4.70 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 28.0 (2.5) (25.1 – 35.4) | 25.8 (1.6) (22.3 – 29.8) | 2.2 | -8.1 12.5 | NS | 25.1 – 32.7 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 1406 (90) (1150 – 1560) | 1313 (144) (1010 – 1700) | 93 | -185 370 | NS | 1250 – 1761.4 | 60.0 – 2453.5 |
| Genistein | 1696 (89) (1455 – 1832) | 1649 (152) (1330 – 2060) | 47 | -270 365 | NS | 1220 – 1782.4 | 144.3 – 2837.2 |
| Glycitein | 362 (11) (347 – 394) | 278 (37) (170 – 340) | 84 | -41 209 | NS | 168.1 – 330 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-40. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site OH2.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 1.36 (0.04) (1.27 – 1.48) | 1.38 (0.04) (1.28 – 1.48) | -0.02 | -0.05 0.01 | NS | 1.24 – 1.41 | 0.63 – 1.96 |
| Lectins | 4.61 (0.25) (4.20 – 5.33) | 4.44 (0.30) (3.76 – 5.20) | 0.17 | -1.52 1.86 | NS | 2.55 – 3.22 | 0.11 – 9.04 |
| Raffinose | 0.74 (0.02) (0.70 – 0.80) | 0.79 (0.02) (1.28 – 1.48) | -0.05 | -0.10 0.00 | NS | 0.60 – 0.78 | 0.21 – 0.66 |
| Stachyose | 3.54 (0.11) (3.21 – 3.69) | 3.64 (0.04) (3.54 – 3.72) | -0.10 | -0.55 0.35 | NS | 3.83 – 4.34 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 21.4 (1.1) (18.6 – 23.3) | 21.4 (1.0) (20.0 – 24.2) | 0 | -4.7 4.7 | NS | 18.6 – 20.4 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 1690 (46) (1584 – 1778) | 1546 (64) (1355 – 1628) | 144 | -43 330 | NS | 1430 – 2047 | 60.0 – 2453.5 |
| Genistein | 1927 (46) (1824 – 2017) | 1829 (66) (1638 – 1935) | 98 | -78 274 | NS | 1400 – 1775 | 144.3 – 2837.2 |
| Glycitein | 359 (20) (316 – 412) | 317 (13) (282 – 344) | 42 | -11 95 | NS | 139 – 307 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-41. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site IA.

| Component (Units)¹ | HB4 Mean (SE)² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig³ | Commercial Reference Range | Literature Range (ILSI)⁴ |
|---|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 1.08 (0.02) (1.03 – 1.13) | 1.16 (0.04) (1.07 – 1.24) | -0.08 | -0.25 0.09 | NS | 0.95 – 1.22 | 0.63 – 1.96 |
| Lectins | 5.44 (0.24) (5.06 – 6.14) | 5.47 (0.34) (4.85 – 6.06) | -0.03 | -1.77 1.71 | NS | 3.44 – 5.01 | 0.11 – 9.04 |
| Raffinose | 0.89 (0.01) (0.88 – 0.90) | 0.84 (0) (0.83 – 0.85) | 0.05 | 0.03 0.07 | * | 0.79 – 0.89 | 0.21 – 0.66 |
| Stachyose | 4.01 (0.02) (3.98 – 4.05) | 3.87 (0.03) (3.80 – 3.93) | 0.14 | 0.10 0.18 | * | 3.84 – 4.62 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 29.1 (1.9) (23.9 – 32.7) | 29.4 (0.6) (28.1 – 30.5) | -0.3 | -7.0 6.4 | NS | 21.5 – 31.3 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 1468 (76) (1340 – 1680) | 1062 (61) (969 – 1240) | 406 | 336 476 | * | 1310 – 1720 | 60.0 – 2453.5 |
| Genistein | 1745 (57) (1650 – 1910) | 1348 (59) (1270 – 1520) | 398 | 370 425 | * | 1320 – 1740 | 144.3 – 2837.2 |
| Glycitein | 298 (12) (275 – 331) | 273 (10) (251 – 300) | 25 | -34 83 | NS | 126 – 296 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-42. Summary of Soybean Seed Anti-Nutrients for HB4 vs. Williams 82 Site KS.

| Component (Units) ¹ | HB4 Mean (SE) (Range) ² | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|---|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Anti-Nutrients (% dwt) | | | | | | | |
| Phytic acid | 1.28 (0.05) (1.17 – 1.40) | 1.28 (0.04) (1.18 – 1.34) | 0.00 | -0.17 0.17 | NS | 1.22 – 1.49 | 0.63 – 1.96 |
| Lectins | 5.06 (0.14) (4.79 – 5.45) | 5.27 (0.37) (4.46 – 6.12) | -0.21 | -1.33 0.91 | NS | 2.98 – 4.47 | 0.11 – 9.04 |
| Raffinose | 0.83 (0.01) (0.81 – 0.87) | 0.81 (0) (0.80 – 0.81) | 0.02 | -0.02 0.06 | NS | 0.72 – 0.90 | 0.21 – 0.66 |
| Stachyose | 3.95 (0.01) (3.91 – 3.97) | 3.92 (0.04) (3.87 – 4.04) | 0.03 | -0.08 0.14 | NS | 4.18 – 4.52 | 1.21 – 3.50 |
| Trypsin Inhibitor (TIU/mg dwt) ⁵ | 23.9 (1.2) (20.8 – 26.4) | 23.68 (0.5) (22.3 – 24.7) | 0.2 | -4.3 4.7 | NS | 22.0 – 27.8 | 19.59 – 118.68 |
| Isoflavones (mg/kg dwt) | | | | | | | |
| Daidzein | 1780 (41) (1680 – 1870) | 1526 (71) (1370 – 1690) | 254 | 158 350 | * | 1260 – 2150 | 60.0 – 2453.5 |
| Genistein | 1994 (49) (1904 – 2130) | 1801 (74) (1640 – 1981) | 194 | 98 289 | * | 1390 – 1940 | 144.3 – 2837.2 |
| Glycitein | 290 (5.9) (275 – 303) | 239 (10) (218 – 258) | 52 | 36 68 | * | 141 – 280 | 15.3 – 310.4 |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ TIU = trypsin inhibitor units

Table 11-43. Summary of Combined-Site Soybean Forage Nutrients for HB4 vs. Parental Control Williams 82.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) | | | | | | | |
| Ash | 9.12 (0.28) (6.43 – 15.90) | 9.04 (0.37) (6.50 – 20.50) | 0.08 | -0.26 0.42 | NS | 6.96 – 19.10 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 49.76 (2.69) (30.43 – 75.40) | 50.54 (2.67) (30.72 – 77.30) | -0.78 | -1.81 0.25 | NS | 32.40 – 73.90 | |
| Moisture (% fwt) ⁶ | 76.72 (0.72) (65.31 – 85.50) | 76.94 (0.67) (65.32 – 85.60) | -0.22 | -1.50 1.06 | NS | 64.30 – 84.20 | 73.5 – 81.6 |
| Protein | 20.85 (0.44) (14.80 – 26.90) | 20.68 (0.48) (13.70 – 29.20) | 0.17 | -0.87 1.26 | NS | 15.60 – 24.70 | 14.37 – 24.71 |
| Total Fat | 2.46 (0.13) (1.15 – 4.70) | 2.47 (0.12) (1.32 – 4.33) | -0.01 | -0.22 0.20 | NS | 1.38– 3.48 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 33.08 (0.65) (24.50 – 42.50) | 33.17 (0.55) (27.30 – 41.20) | -0.09 | -1.53 1.35 | NS | 20.30 – 36.78 | |
| Neutral Detergent Fiber | 41.64 (1.01) (29.50 – 52.40) | 41.87 (0.98) (26.30 – 53.70) | -0.23 | -1.72 1.26 | NS | 25.60 – 52.30 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus ⁵ | 0.25 (0.01) (0.20 – 0.35) | 0.26 (0.01) (0.18 – 0.35) | -0.01 | -0.02 0.01 | NS | 0.21 – 0.37 | |
| Calcium ⁵ | 1.21 (0.02) (1.03 – 1.56) | 1.26 (0.03) (0.96 – 1.58) | -0.05 | -0.09 0.02 | NS | 0.97 – 1.51 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at p ≤ 0.05⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

Table 11-44. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site A.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) | | | | | | | |
| Ash | 9.90 (0.20) (9.50 – 10.50) | 9.86 (0.37) (8.96 – 10.63) | 0.04 | -1.22 1.30 | NS | 9.8 – 11.2 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 33.13 (2.24) (30.66 – 38.01) | 35.12 (1.45) (32.22 – 38.52) | -1.99 | -15.86 11.88 | NS | 34.3 – 37.4 | |
| Moisture (% fwt) ⁶ | 79.21 (0.88) (77.05 – 80.53) | 78.79 (0.67) (77.41 – 80.51) | 0.42 | -0.80 1.64 | NS | 77.7 – 80.4 | 73.5 – 81.6 |
| Protein | 23.83 (1.76) (19.80 – 26.40) | 23.88 (1.11) (21.10 – 26.00) | -0.05 | -14.06 11.96 | NS | 19.9 – 22.1 | 14.37 – 24.71 |
| Total Fat | 1.69 (0.07) (1.55 – 2.02) | 1.94 (0.24) (1.61 – 2.63) | -0.25 | -0.54 0.04 | NS | 1.1 – 1.7 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 33.17 (0.71) (31.80 – 35.30) | 33.50 (0.66) (31.90 – 34.90) | -0.33 | -5.17 4.51 | NS | 30.3 – 35.1 | |
| Neutral Detergent Fiber | 43.03 (1.11) (40.90 – 45.70) | 43.45 (0.40) (42.50 – 44.20) | -0.32 | -3.31 2.47 | NS | 40.6 – 45.7 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.28 (0.01) (0.27 – 0.35) | 0.30 (0.02) (0.27 – 0.35) | -0.02 | -0.07 0.04 | NS | 0.28 – 0.37 | |
| Calcium | 1.17 (0.04) (1.11 – 1.56) | 1.27 (0.09) (1.03 – 1.41) | -0.10 | -0.59 0.39 | NS | 1.24 – 1.51 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

Table 11-45. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site D2.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) | | | | | | | |
| Ash | 8.97 (0.13) (8.61 – 9.20) | 8.52 (0.22) (8.10 – 8.91) | 0.45 | -0.03 0.88 | NS | 8.4 – 9.9 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 33.88 (1.17) (31.80 – 36.07) | 33.51 (1.41) (31.08 – 37.56) | 0.37 | -3.35 4.09 | NS | 30.2 – 35.0 | |
| Moisture (% fwt) ⁶ | 78.79 (0.19) (78.30 – 79.22) | 78.65 (0.19) (78.32 – 79.07) | 0.14 | -0.83 1.11 | NS | 78.4 – 81.0 | 73.5 – 81.6 |
| Protein | 21.75 (0.81) (20.20 – 24.00) | 24.20 (1.79) (21.50 – 29.20) | -2.45 | -5.73 0.83 | NS | 21.4 – 26.4 | 14.37 – 24.71 |
| Total Fat | 1.68 (0.22) (1.24 – 2.21) | 1.71 (0.20) (1.32 – 2.28) | -0.03 | -1.22 1.16 | NS | 1.4 – 1.7 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 35.35 (1.09) (32.50 – 37.20) | 34.73 (1.31) (31.10 – 36.80) | 0.62 | -2.17 3.41 | NS | 33.7 – 37.3 | |
| Neutral Detergent Fiber | 47.00 (0.82) (44.80 – 48.30) | 46.15 (1.65) (42.00 – 49.70) | 0.85 | -3.59 5.29 | NS | 40.2 – 50.5 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.27 (0.01) (0.25 – 0.27) | 0.30 (0.02) (0.26 – 0.33) | -0.03 | -0.09 0.03 | NS | 0.25 – 0.32 | |
| Calcium | 1.23 (0.03) (1.17 – 1.32) | 1.34 (0.08) (1.12 – 1.46) | -0.11 | -0.32 0.09 | NS | 1.21 – 1.33 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

Table 11-46. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site G1.

| Component (Units)¹ | HB4 Mean (SE)² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig³ | Commercial Reference Range | Literature Range (ILSI)⁴ |
|--------------------------------------|--|--------------------------------------|--|------------------------------|------------------------|-----------------------------------|--|
| Proximates (% dwt) | | | | | | | |
| Ash | 8.93 (0.21) (8.51 – 9.45) | 8.90 (0.15) (8.51 – 9.22) | 0.03 | -0.95 1.01 | NS | 8.4 – 9.4 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 35.13 (1.65) (30.43 – 38.14) | 38.67 (1.44) (34.42 – 40.57) | -3.54 | -4.73 -2.35 | NS | 30.7 – 40.2 | |
| Moisture (% fwt) ⁶ | 70.49 (1.50) (66.76 – 73.27) | 76.12 (2.96) (71.96 – 84.67) | -5.62 | - 19.6 4 | NS | 71.9 – 74.9 | 73.5 – 81.6 |
| Protein | 22.20 (1.32) (20.10 – 25.90) | 19.85 (0.30) (19.10 – 20.50) | 2.35 | -1.37 6.07 | NS | 21.2 – 24.7 | 14.37 – 24.71 |
| Total Fat | 1.89 (0.06) (1.78 – 2.06) | 2.18 (0.14) (1.77 – 2.38) | -0.29 | -0.73 0.15 | NS | 1.7 – 2.4 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 32.53 (0.87) (30.80 – 34.30) | 29.43 (0.65) (27.50 – 30.40) | 3.10 | 0.99 5.21 | * | 31.3 – 35.4 | |
| Neutral Detergent Fiber | 47.88 (1.27) (45.30 – 51.00) | 45.03 (1.41) (41.50 – 47.50) | 2.85 | -0.20 5.50 | * | 46.8 – 57.2 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.22 (0.01) (0.20 – 0.24) | 0.23 (0.02) (0.18 – 0.28) | -0.01 | -0.06 0.05 | NS | 0.21 – 0.25 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

| Component (Units)¹ | HB4 Mean (SE)² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig³ | Commercial Reference Range | Literature Range (ILSI)⁴ |
|--|--|--|--|--------------------------------------|------------------------|---|--|
| Calcium | 1.25 (0.03) (1.16 – 1.30) | 1.26 (0.02) (1.20 – 1.31) | -0.01 | -0.17 0.15 | NS | 1.08 – 1.18 | |

Table 11-47. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site Q1.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|--|-------------------------------------|---|--------------------------|------------------|----------------------------------|--|
| Proximates (% dwt) | | | | | | | |
| Ash | 8.80 (0.17) (8.44 – 9.20) | 8.52 (0.17) (8.10 – 8.91) | 0.28 | -0.35 0.91 | NS | 8.7 – 9.7 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 32.21 (0.52) (31.01 – 33.27) | 33.06 (1.21) (30.72 – 35.76) | -0.85 | -5.76 4.06 | NS | 29.3 – 34.3 | |
| Moisture (% fwt) ⁶ | 77.38 (0.45) (76.28 – 78.45) | 76.97 (0.41) (75.83 – 77.79) | 0.41 | -1.76 2.58 | NS | 76.7 – 79.1 | 73.5 – 81.6 |
| Protein | 23.15 (0.26) (22.60 – 23.80) | 22.53 (0.06) (22.40 – 22.70) | 0.62 | -0.26 1.50 | NS | 20.4 – 25.6 | 14.37 – 24.71 |
| Total Fat | 1.56 (0.17) (1.15 – 1.91) | 1.53 (0.12) (1.33 – 1.89) | 0.03 | -0.54 0.60 | NS | 0.9 – 2.1 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 37.98 (0.43) (37.40 – 39.20) | 38.40 (0.96) (36.50 – 40.20) | -0.42 | -3.21 2.37 | NS | 31.6 – 40.1 | |
| Neutral Detergent Fiber | 48.98 (1.01) (47.20 – 51.70) | 48.23 (1.08) (46.20 – 50.80) | 0.75 | -4.51 6.01 | NS | 47.1 – 50.2 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.21 (0.004) (0.20 – 0.22) | 0.20 (0.01) (0.18 – 0.21) | 0.01 | -0.01 0.04 | NS | 0.21 – 0.26 | |
| Calcium | 1.12 (0.03) (1.03 – 1.18) | 1.13 (0.05) (1.03 – 1.22) | -0.01 | -0.09 0.06 | NS | 0.97 – 1.14 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

Table 11-48. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site Q2.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|--|-------------------------------------|---|--------------------------|------------------|----------------------------------|--|
| Proximates (% dwt) | | | | | | | |
| Ash | 8.96 (0.40) (8.43 – 8.94) | 7.97 (0.35) (7.01 – 8.56) | 0.28 | -0.68 2.66 | NS | 8.7 – 9.7 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 32.39 (0.30) (31.54 – 33.28) | 34.02 (1.04) (31.10 – 35.96) | -1.63 | -3.74 0.48 | NS | 29.0 – 35.6 | |
| Moisture (% fwt) ⁶ | 77.87 (0.21) (78.02 – 78.16) | 77.13 (0.44) (76.02 – 78.19) | 0.74 | -0.40 1.88 | NS | 77.5 – 79.4 | 73.5 – 81.6 |
| Protein | 24.46 (0.90) (22.60 – 26.90) | 23.25 (0.50) (21.80 – 24.00) | 1.21 | -2.20 4.62 | NS | 19.7 – 23.7 | 14.37 – 24.71 |
| Total Fat | 1.59 (0.12) (1.34 – 1.60) | 1.60 (0.11) (1.40 – 1.89) | -0.01 | -0.47 0.45 | NS | 1.0 – 1.5 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 38.06 (1.01) (36.90 – 41.40) | 38.10 (0.46) (37.30 – 39.40) | -0.04 | -2.19 2.11 | NS | 36.4 – 39.5 | |
| Neutral Detergent Fiber | 45.40 (0.61) (43.20 – 46.90) | 47.75 (0.89) (46.20 – 49.80) | -2.35 | -4.79 0.09 | * | 44.0 – 50.9 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.25 (0.03) (0.20 – 0.24) | 0.25 (0.02) (0.22 – 0.28) | 0 | -0.11 0.12 | NS | 0.24 – 0.27 | |
| Calcium | 1.22 (0.09) (1.07 – 1.21) | 1.20 (0.08) (0.96 – 1.30) | 0.02 | -0.37 0.42 | NS | 1.10 – 1.28 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

Table 11-49. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site W1.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) | | | | | | | |
| Ash | 8.58 (0.49) (7.71 – 9.93) | 8.86 (0.46) (8.09 – 9.98) | -0.28 | -2.71 2.15 | NS | 8.1 – 9.1 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 37.62 (1.42) (34.61 – 40.42) | 35.47 (0.23) (34.86 – 35.92) | 2.12 | -2.15 6.45 | NS | 32.8 – 39.4 | |
| Moisture (% fwt) ⁶ | 66.43 (0.72) (65.31 – 68.53) | 67.21 (1.58) (65.32 – 71.94) | -0.78 | -6.93 5.37 | NS | 64.3 – 67.9 | 73.5 – 81.6 |
| Protein | 20.83 (0.55) (19.60 – 22.20) | 22.78 (0.99) (20.30 – 24.60) | -1.95 | -4.15 0.25 | NS | 21.0 – 22.8 | 14.37 – 24.71 |
| Total Fat | 2.23 (0.14) (1.97 – 2.58) | 2.50 (0.09) (2.25 – 2.62) | -0.27 | -0.60 0.06 | NS | 1.8 – 2.4 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 31.20 (0.34) (30.50 – 32.00) | 32.60 (0.66) (30.80 – 33.90) | -1.40 | -4.08 1.28 | NS | 28.7 – 34.1 | |
| Neutral Detergent Fiber | 47.28 (1.89) (43.60 – 52.40) | 49.83 (1.36) (47.90 – 53.70) | -2.55 | -6.53 1.43 | NS | 43.8 – 52.3 | |
| Minerals (% dwt) | | | | | | | |
| Phosphorus | 0.27 (0.01) (0.25 – 0.30) | 0.28 (0.02) (0.24 – 0.33) | -0.01 | -0.09 0.07 | NS | 0.22 – 0.29 | |
| Calcium | 1.30 (0.03) (1.24 – 1.35) | 1.36 (0.08) (1.25 – 1.58) | -0.06 | -0.37 0.25 | NS | 1.26 – 1.31 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

Table 11-50. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site IL3.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) | | | | | | | |
| Ash | 8.76 (0.29) (7.99 – 9.40) | 8.76 (0.32) (8.44 – 9.73) | 0 | -1.22 | 1.22 | 7.92 – 9.29 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 67.80 (0.43) (66.7 – 68.8) | 68.13 (0.51) (66.9 – 69.3) | -0.33 | -1.61 | 0.95 | 63.7 – 68.3 | |
| Moisture (% fwt) ⁶ | 76.55 (0.89) (74.1 – 78.3) | 75.35 (0.46) (74.2 – 76.4) | 1.20 | -1.57 | 3.97 | 75.9 – 78.8 | 73.5 – 81.6 |
| Protein | 20.23 (0.31) (19.4 – 20.9) | 19.70 (0.60) (18.7 – 21.4) | 0.53 | -1.63 | 2.69 | 21.0 – 23.1 | 14.37 – 24.71 |
| Total Fat | 3.02 (0.26) (2.34 – 3.61) | 3.33 (0.05) (3.20 – 3.45) | -0.31 | -1.23 | 0.61 | 2.79 – 3.85 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 29.33 (1.47) (27.2 – 33.5) | 28.75 (0.85) (27.3 – 31.0) | 0.58 | -2.65 | 3.81 | 25.10 – 30.70 | |
| Neutral Detergent Fiber | 34.40 (0.51) (33.0 – 35.0) | 31.80 (1.34) (27.3 – 31.0) | 2.60 | -1.91 | 7.11 | 28.80 – 34.20 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

Table 11-51. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site IN.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|------------------------------------|---------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) | | | | | | | |
| Ash | 7.25 (0.31) (6.43 – 7.80) | 6.80 (0.13) (6.50 – 7.13) | 0.45 | -0.54 1.44 | NS | 6.82 – 7.94 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 73.28 (0.75) (71.9 – 75.4) | 75.18 (0.75) (73.8 – 77.3) | -1.90 | -5.03 1.23 | NS | 70.3 – 74.2 | |
| Moisture (% fwt) ⁶ | 76.33 (0.27) (75.6 – 76.9) | 75.10 (0.44) (74.0 – 76.0) | 1.23 | -0.02 2.48 | NS | 74.3 – 76.7 | 73.5 – 81.6 |
| Protein | 16.25 (0.55) (14.8 – 17.3) | 15.60 (0.67) (13.7 – 16.8) | 1.25 | -2.20 3.50 | NS | 15.6 – 18.6 | 14.37 – 24.71 |
| Total Fat | 3.27 (0.03) (3.21 – 3.36) | 2.46 (0.03) (2.38 – 2.54) | 0.81 | 0.61 1.01 | * | 3.02 – 3.54 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 30.83 (1.65) (27.0 – 34.9) | 30.75 (1.28) (30.80 – 33.90) | 0.08 | -4.59 4.75 | NS | 27.3 – 35.8 | |
| Neutral Detergent Fiber | 35.08 (1.80) (32.5 – 40.4) | 37.40 (1.57) (35.6 – 42.1) | -2.32 | - 11.68 | NS | 31.1 – 41.8 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

Table 11-52. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site OH2.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) | | | | | | | |
| Ash | 8.82 (0.05) (8.73 – 8.97) | 8.45 (0.23) (7.85 – 8.83) | 0.37 | -0.25 | 0.99 | 8.32 – 9.17 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 67.90 (0.44) (67.0 – 68.8) | 68.45 (0.32) (67.9 – 69.3) | -0.55 | -1.09 | -0.01 | 65.0 – 68.8 | |
| Moisture (% fwt) ⁶ | 79.90 (0.26) (79.5 – 80.6) | 80.35 (0.35) (79.5 – 81.2) | -0.45 | -1.50 | 0.60 | 78.4 – 79.4 | 73.5 – 81.6 |
| Protein | 20.25 (0.31) (19.7 – 21.0) | 20.05 (0.23) (19.5 – 20.6) | 0.20 | -0.34 | 0.74 | 19.7 – 21.3 | 14.37 – 24.71 |
| Total Fat | 3.06 (0.21) (2.64 – 3.55) | 3.03 (0.07) (2.84 – 3.16) | 0.03 | -0.65 | 0.71 | 2.48 – 4.40 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 34.25 (1.12) (31.1 – 35.9) | 34.45 (2.32) (31.0 – 41.2) | -0.20 | -5.84 | 5.64 | 26.0 – 31.6 | |
| Neutral Detergent Fiber | 39.23 (3.02) (30.8 – 45.2) | 39.80 (0.81) (37.4 – 41.0) | -0.57 | - | 11.18 | 26.1 – 41.6 | |
| | | | | 12.32 | | | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

Table 11-53. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site IA.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) | | | | | | | |
| Ash | 7.51 (0.29) (6.74 – 8.08) | 7.70 (0.44) (6.95 – 8.97) | -0.19 | -1.45 1.07 | NS | 8.14 – 8.84 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 73.25 (0.60) (72.1 – 74.6) | 72.23 (1.22) (68.6 – 73.8) | 1.02 | -2.00 4.04 | NS | 67.9 – 68.9 | |
| Moisture (% fwt) ⁶ | 76.45 (0.35) (75.5 – 77.1) | 76.38 (0.45) (75.7 – 77.7) | 0.07 | -1.19 1.33 | NS | 77.1 – 78.4 | 73.5 – 81.6 |
| Protein | 15.95 (0.24) (15.3 – 16.4) | 16.65 (0.67) (15.4 – 18.5) | -0.70 | -2.90 1.50 | NS | 19.3 – 20.9 | 14.37 – 24.71 |
| Total Fat | 3.31 (0.17) (2.82 – 3.55) | 3.30 (0.29) (2.54 – 3.84) | 0.01 | -0.95 0.97 | NS | 2.90 – 3.79 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 26.98 (1.36) (24.5 – 30.6) | 32.60 (1.43) (29.4 – 36.3) | -5.62 | -9.78 -1.46 | * | 25.6 – 31.2 | |
| Neutral Detergent Fiber | 32.93 (1.95) (29.5 – 36.5) | 36.45 (1.39) (32.4 – 38.0) | -3.52 | - 13.55 | NS | 29.6 – 36.6 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

Table 11-54. Summary of Soybean Forage Nutrients for HB4 vs. Williams 82 in Site KS.

| Component (Units) ¹ | HB4 Mean (SE) ² (Range) | Williams 82 Mean (SE) (Range) | Mean Difference (HB4 minus Williams 82) | 95% Confidence Limits | Sig ³ | Commercial Reference Range | Literature Range (ILSI) ⁴ |
|--------------------------------|------------------------------------|-------------------------------|---|-----------------------|------------------|----------------------------|--------------------------------------|
| Proximates (% dwt) | | | | | | | |
| Ash | 14.10 (1.10) (10.9 – 15.9) | 15.10 (2.19) (11.3 – 20.5) | -1.00 | -6.14 4.14 | NS | 12.20 – 19.10 | 6.71 – 10.78 |
| Carbohydrates ⁵ | 60.93 (0.86) (59.5 – 63.4) | 62.08 (2.56) (56.7 – 67.8) | -1.15 | -7.69 5.39 | NS | 56.9 – 62.1 | |
| Moisture (% fwt) ⁶ | 84.88 (0.50) (83.4 – 85.5) | 84.28 (0.88) (81.7 – 85.1) | 0.60 | -2.87 4.07 | NS | 82.8 – 85.7 | 73.5 – 81.6 |
| Protein | 21.08 (0.49) (19.9 – 22.3) | 19.05 (0.83) (16.8 – 20.8) | 2.03 | -1.71 5.77 | NS | 18.30 – 22.30 | 14.37 – 24.71 |
| Total Fat | 3.80 (0.48) (2.75 – 4.70) | 3.64 (0.26) (3.22 – 4.33) | 0.16 | -1.23 1.55 | NS | 2.87 – 4.30 | 1.30 – 5.13 |
| Fiber (% dwt) | | | | | | | |
| Acid Detergent Fiber | 33.00 (3.71) (25.5 – 42.5) | 31.58 (1.07) (29.6 – 34.4) | 1.42 | - 11.92 | NS | 20.3 – 41.8 | |
| Neutral Detergent Fiber | 36.23 (3.32) (32.1 – 46.1) | 34.70 (3.16) (26.3 – 39.9) | 1.53 | - 14.89 | NS | 25.6 – 47.0 | |

¹ dwt = dry weight² SE = standard error of the mean³ Sig = Significance; NS = not significant; asterisk (*) = significant difference at $p \leq 0.05$ ⁴ Reference range of values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014)⁵ Determined by calculation⁶ fwt = fresh weight

L. Appendix 12. Phenotypic, Agronomic, and Environmental Interactions Assessment of Event HB4.

Introduction

The objectives of the phenotypic, agronomic and environmental studies were to evaluate the overall performance of soybean transgenic event HB4 in comparison with the conventional variety Williams 82 and commercial comparators. HB4 soybean characteristics measured included the following: 1) seed germination and dormancy; 2) pollen morphology and pollen fertility; 3) agronomic and phenotypic evaluations, including vegetative growth, seed retention and lodging; and 4) ecological evaluations, including disease susceptibility, insect interactions, abiotic stress and plant-symbiont characteristics. The results of these studies support the conclusion that the soybean HB4 event is comparable to conventional soybeans and does not pose a specific plant pest risk.

To further characterize the soybean event HB4, phenotypic, agronomic and environmental interactions were observed and collected during the growing seasons in Argentina 2012-2013 and the US 2013. A series of experts in plant pathology, breeding, and entomology evaluated the data to determine whether differences existed between the selected event and the Williams 82 control or the conventional varieties. These analyses were made both on an individual site and a global basis.

Twenty-one agronomic and selected ecological interaction data were collected during the growing season for all entries at each site. Results from the Argentina and US multi-site, replicated field trials demonstrated that HB4 soybeans could provide an increased yield opportunity as compared with Williams 82.

As expected, the most relevant difference among the agronomic characteristics that demonstrated the improvement of soybean event HB4 over the control was yield. The absence of significant variances measured for qualities such as disease susceptibility, arthropod infestation, and general soybean growth characteristics supports the conclusion soybean event HB4 is comparable to conventional and control soybean varieties.

HB4 Soybean Germination and Dormancy Evaluation

Test Materials

Soybean seed germination experiments were conducted to test germination and seed dormancy at the optimal temperature range for soybeans of 20/30°C using Association of Official Seed Analysts rules (AOSA 2013a). To have a more complete picture on the germination performance of this seed, five additional temperatures regimes were also tested. Assessments were conducted on the soybean event HB4 and the non-transgenic near-isogenic parent Williams 82. Results were also obtained from 3 commercial reference lines with similar maturity zone ratings as Williams 82, providing a range of comparative values that are representative of commercial soybean varieties. The data demonstrated seed from soybean event IND-00410-5 was not fundamentally different than the Williams 82 soybean seed control.

Sampling and analysis

The seed germination testing was conducted by SGS Agricultural Services (SGS North America, Brookings, SD) using seed harvested from HB4, Williams 82 and three commercial varieties (Dow 32R280, Pioneer 93Y82 and Asgrow AG3832) grown together in the same 2013 replicated trials at two sites in the US in Pemberton, OH and Richland, IA. The two varieties, Dow 32R280 and Pioneer 93Y84 were grown at every site as HB4 soybean and represent broadly used commercial soybean varieties.

The seed was stored at room temperature until laboratory tests were performed. The five soybean lines from each of the two locations were evaluated utilizing the AOSA standard warm germination method (AOSA 2013a)(20-30°C, evaluation at 5 and 8 days) and additional five temperature regimes (Table 12-1). The five seed lines per site were germinated using the rolled towel method. Eight 50-seed replicates per line (n=400 seeds/line/temperature/site) were placed on paper towels moistened with water and the towels were then placed under one of the six temperature regimes (Table 12-1).

AOSA (2013b) rules were followed for seedling evaluations. Two evaluation counts were performed for each regime. The first evaluation was performed five days after planting for all temperature regimes except constant 10°C. For samples planted at 10°C, the first count was performed seven days after planting due to slower germination at that temperature. At the first count, normal seedlings as defined by AOSA as seedlings possessing the essential structures indicative of their ability to produce plants under favorable conditions, were removed. The remaining non-germinated seed, seedlings too small to evaluate, abnormal seedlings (defined by AOSA as all seedlings that cannot be classified as normal seedlings), and any hard or potentially dead seed were returned to the proper germination temperature. Towels were rewetted as needed. The final evaluation was performed at eight days for testing regimes excluding the 10°C regime. The 10°C regime had a final evaluation performed at fourteen days. At final count, normal seedlings, abnormal seedlings and dead seeds, as well as any hard seed, were scored. Dead seeds are defined by AOSA as seeds that are neither hard nor dormant nor have produced any part of a seedling at the end of the test period.

Germination scores from the eight replications of 50 seed each were collected and an analysis of variance (ANOVA) was performed on the transformed data at $p=0.05$ when acceptable. Least significant sets (LSD) were determined. Statistics were performed utilizing SAS Proprietary Software version 9.4 (SAS Institute, 2015).

Results

Overall, the Iowa sourced seed had a higher germination rate as seen in the commercial control range and the lower amount of abnormal seedlings, likely due to the ideal plant growth conditions in Iowa during pod filling. Germination rates are shown in Table 12-2 and Table 12-3. In most treatments HB4 had the same germination performance as Williams 82 and in all cases performance of HB4 and Williams 82 was within the range of the 3 commercial cultivars. In the AOSA standard germination conditions of 20-30°C for 8 days (AOSA 2013a), there were no significant differences between the lines. These data support the conclusion that the germination rate of soybean event HB4 does not differ from the Williams 82 control. Additionally, hard seed can be an indicator of increased weediness in

soybean germination. The lack of hard seed (Table 12-2) in event HB4 demonstrated the event does not possess increased weediness potential.

Table 12-1. The temperature regimes used for germination testing.

| Temperature | Duration | Sampling (days) |
|-------------|---------------------------|-----------------|
| 10°C | 14 days | 7, 14 |
| 20°C | 8 days | 5, 8 |
| 30°C | 8 days | 5, 8 |
| 10-20°C | (16:8) ¹ hours | 5, 8 |
| 10-30°C | (16:8) ¹ hours | 5, 8 |
| 20-30°C | (16:8) ¹ hours | 5, 8 |

¹The seeds were held 16 hours at the lower temperature, then 8 hours at the higher temperature.

Table 12-2. ANOVA Probability Values (p value) by temperature and count from the seed harvested from the OHIO field trial.

| Temp (°C) | Seed Data | HB4 (%) | Williams 82 (%) | p value | R ² | CV (%) | Root mean square error | Mean | Commercial Checks (%) | |
|-----------|-----------|---------|-----------------|---------|----------------|--------|------------------------|------|-----------------------|-----|
| | | | | | | | | | Min | Max |
| 10 | 1st Count | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Normal | 85 | 85 | 0.99 | 0.5462 | 5 | 1.17 | 88 | 82 | 98 |
| | Abnormal | 10 | 10 | 0.919 | 0.4194 | 20.6 | 0.31 | 8 | 2 | 14 |
| | Hard | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 2 |
| | Dead | 6 | 5 | 0.931 | 0.8657 | 18.4 | 0.041 | 4 | 0 | 10 |
| | Swollen | 0 | 0 | 0.351 | 0.5333 | 400 | 0.035 | 0 | 0 | 2 |
| 20 | 1st Count | 74 | 76 | 0.73 | 0.431 | 7.9 | 1.05 | 79 | 70 | 92 |
| | Normal | 79 | 79 | 0.946 | 0.6365 | 4.8 | 1.1 | 84 | 76 | 96 |
| | Abnormal | 16 | 18 | 0.36 | 0.6984 | 12.9 | 0.42 | 13 | 2 | 18 |
| | Hard | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Dead | 5 | 3 | 0.148 | 0.5088 | 38.8 | 0.073 | 3 | 0 | 8 |
| | Swollen | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| 30 | 1st Count | 86 | 85 | 0.756 | 0.6983 | 5.9 | 1.19 | 88 | 80 | 98 |
| | Normal | 91 | 90 | 0.702 | 0.5603 | 6.1 | 1.26 | 92 | 86 | 98 |
| | Abnormal | 6 | 7 | 0.785 | 0.4854 | 33.7 | 0.24 | 5 | 0 | 10 |
| | Hard | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Dead | 3 | 4 | 0.258 | 0.5667 | 46.2 | 0.079 | 3 | 0 | 14 |
| | Swollen | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| 10-20 | 1st Count | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Normal | 81 | 78 | 0.205 | 0.4833 | 5.9 | 1.1 | 85 | 76 | 98 |
| | Abnormal | 13 | 17 | 0.074 | 0.6363 | 11.8 | 0.39 | 12 | 0 | 16 |
| | Hard | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Dead | 6 | 6 | 0.83 | 0.6254 | 30.6 | 0.071 | 3 | 0 | 8 |
| | Swollen | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| 10-30 | 1st Count | 5 | 5 | 0.803 | 0.5359 | 45.2 | 0.2 | 6 | 2 | 14 |
| | Normal | 94 | 83 | 0 | 0.9012 | 4.2 | 1.23 | 89 | 72 | 96 |

| Temp (°C) | Seed Data | HB4 (%) | Williams 82 (%) | p value | R ² | CV (%) | Root mean square error | Mean | Commercial Checks (%) | |
|--------------|--------------|---------|--------------------|------------|----------------|-----------|---------------------------------|------|--------------------------|-----|
| | | | | | | | | | Min | Max |
| 20-30 | Abnormal | 6 | 10 | 0.038 | 0.6275 | 25.1 | 0.27 | 8 | 4 | 22 |
| | Hard | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Dead | 1 | 7 | 0.014 | 0.6427 | 92.4 | 0.13 | 3 | 0 | 8 |
| | Swollen | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | 1st Count | 59 | 65 | 0.175 | 0.6776 | 9.7 | 0.91 | 65 | 40 | 98 |
| | Normal | 68 | 71 | 0.341 | 0.4917 | 7.3 | 0.99 | 79 | 66 | 100 |
| | Abnormal | 26 | 19 | 0.052 | 0.6545 | 15.5 | 0.49 | 16 | 0 | 32 |
| | Hard | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Dead | 6 | 10 | 0.077 | 0.5732 | 27.4 | 0.077 | 5 | 0 | 26 |
| | Swollen | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |

Table 12-3. ANOVA Probability Values (p value) by temperature and count from the seed harvested from the IOWA field trial.

| Temp | Seed Data | HB4 (%) | Williams 82 (%) | p value | R ² | CV (%) | RMSE | Mean | Commercial Checks (%) | |
|-------|--------------|---------|--------------------|---------|----------------|-----------|-------|------|--------------------------|-----|
| | | | | | | | | | Min | Max |
| 10 | 1st Count | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Normal | 96 | 95 | 0.378 | 0.8959 | 3.1 | 1.37 | 95 | 88 | 98 |
| | Abnormal | 4 | 3 | 0.26 | 0.6689 | 46.7 | 0.16 | 4 | 2 | 8 |
| | Hard | 0 | 0 | 0.351 | 0.5333 | 400 | 0.035 | 0 | 0 | 2 |
| | Dead | | 2 | 0.085 | 0.4489 | 130.7 | 0.096 | 1 | 0 | 6 |
| | Swollen | 0 | 1 | 0.08 | 0.6154 | 195.2 | 0.052 | 0 | 0 | 2 |
| 20 | 1st Count | 97 | 92 | 0.042 | 0.7027 | 6.9 | 1.35 | 95 | 88 | 100 |
| | Normal | 99 | 98 | 0.071 | 0.6981 | 5 | 1.48 | 98 | 92 | 100 |
| | Abnormal | 1 | 2 | 0.223 | 0.5573 | 108.9 | 0.08 | 2 | 0 | 8 |
| | Hard | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Dead | 0 | 0 | 0.351 | 0.5333 | 400 | 0.035 | 0 | 0 | 6 |
| | Swollen | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| 30 | 1st Count | 94 | 90 | 0.017 | 0.7476 | 3.9 | 1.3 | 93 | 82 | 100 |
| | Normal | 96 | 93 | 0.011 | 0.8418 | 3.6 | 1.34 | 95 | 88 | 100 |
| | Abnormal | 4 | 4 | 0.687 | 0.7805 | 28 | 0.18 | 4 | 0 | 12 |
| | Hard | 0 | 2 | <.0001 | 1 | 0 | 0 | 0 | 0 | 0 |
| | Dead | 1 | 2 | 0.016 | 0.8474 | 60.8 | 0.05 | 1 | 0 | 6 |
| | Swollen | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| 10-20 | 1st Count | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Normal | 95 | 94 | 0.188 | 0.6496 | 3.9 | 1.34 | 94 | 86 | 100 |
| | Abnormal | 5 | 5 | 0.95 | 0.6136 | 25 | 0.21 | 5 | 0 | 14 |
| | Hard | 0 | 1 | 0.351 | 0.4167 | 200 | 0.071 | 0 | 0 | 2 |
| | Dead | 0 | 1 | 0.033 | 0.6667 | 151.2 | 0.054 | 1 | 0 | 4 |

| Temp | Seed Data | HB4 (%) | Williams 82 (%) | p value | R2 | CV (%) | RMSE | Mean | Commercial Checks (%) | |
|-------|-----------|---------|-----------------|---------|--------|--------|-------|------|-----------------------|-----|
| | | | | | | | | | Min | Max |
| 10-30 | Swollen | 0 | 0 | 0.351 | 0.5333 | 400 | 0.035 | 0 | 0 | 0 |
| | 1st Count | 9 | 9 | 0.83 | 0.3397 | 30.4 | 0.29 | 8 | 2 | 14 |
| | Normal | 96 | 94 | 0.473 | 0.4959 | 4.9 | 1.35 | 95 | 90 | 100 |
| | Abnormal | 4 | 6 | 0.241 | 0.6678 | 24.4 | 0.21 | 5 | 0 | 10 |
| | Hard | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Dead | 1 | 0 | 0.598 | 0.4103 | 241.7 | 0.064 | 0 | 0 | 2 |
| 20-30 | Swollen | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | 1st Count | 91 | 89 | 0.5 | 0.5595 | 9.8 | 1.27 | 87 | 56 | 98 |
| | Normal | 98 | 98 | 0.511 | 0.4494 | 7 | 1.46 | 97 | 90 | 100 |
| | Abnormal | 2 | 1 | 0.362 | 0.6815 | 89.6 | 0.08 | 3 | 0 | 10 |
| | Hard | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |
| | Dead | 1 | 1 | 1 | 0.3333 | 213.8 | 0.076 | 1 | 0 | 4 |
| | Swollen | 0 | 0 | . | 0 | . | 0 | 0 | 0 | 0 |

C. HB4 Pollen Morphology and Pollen Fertility.

The results presented in this study demonstrate that the introduction of *HaHB4v* gene into the soybean genome did not alter the overall pollen morphology or pollen fertility of HB4 as compared to the parental control Williams 82.

Introduction

The purpose of this study was to determine whether the presence of the *HaHB4v* transgene affected pollen morphology or fertility in HB4 soybeans as compared to the the parental control Williams 82.

General pollen morphology, pollen diameter and fertility were examined to compare potential differences between soybean event HB4 and its parental control Williams 82. Flowers were collected from replicated HB4 and Williams 82 plants grown under similar conditions in an environmentally controlled growth chamber, similar to the conditions encountered by plants across typical commercial soybean production areas. Pollen fertility was assessed by the iodine potassium iodide (I₂-KI) staining test developed by Jensen (1962). Fertile pollen grains exposed to iodine potassium iodide turn dark blue due to the presence of starch-amylose, while infertile pollen grains display the initial honey-brown color of the iodine solution and may appear collapsed depending on the degree of infertility. This test has been used to assess the pollen fertility in a number of crops (Dalmicio *et al.* 1995; Wei *et al.* 2012) including soybean (Smith *et al.* 2001; Palmer *et al.* 2012). Pollen diameter and general morphology were assessed using microscope and digital images, respectively. The purpose of this study was to determine whether the presence of the *HaHB4v* gene affected pollen morphology or fertility in HB4 event when compared to Williams 82.

Materials and Methods

Soybean plants from the transgenic event HB4 were grown along with parental cultivar Williams 82 under the following conditions: 28/21°C day/night temperature, 16 h photoperiod (incandescent/fluorescent lights $230 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), in an environmentally controlled growth chamber (Convicon GC20, Convicon, Winnipeg, Manitoba, Canada R3H 0R9) at Arcadia Biosciences, Davis, CA. Five six-inch pots for each genotype were filled with Sunshine mix #3 potting soil (Sun Gro Horticulture Canada Ltd, Agawam, MA 01001). Initially, two seeds per pot were planted and seedlings were thinned to one plant per pot one week after germination. Fertilizer (100 ppm CaNO_3 , 100 ppm K) was applied once a week.

Five flowers were collected from each plant following the stem from ground up: one flower from the bottom node, three from the middle nodes and one from the top flowering node, for a total of 25 flowers for each genotype, HB4 and Williams 82. Flowers from each plant were placed in 15 ml polypropylene tubes and kept on wet ice until the pollen was prepared and stained.

Microscope slides were labeled with plant number, and a square of approximately 1 cm in size was drawn in the center of each slide with a permanent marker. Five slides from each replicate, one slide per flower, were prepared. After removing sepals and petals using fine tweezers, the pollen was brushed from the anthers into the square drawn on the slide. The tweezers were cleaned with ethanol between each pollen collection. Pollen grains were stained with 20 μl of aqueous 1% iodine potassium iodide (I_2KI) solution according to a previously published protocol (Jensen 1962; Smith *et al.* 2001). A cover slip was placed over the pollen and the slides were left at room temperature for at least 10 minutes prior to microscopic examination. Pollen samples were evaluated on the same day. Ten (10) microscopic fields per flower were examined for a total of 250 fields per genotype.

The slides were viewed with a microscope (Leica DMLB2) equipped with a Leica DC480 digital color camera connected to a computer installed with both Microsoft Windows 2003 Professional and with DC Twain v5.1.1 camera software. The images were archived.

When exposed to iodine potassium iodide, fertile pollen grains stained dark blue due to the presence of starch-amylose. They were round in shape and stained completely. Sterile pollen kept the initial stain color (honey brown) and could appear collapsed in shape, depending on the stage of pollen development reached and the fertility. For each sample, the number of fertile (stained and round = fertile) and sterile pollen grains (unstained, withered = sterile; unstained, spherical = sterile; partially stained and spherical = sterile) were counted from 10 microscopic field pictures. Dense clusters of pollen grains adhering to flower parts such as, for example, the anther sac, were excluded, as they may not have absorbed the stain uniformly.

Micrographs (400x resolution) were taken from 5 selected pollen grains per plant examined, for a total of 25 pollen grains per genotype. The microscope eyepiece with inserted graticule was used to measure the diameters from both perpendicular axes for each selected pollen grain.

General pollen morphology (appearance) was assessed from digital images of both HB4 and Williams 82 pollen grains that were also used for pollen fertility assessments.

An analysis of variance was conducted according to a completely randomized design using SAS® (2008). Genotypes and plants nested within genotypes were included in the model. The level of significance was determined at 5% level ($\alpha=0.05$) by one way ANOVA. Duncan's multiple range test was used as a *post hoc* comparison between HB4 and Williams 82. Fertility is reported as percent (%) and the diameter of pollen grains in micrometer (μm). General pollen morphology assessment was qualitative; therefore, no statistical analysis was conducted on this observation.

Results

Pollen grains from event HB4 and Williams 82 were assessed for percentage of fertile pollen grains and mean diameter of pollen grains. There was no significant difference in pollen fertility or in mean diameter between HB4 and Williams 82 (Table 12-4).

The examination of the exine under the microscope did not reveal any noticeable visual difference in pollen morphology in terms of appearance between pollen grains from event HB4 and those from Williams 82 (Figure 12-1).

Table 12-4. Pollen Characteristics of HB4 Compared to the Conventional Control Williams 82.

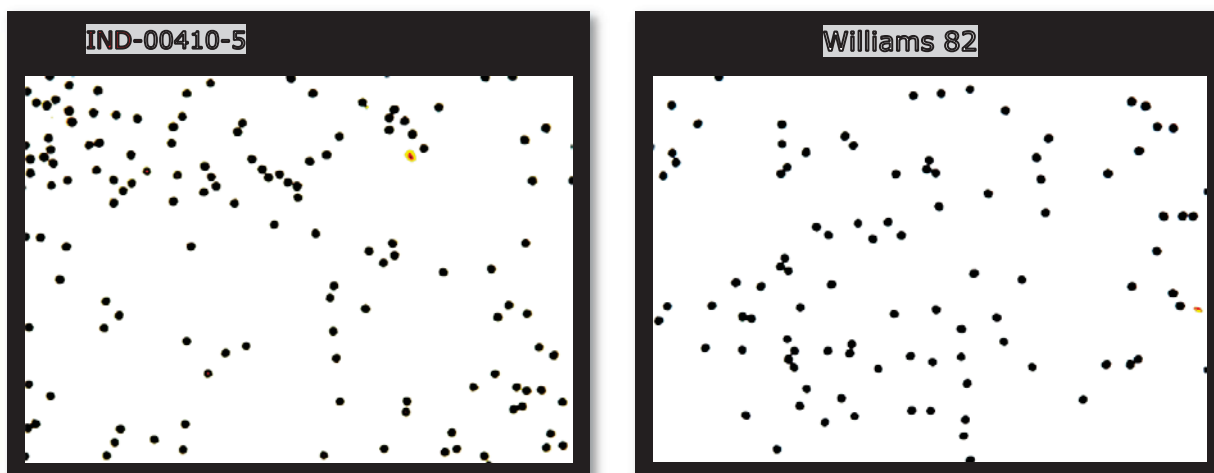
| Pollen Characteristic | HB4 (Mean \pm SE) ¹ | Williams 82 (Mean \pm SE) ¹ |
|----------------------------|----------------------------------|--|
| Fertility (%) | 99.52 \pm 0.08 | 99.50 \pm 0.07 |
| Diameter (μm) | 29.24 \pm 0.72 | 29.40 \pm 1.10 |

Note: There was no significant difference in pollen fertility and in pollen diameter between HB4 and Williams 82 ($p>0.05$).

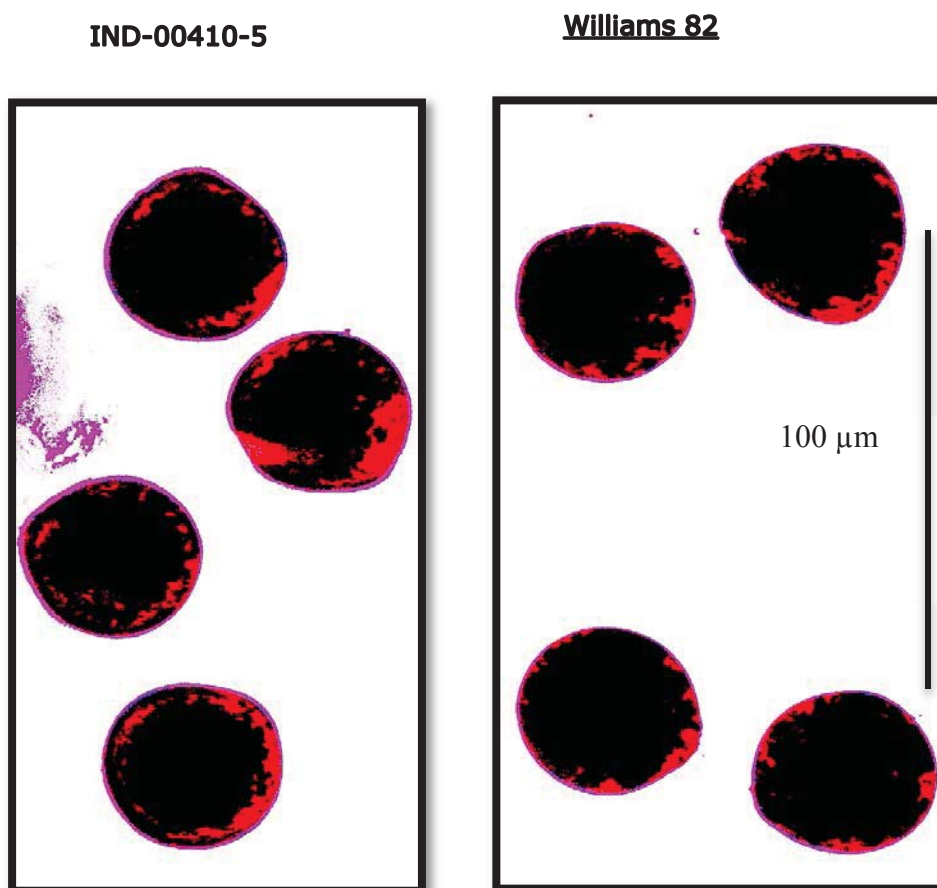
¹Means (n=25) and SE (Standard Error).

Figure 12-1. Pollen Morphology of HB4 Compared to Parental Control Williams 82.

a) Microscopic Field View (10x).



b) Close up view of pollen grains (400x).



HB4 Agronomic and Phenotypic Evaluations

Description of Trial Locations and Entries

The transgenic soybean event HB4 along with conventional control variety (Williams 82) and multiple commercial varieties specific for the growing region were field tested at 15 locations in Argentina and at 10 locations in the US during the 2012-2013 seasons. Both countries have similar temperate growing seasons, environmental conditions and cultivation practices, including maturity groups. The trial locations provided a range of environmental and agronomic conditions representative of the major soybean growing regions where HB4 soybean is expected to be grown commercially in Argentina and the United States (US). The details of trial locations, planting and harvest dates and cropping history are presented in Table 12-5. The location of trials at the soybean growing states is represented (Figure 12-2).

The transgenic event HB4 and conventional control variety Williams 82 were tested in all of the locations in both countries (Table 12-6). HB4 and the parental line Williams 82 have the same genetic background but the latter does not possess any transgenic elements. At each location, two commercial check varieties (DM 3810, SRM 3970 in AR; Dow 32R280, Pioneer 93Y82 in US) were also tested. In one Argentina location (Location P), SRM 3970 was not available. The inclusion of commercial check varieties provided a range of comparative values and an estimate of normal variation among soybean lines currently commercially grown. Three additional commercial varieties, typically grown in the Argentina locations, were also included (Table 12-6). The source and characteristics of each seed is summarized in Table 12-7. The agronomic evaluation of transgenic soybean event along with commercial and local check varieties will give the appropriate context for the interpretation of the experimental results in terms of their biological significance.

Agronomic and phenotypic data were collected throughout the growing seasons. The data included germination information, vegetative and reproductive parameters, and ecological interactions as a reflection of plant pest potential and are discussed in detail below and the data are summarized in Tables 12-13 through 12-53. These findings support the conclusion HB4 is comparable to its non-transgenic parental line, Williams 82 and does not possess unexpected phenotypic qualities.

Table 12-5. Field trial site and planting information.

| Argentina | | | | | | |
|---------------|------------------|--------------|---------------|--------------|------------|------------|
| Site ID | Site Location | Province | Planting Date | Harvest Date | Longitude | Latitude |
| A | Monte Buey | Córdoba | 11/7/2012 | 3/28/2013 | -62.438099 | -33.00351 |
| C | Chilibroste | Córdoba | 11/21/2012 | 4/9/2013 | -62.52075 | -32.256694 |
| D1 | Corral de Bustos | Córdoba | 11/8/2012 | 3/30/2013 | -62.129861 | -33.279616 |
| D2 | Corral de Bustos | Córdoba | 12/10/2012 | 4/18/2013 | -62.129883 | -33.279735 |
| F | Villa Saboya | Buenos Aires | 12/27/2012 | 4/22/2013 | -62.7076 | -34.494133 |
| G1 | Carmen de Areco | Buenos Aires | 11/20/2012 | 4/15/2013 | -59.831067 | -34.329617 |
| G2 | Carmen de Areco | Buenos Aires | 12/14/2012 | 4/24/2013 | -59.831067 | -34.329283 |
| H | Daireaux | Buenos Aires | 11/13/2012 | 4/23/2013 | -62.09095 | -36.6273 |
| I1 | San Agustín | Buenos Aires | 12/3/2012 | 5/8/2013 | -58.375611 | -37.990167 |
| I2 | San Agustín | Buenos Aires | 12/10/2013 | 5/8/2013 | -58.326083 | -37.9119 |
| P | Landeta | Santa Fe | 1/14/2013 | 5/6/2013 | -61.982861 | -32.0425 |
| Q1 | Hughes | Santa Fe | 11/10/2012 | 3/27/2013 | -61.302081 | -33.816275 |
| Q2 | Hughes | Santa Fe | 12/11/2012 | 4/17/2013 | -61.301778 | -33.816583 |
| W1 | Aranguren | Entre Rios | 11/16/2012 | 4/4/2013 | -60.153183 | -32.243267 |
| W2 | Aranguren | Entre Rios | 12/26/2012 | 4/18/2013 | -60.153633 | -32.243583 |
| United States | | | | | | |
| Site ID | Site Location | State | Planting Date | Harvest Date | Longitude | Latitude |
| IA | Richland | IA | 6/7/2013 | 10/18/2013 | -92.00401 | 41.14789 |
| IL1 | Highland | IL | 6/11/2013 | 10/11/2013 | -89.58002 | 38.75872 |
| IL2 | Carlyle | IL | 6/22/2013 | 10/21/2013 | -89.37655 | 38.67259 |
| IL3 | Effingham | IL | 7/13/2013 | 11/4/2013 | -88.64983 | 38.96652 |
| IN | Ladoga | IN | 6/6/2013 | 10/11/2013 | -86.862797 | 39.88668 |
| KS | Troy | OH | 6/7/2013 | 10/27/2013 | -84.15379 | 40.08056 |
| NE | York | NE | 6/6/2013 | 10/10/2013 | -97.672848 | 40.902742 |
| OH1 | Troy | KS | 6/21/2013 | 11/11/2013 | -95.21008 | 39.87382 |
| OH2 | Pemberton | OH | 6/4/2013 | 10/26/2013 | -84.07401 | 40.28575 |
| OK | Hinton | OK | 5/23/2013 | 10/8/2013 | -98.46366 | 35.49393 |

Figure 12-2. Geographic distribution of 2012-2013 field locations for agronomic, phenotypic and environmental evaluation in 1A) Argentina and 1B) the United States.

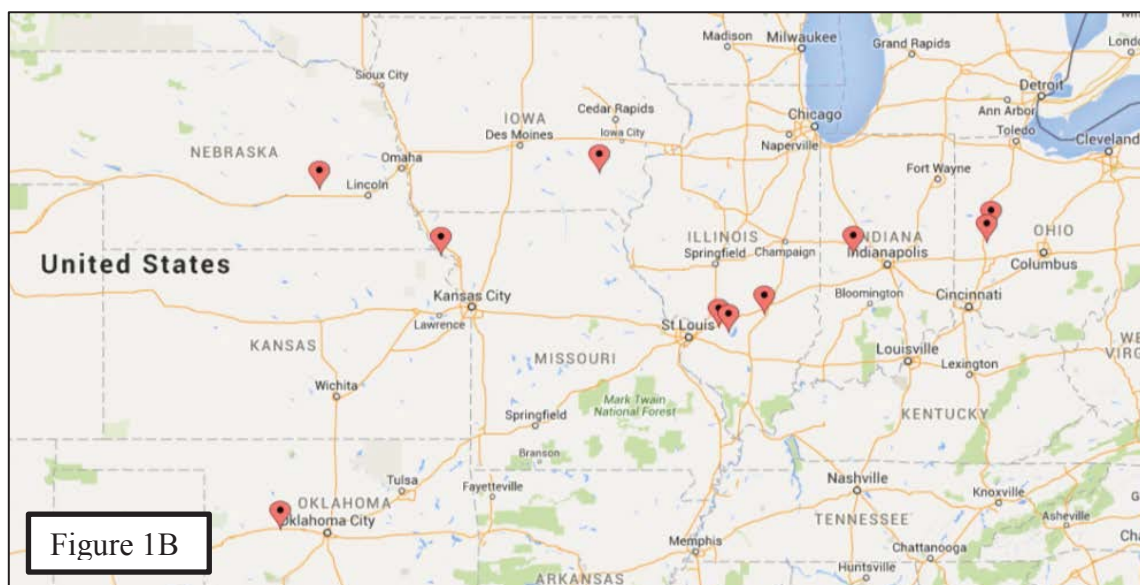


Table 12-6. Planting location of each seed variety used in the field trials in Argentina and the US.

| Argentina Site | HB4 | Williams 82 | DM 3810 | SRM 3970 | SRM 3410 | A 3731 RG | FN 3.85 | SPS 3900 | NS 4009 | DM 4210 | DM 4670 | Biosoja 4.6 |
|----------------|-----|-------------|---------|----------|----------|-----------|---------|----------|---------|---------|---------|-------------|
| A | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | ✓ | ✓ | |
| C | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | | ✓ | ✓ |
| D1 | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | ✓ | | ✓ |
| D2 | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | ✓ | | |
| F | ✓ | ✓ | ✓ | ✓ | | | ✓ | | | ✓ | | |
| G1 | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | | | ✓ | | |
| G2 | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | | | ✓ | | |
| H | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | | | |
| I1 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | |
| I2 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | |
| P | ✓ | ✓ | ✓ | | | | | ✓ | ✓ | ✓ | ✓ | ✓ |
| Q1 | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | ✓ | | |
| Q2 | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | ✓ | | |
| W1 | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | ✓ | ✓ | ✓ |
| W2 | ✓ | ✓ | ✓ | ✓ | | | | | | ✓ | ✓ | ✓ |

| United States Site | HB4 | Williams 82 | Dow 32R280 | Pioneer 93Y82 | Asgrow 3431 | Asgrow 3533 | Asgrow 3731 | Asgrow AG3832 | Asgrow AG3931 | Big cob B38LL | Channel 3806 | DynaGro 36RY38 | Hoffman H38-17CR? | NK S38-S4 | NK S39-U2 | NK S44-K7 | Pioneer 93M94 | Pioneer 93Y84 | Pioneer 93Y92 | Stine 39LD02 |
|--------------------|-----|-------------|------------|---------------|-------------|-------------|-------------|---------------|---------------|---------------|--------------|----------------|-------------------|-----------|-----------|-----------|---------------|---------------|---------------|--------------|
| IA | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | | | | | | | ✓ |
| IL1 | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | | ✓ | | ✓ | | ✓ | | | | | |
| IL2 | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | | | ✓ | | ✓ | | | | | |
| IL3 | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | | | ✓ | | ✓ | | | | | |
| IN | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | | | | | | | | | |
| KS | ✓ | ✓ | ✓ | ✓ | | | | | | | | | | | | | ✓ | | | |
| NE | ✓ | ✓ | ✓ | ✓ | | | | | | ✓ | | | | | | | | | | |
| OHI | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | | | ✓ | | | | | | ✓ | | |
| OH2 | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | | | ✓ | | | | | | ✓ | | |
| OK | ✓ | ✓ | ✓ | ✓ | | | | | | | | | | | | | | | | ✓ |

Table 12-7. Seed characteristics of HB4, the conventional control and commercial reference varieties.

| Variety | Country | Material Type | Phenotype | Source | Origin |
|---|----------------|----------------------|--------------------------------|--------------------------|-------------------------|
| HB4 | AR, US | Test | Test | - | - |
| Williams 82 | AR, US | Control | Conventional | - | - |
| A3731RG, NS 4009 | AR | Reference | Glyphosate-tolerant | Nidera Semillas | Buenos Aires, Argentina |
| Biosoja 4.6 | AR | Reference | Glyphosate-tolerant | Bioceres Semillas | Buenos Aires, Argentina |
| DM 3810, 4210, 4670 | AR | Reference | Glyphosate-tolerant | Don Mario Semillas | Buenos Aires, Argentina |
| FN 3.85 | AR | Reference | Glyphosate-tolerant | FN Semillas | Buenos Aires, Argentina |
| SPS 3900 | AR | Reference | Glyphosate-tolerant | Syngenta | Basel, Switzerland |
| SRM 3410, 3970 | AR | Reference | Glyphosate-tolerant | Sursem | Buenos Aires, Argentina |
| Asgrow 3431, 3533, 3731, AG3832, AG3931 | US | Reference | Glyphosate-tolerant | Asgrow (Monsanto) | St. Louis, Missouri |
| Big Cob B38LL | US | Reference | Glufosinate-tolerant | Big Cob Hybrids | Seward, Nebraska |
| Channel 3806 | US | Reference | Glufosinate/Synchrony-tolerant | Channel Seeds (Monsanto) | St. Louis, Missouri |
| Dow 32R280 | US | Reference | Glyphosate-tolerant | Brodbeck Seeds | Wabash, Indiana |
| DynaGro 36RY38 | US | Reference | Glyphosate-tolerant | Dyna-Gro Seed | Geneseo, Illinois |
| Hoffman H38-12CR2 | US | Reference | Glyphosate-tolerant | Hoffman Seed | Hoffman, Illinois |
| NK S38-S4, S39-U2, S44-K7 | US | Reference | Glyphosate-tolerant | Syngenta | Basel, Switzerland |
| Pioneer 93M94, 93Y82, 93Y84, 93Y92 | US | Reference | Glufosinate/Synchrony-tolerant | Pioneer | Johnston, Iowa |

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IND-00410-5 Soybean

| Variety | Country | Material Type | Phenotype | Source | Origin |
|----------------|----------------|----------------------|----------------------|---------------|---------------|
| Stine 39LD02 | US | Reference | Glufosinate-tolerant | Stine Seed | Adel, Iowa |

Climate and Soil Characteristics of Trial Locations

Climatic conditions can affect the growing potential of soybean. Additionally, because soybean event HB4 is under development for improved yield under a range of typical soybean production areas, the soil conditions as well as the cumulative rainfall and temperatures throughout the season were measured. The objective of the multiple locations was to cover a broad area of commercial soybean production.

Data on general climate including rainfall (Table 12-8) and temperature (Table 12-9) were collected throughout the growing season at all of the trial locations in both regions. A detailed soil characterization protocol was performed for each selected site. Soil cores were collected from all locations at planting dates and at two soil depths (0-30 cm and 30-60 cm) in Argentina and at one soil depth at the US sites. The samples were analyzed for soil physical properties such as soil type and texture; soil moisture, pH, electrical conductivity (EC), and contents of organic matter, nitrate, and phosphorus (Table 12-10).

Table 12-8. Rainfall (in mm) at each trial location in Argentina and the United States.

| Month/Site (Argentina) | A | C | D | F | G | H | I | P | Q | W |
|---------------------------|-------|-------|--------|-------|--------|-------|-------|-------|--------|-------|
| September | 135 | 125 | 132.5 | 65 | 72.5 | 70 | 41.8 | 285 | 123 | 110 |
| October | 321 | 285 | 291 | 245 | 285 | 251.5 | 27 | 295 | 396 | 270.9 |
| November | 73 | 33 | 154.5 | 119 | 98 | 87 | 77 | 245 | 118 | 64 |
| December | 128 | 170 | 155.5 | 0 | 191.5 | 179 | 150 | 180 | 156 | 173 |
| January | 35 | 23 | 25 | 0 | 46 | 32 | 69 | 45 | 45 | 20 |
| February | 87 | 148 | 159 | 0 | 77 | 10 | 33 | 35 | 131 | 160.1 |
| March | 78 | 50 | 42.9 | 72 | 112 | 45 | 98 | 110 | 98.5 | 122.5 |
| April | 106 | 176 | 83 | 30 | 30.5 | 63 | 45 | 85 | 42 | 157 |
| May | 44 | 40 | 32.5 | 28 | 90 | 0 | 10 | 50 | 47 | 94.5 |
| TOTAL | 1007 | 1050 | 1075.9 | 559 | 1002.5 | 737.5 | 550.8 | 1330 | 1156.5 | 1172 |
| Month/Site (US) | OH2 | OH1 | IN | IL3 | IL1 | IA | KS | NE | OK | IL2 |
| June | 82.8 | 127 | 56.1 | 244.9 | 235.7 | 66.3 | 53.1 | 55.4 | 77.2 | 162.3 |
| July | 206.5 | 145.5 | 40.4 | 102.6 | 149.4 | 43.2 | 57.7 | 39.9 | 198.1 | 73.7 |
| August | 44.2 | 17 | 63.8 | 14.7 | 19.3 | 15.5 | 32 | 42.9 | 54.9 | 86.6 |
| September | 82.3 | 58.4 | 48 | 36.3 | 52.1 | 34.5 | 90.4 | 49.3 | 67.8 | 27.2 |
| October | 117.1 | 147.3 | 117.1 | 99.1 | 70.1 | 120.4 | 144 | 115.1 | 57.9 | 65.3 |
| TOTAL | 532.9 | 495.3 | 325.4 | 497.6 | 526.5 | 279.9 | 377.2 | 302.5 | 455.9 | 415 |

Table 12-9. Temperature range at each trial location in Argentina 2012-13 and the US in 2013.

| Site ID | Weather station location | Month | Temperature (°Celsius) | | |
|--------------------|--------------------------|----------|------------------------|------|------|
| | | | Max | Min | Mean |
| A, C, D1, D2 and P | Marcos Juarez, Córdoba | November | 35.4 | 11 | 22.3 |
| | | December | 37.2 | 11.8 | 24.1 |
| | | January | 37 | 8.8 | 24.3 |
| | | February | 36.8 | 8.5 | 20.9 |
| | | March | 33.4 | 6.4 | 19.5 |
| | | April | 29.4 | 2.8 | 18 |
| | | May | 26.8 | -1.5 | 14.3 |
| F | Rufino, Buenos Aires | November | 35.2 | 10.6 | 21.6 |
| | | December | 37.2 | 13.3 | 23.2 |
| | | January | 35.6 | 9.3 | 24.1 |
| | | February | 37.6 | 9.7 | 23.1 |
| | | March | 32.2 | 9.1 | 19.1 |
| | | April | 30.3 | 3.1 | 17.9 |
| | | May | ND ¹ | ND | ND |
| G1, G2, Q1 and Q2 | Pergamino, Buenos Aires | November | 34.5 | 8 | 20.6 |
| | | December | 36.2 | 10 | 22.1 |
| | | January | 37.2 | 5 | 22.2 |
| | | February | 35.8 | 7 | 21.3 |
| | | March | 29.2 | 4 | 18.2 |
| | | April | 28.6 | -0.5 | 16.9 |
| | | May | 24.8 | -1.7 | 12.8 |
| H | Daireaux, Buenos Aires | November | 32.7 | 7.9 | 20.2 |
| | | December | 35.3 | 11.1 | 21.4 |
| | | January | 34.7 | 7.9 | 22.5 |
| | | February | 34.1 | 4 | 21.5 |
| | | March | ND | ND | ND |
| | | April | 30.1 | -0.1 | 16.2 |
| | | May | 24.9 | -5.3 | 10.4 |

| Site ID | Weather station location | Month | Temperature (°Celsius) | | |
|---------|--------------------------|----------|------------------------|------|-------|
| | | | Max | Min | Mean |
| I1, I2 | Balcarce, Buenos Aires | November | 27 | 11 | 17.8 |
| | | December | 31 | 9 | 20.2 |
| | | January | 37.3 | 7.6 | 21.1 |
| | | February | 34.2 | 8.1 | 21.5 |
| | | March | 30.2 | 2.7 | 16.35 |
| | | April | 32 | 2.9 | 17.18 |
| | | May | 23.8 | 0.9 | 11.7 |
| W1, W2 | Nogoyá, Entre Rios | November | 35.5 | 11.4 | 22.4 |
| | | December | 36.1 | 12.2 | 23.5 |
| | | January | 39.3 | 9.1 | 24.9 |
| | | February | 37.9 | 10.2 | 22.8 |
| | | March | 33.7 | 9 | 19.2 |
| | | April | 30.8 | 3.2 | 17.9 |
| | | May | 27.6 | 0.6 | 14.1 |

¹ ND = no data

| Site ID | Weather station location | Month | Temperature (°Fahrenheit) | | |
|---------|--------------------------|-----------|---------------------------|------|------|
| | | | Max | Min | Mean |
| OH2 | Pemberton, OH | June | 88.6 | 46.6 | 70.2 |
| | | July | 91.5 | 50.2 | 72.0 |
| | | August | 87.9 | 43.1 | 69.9 |
| | | September | 96.5 | 38.4 | 65.9 |
| | | October | 82.7 | 26.5 | 54.4 |
| OH1 | Troy, OH | June | 88.9 | 47.8 | 70.3 |
| | | July | 93.5 | 50.2 | 72.8 |
| | | August | 88.4 | 43.2 | 70.2 |
| | | September | 96.8 | 36.3 | 65.7 |
| | | October | 81.5 | 25.4 | 53.8 |
| IN | Ladoga, IN | June | 89.6 | 48.1 | 71.4 |
| | | July | 92.5 | 48.1 | 72.0 |
| | | August | 95.6 | 46.7 | 72.4 |
| | | September | 94.1 | 40.8 | 68.0 |
| | | October | 82.3 | 23.7 | 54.1 |
| IL3 | Effingham, IL | June | 73.0 | 68.0 | 72.5 |
| | | July | 74.0 | 66.0 | 73.2 |

| Site ID | Weather station location | Month | Temperature (°Fahrenheit) | | |
|---------|--------------------------|-----------|---------------------------|------|------|
| | | | Max | Min | Mean |
| | | August | 69.0 | 72.0 | 73.5 |
| | | September | 71.0 | 69.0 | 70.1 |
| | | October | 67.0 | 44.0 | 55.4 |
| IL1 | Highland, IL | June | 92.5 | 53.1 | 74.2 |
| | | July | 94.8 | 50.3 | 74.8 |
| | | August | 97.9 | 52.4 | 75.2 |
| | | September | 96.4 | 44.5 | 71.0 |
| | | October | 85.2 | 22.0 | 54.5 |
| IA | Richland, IA | June | 91.3 | 45.8 | 71.1 |
| | | July | 95.2 | 48.8 | 73.1 |
| | | August | 102.1 | 46.0 | 73.9 |
| | | September | 101.0 | 41.3 | 68.4 |
| | | October | 85.7 | 24.4 | 53.1 |
| KS | Troy, KS | June | 96.0 | 44.0 | 73.4 |
| | | July | 100.0 | 48.0 | 75.9 |
| | | August | 99.0 | 51.0 | 75.2 |
| | | September | 98.0 | 41.0 | 69.8 |
| | | October | 89.0 | 21.0 | 53.8 |
| NE | York, NE | June | 98.7 | 45.6 | 72.4 |
| | | July | 97.8 | 50.0 | 75.3 |
| | | August | 96.0 | 56.4 | 75.0 |
| | | September | 97.8 | 44.5 | 69.8 |
| | | October | 85.3 | 29.4 | 52.2 |
| OK | Hinton, OK | June | 105.0 | 53.0 | 78.7 |
| | | July | 103.0 | 54.0 | 79.8 |
| | | August | 105.0 | 63.0 | 80.5 |
| | | September | 100.0 | 49.0 | 75.2 |
| | | October | 90.0 | 33.0 | 60.6 |
| IL2 | Carlyle, IL | June | 92.5 | 53.1 | 74.2 |
| | | July | 94.8 | 50.3 | 74.8 |
| | | August | 97.9 | 52.4 | 75.2 |
| | | September | 96.4 | 44.5 | 71.0 |
| | | October | 85.2 | 22.0 | 54.5 |

Table 12-10. Soil characterization at two depths (AR) and one depth (US) at each evaluation site.

| Site ID (AR) | Site location | Province | Soil type | Soil Depth | Organic Matter | | Nitrates | Moisture | Phosphorus | pH | Electrical conductance | Texture | |
|--------------|------------------|--------------|-----------------|------------|----------------|-------|----------|----------|------------|-----|------------------------|---------|-------|
| | | | | | % | ppm | | | | | | % | mS/cm |
| A | Monte Buey | Cordoba | Typic Argiudoll | 0-30 | 2.36 | 46 | 21.1 | 140 | 6.2 | 0.1 | 22.5 | 43.5 | 32.5 |
| C | Chilibroste | Cordoba | Udic Argiustol | 0-30 | 1.14 | 29.5 | 20 | 46.5 | 7 | 0.1 | 26.5 | 43.5 | 30.5 |
| D1 | Corral de Bustos | Cordoba | Typic Argiudoll | 0-30 | 1.84 | 128 | 19.7 | 37 | 7 | 0.3 | 12.5 | 56.5 | 33 |
| D2 | Corral de Bustos | Cordoba | Typic Argiudoll | 0-30 | 2.21 | 128.5 | 19.2 | 40 | 6.6 | 0.2 | 13 | 53.5 | 33 |
| F | Villa Saboya | Buenos Aires | Entic Hapludoll | 0-30 | 2.75 | 69 | 20.7 | 19.5 | 6.1 | 0.1 | 23 | 48 | 27 |
| G1 | Carmen de Areco | Buenos Aires | Typic Argiudoll | 0-30 | 1.1 | 38 | 19.8 | 6.5 | 6.7 | 0.1 | 25 | 41.5 | 30 |
| G2 | Carmen de Areco | Buenos Aires | Typic Argiudoll | 0-30 | 2.39 | 81.5 | 15.1 | 27.5 | 5.9 | 0.1 | 23.5 | 44.5 | 28 |
| H | Daireaux | Buenos Aires | Entic Hapludoll | 0-30 | 1.05 | 60 | 17.3 | 10 | 6.6 | 0.1 | 28.5 | 37 | 32.5 |
| I1 | San Agustín | Buenos Aires | Typic Argiudoll | 0-30 | 1.7 | 143 | 13.8 | 19.5 | 6 | 0.2 | 66.5 | 19.5 | 12.5 |
| I2 | San Agustín | Buenos Aires | Typic Argiudoll | 0-30 | 0.8 | 53.5 | 16.5 | 10.5 | 6.7 | 0.1 | 68.5 | 17 | 14 |
| P | Landeta | Santa Fe | Typic Argiudoll | 0-30 | 2.17 | 62 | 14.3 | 11 | 6.5 | 0.1 | 17 | 48.5 | 33 |
| Q1 | Hughes | Santa Fe | Typic Argiudoll | 0-30 | 1.1 | 9.5 | 19.2 | 2.5 | 6.4 | 0.1 | 14 | 35 | 51 |
| Q2 | Hughes | Santa Fe | Typic Argiudoll | 0-30 | 2.44 | 63.5 | 14.5 | 11 | 6.5 | 0.1 | 17 | 48 | 31 |
| | | | | 0-30 | 1.11 | 17 | 19.1 | 3 | 6.7 | 0.1 | 13 | 40.5 | 45.5 |
| | | | | 0-30 | 1.37 | 54 | 11.2 | 93.5 | 6.5 | 0.1 | 86 | 8.5 | 4 |
| | | | | 0-30 | 0.61 | 20 | 9 | 15 | 6.9 | 0 | 87.5 | 7 | 3 |
| | | | | 0-30 | 4.35 | 85.5 | 18.9 | 24 | 6.2 | 0.1 | 24 | 38.5 | 34 |
| | | | | 0-30 | 2.25 | 57.5 | 20.2 | 5 | 6.7 | 0.1 | 22 | 31 | 45 |
| | | | | 0-30 | 5.13 | 84.5 | 20.8 | 29 | 5.9 | 0.1 | 28 | 37 | 29.5 |
| | | | | 0-30 | 3.22 | 47 | 18.9 | 9 | 6.3 | 0.1 | 27 | 35 | 37 |
| | | | | 0-30 | 2.5 | 77 | 18.8 | 37 | 6.1 | 0.1 | 3.5 | 63.5 | 34 |
| | | | | 0-30 | 1.02 | 46 | 20.5 | 10.5 | 7.2 | 0.2 | 3 | 47 | 50 |
| | | | | 0-30 | 3.47 | 53 | 19.8 | 23 | 6.3 | 0.1 | 20.5 | 51.5 | 24.5 |
| | | | | 0-30 | 1.38 | 31 | 21.2 | 5 | 6.6 | 0.1 | 18.5 | 41.5 | 40 |
| | | | | 0-30 | 3.26 | 117 | 9.6 | 13.5 | 6.3 | 0.1 | 19 | 53 | 23 |
| | | | | 0-30 | 1.36 | 56.5 | 19.6 | 3 | 6.7 | 0.1 | 17.5 | 40 | 39 |

| Site ID (AR) | Site location | Province | Soil type | Soil Depth | Organic Matter | | Nitrites | Moisture | Phosphorus | pH | Electrical conductance | Texture | |
|--------------|---------------|------------|-----------------|------------|----------------|------|----------|----------|------------|-----|------------------------|---------|-------|
| | | | | | % | ppm | | | | | | % | mS/cm |
| W1 | Aranguren | Entre Rios | Aquic Argiudoll | 0-30 | 2.44 | 27.5 | 20.3 | 4.5 | 6.7 | 0.1 | 3 | 47 | 50 |
| | | | | 30-60 | 1.48 | 13.5 | 23.8 | 4 | 7.6 | 0.4 | 3 | 37.5 | 60.5 |
| W2 | Aranguren | Entre Rios | Aquic Argiudoll | 0-30 | 2.28 | 26.5 | 20.6 | 4 | 7 | 0.4 | 2 | 41 | 56.5 |
| | | | | 30-60 | 1.36 | 11.5 | 21.8 | 4.5 | 7.6 | 0.3 | 3 | 39 | 59 |

| Site ID (US) | Site location | State | Soil type | Soil Depth | Organic Matter | | Nitrites | Moisture | Phosphorus | pH | Cation Exchange Capacity | Texture | |
|--------------|---------------|-------|--------------------------|------------|----------------|-----|----------|----------|------------|------|--------------------------|---------|-----------|
| | | | | | % | ppm | | | | | | % | Meq/100 g |
| OH2 | Pemberton | OH | Brookston Loam | - | 3 | - | - | - | 6.7 | 15.2 | - | - | - |
| OH1 | Troy | OH | Crosby Silt Loam | - | 2.2 | - | - | - | 6.9 | 10.9 | - | - | - |
| IN | Ladoga | IN | Xenia-Birkbeck Silt Loam | - | 1.5 | - | - | 9.5 | 7.1 | - | - | - | - |
| IL3 | Effingham | IL | Bluford Silt Loam | - | 2 | - | - | 28 | 6 | 6.6 | - | - | - |
| IL1 | Highland | IL | Silt Loam | - | 2.2 | - | - | 181.5 | 6.7 | 9.2 | 20 | 65 | 15 |
| IA | Richland | IA | Silty Clay Loam | - | 3.6 | - | 29.6 | - | 6.8 | 21.6 | 14 | 53 | 33 |
| KS | Troy | KS | Silt Loam | - | 2.7 | - | - | 22.5 | 5.2 | 15.5 | - | - | - |
| NE | York | NE | Silt Loam | - | 2.8 | - | - | 22.5 | 4.8 | 17.5 | - | - | - |
| | | | | - | 2.2 | - | - | 13 | 5.7 | 18 | 20 | 58 | 22 |
| | | | | - | 1.7 | - | - | 10 | 6.4 | 26 | 26 | 48 | 26 |

| Site ID (US) | Site location | State | Soil type | Soil Depth | Organic Matter | Nitrates | Moisture | Phos-phorus | pH | Cation Exchange Capacity | Texture | | |
|--------------|---------------|-------|-----------------|------------|----------------|----------|----------|-------------|------|--------------------------|---------|----|-----|
| | | | | | | | | | | | cm | % | ppm |
| OK | Hinton | OK | Pond Creek Loam | - | 1.1 | - | 12.5 | - | 7.3 | 11.1 | 51 | 34 | 15 |
| IL2 | Carlyle | IL | Silt Loam | - | 2 | - | 26.4 | - | 6.08 | 10 | 15 | 73 | 12 |

Characterization of the Materials for Agronomic and Phenotypic Evaluations

The identities of HB4 and the conventional control Williams 82 seed were verified by polymerase chain reaction analyses.

Data Analyses

Data analysis of the HB4 soybean field trials was a multistep process starting with a close, prudential examination of the raw data to identify/exclude problematic trial location(s) and anomalous data point(s). The next step was to determine the models that will be used for individual and combined location analysis and then implementing those models onto the prepared data set. Commercial reference varieties were also grown at each site and their values used to determine reference ranges for each trait parameter. Any significant differences were assessed to see if the parameter value of the transgenic line was outside of the reference and historical ranges of that parameter and what potential environmental or biological effects might result from that difference.

The first step of the data analysis process was to produce a data set free of errors and minimize statistical noise by excluding trial location(s) with overall poor quality. Poor quality sites were determined by reviewing observation notes from field trials, auditing data from field trial cooperators and examining the trial statistical parameters to see if they fall within acceptable limits. All data was analyzed using a linear mixed model analysis of variance. The locations were analyzed individually as well as grouped by country and a global analysis of all locations combined.

The individual location analysis used the following model:

$$y_{ij} = \mu + g_i + r_j + e_{ij}$$

$$r_j \sim iid N(0, \sigma^2 \text{ Replicate}) \text{ and } e_{ij} \sim iid N(0, \sigma^2 \text{ Plot})$$

- Notation:
 - y_{ij} denotes the unique individual observation
 - μ denotes the overall mean
 - g_i denotes the mean of the i th entry
 - r_j denotes the effect of the j th replicate
 - e_{ij} denotes the effect of the plot assigned the i th entry in the j th replicate (residual error).
 - $\sim iid N(0, \sigma^2)$ indicates random variables that are identically and independently distributed (*iid*) as normal with zero mean and variance σ^2

The combined location analysis used the following model:

$$y_{ijk} = \mu + g_i + l_j + r_{k(j)} + (gl)_{ij} + e_{ijk}$$

$$l_j \sim iid N(0, \sigma^2 \text{ Location}), r_{k(j)} \sim iid N(0, \sigma^2 \text{ Replicate}), (gl)_{ij} \sim iid N(0, \sigma^2 \text{ Location} \sim \text{Entry}),$$

$$\text{and } e_{ijk} \sim iid N(0, \sigma^2 \text{ Plot})$$

- Notation:
 - y_{ijk} denotes the unique individual observation
 - μ denotes the overall mean
 - g_i denotes the mean of the i th entry
 - l_j denotes the effect of the j th location

- $r_{k(j)}$ denotes the effect of the k th replicate within the j th location
- $(gl)_{ij}$ denotes the interaction between the entries and locations
- e_{ijk} denotes the effect of the plot assigned the i th entry in the k th replicate of the j th location (residual error)
- $\sim iid N(0, \sigma^2)$ indicates random variables that are identically and independently distributed (*iid*) as normal with zero mean and variance σ^2

The mixed model analyses of variance were conducted using SAS Proprietary Software version 9.4 (SAS Institute, 2015). All the trials used a randomized complete block design (RCBD). All trials at the Argentina locations used 4 replicates except for 3 locations that had 7 replicates for yield data only. All trial locations in the U.S. used 6 replicates. HB4 was compared to its parental soybean line *cv.* Williams 82 for growth characteristics within each location (*i.e.*, individual-location analyses) and a combined-site analysis in which the data were pooled across countries for comparison. The characteristics analyzed included: days to 50% emergence, early plant stand, initial plant density, seedling vigor, plant height (at V2-V3 and R6-R7), days to 50% flowering, days to 50% maturity, lodging score, shattering score, plant stand at R8, flower color, grain moisture (%), 1000 seed weight at 13% moisture, yield, arthropod counts, pest damage and disease infestation. The level of statistical significance was predetermined to be 5% ($\alpha=0.05$). HB4 was not statistically analyzed against the reference varieties, but the minimum and maximum mean values (reference range) were determined from the reference substances across the various locations. The reference range values were derived from the commercial varieties (Tables 1-1 and 1-2) grown in the same locations for all of the trials.

Once data analysis is complete differences in the data parameters were assessed for their biological significance. Significant differences between HB4 and *cv.* Williams 82 in a single location that were not consistent across many combined locations were not considered likely to have biological significance. Differences in data parameters that were consistent in the combined site analysis needed additional assessment. It was necessary to assess whether the changes were reproducible across multiple sites within the range of natural variability that exists in soybean.

If a data parameter was significantly different between HB4 and *cv.* Williams 82 in the combined location analysis the value for the HB4 was compared to the values in the reference range established by the commercial varieties. If the value for HB4 lay within the reference range, the parameter was not considered biologically significant. If the value for HB4 fell outside the reference range additional assessment was needed to determine whether the variability fell out of reference range with values published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2014).

If the value for HB4 lay within the historical range, the parameter was not considered biologically significant. If the value for HB4 fell outside the historical reference range additional assessment was needed. If the data parameter value for HB4 fell outside of the historical range the effect of the change in the parameter was assessed for its potential to become an environmental or biological hazard and addressed specifically in the text below.

Field Sites and Plot Design

Data were collected from all field sites conducted during the 2012-2013 season in Argentina and throughout the 2013 season in the US. The 25 sites provided a range of typical environmental and agronomic conditions for soybean production in both countries. The trials were managed by cooperators who were familiar with soybean growth and development, production and data collection relevant to evaluating agronomic performance.

Field trials were established at each site following a completely randomized block design. In Argentina, four replications were evaluated at all locations except in Aranguren for the first planting date, Monte Buey, and Carmen de Areco for both planting dates. At these sites, seven (instead of four) replications were evaluated. Only yield data were obtained from the three extra replications at these sites. Plots at the A, C, D1, D2, F, H, P, Q1, Q2, W1, W2 sites consisted of four rows spaced 40 cm apart and five meters in length. Plots at Carmen de Areco (G1 and G2) consisted of four rows spaced 70 cm apart and five meters in length. In sites I1 and I2 the plots consisted of four rows spaced 40 cm apart and six meters in length. All field trials were bordered with plots of 4 rows of a commercial soybean variety.

In the US, six replications were evaluated at all locations for yield. Plots consisted of four rows spaced 2 feet apart and approximately 20 feet in length. Plots at Troy, KS (KS) consisted of four rows spaced 2 feet apart and approximately 15 feet in length. All field trials were bordered with plots of 4 rows of a commercial soybean variety.

Field Phenotypic and Agronomic Observations

The data on seeding rate, depth of planting, and plot size are presented in Table 12-11. The agronomic practices for field preparation, planting were characteristic of those used in each geographical area. Table 12-11 also includes the cropping history for past year (Argentina) and two years (US) in the respective locations. Routine fertilizer and chemical application for control of weeds, insect and diseases were performed at trial sites when required and determined by the trial cooperators (Table 12-12).

Twenty-one agronomic and selected ecological interaction data were collected during the growing season for all entries at each site and are presented in Table 12-13. Disease and insect damage were determined based on the rating scale in Tables 12-21 to 12-24 at Vn, R1, R3 and R5 growth stages in both countries (Fehr and Caviness, 1977).

The results on agronomic differences on growth and development descriptors, and yield components are presented below for a combined-site analysis (Table 12-14). The data on the phenotypic parameters at individual sites in each country are presented in Tables 12-15 to 12-20. The data for plant stand and seedling vigor showed significantly different results when the data are analyzed in a combined site analysis, but not on an individual site level. This can occur because as the sample size increases, the data points and their separate means can become more precise, distinguishing the two different plant lines being compared. The data on yield and its components at individual sites at each country are presented in Table 12-14.

Results from some sites at the Argentina and US multi-site, replicated field trials demonstrated that HB4 soybeans could provide an increased yield opportunity as compared with Williams 82 (Table 12-20). For most measured traits, there were no significant differences between HB4 and Williams 82, including: plant height at V2-V3, days to 50%

flowering, plant height at R6-R7, lodging score, shattering score, flower color, and grain moisture, thereby supporting the conclusion that the *HaHB4v* gene does not confer a selective advantage for HB4 soybean over the parental control Williams 82.

Among the growth descriptors, there was a significant difference between genotypes for days to 50% emergence (Table 12-15), seedling vigor (Table 12-15), initial and final plant stand (plants/square meter; Table 12-16) and days to 50% maturity (Table 12-18) in a global combined analysis. A few individual sites demonstrated significant differences for plant height (table 12-17) and lodging (Table 12-19), however no differences were observed in the combined analyses (Table 12-14). The overall results of growth descriptors are presented in Table 12-14. These traits, taken in light of the overall agronomic, phenotypic and environmental results, are minor and do not support a selective advantage for HB4. Furthermore, the results for these few differences are well within the range of values observed for the commercial soybean varieties.

Several significant differences were observed for the 1000 seed weight measurements at the individual sites and combined analyses. In all instances, the seed weights were higher for Williams 82 than soybean event HB4. It appears while the grain from soybean event HB4 is smaller; it is also compositionally comparable to the control Williams 82 and the reference varieties (Appendix 11). There were also few instances of significant differences in grain yield between HB4 soybean and Williams 82, with a global difference at a p value of 0.062.

Table 12-11. Planting and plot information (Argentinian and US).Argentina Locations

| Sites ID | Argentina Location | Seeding rate (seeds per meter) | Planting depth (cm) | Plot Area (sq ft) | Rows per plot | Crop History | |
|----------|-------------------------------|--------------------------------|---------------------|-------------------|---------------|----------------------|--|
| A | Monte Buey, Córdoba | 17 | 2-3 | 86.1 | 4 | Soybean | |
| C | Chilibroste, Córdoba | 17 | 2-3 | 86.1 | 4 | Corn | |
| D1 | Corral de Bustos, Córdoba | 17 | 2-3 | 86.1 | 4 | Corn | |
| D2 | Corral de Bustos, Córdoba | 17 | 2-3 | 86.1 | 4 | Corn | |
| F | Villa Saboya, Buenos Aires | 17 | 2-3 | 86.1 | 4 | Soybean/Common vetch | |
| G1 | Carmen de Areco, Buenos Aires | 17 | 2-3 | 150.7 | 4 | Soybean | |
| G2 | Carmen de Areco, Buenos Aires | 17 | 2-3 | 150.7 | 4 | Soybean | |
| H | Daireaux, Buenos Aires | 17 | 2-3 | 86.1 | 4 | Corn | |
| I1 | San Agustín, Buenos Aires | 17 | 2-3 | 103.3 | 4 | Soybean | |
| I2 | San Agustín, Buenos Aires | 17 | 2-3 | 103.3 | 4 | Soybean | |
| P | Landeta, Santa Fe | 17 | 2-3 | 86.1 | 4 | Soybean | |
| Q1 | Hughes, Santa Fe | 17 | 2-3 | 86.1 | 4 | Soybean | |
| Q2 | Hughes, Santa Fe | 17 | 2-3 | 86.1 | 4 | Soybean | |
| W1 | Aranguren, Entre Rios | 17 | 2-3 | 86.1 | 4 | Soybean/wheat | |
| W2 | Aranguren, Entre Rios | 17 | 2-3 | 86.1 | 4 | Soybean/wheat | |

US Locations

| Site ID | US Location | Seeding rate (seeds per meter) | Planting depth (cm) | Plot Area (sq ft) | Rows per plot | Crop History | |
|---------|---------------|--------------------------------|---------------------|-------------------|---------------|--------------|-----------------------|
| | | | | | | 2011 | 2012 |
| OH2 | Pemberton, OH | 30 | 3.5 | 106.3 | 4 | Soybean | Corn |
| OH1 | Troy OH | 30 | 3.5 | 106.5 | 4 | Wheat | Corn |
| IN | Ladoga, IN | 26 | 3.175 | 100.1 | 6 | Corn | Corn |
| IL3 | Effingham, IL | 26 | 2.5 | 100.1 | 4 | Soybean | Corn |
| IL1 | Highland, IL | 26 | 3 | 100.1 | 4 | Soybean | Corn |
| IA | Richland, IA | 25 | 1.9 | 98.4 | 4 | Soybean | Field Corn |
| KS | Troy, KS | 26 | 3.175 | 78.2 | 4 | Corn | Soybeans |
| NE | York, NE | 26 | 3.8 | 98.6 | 4 | Soybeans | Soybeans |
| OK | Hinton, OK | 26 | 3.8 | 85.5 | 4 | Wheat | Corn, Soybean, Cotton |
| IL2 | Carlyle, IL | 26 | 3 | 100.1 | 4 | Soybean | Soybeans |

Table 12-12A. Fertilization and chemical inputs for weed and pest control for each site in Argentina.

| Site ID | Chemical | Date | Rate | Units | Purpose |
|---------|--|------------|-------|---------|------------------------|
| A | Glyphosate (Roundup Full II) | 10/15/2012 | 2 | L.ha-1 | Pre-planting herbicide |
| | Diclosulam (Spider) | 10/15/2012 | 0.032 | Kg.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 11/7/2012 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Clethodim (Select) | 12/4/2012 | 1 | L.ha-1 | Herbicide |
| | Alpha-cypermethrin (Fastac) | 12/4/2012 | 0.125 | L.ha-1 | Insecticide |
| | Clethodim (Select) | 2/21/2013 | 1 | L.ha-1 | Herbicide |
| | Rynaxypyr (Coragen) | 2/21/2013 | 0.03 | L.ha-1 | Insecticide |
| C | Glyphosate (Roundup Full II) | 11/1/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| | Diclosulam (Spider) | 11/1/2012 | 0.04 | Kg.ha-1 | Pre-planting herbicide |
| | Fluroxypyr (Starane) | 11/1/2012 | 0.5 | L.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 11/21/2012 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Clethodim (Select) | 12/12/2012 | 1 | L.ha-1 | Herbicide |
| | Imidacloprid (Connect) | 1/24/2013 | 0.6 | L.ha-1 | Insecticide |
| | Clethodim (Select) | 2/5/2013 | 1 | L.ha-1 | Herbicide |
| | Rynaxypyr (Coragen) | 2/5/2013 | 0.03 | L.ha-1 | Insecticide |
| | Clethodim (Select) | 3/22/2013 | 1 | L.ha-1 | Herbicide |
| D1 | Glyphosate (Roundup Full II) | 10/16/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| | Diclosulam (Spider) | 16/10/2012 | 0.04 | Kg.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 11/8/2012 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Clethodim (Centurion) | 11/23/2012 | 0.7 | L.ha-1 | Herbicide |
| | Rynaxypyr (Coragen) | 2/6/2013 | 0.03 | L.ha-1 | Insecticide |
| D2 | Glyphosate (Roundup Full II) | 11/20/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| | Diclosulam (Spider) | 11/20/2012 | 0.04 | Kg.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 12/10/2012 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Rynaxypyr (Coragen) | 2/6/2013 | 0.03 | L.ha-1 | Insecticide |
| | Pyraclostrobin + Epoxiconazole (Opera) | 3/20/2013 | 0.5 | L.ha-1 | Fungicide |
| | Rynaxypyr (Coragen) | 3/20/2013 | 0.035 | L.ha-1 | Insecticide |
| F | Glyphosate (Roundup Full II) | 11/5/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| | Fluroxypyr (Starane) | 11/5/2012 | 0.5 | L.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 12/27/2012 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Chlorpyrifos | 2/1/2013 | 0.5 | L.ha-1 | Insecticide |
| | Methoxyfenozide (Intrepid) | 2/1/2013 | 0.15 | L.ha-1 | Insecticide |
| | Pyraclostrobin + Epoxiconazole (Opera) | 2/23/2013 | 0.5 | L.ha-1 | Fungicide |
| | Rynaxypyr (Coragen) | 2/23/2013 | 0.03 | Kg.ha-1 | Insecticide |
| G1 | Glyphosate (Roundup Full II) | 11/1/2012 | 3 | L.ha-1 | Pre-planting herbicide |

| Site ID | Chemical | Date | Rate | Units | Purpose |
|---------|--|------------|------|---------|------------------------|
| G2 | Glyphosate (Roundup Full II) | 12/14/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| H | Glyphosate (Roundup Full II) | 10/25/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| | Diclosulam (Spider) | 10/25/2012 | 0.04 | Kg.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 11/13/2012 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Chlorpyrifos | 2/1/2013 | 0.8 | L.ha-1 | Insecticide |
| | Paraquat | 4/19/2013 | 2 | L.ha-1 | Desiccant |
| I1 | Glyphosate (Roundup Full II) | 11/15/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| | Imazetapir (Pivot) | 11/15/2012 | 1 | L.ha-1 | Pre-planting herbicide |
| | Diammonium phosphate | 12/3/2012 | 80 | Kg.ha-1 | Fertilizer at planting |
| I2 | Glyphosate (Roundup Full II) | 11/20/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| | Imazetapir (Pivot) | 11/20/2012 | 1 | L.ha-1 | Pre-planting herbicide |
| | Diammonium phosphate | 12/10/2012 | 80 | Kg.ha-1 | Fertilizer at planting |
| P | Glyphosate (Roundup Full II) | 12/20/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 1/14/2013 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Chlorpyrifos 25% | 2/5/2013 | 0.7 | L.ha-1 | Insecticide |
| | Flubendiamide (Belt) | 3/16/2013 | 0.08 | L.ha-1 | Insecticide |
| | Tebuconazole 200 g/l + Azoxystrobin 120 g/l (Custodia) | 3/16/2013 | 0.5 | L.ha-1 | Fungicide |
| | Chlorpyrifos | 3/16/2013 | 0.5 | L.ha-1 | Insecticide |
| Q1 | Glyphosate (Roundup Full II) | 10/20/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| | Diclosulam (Spider) | 10/20/2012 | 0.04 | Kg.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 11/10/2012 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Clethodim (Select) | 1/16/2013 | 1.5 | L.ha-1 | Herbicide |
| | Rynaxypyr (Coragen) | 1/16/2013 | 0.03 | L.ha-1 | Insecticide |
| | Alpha-cypermethrin (Fastac) | 1/16/2013 | 0.12 | L.ha-1 | Insecticide |
| Q2 | Glyphosate (Roundup Full II) | 11/20/2012 | 3 | L.ha-1 | Pre-planting herbicide |
| | Diclosulam (Spider) | 11/20/2012 | 0.04 | Kg.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 12/11/2012 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Clethodim (Select) | 1/16/2013 | 1.5 | L.ha-1 | Herbicide |
| | Rynaxypyr (Coragen) | 1/16/2013 | 0.03 | L.ha-1 | Insecticide |
| | Alpha-cypermethrin (Fastac) | 1/16/2013 | 0.12 | L.ha-1 | Insecticide |
| W1 | Glyphosate | 11/30/2012 | 2 | L.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 11/16/2012 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Clethodim (Centurion) | 12/2/2012 | 0.7 | L.ha-1 | Herbicide |
| | Clethodim (Select) | 2/7/2013 | 1 | L.ha-1 | Herbicide |
| | Clethodim (Select) | 2/22/2013 | 1 | L.ha-1 | Herbicide |
| | Rynaxypyr (Coragen) | 2/22/2013 | 0.03 | L.ha-1 | Insecticide |

| Site ID | Chemical | Date | Rate | Units | Purpose |
|---------|------------------------------|------------|-------|---------|------------------------|
| W2 | Glyphosate (Roundup Full II) | 12/5/2012 | 2 | L.ha-1 | Pre-planting herbicide |
| | Diclosulam (Spider) | 12/5/2012 | 0.032 | Kg.ha-1 | Pre-planting herbicide |
| | Monoammonium phosphate | 12/26/2012 | 110 | Kg.ha-1 | Fertilizer at planting |
| | Clethodim (Select) | 2/22/2013 | 1 | L.ha-1 | Herbicide |
| | Rynaxypyr (Coragen) | 2/22/2013 | 0.03 | L.ha-1 | Insecticide |

Table 12-12B. Fertilization and chemical inputs for weed and pest control for each site in the US.

| Site ID | Chemical | Date | Rate | Units | Purpose |
|---------|----------------------|------------|--------|-------------|-----------------------|
| OH2 | Potash | 11/30/2012 | 90 | Kg.ha-1 | Fertilizer |
| | Buccaneer Plus | 6/9/2013 | 0.94 | l.ha-1 | All Weeds |
| | Sonic | 6/9/2013 | 0.11 | Kg.ha-1 | All Weeds |
| | Glory | 6/9/2013 | 0.11 | Kg.ha-1 | All Weeds |
| | Marvel | 7/16/2013 | 0.15 | l.ha-1 | All Weeds |
| | Tide | 7/16/2013 | 0.3 | l.ha-1 | All Weeds |
| | First Rate | 7/16/2013 | 14 | g.ha-1 | All Weeds |
| OH1 | Diammonium phosphate | 1/23/2013 | 79 | Kg.ha-1 | Fertilizer |
| | Potash | 1/23/2013 | 45 | Kg.ha-1 | Fertilizer |
| | Buccaneer Plus II | 6/9/2013 | 0.94 | l.ha-1 | All Weeds |
| | Sonic | 6/9/2013 | 0.11 | Kg.ha-1 | All Weeds |
| | Glory | 6/9/2013 | 0.11 | Kg.ha-1 | All Weeds |
| | Resource | 6/22/2013 | 0.06 | l.ha-1 | All Weeds |
| | Buccaneer Plus | 6/22/2013 | 0.94 | l.ha-1 | All Weeds |
| | First Rate | 6/22/2013 | 8.5 | g.ha-1 | All Weeds |
| | Marvel | 7/18/2013 | 0.15 | l.ha-1 | All Weeds |
| Tide | 7/18/2013 | 0.23 | l.ha-1 | All Weeds | |
| IN | fertilizer (generic) | 4/25/2013 | 300 | lb.ac-1 | Fertilizer |
| | sonic | 6/6/2013 | 6.3 | oz.ac-1 | All Weeds |
| IL3 | potash | 11/1/2012 | 130 | lb.ac-1 | Fertilizer |
| | lime | 11/1/2012 | 3 | US ton.ac-1 | Fertilizer |
| | Gramoxone SL 2.0 | 7/15/2013 | 2 | pints.ac-1 | burndown weed control |
| | Sonic | 7/15/2013 | 4 | oz.ac-1 | residual weed control |
| | Boundary | 7/15/2013 | 2 | pints.ac-1 | residual weed control |
| | Endigo | 10/3/2013 | 5 | fl.oz.ac-1 | stinkbug control |

| Site ID | Chemical | Date | Rate | Units | Purpose |
|---------|-------------------------------|-----------|------|------------|--|
| IL1 | Sonic | 6/13/2013 | 4 | oz.ac-1 | residual weed control |
| | Boundary | 6/13/2013 | 2 | pints.ac-1 | residual weed control |
| | Touchdown | 6/13/2013 | 32 | oz.ac-1 | burndown weed control |
| IA | Valor XLT | 6/8/2013 | 4 | oz.ac-1 | All Weeds |
| | Dual II Magnum | 6/8/2013 | 1.7 | pints.ac-1 | All Weeds |
| | Flexstar | 7/7/2013 | 1.3 | pints.ac-1 | All Weeds |
| | Select | 7/7/2013 | 6 | oz.ac-1 | All Weeds |
| KS | Fertilizer (NPK = 18-46-0) | 4/25/2013 | 100 | lb.ac-1 | Fertilizer; broadcast soil incorporated |
| | Fertilizer (NPK = 18-46-0) | 4/25/2013 | 100 | lb.ac-1 | Fertilizer; broadcast soil incorporated |
| | Cobra | 7/10/2013 | 12.5 | oz.ac-1 | Waterhemp |
| NE | Boundary | 6/6/2013 | 2.25 | pints.ac-1 | All Weeds |
| | Spartan | 6/6/2013 | 8 | oz.ac-1 | All Weeds |
| OK | Fertilizer (NPK = 25-0-0) | 7/24/2013 | 2 | gal.ac-1 | Fertilizer; sprayed on plants |
| | Prowl H2O | 5/21/2013 | 2 | pints.ac-1 | All Weeds |
| | Cobra | 6/13/2013 | 10 | oz.ac-1 | All Weeds |
| IL2 | Sonic | 6/24/2013 | 4 | oz.ac-1 | residual weed control |
| | Boundary | 6/24/2013 | 2 | pints.ac-1 | residual weed control |
| | Touchdown | 6/24/2013 | 32 | oz.ac-1 | burndown weed control |

Table 12-13. Phenotypic, agronomic and environmental interaction characteristics measured at each site, evaluation timing, trait description and evaluation scale.

| Trait | Stage | Description of data | Scale |
|------------------------------------|-------|--|--|
| Days to 50% emergence (days) | VE-V2 | Days at which 50% of plants were emerging | Date recorded when 50% of plants in each plot were emerging; Days since planting calculated |
| Early plant stand (plants.m-1) | V1-V2 | Total number of plants in the two central rows. Total row length of the two central rows | Emerged plants in 1 meter is calculated by the total number of plants of the two central rows divided by the rows length |
| Initial plant density (Plants.m-2) | V1-V2 | Visual estimate of average plant number per square meter | Number of plants in 1 square meter |
| Seedling vigor (1-9 scale) | V1-V2 | Visual estimate of average vigor of plants in each plot | 1-9 Rating score; 1-3 short plant height, small leaves and very low or low vigor; 4-6 medium plant height, average leaf size and medium vigor; 7-9 tall plant height, large leaves and high vigor |
| Plant height at V2-V3 (cm) | V2-V3 | Average plant height: from soil surface to the last node with a fully developed leaf | Recorded the average height of five representative plants of each plot |
| Disease damage at Vn (1-9 scale) | Vn | Visual estimates of disease incidence | 1-9 Rating score; 0 None, no symptoms observed; 1-3 Mild, very little disease injury (<10%) visible; 4-6 Moderate, noticeable plant tissue damage (10%-30%); 7-9 Severe, significant plant tissue damage (>30%) |
| Disease damage at R1 (0-9 scale) | R1 | Visual estimates of disease incidence | 1-9 Rating score; 0 None, no symptoms observed; 1-3 Mild, very little disease injury (<10%) visible; 4-6 Moderate, noticeable plant tissue damage (10%-30%); 7-9 Severe, significant plant tissue damage (>30%) |
| Insect damage at Vn (0-5) | Vn | Visual estimates of insect damage | 0-5 Rating score; 0 None, no symptoms observed; 1 (1-20%) slight, symptoms not damaging to plant development; 2 (21-40%) and 3 (41-60%) moderate (intermediate between slight and severe); 4 (61-80%) and 5 (>80%) severe (symptoms damaging to plant development) |
| Insect damage at R1 (0-5) | R1 | Visual estimates of insect damage | 0-5 Rating score; 0 None, no symptoms observed; 1 (1-20%) slight, symptoms not damaging to plant development; 2 (21-40%) and 3 (41-60%) moderate (intermediate between slight and severe); 4 (61-80%) and 5 (>80%) severe (symptoms damaging to plant development) |
| Days to 50% flowering (days) | R1 | Days at which 50% of plants were flowering | Date recorded when 50% of plants in each plot were flowering; Days since planting calculated. |
| Disease damage at R3 (0-9 scale) | R3 | Visual estimates of disease incidence | 1-9 Rating score; 0 None, no symptoms observed; 1-3 Mild, very little disease injury (<10%) visible; 4-6 Moderate, noticeable plant tissue damage (10%-30%); 7-9 Severe, significant plant tissue damage (>30%). |

| Trait | Stage | Description of data | Scale |
|----------------------------------|------------|---|--|
| Insect damage at R3 (0-5) | R3 | Visual estimates of insect damage | 0-5 Rating score; 0 None, no symptoms observed; 1 (1-20%) slight, symptoms not damaging to plant development; 2 (21-40%) and 3 (41-60%) moderate (intermediate between slight and severe); 4 (61-80%) and 5 (>80%) severe (symptoms damaging to plant development) |
| Disease damage at R5 (0-9 scale) | R5 | Visual estimates of disease incidence | 1-9 Rating score; 0 None, no symptoms observed; 1-3 Mild, very little disease injury (<10%) visible; 4-6 Moderate, noticeable plant tissue damage (10%-30%); 7-9 Severe, significant plant tissue damage (>30%) |
| Insect damage at R5 (0-5) | R5 | Visual estimates of insect damage | 0-5 Rating score; 0 None, no symptoms observed; 1 (1-20%) slight, symptoms not damaging to plant development; 2 (21-40%) and 3 (41-60%) moderate (intermediate between slight and severe); 4 (61-80%) and 5 (>80%) severe (symptoms damaging to plant development) |
| Plant height at R6-R7 (cm) | R6-R7 | Average plant height: from soil surface to the last node with a fully developed leaf | Recorded the average height of five representative plants of each plot |
| Days to 50% maturity (days) | R8 | Days at which 50% of plants reached maturity | Date recorded when 50% of plants in each plot reach maturity. Days since planting calculated |
| Lodging Score (1-9) | R8 | Visual estimates of lodging severity | 1-9 Rating score; 1 more than 90% of plant lying flat; 2-3 more than 75% of plants lying flat; 4-5 more than 50% of plants lying flat; 6-7 more than 25% of plants lying flat; 8 less than 10% of plants lying flat and 9 all plants erect |
| Shattering Score (1-9) | R8 | Visual estimates of pod shattering | 1-9 Rating score; 1 more than 90% of pods shattered; 2-3 more than 75% of pod shattered; 4-5 more than 50% of pods shattered; 6-7 more than 25% of pod shattered; 8 less than 10% of pod shattered and 9 no pods shattered |
| Plant Stand at R8 (plants.m-2) | R8 | Total number of plants in one square meter | Plants in 1 square meter |
| Grain moisture (%) | At harvest | Grain moisture from bulk yield sample | Moisture (%) of a sample from bulk yield |
| 1000 seed weight (g) | At harvest | Weight in grams for 100 randomly chosen seeds from bulk yield sample | Weight in grams |
| Yield (Kg.ha-1) | At harvest | Weight of grain harvested from the two middle rows of each plot corrected by harvested area and grain moisture (13%). | Recorded the weight in grams of grain harvested from the two middle rows of each plot. Yield (Kg/ha) calculated |

Table 12-14. Global analysis of growth and yield descriptors of HB4 and Williams 82 control.

| Data Type | Data Parameter | Location | HB4 mean | HB4 SE [^] | Williams 82 mean | Williams 82 SE | Ref | Ref | Significance |
|------------------------------------|------------------------------|----------|----------|---------------------|------------------|----------------|-------|-------|--------------|
| | | | | | | | Min | Max | |
| Growth Descriptors | Days to 50% emergence (days) | AR | 6.4 | 0.44 | 6.9 | 0.48 | 5.9 | 8.8 | 0.0323 |
| | | US | 7.5 | 0.54 | 7.6 | 0.53 | 5 | 16 | ns* |
| Early plant stand (plants m-1) | | All | 6.9 | 0.35 | 7.2 | 0.36 | 5 | 16 | 0.0502 |
| | | AR | 20.1 | 0.51 | 16.9 | 0.49 | 16.5 | 21.6 | <.0001 |
| | | US | 19.5 | 0.64 | 16.9 | 0.7 | 15.8 | 23.5 | 0.0029 |
| | | All | 19.8 | 0.4 | 16.9 | 0.4 | 15.8 | 23.5 | <.0001 |
| Initial plant density (Plants m-2) | | AR | 46.2 | 0.8 | 39.1 | 0.97 | 39.5 | 52.3 | <.0001 |
| | | US | 48.7 | 1.61 | 42.3 | 1.76 | 39.6 | 58.8 | 0.0029 |
| | | All | 47.2 | 0.81 | 40.4 | 0.92 | 39.5 | 58.8 | <.0001 |
| | | AR | 6.2 | 0.19 | 5.7 | 0.2 | 4.5 | 6.4 | 0.008 |
| Seedling vigor (1-9 scale) | | US | 6.9 | 0.24 | 6.5 | 0.23 | 4.3 | 8.3 | 0.0384 |
| | | All | 6.5 | 0.15 | 6 | 0.15 | 4.3 | 8.3 | 0.0005 |
| | | AR | 12.5 | 0.61 | 12.4 | 0.61 | 9.4 | 16.8 | ns |
| | | US | 13.5 | 0.54 | 13.5 | 0.56 | 6.5 | 16.8 | ns |
| Plant height at V2-V3 (cm) | | All | 12.9 | 0.43 | 12.8 | 0.43 | 6.5 | 16.8 | ns |
| | | AR | 41.5 | 1.08 | 41.5 | 1.09 | 38.7 | 53.7 | ns |
| | | US | 42.5 | 1.08 | 42.4 | 1.1 | 32 | 71.7 | ns |
| | | All | 41.9 | 0.78 | 41.9 | 0.78 | 32 | 71.7 | ns |
| Plant height at R6-R7 (cm) | | AR | 73.3 | 3.64 | 72.6 | 3.67 | 50.1 | 86.5 | ns |
| | | US | 82.6 | 1.79 | 84.4 | 1.96 | 64.6 | 84.5 | ns |
| | | All | 77 | 2.34 | 77.3 | 2.4 | 50.1 | 86.5 | ns |
| | | AR | 117.8 | 1.58 | 116.8 | 1.52 | 112.8 | 132.2 | 0.0064 |
| Days to 50% maturity (days) | | US | 108.9 | 1.76 | 108.8 | 1.78 | 100.3 | 131 | ns |
| | | All | 114.2 | 1.25 | 113.6 | 1.22 | 100.3 | 132.2 | 0.0095 |
| | | AR | 7.6 | 0.35 | 8.1 | 0.26 | 6.9 | 9 | ns |
| | | US | 8.5 | 0.15 | 8.5 | 0.15 | 8 | 9 | ns |
| Lodging Score (1-9) | | All | 8 | 0.22 | 8.2 | 0.16 | 6.9 | 9 | ns |

| Data Type | Data Parameter | Location | HB4 mean | HB4 SE [^] | Williams 82 mean | Williams 82 SE | Ref Min | Ref Max | Significance |
|---|--------------------|----------|----------|---------------------|------------------|----------------|---------|---------|--------------|
| Shattering Score (1-9) | | AR | 9 | 0.03 | 8.9 | 0.03 | 8.7 | 9 | ns |
| | | US | 8.8 | 0.06 | 8.8 | 0.06 | 8 | 9 | ns |
| | | All | 8.9 | 0.03 | 8.9 | 0.03 | 8 | 9 | ns |
| Plant Stand at R8 (plants m ⁻²) | | AR | 41.4 | 1.18 | 35.8 | 1.02 | 33.7 | 47.5 | 0.0007 |
| | | US | 48.3 | 1.92 | 43.2 | 1.81 | 33.8 | 69.4 | 0.0091 |
| | | All | 44.4 | 1.12 | 39 | 1.04 | 33.7 | 69.4 | <.0001 |
| Flower Color | | AR | . | . | . | . | . | . | . |
| | | US | White | . | White | . | . | . | . |
| | | All | . | . | . | . | . | . | . |
| Yield Data | Grain moisture (%) | AR | 12.1 | 0.25 | 12 | 0.25 | 11 | 14.6 | ns |
| | | US | 12.2 | 0.34 | 12.3 | 0.32 | 7.7 | 13.8 | ns |
| | | All | 12.2 | 0.2 | 12.2 | 0.2 | 7.7 | 14.6 | ns |
| 1000 seed weight (g) @13% moisture | | AR | 179.2 | 2.75 | 191.9 | 2.96 | 147.6 | 178.1 | <.0001 |
| | | US | 148.3 | 4.18 | 157.3 | 3.9 | 95.1 | 204.9 | 0.0005 |
| | | All | 166 | 2.74 | 177.4 | 2.82 | 95.1 | 204.9 | <.0001 |
| Yield @ 13% moisture (Kg ha ⁻¹) | | AR | 3100.1 | 170.76 | 3027.7 | 168.8 | 1911.7 | 5500.8 | ns‡ |
| | | US | 2352.8 | 87.77 | 2218.6 | 81 | 1549.9 | 4291.9 | ns |
| | | All | 2760.4 | 106.08 | 2666.7 | 105.99 | 1549.9 | 5500.8 | ns§ |

[^] SE = standard error

* = not significant

‡ Value determined at 0.1286

§ Value determined at 0.062

Table 12-15. Data results and analysis of days to 50% emergence and seedling vigor of HB4 compared to Williams 82 control in the US and Argentina (AR).

| Country | Site | Days to 50% emergence (days) | | | | Seedling vigor (1-9 scale) | | | | Sig. | Ref. range | Sig. | | | | | |
|-----------------|-------------|------------------------------|------|------|------|----------------------------|------|------|------|------|------------|------|------|------|--------|--------|----|
| | | HB4 | W82 | SE | Mean | Min | Max | HB4 | W82 | | | | SE | Mean | Min | Max | |
| US sites | IA | 6.0 | 6.3 | 0 | 6.3 | 0.25 | 6.0 | 6.8 | 5.0 | 4.8 | 0.41 | 4.8 | 0.25 | 4.0 | 5.5 | ns | |
| | IL1 | 6.0 | 6.0 | 0 | 6.0 | 0 | 6.0 | 6.0 | 8.0 | 7.5 | 0.41 | 7.5 | 0.29 | 7.3 | 8.0 | ns | |
| | IL2 | 12 | 14 | 0.58 | 14 | 0.58 | 11.5 | 13.5 | 8.8 | 7.8 | 0.25 | 7.8 | 0.25 | 8.3 | 9.0 | ns | |
| | IL3 | 5.0 | 5.0 | 0 | 5.0 | 0 | 5.0 | 5.0 | 8.0 | 8.3 | 0.41 | 8.3 | 0.25 | 7.8 | 8.3 | ns | |
| | IN | 5.8 | 6.0 | 0.25 | 6.0 | 0.41 | 5.8 | 6.0 | 7.3 | 6.5 | 0.25 | 6.5 | 0.29 | 6.3 | 6.8 | ns | |
| | KS | 5.0 | 5.0 | 0 | 5.0 | 0 | 5.0 | 5.0 | 7.0 | 7.0 | 0 | 7.0 | 0 | 7.0 | 7.0 | ns | |
| | NE | 6.0 | 6.0 | 0 | 6.0 | 0 | 6.0 | 6.0 | 8.0 | 8.0 | 0 | 8.0 | 0 | 8.0 | 8.0 | ns | |
| | OH1 | 6.5 | 6.8 | 0.29 | 6.8 | 0.25 | 5.8 | 8.5 | 5.8 | 5.8 | 0.63 | 5.8 | 0.25 | 6.5 | 7.5 | ns | |
| | OH2 | 7.0 | 7.3 | 0 | 7.3 | 0.25 | 6.8 | 8.8 | 6.8 | 5.5 | 0.48 | 5.5 | 0.29 | 6.0 | 7.8 | ns | |
| | OK | 15.5 | 14 | 1.19 | 14 | 0 | 14 | 20.8 | 4.5 | 4.3 | 0.5 | 4.3 | 0.48 | 2.0 | 5.5 | ns | |
| | US combined | 7.48 | 7.63 | 0.54 | 7.63 | 0.53 | 5 | 16 | 6.9 | 6.53 | 0.24 | 6.53 | 0.23 | 4.25 | 8.33 | 0.0384 | |
| | AR sites | A | 8.8 | 9.3 | 0.25 | 9.3 | 0.48 | 8.3 | 10 | 6.3 | 5.5 | 0.25 | 5.5 | 0.29 | 4.5 | 5.8 | ns |
| | | C | . | . | . | . | . | . | . | 6.8 | 6.3 | 0.48 | 6.3 | 0.48 | 3.8 | 6.8 | ns |
| | | D1 | 4.0 | 4.0 | 0 | 4.0 | 0 | 4.0 | 4.0 | 6.3 | 6.5 | 0.63 | 6.5 | 0.29 | 4.5 | 7.0 | ns |
| D2 | | 3.5 | 3.8 | 0.5 | 3.8 | 0.25 | 3.8 | 4.8 | 4.8 | 5.0 | 0.48 | 5.0 | 0 | 4.5 | 6.5 | ns | |
| F | | 5.5 | 5.5 | 0.29 | 5.5 | 0.29 | 5.3 | 5.5 | 8.3 | 7.8 | 0.25 | 7.8 | 0.63 | 5.5 | 7.5 | ns | |
| G1 | | . | . | . | . | . | . | . | 7 | 6.3 | 0 | 6.3 | 0.48 | 6.8 | 6.8 | ns | |
| G2 | | . | . | . | . | . | . | . | 6.3 | 5.3 | 0.25 | 5.3 | 0.25 | 5.0 | 5.8 | ns | |
| H | | 4.0 | 4.0 | 0 | 4.0 | 0 | 4.0 | 4.0 | 4.8 | 5 | 1.38 | 5 | 2.04 | 3.5 | 6.3 | ns | |
| I1 | | 8.5 | 10.3 | 0.65 | 10.3 | 0.48 | 9.5 | 11.3 | . | . | . | . | . | . | . | . | |
| I2 | | 8.3 | 9.3 | 0.25 | 9.3 | 0.48 | 8.0 | 8.8 | . | . | . | . | . | . | . | . | |
| P | | 13.3 | 14.3 | 0.25 | 14.3 | 0.48 | 13.3 | 14.5 | 7.3 | 5.5 | 0.48 | 5.5 | 0.29 | 6.0 | 6.8 | ns | |
| Q1 | | 4.0 | 4.0 | 0 | 4.0 | 0 | 4.0 | 4.5 | 6 | 5.3 | 0.41 | 5.3 | 0.25 | 3.3 | 5.5 | ns | |
| Q2 | | 4.0 | 5.75 | 0.41 | 5.75 | 0.25 | 4.5 | 6.8 | 6 | 5.3 | 0 | 5.3 | 0.25 | 4.0 | 5.5 | ns | |
| W1 | | 4.0 | 4.0 | 0 | 4.0 | 0 | 4.0 | 4.0 | 5.5 | 5.3 | 0.29 | 5.3 | 0.48 | 4.5 | 5.3 | ns | |
| W2 | 9.3 | 9.0 | 0.25 | 9.0 | 0 | 9.0 | 9.5 | 5 | 4.8 | 0 | 4.8 | 0.25 | 3.8 | 5.0 | ns | | |
| AR combined | 6.4 | 6.9 | 0.4 | 6.9 | 0.48 | 5.9 | 8.8 | 6.15 | 5.65 | 0.19 | 5.65 | 0.2 | 4.54 | 6.42 | 0.008 | | |
| Global combined | 6.9 | 7.24 | 0.35 | 7.24 | 0.36 | 5 | 16 | 6.48 | 6.03 | 0.15 | 6.03 | 0.15 | 4.25 | 8.33 | 0.0005 | | |

Table 12-16. Data Results and Analysis of initial plant density and plant stand at maturity of HB4 compared to Williams 82 control in the US and Argentina (AR).

| Country | Site | Initial plant density (no. of plants per sq. meter) | | | | | | Plant stand at maturity (R8) (no. of plants per sq. meter) | | | | | | | |
|-----------------|-------------|---|-------|-------|-------|------------|-------|--|-------|-------|-------|------------|-------|--------|--------|
| | | HB4 | | W82 | | Ref. range | | HB4 | | W82 | | Ref. range | | Sig. | |
| | | Mean | SE | Mean | SE | Min | Max | Mean | SE | Mean | SE | Min | Max | | |
| US sites | IA | 59.8 | 1.8 | 56.2 | 2.86 | 42.7 | 58 | 43.39 | 0.66 | 45.47 | 1.73 | 44.53 | 47.97 | ns | |
| | IL1 | 52.8 | 2 | 48.2 | 1.13 | 49.5 | 57.5 | 54.25 | 0.51 | 49.95 | 0.97 | 51.59 | 59.07 | 0.0439 | |
| | IL2 | 29.8 | 1.2 | 18.3 | 1.74 | 24.9 | 34.3 | 28.33 | 2.35 | 17.76 | 0.27 | 24.95 | 35.25 | ns | |
| | IL3 | 47.3 | 1.8 | 41.4 | 2.56 | 41.3 | 51.3 | 49.59 | 2.14 | 48 | 2.13 | 45.13 | 60.6 | ns | |
| | IN | 52.4 | 0.6 | 52.4 | 0.47 | 51.3 | 52.5 | 51.52 | 0.83 | 49.52 | 1.47 | 51.06 | 52.34 | ns | |
| | KS | 53 | 3.1 | 38 | 0.92 | 48.1 | 59 | 69.77 | 4.01 | 53.38 | 1.52 | 60.1 | 73.43 | ns | |
| | NE | 53.7 | 0.8 | 50.5 | 2.23 | 46.3 | 60.5 | 50.2 | 0.62 | 49.23 | 2.07 | 44.93 | 58.04 | ns | |
| | OH1 | 48.3 | 1.2 | 38.2 | 0.79 | 47.5 | 55.5 | 48.31 | 1.54 | 39.41 | 0.35 | 47.08 | 55.34 | ns | |
| | OH2 | 57 | 1.2 | 47.1 | 0.74 | 48 | 60.4 | 57.13 | 1.17 | 48.75 | 0.66 | 49.19 | 61.31 | ns | |
| | OK | 33.2 | 4.8 | 32.9 | 1.02 | 16.5 | 46.7 | 30.78 | 2.97 | 23.96 | 1.83 | 14 | 34.72 | ns | |
| | US combined | | 48.73 | 1.6 | 42.3 | 1.76 | 39.6 | 58.81 | 48.33 | 1.92 | 43.18 | 1.81 | 33.83 | 69.41 | 0.0091 |
| | AR sites | A | 38.7 | 0.6 | 30.4 | 0.67 | 38.2 | 41.5 | 28.58 | 0.63 | 26.61 | 1.44 | 31.12 | 33.23 | ns |
| | | C | 43.8 | 0.9 | 41.6 | 0.47 | 46.1 | 53 | 33.78 | 1.21 | 28.84 | 1.28 | 35.04 | 39.48 | 0.0361 |
| | | D1 | 46.9 | 1.5 | 43.9 | 1.1 | 50.4 | 53.9 | 41.57 | 1.92 | 38.69 | 1.02 | 45.26 | 51.13 | ns |
| | | D2 | 48.7 | 3.2 | 45.1 | 0.38 | 48.7 | 60.4 | 44.88 | 3.07 | 38.69 | 0.82 | 45.51 | 54.72 | ns |
| | | F | 51.1 | 1.2 | 46.9 | 1.09 | 46.7 | 56 | 41.31 | 2.41 | 37.93 | 0.65 | 39.07 | 48.23 | ns |
| G1 | | 38.5 | 2.4 | 33.6 | 2.1 | 35.8 | 38.7 | | | | | | | | |
| G2 | | 41.7 | 1 | 30.8 | 4.11 | 32.3 | 40.8 | | | | | | | | |
| H | | 48.1 | 2.3 | 42.8 | 1.87 | 49.1 | 55.9 | 51.17 | 3.05 | 43.67 | 2.52 | 57.96 | 60.23 | 0.0146 | |
| I1 | | 39 | 3.2 | 30.6 | 1 | 34.8 | 39.3 | 26.35 | 0.57 | 25.51 | 1.81 | 26.24 | 29.16 | ns | |
| I2 | | 42.6 | 4.1 | 24.1 | 1.52 | 26.7 | 44.4 | 40.2 | 4.02 | 23.43 | 1.34 | 25.31 | 41.98 | ns | |
| P | | 51.3 | 0.7 | 43.8 | 1.25 | 49.4 | 53.8 | 41.61 | 1.87 | 37.46 | 2.41 | 42.74 | 50.49 | ns | |
| Q1 | | 51.2 | 0.5 | 47 | 0.49 | 50.6 | 55.8 | 45.14 | 3.88 | 44.16 | 1.69 | 48.64 | 52.21 | ns | |
| Q2 | | 52.7 | 0.6 | 42.7 | 1.64 | 49.7 | 58.4 | 48.6 | 0.91 | 40.69 | 1.55 | 46.81 | 55.63 | 0.0196 | |
| W1 | | 53.6 | 0.9 | 41.8 | 1.49 | 51.1 | 52.1 | 50.81 | 1.05 | 39.71 | 0.64 | 50.68 | 52.24 | 0.0049 | |
| W2 | | 45.1 | 1.3 | 40.6 | 1.22 | 42.5 | 56.1 | 44.18 | 1.4 | 39.69 | 0.51 | 42.89 | 56.16 | ns | |
| AR combined | | | 46.18 | 0.8 | 39.05 | 0.97 | 39.51 | 52.28 | 41.4 | 1.18 | 35.78 | 1.02 | 33.69 | 47.54 | 0.0007 |
| Global combined | | 47.2 | 0.8 | 40.35 | 0.92 | 39.51 | 58.81 | 44.41 | 1.12 | 38.95 | 1.04 | 33.69 | 69.41 | <.0001 | |

Table 12-17. Data results and analysis of plant height of HB4 compared to Williams 82 control in the US and Argentina (AR).

| Country | Site | Plant height at V2-V3 (cm) | | | | | | Plant height at R6-R7 (cm) | | | | | | | | | | |
|-----------------|-------------|----------------------------|-------|-------|-------|------------|-------|----------------------------|--------|-------|-------|-------|-------|------------|-------|-------|----|----|
| | | HB4 | | W82 | | Ref. range | | Sig. | | HB4 | | W82 | | Ref. range | | Sig. | | |
| | | Mean | SE | Mean | SE | Min | Max | | | Mean | SE | Mean | SE | Min | Max | | | |
| US sites | IA | 12.2 | 0.18 | 12.6 | 0.46 | 11.9 | 13.1 | ns | ns | 86.6 | 0.87 | 91.2 | 2.29 | 71.4 | 87.4 | ns | ns | |
| | IL1 | 16 | 0.59 | 16.8 | 0.98 | 14.2 | 17 | ns | ns | 97.8 | 3.02 | 96.3 | 3.59 | 81.9 | 95.6 | ns | ns | |
| | IL2 | 11.7 | 0.54 | 11.1 | 0.41 | 11.7 | 13.7 | ns | ns | 76.6 | 2.71 | 76 | 1.64 | 69.8 | 83.7 | ns | ns | |
| | IL3 | 17.8 | 0.42 | 17.8 | 0.57 | 15.7 | 17.7 | ns | ns | 64.9 | 2.46 | 64.3 | 2.93 | 59.7 | 65.7 | ns | ns | |
| | IN | 13.2 | 0.42 | 12 | 0.72 | 12.3 | 13.3 | ns | ns | 82.9 | 3.48 | 88 | 1.88 | 72.2 | 89.9 | ns | ns | |
| | KS | 7.8 | 0.25 | 7.4 | 0.56 | 6.5 | 7.9 | ns | ns | 86.9 | 3.48 | 92 | 1.88 | 76.2 | 93.9 | ns | ns | |
| | NE | 13.5 | 0.3 | 13.3 | 0.22 | 13.2 | 13.9 | ns | ns | 79.5 | 7.09 | 88.3 | 9.75 | 77 | 85.9 | ns | ns | |
| | OH1 | 15 | 0.74 | 15 | 0.74 | 14.9 | 17.9 | ns | ns | 82.1 | 3.41 | 79.9 | 2.03 | 67.1 | 82.9 | ns | ns | |
| | OH2 | 18.6 | 0.22 | 18.5 | 0.46 | 16.9 | 17.6 | ns | ns | 95.5 | 3.61 | 96.2 | 2.32 | 81.6 | 95.9 | ns | ns | |
| | OK | 9.3 | 0.51 | 10.3 | 0.41 | 8.5 | 10.4 | ns | ns | 73.8 | 2.55 | 72.4 | 2.69 | 57.4 | 70.1 | ns | ns | |
| | US combined | | 13.49 | 0.54 | 13.46 | 0.56 | 6.54 | 16.78 | ns | ns | 82.64 | 1.79 | 84.43 | 1.96 | 64.55 | 84.52 | ns | ns |
| | AR sites | A | 9.5 | 0.17 | 9.5 | 0.4 | 7.7 | 10.2 | ns | ns | 67.3 | 3.13 | 70.7 | 3.46 | 64.6 | 75.4 | ns | ns |
| | | C | 8.6 | 0.2 | 8.4 | 0.16 | 7.52 | 9.3 | ns | ns | 90.6 | 4.45 | 91.3 | 7.32 | 71 | 86.4 | ns | ns |
| | | D1 | 7.2 | 0.37 | 8.6 | 0.63 | 6.7 | 8.6 | ns | ns | 131.7 | 3.21 | 121.1 | 10.61 | 108 | 142.6 | ns | ns |
| D2 | | 10.2 | 0.71 | 9.8 | 0.15 | 9.5 | 11.8 | ns | ns | 103.9 | 4.57 | 108.8 | 5.91 | 79.7 | 96.9 | ns | ns | |
| F | | 15.3 | 0.88 | 13.9 | 0.62 | 11.6 | 15.2 | ns | ns | 69.8 | 5.84 | 71.7 | 3.19 | 53.3 | 70.8 | ns | ns | |
| G1 | | 18.2 | 0.62 | 16.8 | 0.88 | 16.9 | 18.4 | 0.033 | 0.033 | 85.6 | 5.64 | 83 | 4.68 | 74.3 | 82.5 | ns | ns | |
| G2 | | 17.6 | 0.71 | 16.2 | 0.89 | 14.7 | 16.9 | 0.0176 | 0.0176 | 72.1 | 0.95 | 67 | 4.46 | 52.1 | 67.5 | ns | ns | |
| H | | 7.08 | 0.28 | 6.38 | 0.69 | 5.8 | 6.85 | ns | ns | 24.2 | 1.94 | 24.5 | 2.6 | 20.6 | 27.6 | ns | ns | |
| I1 | | 21.8 | 0.5 | 22.8 | 0.29 | 20.8 | 23.2 | ns | ns | 67.3 | 2.14 | 65.8 | 2.59 | 60.3 | 69.3 | ns | ns | |
| I2 | | 17.2 | 0.38 | 17.3 | 0.25 | 15.3 | 16.7 | ns | ns | 73.3 | 4.5 | 66.5 | 1.76 | 60.3 | 71.3 | ns | ns | |
| P | | 7.54 | 0.47 | 7.19 | 0.1 | 7.3 | 7.98 | ns | ns | 48.4 | 3.38 | 41.5 | 4.73 | 35.7 | 44.6 | ns | ns | |
| Q1 | | 16.3 | 0.42 | 17.6 | 0.55 | 15.2 | 17.7 | ns | ns | 87.9 | 4.71 | 92.2 | 8.34 | 88.8 | 97.1 | ns | ns | |
| Q2 | | 9.7 | 0.34 | 10.4 | 0.38 | 8.6 | 10 | 0.006 | 0.006 | 98.3 | 6.56 | 102.7 | 3.15 | 78.7 | 95.2 | ns | ns | |
| W1 | | 10.6 | 0.42 | 10.6 | 0.7 | 9.8 | 10.8 | ns | ns | 41.2 | 2.43 | 42.6 | 0.95 | 34 | 41.1 | ns | ns | |
| W2 | 10.2 | 0.24 | 11 | 0.86 | 9.5 | 11 | ns | ns | 38.4 | 2.16 | 39.7 | 1.14 | 29.9 | 40.9 | ns | ns | | |
| AR combined | | 12.45 | 0.61 | 12.42 | 0.61 | 9.44 | 16.75 | ns | ns | 73.31 | 3.64 | 72.58 | 3.67 | 50.06 | 86.45 | ns | ns | |
| Global combined | | 12.87 | 0.43 | 12.84 | 0.43 | 6.54 | 16.78 | ns | ns | 77.04 | 2.34 | 77.32 | 2.4 | 50.06 | 86.45 | ns | ns | |

Table 12-18. Data results and analysis of days to flowering and days to 50% maturity of HB4 compared to Williams 82 control in the US and Argentina (AR).

| Country | Site | Days to 50% maturity (days) | | | | | | | | | | | | | | | | | |
|-----------------|-------------|-----------------------------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----|
| | | Flowering (days) | | | | | | HB4 | | | | | | W82 | | | | | |
| | | Mean | SE | Mean | SE | Min | Max | Sig. | Mean | SE | Min | Max | Sig. | Mean | SE | Min | Max | Sig. | |
| US sites | IA | 48.5 | 0.29 | 49.25 | 0.95 | 47.5 | 49.75 | ns | 114.0 | 0.41 | 113.8 | 117.8 | ns | 113.8 | 0.25 | 115.5 | 117.8 | ns | |
| | IL1 | 42.0 | 0 | 42.0 | 0 | 42.0 | 42.0 | ns | 106.0 | 0 | 106.3 | 108.5 | ns | 106.3 | 0.25 | 106.8 | 108.5 | ns | |
| | IL2 | 44.25 | 0.25 | 44.75 | 0.25 | 44.0 | 44.5 | ns | 96.8 | 0.75 | 97.5 | 100.0 | ns | 97.5 | 0.65 | 98.5 | 100.0 | ns | |
| | IL3 | 38.0 | 0 | 37.5 | 0.29 | 36.0 | 129 | ns | 88.8 | 0.25 | 87.5 | 185.8 | ns | 87.5 | 0.29 | 92.8 | 185.8 | ns | |
| | IN | 34.0 | 0 | 34.0 | 0 | 34.0 | 34.0 | ns | 107.8 | 1.03 | 107.8 | 116.0 | ns | 107.8 | 1.03 | 109.5 | 116.0 | ns | |
| | KS | 32.0 | 0 | 32.0 | 0 | 32.0 | 32.0 | ns | 111.0 | 0 | 111.0 | 111.0 | ns | 111.0 | 0 | 111 | 111.0 | ns | |
| | NE | 57.0 | 0 | 57.0 | 0 | 56.0 | 57.0 | ns | 105.0 | 0 | 105.5 | 110.0 | ns | 105.5 | 0.5 | 108.5 | 110.0 | ns | |
| | OH1 | 43.0 | 0 | 42.5 | 0.29 | 40.0 | 42.0 | ns | 122.0 | 0.41 | 122.3 | 125.8 | ns | 122.3 | 0.48 | 124.3 | 125.8 | ns | |
| | OH2 | 42.5 | 0.29 | 41.75 | 0.25 | 39.0 | 43.75 | ns | 129.0 | 0 | 129.0 | 132.0 | ns | 129.0 | 0.41 | 129.8 | 132.0 | ns | |
| | OK | 43.25 | 0.48 | 43.25 | 0.48 | 42.25 | 46.25 | ns | 108.5 | 2.06 | 107.8 | 112.0 | ns | 107.8 | 2.14 | 105.3 | 112.0 | ns | |
| | US combined | 42.45 | 1.08 | 42.4 | 1.1 | 32.0 | 71.67 | ns | 108.88 | 1.76 | 108.83 | 131.0 | ns | 108.83 | 1.78 | 100.25 | 131.0 | ns | |
| | AR sites | A | 37.0 | 0 | 37.0 | 0 | 36.0 | 37.5 | ns | 130.5 | 0.5 | 129.5 | 136.3 | ns | 129.5 | 0.29 | 129.0 | 136.3 | ns |
| | | C | 36.75 | 0.25 | 36.75 | 0.25 | 36.75 | 38.25 | ns | 119.0 | 0.41 | 118.3 | 123.3 | ns | 118.3 | 0.25 | 120.5 | 123.3 | ns |
| | | D1 | 40.5 | 0.5 | 39.5 | 0.5 | 39.5 | 42.0 | ns | 126.5 | 1.04 | 124.8 | 135.0 | ns | 124.8 | 0.25 | 125.5 | 135.0 | ns |
| | | D2 | 34.5 | 0.5 | 34.5 | 0.5 | 32.5 | 36.0 | ns | 113.8 | 0.48 | 111.8 | 116.3 | ns | 111.8 | 1.31 | 115.0 | 116.3 | ns |
| | | F | 36.5 | 0.29 | 36.5 | 0.29 | 36.75 | 37.25 | ns | 106.3 | 0.48 | 104.0 | 113.5 | ns | 104.0 | 1.22 | 108.8 | 113.5 | ns |
| G1 | | 44.0 | 0 | 44.0 | 0 | 44.0 | 44.0 | ns | 121.5 | 1.26 | 119.3 | 124.8 | ns | 119.3 | 2.5 | 117.3 | 124.8 | ns | |
| G2 | | 37.0 | 0 | 37.0 | 0 | 36.33 | 42.0 | ns | 110.5 | 0.87 | 109.5 | 119.0 | ns | 109.5 | 0.65 | 115.5 | 119.0 | ns | |
| H | | 39.0 | 0 | 39.0 | 0 | 39.0 | 39.0 | ns | 142.0 | 0 | 140.75 | 139.5 | ns | 140.75 | 1.25 | 138.3 | 139.5 | ns | |
| I1 | | 59.0 | 0 | 59.0 | 0 | 59.0 | 59.0 | ns | 126.0 | 0.41 | 125.5 | 130.8 | ns | 125.5 | 0.5 | 128.3 | 130.8 | ns | |
| I2 | | 63.0 | 0 | 63.0 | 0 | 63.0 | 63.0 | ns | 125.5 | 0.87 | 124.8 | 127.3 | ns | 124.8 | 0.25 | 126.0 | 127.3 | ns | |
| P | | 40.0 | 0 | 41.5 | 0.5 | 41.0 | 42.0 | ns | 94.0 | 0 | 96.3 | 99.3 | ns | 96.3 | 0.75 | 95.5 | 99.3 | ns | |
| Q1 | | 38.0 | 0 | 38.0 | 0 | 37.5 | 39.25 | ns | 126.0 | 0.91 | 123.8 | 130.3 | ns | 123.8 | 0.85 | 127.3 | 130.3 | ns | |
| Q2 | | 35.5 | 0.29 | 35.0 | 0 | 34.5 | 36.0 | ns | 115.3 | 0.25 | 114.0 | 117.3 | ns | 114.0 | 0.58 | 116.0 | 117.3 | ns | |
| W1 | | 38.75 | 0.48 | 39.5 | 0.5 | 38.5 | 40.0 | ns | 110.5 | 0.5 | 110.0 | 122.5 | ns | 110.0 | 0 | 111.8 | 122.5 | ns | |
| W2 | | 40.75 | 0.25 | 40.5 | 0.29 | 40.5 | 43.0 | ns | 99.5 | 0.29 | 100.0 | 107.0 | ns | 100.0 | 0.41 | 101.8 | 107.0 | ns | |
| AR combined | | 41.5 | 1.08 | 41.53 | 1.09 | 38.68 | 53.67 | ns | 117.78 | 1.58 | 116.8 | 132.17 | ns | 116.8 | 1.52 | 112.81 | 132.17 | 0.0064 | |
| Global combined | 41.89 | 0.78 | 41.89 | 0.78 | 32.0 | 71.67 | ns | 114.22 | 1.25 | 113.61 | 132.17 | ns | 113.61 | 1.22 | 100.25 | 132.17 | 0.0095 | | |

Table 12-19. Data results and analysis of lodging score and pod shattering of HB4 compared to Williams 82 control in the US and Argentina (AR).

| Country | Site | Lodging (1-9 scale) | | | | Pod shattering (1-9 scale) | | | | Ref. range | Sig. | Max | | | |
|-----------------|-------------|---------------------|------|------|------|----------------------------|------|------|------|------------|------|-----|-----|----|----|
| | | HB4 | W82 | SE | Mean | HB4 | W82 | SE | Mean | | | | | | |
| US sites | IA | 8.8 | 8.0 | 0.0 | 8.5 | 9.0 | 0.0 | 8.0 | 0.0 | 8.0 | 8.0 | 9.0 | ns | ns | |
| | IL1 | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| | IL2 | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| | IL3 | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| | IN | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| | KS | 8.0 | 8.0 | 0.0 | 8.0 | 8.0 | 0.0 | 8.0 | 0.0 | 8.0 | 8.0 | 8.0 | ns | ns | |
| | NE | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 8.3 | 9.0 | ns | ns | |
| | OH1 | 8.0 | 8.0 | 0.0 | 8.3 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| | OH2 | 6.0 | 6.8 | 0.25 | 7.3 | 8.3 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| | OK | 8.8 | 8.8 | 0.25 | 8.8 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 7.8 | 9.0 | ns | ns | |
| | US combined | 8.45 | 8.45 | 0.15 | 8.0 | 9.0 | 0.06 | 8.8 | 0.06 | 8.8 | 8.0 | 9.0 | ns | ns | |
| | AR sites | A | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns |
| | | C | 8.3 | 7.8 | 0.25 | 6.8 | 8.5 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns |
| | | D1 | 1.5 | 3.3 | 1.25 | 1.3 | 1.8 | 0.25 | 9.0 | 0.25 | 9.0 | 8.8 | 9.0 | ns | ns |
| | | D2 | 2.3 | 3.8 | 0.48 | 2.8 | 4.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns |
| | | F | 9.0 | 9.0 | 0.0 | 8.8 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns |
| G1 | | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| G2 | | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| H | | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 8.3 | 0.29 | 8.3 | 8.0 | 8.8 | ns | ns | |
| I1 | | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| I2 | | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| P | | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| Q1 | | 8.8 | 8.8 | 0.25 | 6.5 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| Q2 | | 3.8 | 7.5 | 0.65 | 7.3 | 8.3 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| W1 | | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 8.8 | 0.25 | 8.8 | 9.0 | 9.0 | ns | ns | |
| W2 | | 9.0 | 9.0 | 0.0 | 9.0 | 9.0 | 0.0 | 9.0 | 0.0 | 9.0 | 9.0 | 9.0 | ns | ns | |
| AR combined | | 7.63 | 8.07 | 0.35 | 6.93 | 9.0 | 0.03 | 8.93 | 0.03 | 8.93 | 8.67 | 9.0 | ns | ns | |
| Global combined | 7.96 | 8.22 | 0.22 | 6.93 | 9.0 | 0.03 | 8.88 | 0.03 | 8.88 | 8.0 | 9.0 | ns | ns | | |

Table 12-20. Data results and analysis of seed weight and yield of HB4 compared to Williams 82 control in the US and Argentina (AR).

| Country | Site | Grain yield (kg/ha) | | | | | | | | | | | | | | |
|-----------------|-------------|-----------------------------------|--------|----------|--------|----------------|--------|--------|----------|--------|----------|--------|----------------|--------|--------|----|
| | | 1000 seed weight (g)@13% moisture | | | | | | | W82 | | | | | | | |
| | | HB4 Mean | SE | W82 Mean | SE | Ref. range Min | Max | Sig. | HB4 Mean | SE | W82 Mean | SE | Ref. range Min | Max | Sig. | |
| US sites | IA | 151.9 | 3.78 | 164.7 | 2.47 | 146.2 | 175.8 | 0.0424 | 2546.6 | 28.33 | 2289.9 | 186.45 | 2717.4 | 3327.3 | ns | |
| | IL1 | 131.5 | 7.36 | 138.4 | 5.89 | 120.9 | 145.3 | ns | 2079.1 | 325.04 | 2274.9 | 346.67 | 2981.2 | 3607.6 | ns | |
| | IL2 | 137.1 | 3.5 | 151.4 | 2.05 | 131.2 | 157 | 0.0462 | 2827.7 | 57.74 | 1990.9 | 315.75 | 2768.5 | 3425.0 | 0.0466 | |
| | IL3 | 137.4 | 1.61 | 146.0 | 1.6 | 136.3 | 151.7 | 0.0261 | 2491.8 | 113.98 | 2380.1 | 111.96 | 2662.6 | 3217.5 | ns | |
| | IN | 196.7 | 3.02 | 193.5 | 3.06 | 192.4 | 199.8 | ns | 2832.4 | 133.3 | 2676.3 | 99.88 | 2862.7 | 3392.5 | ns | |
| | KS | 196.0 | 0.96 | 205.9 | 2.79 | 185.3 | 204.9 | 0.0328 | 3106.6 | 83.86 | 2841.9 | 117.4 | 3350.5 | 4291.9 | ns | |
| | NE | 115.1 | 2.21 | 127.6 | 1.98 | 115.7 | 144.3 | 0.0038 | 1090 | 102.06 | 1163.4 | 163.61 | 1365.3 | 2257.1 | ns | |
| | OH1 | 152.8 | 5.94 | 162.6 | 6.23 | 153.7 | 167.2 | 0.0212 | 2476.7 | 215.79 | 2256.7 | 184.48 | 3033 | 3257.7 | ns | |
| | OH2 | 183.2 | 2.73 | 187.6 | 2.67 | 191.7 | 202.2 | ns | 2431.5 | 127.02 | 2465.9 | 84.72 | 3201.9 | 3545.7 | ns | |
| | OK | 110.8 | 1.99 | 121.0 | 3.09 | 95.1 | 136.9 | 0.0083 | 1645.7 | 184.2 | 1770.5 | 123.2 | 1445.6 | 2244.8 | ns | |
| | US combined | | 148.34 | 4.18 | 157.25 | 3.9 | 95.06 | 204.88 | 0.0005 | 2352.8 | 87.77 | 2218.6 | 81.0 | 1549.9 | 4291.9 | ns |
| | AR sites | A | 174.4 | 5.14 | 179.7 | 5.67 | 148.7 | 174.6 | ns | 4762.2 | 266.45 | 4440.5 | 218.31 | 5572.5 | 6225.3 | ns |
| | | C | 203.1 | 4.58 | 203.9 | 1.18 | 160.6 | 181.9 | ns | 4362.6 | 129.96 | 4762.8 | 56.86 | 5203.5 | 6185.6 | ns |
| D1 | | 197.4 | 1.59 | 215.6 | 1.9 | 176.6 | 205.5 | 0.0026 | 4890.5 | 282.52 | 4853.9 | 264.94 | 5174.2 | 6419.4 | ns | |
| D2 | | 202.1 | 2.02 | 222.3 | 1.29 | 178.6 | 215.3 | 0.0004 | 4659.0 | 118.65 | 4212.7 | 159.49 | 5193.5 | 5596.8 | 0.0287 | |
| F | | 196.1 | 3.64 | 200.0 | 4.43 | 146.5 | 173.4 | ns | 3584.9 | 242.75 | 3368.9 | 55.13 | 3545.5 | 4445.9 | ns | |
| G1 | | 177.3 | 1.72 | 201.8 | 3.89 | 162.2 | 183.1 | 0.0014 | 2549.8 | 75.75 | 2638.6 | 147.38 | 3365.6 | 4213.4 | ns | |
| G2 | | 205.1 | 2.77 | 221.5 | 3.14 | 171.6 | 196.8 | 0.0012 | 2316.6 | 148.69 | 2506.5 | 153.37 | 2624.1 | 3237.5 | ns | |
| H | | 138.9 | 3.3 | 140.0 | 1.53 | 127.8 | 144.5 | ns | 711.8 | 56.73 | 786.1 | 42.45 | 743.91 | 1025.8 | ns | |
| I1 | | 147.8 | 2.28 | 161.8 | 5.08 | 138.2 | 165.3 | ns | 1470.2 | 232.09 | 1329.6 | 198.28 | 1808.1 | 2438.2 | ns | |
| I2 | | 148.0 | 1.13 | 157.4 | 1.92 | 123.9 | 135.7 | 0.0021 | 2453.6 | 128.63 | 2349.9 | 189.7 | 2940.7 | 3181.3 | ns | |
| P | | 183.3 | 5.7 | 204.9 | 4.64 | 166.7 | 180.8 | ns | 2543 | 49.18 | 2096.3 | 266.9 | 2339.3 | 2859.4 | ns | |
| Q1 | | 174.2 | 7.73 | 198.6 | 2.45 | 147.3 | 175.7 | ns | 5450.4 | 302.86 | 5278.7 | 294.35 | 6046.1 | 6837.5 | ns | |
| Q2 | | 207.2 | 2.4 | 214.4 | 3.48 | 162.4 | 194.3 | ns | 4148.1 | 123.17 | 4406.3 | 194.24 | 4953.2 | 5656.3 | ns | |
| W1 | 177.0 | 4.5 | 180.0 | 3.79 | 173.3 | 190.1 | ns | 1763 | 100.07 | 1497.6 | 55.02 | 1848.8 | 2197.0 | 0.0188 | | |
| W2 | 143.7 | 1.2 | 165.5 | 2.97 | 134.1 | 158.9 | 0.0067 | 1591.6 | 121.45 | 1658.2 | 57.69 | 1742 | 2225.1 | ns | | |
| AR combined | | 179.19 | 2.75 | 191.92 | 2.96 | 147.6 | 178.14 | <.0001 | 3100.1 | 170.76 | 3027.7 | 168.8 | 1911.7 | 5500.8 | 0.1286 | |
| Global combined | | 165.97 | 2.74 | 177.38 | 2.82 | 95.06 | 204.88 | <.0001 | 2760.4 | 106.08 | 2666.7 | 105.99 | 1549.9 | 5500.8 | 0.062 | |

HB4 Ecological Evaluations: Disease Susceptibility, Insect Interactions and Abiotic Stress.

In both Argentina and the US, multiple samples were collected and observations made throughout development to obtain information regarding plant pest infestation. In Argentina, pest and beneficial arthropods were sampled at D1, D2, Q1 and W1 sites at four different stages (Vn, R1, R3 and R6) for HB4, Williams 82 and the five commercial varieties. Pest and beneficial arthropods were sampled at R5/R6 stage across all US trial locations. Arthropods were collected using a 1 square meter vertical beat sheet. Plants were shaken against the beat sheet and arthropods collected were stored in plastic flasks with alcohol and sent to a specialized laboratory for identification (INTA, Marcos Juarez, Córdoba for all Argentina locations; UC Davis, CA for all US locations).

HB4 and Williams 82 were observed for diseases and pest damage at Vn, R1/R2, R3/R4, and R5/R6 at all locations in Argentina and at R1/R2, R3/R4, and R5/R6 at all locations in the US. Stress and disease damage observations were collected from each plot using a continuous 0-9 scale (0 being no damage and 9 being severe, Table 12-21). Plant insect damage was similarly observed and rated. Insect damage to plants was evaluated on all entries. Insect susceptibility of the transgenic plants was compared with controls as one element of demonstrating equivalency between the event and the controls. In each plot, 10 random interior plants were observed per plot and the plant damage was rated according to the scale provided (Tables 12-21 to 12-24). Defoliation damage was not collected for any of the US sites at the Vn stage and pod damage was not observed at the Argentina sites at the R1/R2 stage. Additionally, aphid damage, leafhopper and unknown insect damage were not collected at any of the Argentina sites at any of the growth stages. The data collected and analysis for significance at the individual, country and global levels for all plant pests and diseases are summarized in Tables 12-25 to 12-51. The values in the reference variety columns represent the range of all the commercial varieties that were grown in the same trials at each location as listed in Appendix 1. The significant differences are calculated as described under the section above labeled, "Characterization of the Materials for Agronomic and Phenotypic Evaluations."

At all locations in Argentina and in the US, there were no significant differences between genotypes throughout the developmental stages for arthropod counts, plant diseases and plant pests both at the individual site level as well as in the combined global analysis, except for a single combined site analysis for septoria brown spot at one developmental stage. It was not repeated at any other developmental time point and does not confer a change in susceptibility different than traditional soybean varieties. These data demonstrated that soybean event HB4 has equivalent plant-environment interactions as compared to Williams 82 and did not demonstrate improved pest or disease tolerance.

Table 12-21. Category scale for rating disease damage of soybean plants in the field.

| Rating Range | Extent of plant damage |
|---------------------|--|
| 0 | None – no symptoms observed |
| 1-3 | Mild – very little disease injury (<10%) visible |
| 4-6 | Moderate – noticeable plant tissue damage (10% to 30%) |
| 7-9 | Severe – significant plant tissue damage (>30%) |

Table 12-22. Category scale for rating arthropod damage of soybean plants in the field.

| Defoliating arthropods (e.g., corn earworm, bean leaf beetle, Japanese beetle, soybean looper) and Pod feeding arthropods (e.g., corn earworm, bean leaf beetle, stink bug, Lygus bug on reproductive plant parts) | | |
|--|-----------------|---|
| Rating | Defoliation (%) | Severity of plant damage |
| 0 | none | none (no symptoms observed) |
| 1 | 1 – 20 % | slight (symptoms not damaging to plant development) |
| 2 | 21 – 40% | moderate (intermediate between slight and severe) |
| 3 | 41 – 60% | |
| 4 | 61 – 80% | severe (symptoms damaging to plant development) |
| 5 | > 80% | |

Table 12-23. Category scale for rating aphid damage of soybean plants in the field.

| Aphids (e.g., soybean aphid) | | |
|------------------------------|---|---|
| Rating | Aphids present | Severity of plant damage |
| 0 | none | none (no symptoms observed) |
| 1 | 1 – 100 aphids per plant; no leaf puckering | slight (symptoms not damaging to plant development) |
| 2 | 101 – 250 aphids per plant; no leaf puckering | moderate (intermediate between slight and severe) |
| 3 | ≥ 250 aphids per plant with leaf puckering | |
| 4 | ≥ 250 aphids per plant with leaf puckering and leaf yellowing and/or necrosis | severe (symptoms damaging to plant development) |

Table 12-24. Category scale for rating leafhopper damage of soybean plants in the field.

| Leafhoppers (e.g., potato leafhopper) | | |
|--|--|---|
| Rating | Foliar damage (%) | Severity of plant damage |
| 0 | none | none (no symptoms observed) |
| 1 | 1 – 50% of foliage with leaf yellowing; no leaf puckering or leaf margin necrosis | slight (symptoms not damaging to plant development) |
| 2 | 1 – 50% of foliage with leaf yellowing, leaf puckering and/or leaf margin necrosis | moderate (intermediate between slight and severe) |
| 3 | > 50% of foliage with leaf yellowing; no leaf puckering or leaf margin necrosis | |
| 4 | > 50% of foliage with leaf yellowing, leaf puckering, and/or leaf margin necrosis | severe (symptoms damaging to plant development) |

Table 12-25. Data results and analysis of the incidence of septoria brown spot in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | | | | |
|-------------|------|-------------|-------------|------------|------|-------------|-------------|-------------|------|-------------|------|-------------|-------------|------|------|------|
| | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | | | | |
| | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| US sites | IA | 1.25 (0.25) | 1.00 (0.00) | ns | 1.00 | 1.25 | 1.75 (0.25) | 1.25 (0.25) | ns | 1.00 | 1.5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | IL1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 0.50 | 0.75 |
| | IL2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 2.00 (0.00) | 1.50 (0.29) | ns | 1.25 | 2.00 |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 2.00 (0.00) | 2.00 (0.00) | ns | 3.00 | 3.00 |
| | IN | 2.00 (0.00) | 2.00 (0.00) | ns | 2.00 | 2.00 | 2.50 (0.65) | 2.25 (0.48) | ns | 2.25 | 3.00 | 2.50 (0.65) | 2.25 (0.48) | ns | 2.25 | 3.00 |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OHI | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| US combined | | 0.33 (0.11) | 0.30 (0.1) | ns | 0.00 | 2.00 | 0.63 (0.15) | 0.55 (0.13) | ns | 0.00 | 3.00 | 0.85 (0.19) | 0.78 (0.18) | ns | 0.00 | 3.00 |
| AR sites | A | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.50 (0.29) | 0.50 (0.29) | ns | 0.00 | 0.50 |
| | C | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 1.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.75 (0.75) | 0.25 (0.25) | ns | 0.00 | 0.25 |
| | D1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | D2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | F | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.25 | 1.75 (0.25) | 1.50 (0.29) | ns | 2.00 | 2.25 | 1.00 (0.00) | 1.00 (0.00) | ns | 0.00 | 0.75 |
| | G1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |

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| Country | Site | R3/R4 Stage | | | | R5/R6 Stage | | | | | | | | | | |
|---------|-----------------|-------------|-------------|---------|------------|-------------|-------------|-------------|------------|------|------|-------------|-------------|------|------|------|
| | | Entries | Ref. range | Entries | Ref. range | Entries | Ref. range | Entries | Ref. range | | | | | | | |
| | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| | G2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | H | 0.63 (0.24) | 0.25 (0.14) | ns | 0.00 | 0.63 | 2.13 (0.29) | 1.48 (0.06) | ns | 1.40 | 1.48 | 5.00 (0.00) | 5.75 (0.48) | ns | 4.50 | 5.50 |
| | I1 | 1.00 (0.41) | 0.75 (0.25) | ns | 0.25 | 1.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | I2 | 0.25 (0.25) | 0.25 (0.25) | ns | 0.00 | 0.50 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | P | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.25 (0.25) | 1.00 (0.00) | ns | 1.00 | 1.25 |
| | Q1 | 0.75 (0.25) | 0.25 (0.25) | ns | 0.00 | 1.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 2.00 (0.41) | 1.75 (0.48) | ns | 0.88 | 1.75 |
| | Q2 | 1.00 (0.00) | 0.88 (0.13) | ns | 0.50 | 0.75 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 2.00 (0.00) | 2.00 (0.00) | ns | 1.00 | 1.50 |
| | W1 | 0.25 (0.25) | 1.25 (0.95) | ns | 0.00 | 1.75 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 0.75 (0.25) | 1.25 (0.25) | ns | 0.63 | 1.00 |
| | W2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.25 (0.25) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 0.75 (0.25) | ns | 1.00 | 1.00 |
| | AR combined | 0.33 (0.06) | 0.33 (0.08) | ns | 0.06 | 0.54 | 0.54 (0.09) | 0.47 (0.08) | ns | 0.40 | 0.57 | 0.95 (0.18) | 0.95 (0.19) | ns | 0.00 | 1.83 |
| | Global combined | 0.33 (0.06) | 0.32 (0.06) | ns | 0.00 | 2.00 | 0.58 (0.08) | 0.5 (0.07) | ns | 0.00 | 3.00 | 0.91 (0.13) | 0.88 (0.14) | ns | 0.00 | 3.00 |

Table 12-26. Data results and analysis of the incidence of frog eye in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | | R3/R4 Stage | | | | | R5/R6 Stage | | | | |
|-------------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-------------|-----------|-----------|------------|
| | | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range |
| US sites | IA | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | IL1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.50 (0.29) | 0.25 (0.25) | 0.25 (0.25) | ns | 0.00 0.50 | |
| | IL2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.25 (0.25) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.50 | |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | |
| | IN | 1.75 (0.25) | 1.25 (0.48) | ns | 1.50 2.00 | 3.00 (0.00) | 3.00 (0.00) | 3.00 (0.00) | ns | 3.00 3.00 | 4.00 (0.00) | 4.00 (0.00) | 4.00 (0.00) | ns | 4.00 4.00 | |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | OH1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.75 | |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 1.00 (0.00) | 1.25 (0.25) | 1.25 (0.25) | ns | 1.50 2.50 | |
| | OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| US combined | 0.18 (0.09) | 0.13 (0.07) | ns | 0.00 2.00 | 0.30 (0.14) | 0.30 (0.14) | 0.30 (0.14) | ns | 0.00 3.00 | 0.78 (0.19) | 0.75 (0.19) | 0.75 (0.19) | ns | 0.00 4.00 | | |
| AR sites | A | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | C | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | D1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | D2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | F | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | G1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | G2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |

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| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | | |
|-------------|------|-------------|-------------|------|------------|-------------|-------------|----------|-----------|-------------|-------------|-------------|----------|-----------|
| | | Entries | W82 (SE) | Sig. | Ref. range | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range | Entries | HB4 (SE) | W82 (SE) | Sig. |
| | H | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | I1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | I2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | P | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | Q1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | Q2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 |
| | W1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | W2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| AR combined | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.07 (0.03) | 0.07 (0.03) | 0.07 (0.03) | ns | 0.00 0.14 |

Table 12-27. Data results and analysis of the incidence of downy mildew in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | | | R3/R4 Stage | | | | | | R5/R6 Stage | | | | | | | |
|-------------|-------------|-------------|-------------|------------|------|-------------|-------------|-------------|------|---------|-------------|-------------|-------------|-------------|------|-------------|-------------|-------------|------|------------|------|
| | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | |
| | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| US sites | IA | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.25 (0.25) | 1.00 (0.00) | ns | 1.00 | 1.25 | 1.75 (0.25) | 2.25 (0.25) | ns | 1.00 | 1.25 | 1.75 (0.25) | 2.25 (0.25) | ns | 1.00 | 1.25 |
| | IL1 | 0.75 (0.25) | 0.75 (0.25) | ns | 0.00 | 0.25 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.5 | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.5 |
| | IL2 | 1.00 (0.00) | 1.00 (0.00) | ns | 0.00 | 0.25 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | IN | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.75 (0.25) | 1.75 (0.25) | ns | 1.00 | 1.25 |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 2.75 (0.48) | 2.75 (0.25) | ns | 1.00 | 1.5 |
| | OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| US combined | 0.18 (0.06) | 0.18 (0.06) | ns | 0.00 | 0.00 | 0.23 (0.08) | 0.20 (0.06) | ns | 0.00 | 1.00 | 0.63 (0.17) | 0.70 (0.17) | ns | 0.00 | 1.25 | 0.63 (0.17) | 0.70 (0.17) | ns | 0.00 | 1.25 | |
| AR sites | A | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | C | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | D1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | D2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | F | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | G1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | G2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |

Verdeca LLC CBI-DELETED COPY IND-00410-5 Soybean

| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | |
|---------|-----------------|-------------|-------------|------|------------|-------------|-------------|------|------------|-------------|-------------|------|------------|
| | | HB4 (SE) | W82 (SE) | Sig. | Ref. range | HB4 (SE) | W82 (SE) | Sig. | Ref. range | HB4 (SE) | W82 (SE) | Sig. | Ref. range |
| | | | (0.00) | | | (0.00) | | | | (0.00) | | | |
| | H | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | I1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | I2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | P | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | Q1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | Q2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | W1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | W2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | AR combined | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | Global combined | 0.07 (0.03) | 0.07 (0.03) | ns | 0.00 0.00 | 0.09 (0.03) | 0.08 (0.03) | ns | 0.00 1.00 | 0.25 (0.07) | 0.28 (0.08) | ns | 0.00 1.25 |

Table 12-28. Data results and analysis of the incidence of soybean vein necrosis virus in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | | Ref. range | | | | | |
|-------------|-------------|-------------|-------------|----------|------|-------------|-------------|-------------|-------------|-------------|------|-------------|-------------|-------------|-------------|----------|----------|------|------|
| | | Entries | HB4 (SE) | W82 (SE) | Sig. | Min | Max | Entries | HB4 (SE) | W82 (SE) | Sig. | Min | Max | | Entries | HB4 (SE) | W82 (SE) | Sig. | Min |
| US sites | IA | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 1.00 (0.00) | 1.25 (0.25) | ns | 1.00 | 1.00 | 1.75 |
| | IL1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | IL2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | IN | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | OH1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| US combined | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.10 (0.05) | 0.10 (0.05) | 0.13 (0.06) | ns | 0.00 | 0.00 | 1.00 | |
| AR sites | A | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | C | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | D1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | D2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | F | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | G1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | G2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |

Verdeca LLC CBI-DELETED COPY IND-00410-5 Soybean

| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | |
|---------|-----------------|-------------|----------|------|------------|-------------|----------|------|------------|-------------|----------|------|------------|
| | | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range |
| | | HB4 (SE) | | Min | Max | HB4 (SE) | | Min | Max | HB4 (SE) | | Min | Max |
| | | | (0.00) | | | | (0.00) | | | | (0.00) | | |
| | H | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | I1 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | I2 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | P | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | Q1 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | Q2 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | W1 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | W2 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | AR combined | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | Global combined | 0.04 (0.02) | 0.04 | 0.00 | 1.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.04 (0.02) | ns | 0.00 | 1.00 |

Table 12-29. Data results and analysis of the incidence of white mold in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | | | R3/R4 Stage | | | | | | R5/R6 Stage | | | | | | | | |
|-------------|-------------|-------------|-------------|------------|------|-------------|-------------|-------------|------|---------|-------------|-------------|-------------|-------------|------|-------------|-------------|-------------|------|------------|------|------|
| | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | | |
| | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | |
| US sites | IA | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | IL1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | IL2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | IN | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.50 (0.50) | 1.50 (0.65) | ns | 0.50 | 1.00 | 3.75 (0.25) | 3.75 (0.25) | ns | 0.50 | 1.00 | 3.75 (0.25) | 3.75 (0.25) | ns | 0.50 | 1.00 | 3.75 |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | OHI | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | |
| US combined | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.15 (0.08) | 0.15 (0.09) | ns | 0.00 | 0.75 | 0.38 (0.18) | 0.38 (0.18) | ns | 0.00 | 0.75 | 0.38 (0.18) | 0.38 (0.18) | ns | 0.00 | 3.75 | |
| AR sites | A | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | C | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | D1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | D2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | F | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | G1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | G2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |

Verdeca LLC CBI-DELETED COPY IND-00410-5 Soybean

| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | |
|---------|-----------------|-------------|----------|------|-------------|-------------|----------|-------------|------------|-------------|-------------|------|------------|
| | | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range |
| | | HB4 (SE) | Min | Max | HB4 (SE) | Min | Max | HB4 (SE) | Min | Max | HB4 (SE) | Min | Max |
| | | (0.00) | | | (0.00) | | | (0.00) | | | (0.00) | | |
| | H | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 | 0.00 |
| | I1 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 | 0.00 |
| | I2 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 | 0.00 |
| | P | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 | 0.00 |
| | Q1 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 | 0.00 |
| | Q2 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 | 0.00 |
| | W1 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 | 0.00 |
| | W2 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 | 0.00 |
| | AR combined | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 | 0.00 |
| | Global combined | 0.00 (0.00) | 0.00 | 0.00 | 0.06 (0.03) | 0.06 | 0.75 | 0.15 (0.07) | ns | 0.00 | 0.15 (0.08) | 0.15 | 3.75 |

Table 12-30. Data results and analysis of the incidence of rust in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | | Ref. range | | |
|-------------|------|-------------|-------------|------|------------|-------------|-------------|------|------------|-------------|-------------|------|------------|------------|------|------|
| | | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range | | | |
| | | HB4 (SE) | | Min | Max | HB4 (SE) | | Min | Max | HB4 (SE) | | Min | Max | Min | Max | |
| US sites | IA | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | |
| | IL1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | |
| | IL2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | |
| | IN | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | |
| | OH1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 1.00 | 0.00 | 0.00 | 1 | 1.25 |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| US combined | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.10 (0.05) | 0.10 | 0.00 | 0.00 | 0.5 | |
| AR sites | A | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | |
| | C | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | |
| | D1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | |
| | D2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | F | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | G1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | G2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Verdeca LLC CBI-DELETED COPY IND-00410-5 Soybean

| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | | | |
|---------|-----------------|-------------|------|------|------|-------------|----------|------|------|-------------|-------------|----------|------|------|------|
| | | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| | H | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | I1 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | I2 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | P | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | Q1 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | Q2 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | W1 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | W2 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | AR combined | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 |
| | Global combined | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.04 (0.02) | ns | 0.00 | 0.00 | 0.5 |

Table 12-31. Data results and analysis of the incidence of bacterial brown rot in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | | R3/R4 Stage | | | | | R5/R6 Stage | | | | |
|-------------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-------------|-----------|-----------|------------|
| | | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range |
| US sites | IA | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | IL1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | IL2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | IN | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | OH1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.25 | |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | | |
| US combined | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 0.50 | | |
| AR sites | A | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | C | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | D1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | D2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | F | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | G1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | G2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |

Verdeca LLC CBI-DELETED COPY IND-00410-5 Soybean

| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | | | | |
|---------|-----------------|-------------|-------------|---------|------------|-------------|-------------|-------------|------------|-------------|------------|-------------|-------------|------|------|------|
| | | Entries | Ref. range | Entries | Ref. range | Entries | Ref. range | Entries | Ref. range | Entries | Ref. range | Entries | Ref. range | | | |
| | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| | | | (0.00) | | | | | (0.00) | | | | | (0.00) | | | |
| | H | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | I1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | I2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | P | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 |
| | Q1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 0.63 (0.00) | ns | 0.13 | 0.63 |
| | Q2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | W1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.88 (0.43) | 0.00 (0.00) | ns | 0.00 | 0.50 |
| | W2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.25 (0.25) | 0.00 (0.00) | ns | 0.00 | 0.50 |
| | AR combined | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.21 (0.06) | 0.11 (0.04) | ns | 0.00 | 0.40 |
| | Global combined | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.13 (0.04) | 0.07 (0.02) | ns | 0.00 | 0.40 |

Table 12-32. Data results and analysis of the incidence of pod damage in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | | R3/R4 Stage | | | | | R5/R6 Stage | | | | |
|-------------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-------------|-----------|-----------|------------|
| | | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range |
| US sites | IA | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 1.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | |
| | IL1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | IL2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 2.00 (0.00) | 2.00 (0.00) | ns | 2.00 2.00 | |
| | IN | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | OH1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | |
| | OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| US combined | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 1.00 | 0.20 (0.06) | 0.20 (0.06) | 0.20 (0.06) | ns | 0.00 1.00 | 0.60 (0.11) | 0.60 (0.11) | 0.60 (0.11) | ns | 0.00 1.00 | | |
| AR sites | A | . | . | . | . | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | C | . | . | . | . | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | D1 | . | . | . | . | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | D2 | . | . | . | . | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | F | . | . | . | . | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | G1 | . | . | . | . | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |

Verdeca LLC CBI-DELETED COPY IND-00410-5 Soybean

| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | |
|---------|-----------------|-------------|----------|------|------------|-------------|----------|------|------------|-------------|----------|------|------------|
| | | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range |
| | | HB4 (SE) | | Min | Max | HB4 (SE) | | Min | Max | HB4 (SE) | | Min | Max |
| | G2 | . | . | . | . | 0.00 (0.00) | | 0.00 | 0.00 | 0.00 (0.00) | | 0.00 | 0.00 |
| | H | . | . | . | . | 0.00 (0.00) | | 0.00 | 0.00 | 0.00 (0.00) | | 0.00 | 0.00 |
| | I1 | . | . | . | . | 0.00 (0.00) | | 0.00 | 0.00 | 0.00 (0.00) | | 0.00 | 0.00 |
| | I2 | . | . | . | . | 0.00 (0.00) | | 0.00 | 0.00 | 0.00 (0.00) | | 0.00 | 0.00 |
| | P | . | . | . | . | 0.00 (0.00) | | 0.00 | 0.00 | 1.25 (0.25) | | 1.25 | 2.00 |
| | Q1 | . | . | . | . | 0.00 (0.00) | | 0.00 | 0.00 | 0.00 (0.00) | | 0.00 | 0.00 |
| | Q2 | . | . | . | . | 0.00 (0.00) | | 0.00 | 0.00 | 0.00 (0.00) | | 0.00 | 0.00 |
| | W1 | . | . | . | . | 0.00 (0.00) | | 0.00 | 0.00 | 0.75 (0.25) | | 0.50 | 0.63 |
| | W2 | . | . | . | . | 0.00 (0.00) | | 0.00 | 0.00 | 0.63 (0.13) | | 0.50 | 0.88 |
| | AR combined | . | . | . | . | 0.00 (0.00) | | 0.00 | 0.00 | 0.18 (0.05) | | 0.15 | 0.75 |
| | Global combined | 0.10 (0.05) | ns | 0.00 | 1.00 | 0.08 (0.03) | | 0.08 | 1.00 | 0.35 (0.06) | | 0.33 | 1.00 |

Table 12-33. Data results and analysis of the incidence of aphid damage in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | | R3/R4 Stage | | | | | R5/R6 Stage | | | | |
|---|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-------------|-----------|-----------|------------|
| | | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range | Entries | HB4 (SE) | W82 (SE) | Sig. | Ref. range |
| US sites | IA | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | |
| | IL1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.25 0.67 | |
| | IL2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | IN | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.25) | 0.25 (0.25) | 0.00 (0.00) | ns | 0.00 0.25 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | OH1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| | OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | |
| US combined | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.07) | 0.23 (0.07) | 0.20 (0.06) | ns | 0.00 1.00 | 1.00 (0.06) | 0.20 (0.06) | 0.20 (0.06) | ns | 0.00 1.00 | | |
| Aphid damage was not rated in the Argentina Sites | | | | | | | | | | | | | | | | |

Table 12-34. Data results and analysis of the incidence of leafhopper damage in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | R3/R4 Stage | | | | R5/R6 Stage | | | |
|--|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-----------|------------|
| | | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range |
| US sites | IA | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 |
| | IL1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 0.25 1.00 |
| | IL2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.25 0.5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 1.25 (0.25) | 1.25 (0.25) | ns | 1.25 1.50 |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | IN | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | OH1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| US combined | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 1.00 | 0.30 (0.07) | 0.30 (0.07) | ns | 0.00 1.00 | 0.43 (0.09) | 0.43 (0.09) | ns | 0.00 1.00 | |
| Leafhopper damage was not rated in the Argentina Sites | | | | | | | | | | | | | |

Table 12-35. Data results and analysis of the incidence of damage caused by unknown insects in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R1/R2 Stage | | | | | R3/R4 Stage | | | | | R5/R6 Stage | | | | |
|--|-------------|-------------|-------------|------|------|-------------|-------------|-------------|------|------|-------------|-------------|-------------|------|------|------|
| | | Entries | W82 (SE) | Sig. | Min | Max | Entries | W82 (SE) | Sig. | Min | Max | Entries | W82 (SE) | Sig. | Min | Max |
| US sites | IA | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | IL1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | IL2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | IL3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | IN | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 |
| | KS | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OK | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| US combined | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 | 1.00 | |
| Unknown insect damage was not rated in the Argentina Sites | | | | | | | | | | | | | | | | |

Table 12-36A. Data results and analysis of the incidence of defoliation damage at growth stages Vn and R1/R2 in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | Vn Stage | | | | R1/R2 Stage | | | |
|-------------|------|-------------|-------------|------|-------------|-------------|-------------|-----------|------------|
| | | HB4 (SE) | W82 (SE) | Sig. | Ref. range | HB4 (SE) | W82 (SE) | Sig. | Ref. range |
| US sites | IA | . | . | . | . | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 |
| | IL1 | . | . | . | . | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 |
| | IL2 | . | . | . | . | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 |
| | IL3 | . | . | . | . | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 |
| | IN | . | . | . | . | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 |
| | KS | . | . | . | . | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | NE | . | . | . | . | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | OHI | . | . | . | . | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | OH2 | . | . | . | . | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 |
| | OK | . | . | . | . | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| US combined | . | . | . | . | 0.60 (0.08) | 0.60 (0.08) | ns | 0.00 1.00 | |
| AR sites | A | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | C | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | D1 | 1.75 (0.25) | 1.75 (0.25) | ns | 0.75 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | D2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 1.00 |
| | F | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 1.50 (0.29) | 1.25 (0.25) | ns | 1.50 2.00 |
| | G1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |
| | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 0.00 |

| Country | Site | Vn Stage | | | R1/R2 Stage | | | Ref. range | | | |
|---------|-----------------|-------------|-------------|------|-------------|-------|-------------|-------------|----------|------|------|
| | | Entries | W82 (SE) | Sig. | Min | Max | Entries | | W82 (SE) | Sig. | Min |
| | G2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | H | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.75 (0.25) | 0.50 (0.29) | ns | 0.38 | 0.63 |
| | I1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | I2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | P | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 |
| | Q1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | Q2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.75 (0.14) | 0.63 (0.13) | ns | 0.5 | 1.00 |
| | W1 | 0.25 (0.25) | 0.25 (0.25) | ns | 0.00 | 0.75 | 0.50 (0.20) | 0.63 (0.24) | ns | 0.25 | 0.88 |
| | W2 | 0.13 (0.13) | 0.13 (0.13) | ns | 0.00 | 0.13 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.13 |
| | AR combined | 0.14 (0.06) | 0.14 (0.06) | ns | 0.00 | 0.45 | 0.37 (0.07) | 0.33 (0.06) | ns | 0.21 | 0.48 |
| | Global combined | 0.14 (0.06) | 0.14 (0.06) | ns | 0.00 | 60.00 | 0.46 (0.05) | 0.44 (0.05) | ns | 0.00 | 1.00 |

Table 12-36B. Data results and analysis of the incidence of defoliation damage at growth stages R3/R4 and R5/R6 in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | Site | R3/R4 Stage | | | | R5/R6 Stage | | | | | | | | | |
|-------------|------|-------------|-------------|------------|---------|-------------|-------------|-------------|-------------|-------------|------|------|------|------|------|
| | | Entries | | Ref. range | Entries | | Ref. range | | | | | | | | |
| | | HB4 (SE) | W82 (SE) | | Sig. | Min | | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | | |
| US sites | IA | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 | |
| | IL1 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 | |
| | IL2 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 0.75 | 1.00 | 1.00 | 1.00 | |
| | IL3 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 | |
| | IN | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 | |
| | KS | 1.50 (0.65) | 1.25 (0.63) | ns | 0.75 | 1.5 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 |
| | NE | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 |
| | OH1 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 |
| | OH2 | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 |
| | OK | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.25 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 |
| US combined | | 0.95 (0.08) | 0.93 (0.08) | ns | 0.00 | 1.25 | 0.80 (0.06) | 0.80 (0.06) | 0.80 (0.06) | ns | 0.00 | 0.00 | 0.00 | 1.00 | |
| AR sites | A | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 1.00 | 1.00 | 1.00 | 1.00 | |
| | C | 2.00 (0.00) | 2.00 (0.00) | ns | 1.00 | 2.00 | 2.00 (0.00) | 2.00 (0.00) | 2.00 (0.00) | ns | 1.00 | 1.00 | 2.00 | 2.00 | |
| | D1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 |
| | D2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 |
| | F | 0.5 (0.29) | 0.25 (0.25) | ns | 0.00 | 0.50 | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | 1.00 (0.00) | ns | 0.5 | 0.5 | 0.75 | 0.75 |
| | G1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | | | | | | | | | | | | |

Verdeca LLC CBI-DELETED COPY IND-00410-5 Soybean

| Country | Site | R3/R4 Stage | | | | R5/R6 Stage | | | | |
|---------|-----------------|-------------|-------------|------|------------|-------------|-------------|-------------|------------|------|
| | | Entries | W82 (SE) | Sig. | Ref. range | Entries | W82 (SE) | Sig. | Ref. range | |
| | | HB4 (SE) | HB4 (SE) | Max | Min | Max | Min | Max | Min | Max |
| | G2 | 0.00 (0.00) | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | H | 1.25 (0.25) | 1.50 (0.29) | 1.15 | 1.00 | 1.15 | 0.75 (0.25) | 0.75 (0.25) | 0.38 | 1.00 |
| | I1 | 0.00 (0.00) | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 | 0.00 |
| | I2 | 0.00 (0.00) | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 | 0.00 |
| | P | 1.00 (0.00) | 1.00 (0.00) | 1.00 | 1.00 | 1.00 | 1.00 (0.00) | 1.00 (0.00) | 1.00 | 1.00 |
| | Q1 | 0.00 (0.00) | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | 0.00 | 0.00 |
| | Q2 | 1.00 (0.00) | 1.00 (0.00) | 1.00 | 1.00 | 1.00 | 1.75 (0.25) | 1.75 (0.25) | 1.00 | 1.50 |
| | W1 | 1.00 (0.00) | 1.00 (0.00) | 1.00 | 1.00 | 1.00 | 0.75 (0.14) | 0.75 (0.14) | 0.75 | 1.00 |
| | W2 | 0.75 (0.14) | 0.50 (0.20) | 0.38 | 0.00 | 0.38 | 0.88 (0.13) | 0.88 (0.13) | 0.63 | 1.00 |
| | AR combined | 0.57 (0.08) | 0.55 (0.09) | 1.03 | 0.10 | 1.03 | 0.61 (0.09) | 0.61 (0.09) | 0.10 | 1.22 |
| | Global combined | 0.72 (0.06) | 0.70 (0.06) | 1.25 | 0.00 | 1.25 | 0.69 (0.06) | 0.69 (0.06) | 0.00 | 1.22 |

Table 12-37. Combined site analysis of the incidence of all diseases in HB4 and Williams 82 control.

| Disease | Stage | Combined US Sites | | | | | | Combined AR Sites | | | | | | Combined Global Sites | | | | | | | |
|-------------------------------------|-------|-------------------|----------------|------------|------|---------|----------------|-------------------|------|---------|------|----------------|----------------|-----------------------|------|------------|----------------|----------------|-------|------|------|
| | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | | | | | |
| | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| Septoria Brown spot | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1/R2 | 0.33 (0.11) | 0.30 (0.10) | ns | 0.00 | 2.00 | 0.33 (0.06) | 0.33 (0.08) | ns | 0.06 | 0.54 | 0.33 (0.06) | 0.32 (0.06) | ns | 0.00 | 0.54 | 0.33 (0.06) | 0.32 (0.06) | ns | 0.00 | 2.00 |
| | R3/R4 | 0.63 (0.15) | 0.55 (0.13) | ns | 0.00 | 3.00 | 0.54 (0.09) | 0.47 (0.08) | ns | 0.4 | 0.57 | 0.58 (0.08) | 0.50 (0.07) | 0.038 | 0.00 | 0.57 | 0.58 (0.08) | 0.50 (0.07) | 0.038 | 0.00 | 3.00 |
| | R5/R6 | 0.85 (0.19) | 0.78 (0.18) | ns | 0.00 | 3.00 | 0.95 (0.18) | 0.95 (0.19) | ns | 0.00 | 1.83 | 0.91 (0.13) | 0.88 (0.14) | ns | 0.00 | 1.83 | 0.91 (0.13) | 0.88 (0.14) | ns | 0.00 | 3.00 |
| | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| Xanthomonas Campestris ¹ | R1/R2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3/R4 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5/R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1/R2 | 0.18 (0.09) | 0.13 (0.07) | ns | 0.00 | 2.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 2.00 |
| | R3/R4 | 0.30 (0.14) | 0.30 (0.14) | ns | 0.00 | 3.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 3.00 |
| Downy Mildew | R5/R6 | 0.78 (0.19) | 0.75 (0.19) | ns | 0.00 | 4.00 | 0.07 (0.03) | 0.07 (0.03) | ns | 0.00 | 0.14 | 0.35 (0.08) | 0.34 (0.09) | ns | 0.00 | 0.14 | 0.35 (0.08) | 0.34 (0.09) | ns | 0.00 | 4.00 |
| | R1/R2 | 0.18 (0.06) | 0.18 (0.06) | ns | 0.00 | 0.08 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.08 |
| | R3/R4 | 0.23 (0.08) | 0.20 (0.06) | ns | 0.00 | 1.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 1.00 |
| | R5/R6 | 0.63 (0.17) | 0.70 (0.17) | ns | 0.00 | 1.25 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 1.25 |
| | R1/R2 | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 | 1.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 1.00 |
| Soybean vein necrosis virus | R3/R4 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5/R6 | 0.10 (0.00) | 0.13 (0.00) | ns | 0.00 | 1.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 1.00 |
| | R1/R2 | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 | 1.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 1.00 |

¹ No incidence of Xanthomonas campestris was observed at any location during the developmental stages collected; therefore, individual site data were not presented.

| Disease | Stage | Combined US Sites | | | | | | Combined AR Sites | | | | | | Combined Global Sites | | | | | | | |
|---------------------|-------|-------------------|----------------|------------|------|---------|----------------|-------------------|------|---------|------|----------------|----------------|-----------------------|------|------------|----------------|----------------|------|------|------|
| | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | | Entries | | Ref. range | | | | | |
| | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| White mold | R1/R2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3/R4 | 0.15 (0.08) | 0.15 (0.09) | ns | 0.00 | 0.75 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.06 (0.03) | 0.06 (0.04) | ns | 0.00 | 0.00 | 0.06 (0.03) | 0.06 (0.04) | ns | 0.00 | 0.75 |
| | R5/R6 | 0.38 (0.18) | 0.38 (0.18) | ns | 0.00 | 3.75 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.15 (0.07) | 0.15 (0.08) | ns | 0.00 | 0.00 | 0.15 (0.07) | 0.15 (0.08) | ns | 0.00 | 3.75 |
| Rust | R1/R2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3/R4 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5/R6 | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 | 0.50 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.04 (0.02) | 0.04 (0.02) | ns | 0.00 | 0.00 | 0.04 (0.02) | 0.04 (0.02) | ns | 0.00 | 0.50 |
| Bacterial Brown rot | R1/R2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3/R4 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5/R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.21 (0.06) | 0.21 (0.06) | ns | 0.00 | 0.00 | 0.13 (0.04) | 0.13 (0.04) | ns | 0.00 | 0.40 | 0.13 (0.04) | 0.07 (0.02) | ns | 0.00 | 0.40 |

Table 12-38. Combined site analysis of the rate of all insect damage in HB4 and Williams 82 control.

| Damage | Stage | Combined US Sites | | | | | Combined AR Sites | | | | | Combined Global Sites | | | | | | |
|-----------------------|-------|---------------------|----------------|------|-----------------------|---------------------|-------------------|------|-----------------------|---------------------|----------------|-----------------------|-----------------------|---------------------|----------------|------|-----------------------|------|
| | | Entries HB4 (SE) | W82 (SE) | Sig. | Ref. Range Min Max | Entries HB4 (SE) | W82 (SE) | Sig. | Ref. Range Min Max | Entries HB4 (SE) | W82 (SE) | Sig. | Ref. Range Min Max | Entries HB4 (SE) | W82 (SE) | Sig. | Ref. Range Min Max | |
| Defoliation | Vn | . | . | . | . | 0.14 (0.06) | ns | 0.00 | 0.45 | 0.14 (0.06) | ns | 0.00 | 0.45 | 0.14 (0.06) | 0.14 (0.06) | ns | 0.00 | 0.45 |
| | R1/R2 | 0.60 (0.08) | 0.60 (0.08) | ns | 0.00 | 1.00 | 0.37 (0.07) | ns | 0.21 | 0.48 | 0.46 (0.05) | ns | 0.00 | 1.00 | 0.44 (0.05) | ns | 0.00 | 1.00 |
| | R3/R4 | 0.95 (0.08) | 0.93 (0.08) | ns | 0.00 | 1.25 | 0.57 (0.08) | ns | 0.10 | 1.03 | 0.72 (0.06) | ns | 0.00 | 1.25 | 0.70 (0.06) | ns | 0.00 | 1.25 |
| | R5/R6 | 0.80 (0.06) | 0.80 (0.06) | ns | 0.00 | 1.00 | 0.61 (0.09) | ns | 0.10 | 1.22 | 0.69 (0.06) | ns | 0.00 | 1.22 | 0.69 (0.06) | ns | 0.00 | 1.22 |
| | R1/R2 | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 | 1.00 | . | ns | 0.00 | 0.00 | 0.10 (0.05) | ns | 0.00 | 1.00 | 0.10 (0.05) | ns | 0.00 | 1.00 |
| Pod feeding damage | R3/R4 | 0.20 (0.06) | 0.20 (0.06) | ns | 0.00 | 1.00 | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.08 (0.03) | ns | 0.00 | 1.00 | 0.08 (0.03) | ns | 0.00 | 1.00 |
| | R5/R6 | 0.60 (0.11) | 0.60 (0.11) | ns | 0.00 | 1.00 | 0.18 (0.05) | ns | 0.00 | 0.75 | 0.35 (0.06) | ns | 0.00 | 1.00 | 0.33 (0.06) | ns | 0.00 | 1.00 |
| | R1/R2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | . | ns | . | . | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3/R4 | 0.23 (0.07) | 0.20 (0.06) | ns | 0.00 | 1.00 | . | ns | . | . | 0.23 (0.07) | ns | 0.00 | 1.00 | 0.20 (0.06) | ns | 0.00 | 1.00 |
| | R5/R6 | 0.20 (0.06) | 0.20 (0.06) | ns | 0.00 | 1.00 | . | ns | . | . | 0.20 (0.06) | ns | 0.00 | 1.00 | 0.20 (0.06) | ns | 0.00 | 1.00 |
| Leaf hopper damage | R1/R2 | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 | 1.00 | . | ns | . | . | 0.10 (0.05) | ns | 0.00 | 1.00 | 0.10 (0.05) | ns | 0.00 | 1.00 |
| | R3/R4 | 0.30 (0.07) | 0.30 (0.07) | ns | 0.00 | 1.00 | . | ns | . | . | 0.30 (0.07) | ns | 0.00 | 1.00 | 0.30 (0.07) | ns | 0.00 | 1.00 |
| | R5/R6 | 0.43 (0.09) | 0.43 (0.09) | ns | 0.00 | 1.00 | . | ns | . | . | 0.43 (0.09) | ns | 0.00 | 1.00 | 0.43 (0.09) | ns | 0.00 | 1.00 |
| | R1/R2 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | . | ns | . | . | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3/R4 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | . | ns | . | . | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 | 0.00 |
| Unknown | R5/R6 | 0.10 (0.05) | 0.10 (0.05) | ns | 0.00 | 1.00 | . | ns | . | . | 0.10 (0.05) | ns | 0.00 | 1.00 | 0.10 (0.05) | ns | 0.00 | 1.00 |

Table 12-39. Data results and analysis of the incidence of *Frankliniella occidentalis* and *Megascelis* species in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | | Site | Stage | Entries | Ref. range | | | Megalocelis species | | | Ref. range | | | | | |
|-------------|--|------|-------|-------------|-------------|------|------|---------------------|-------------|------|------------|-------------|-------------|------|------|-------|
| | | | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | Country | Site | Stage | Entries | W82 (SE) | Sig. | Min | Max |
| US sites | | IA | R5-R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US sites | IA | R5-R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | NE | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | NE | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | OH1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | OH2 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH2 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| US Combined | | all | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US Combined | all | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR sites | | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | D1 | Vn | 9.00 (2.74) | 4.75 (1.25) | ns | 3.75 | 11.00 |
| | | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | D2 | Vn | 0.00 (0.00) | 0.08 (0.08) | ns | 0.00 | 0.17 |
| | | R1 | | 0.00 (0.00) | 0.17 (0.17) | ns | 0.00 | 0.08 | | | R1 | 4.00 (0.00) | 4.00 (0.00) | ns | 4.00 | 4.00 |
| | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Q1 | Vn | 0.25 (0.25) | 0.25 (0.25) | ns | 0.00 | 1.00 | | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |

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| Frankliniella occidentalis | | Megascelis species | | | | | | | | | | | | |
|----------------------------|-----------------|--------------------|-------------|-------------|------|------|---------|-----------------|-------|-------------|-------------|------|------|------|
| Country | Site | Stage | Entries | Ref. range | | | Country | Site | Stage | Entries | Ref. range | | | |
| | | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | | | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| | | | | (0.00) | | | | | | (0.00) | | | | |
| | W1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | W1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | AR Combined | all | 0.06 (0.06) | 0.13 (0.09) | ns | 0.00 | 0.25 | AR Combined | all | 3.25 (1.13) | 2.25 (0.65) | ns | 0.00 | 5.50 |
| | AR Combined | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | AR Combined | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | Global Combined | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | Global Combined | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |

Table 12-40. Data results and analysis of the incidence of *Diabrotica speciosa* and *Lagria hirta* in HB4 and Williams 82 control in the US and Argentina (AR).

| Diabrotica speciosa | | Lagria hirta | | | | | | | | | | | | | | |
|---------------------|------|--------------|-------------|-----|-------------|------|------------|------|-------------|------|-------------|-----|-------------|------|------------|------|
| Country | Site | Stage | Entries | | W82 (SE) | Sig. | Ref. range | | W82 (SE) | Sig. | Entries | | W82 (SE) | Sig. | Ref. range | |
| | | | HB4 (SE) | Max | | | Min | Max | | | HB4 (SE) | Max | | | Min | Max |
| US sites | IA | R5- R6 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH1 | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH2 | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OK | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| US Combined | | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR sites | D1 | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | D2 | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | Q1 | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | W1 | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.50 | 0.00 (0.00) | ns | 0.50 (0.29) | ns | 0.00 (0.00) | ns | 0.00 | 0.5 |
| | | R1 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR Combined | | all | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 | 0.00 (0.00) | ns | 0.19 (0.14) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 |
| AR Combined | | R5 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.06 (0.06) | ns | 0.00 (0.00) | ns | 0.00 | 0.08 |
| Global Combined | | R5 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 (0.00) | ns | 0.03 (0.03) | ns | 0.00 (0.00) | ns | 0.00 | 0.08 |

Table 12-41. Data results and analysis of the incidence of *Epinotia aporema* and *Loxostege bifidalis* in HB4 and Williams 82 control in the US and Argentina (AR).

| Epinotia aporema | | Loxostege bifidalis | | | | | | | | | | | | | |
|------------------|-------------|---------------------|-------------|-------------|-------------|------|---------|-----------------|-------------|-------------|-------------|-------------|-------------|------|------|
| Country | Site | Stage | Entries | | Ref. range | | Country | Site | Stage | Entries | | Ref. range | | | |
| | | | HB4 (SE) | W82 (SE) | Sig. | Min | | | | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| US sites | IA | R5-R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US sites | IA | R5-R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | NE | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | OH1 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH2 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | OH2 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | OK | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | US Combined | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US Combined | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 |
| AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1 | | 0.67 (0.30) | 0.42 (0.16) | ns | 0.25 | 0.58 | R1 | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R3 | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R5 | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | D2 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | D2 | Vn | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R1 | | R1 | 2.92 (0.28) | 3.58 (0.83) | ns | 4.33 | 6.08 |
| | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R3 | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R5 | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | Q1 | Vn | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R1 | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R3 | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R5 | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | W1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | W1 | Vn | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R1 | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R3 | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R5 | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| AR Combined | all | | 0.25 (0.14) | 0.19 (0.10) | ns | 0.00 | 0.5 | AR Combined | all | all | 0.81 (0.37) | 1.00 (0.47) | ns | 0.00 | 2.17 |
| AR Combined | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | AR Combined | R5 | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| Global Combined | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | Global Combined | R5 | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |

Table 12-42. Data results and analysis of the incidence of *Rachiplusia nu* and *Anticarsia gemmatalis* in HB4 and Williams 82 control in the US and Argentina (AR).

| Rachiplusia nu | | Anticarsia gemmatalis | | | | | | | | | | | | | | |
|----------------|-----------------|-----------------------|-------------|-------------|-------------|------|---------|-----------------|----------|---------|-------------|-------------|-------------|------|------|------|
| Country | Site | Stage | Entries | | Ref. range | | Country | Site | Stage | Entries | | Ref. range | | | | |
| | | | HB4 (SE) | W82 (SE) | Sig. | Min | | | | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max | |
| US sites | IA | R5-R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US sites | IA | R5-R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | NE | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | NE | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | OH1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | OH1 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | OH2 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | OH2 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | OK | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | US Combined | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US Combined | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | AR sites | D1 | Vn | 0.25 (0.25) | 0.25 (0.25) | ns | 0.00 | 0.50 | AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | | R1 | | 0.83 (0.17) | 1.17 (0.17) | ns | 0.42 | 1.50 | R1 | | | 0.25 (0.16) | 0.00 (0.00) | ns | 0.00 | 0.17 |
| | | R3 | | 1.00 (0.24) | 1.17 (0.29) | ns | 0.83 | 1.00 | R3 | | | 2.00 (0.24) | 1.67 (0.24) | ns | 1.50 | 2.08 |
| | | R5 | | 0.08 (0.08) | 0.17 (0.10) | ns | 0.00 | 0.25 | R5 | | | 1.54 (0.21) | 1.17 (0.10) | ns | 1.00 | 1.50 |
| D2 | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | D2 | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| R1 | | | 3.92 (0.5) | 3.50 (0.92) | ns | 2.67 | 7.08 | R1 | | | 0.67 (0.41) | 0.33 (0.14) | ns | 0.33 | 1.00 | |
| R3 | | | 0.08 (0.08) | 0.00 (0.00) | ns | 0.08 | 0.17 | R3 | | | 1.17 (0.17) | 0.67 (0.00) | ns | 0.50 | 1.08 | |
| R5 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R5 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| Q1 | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | Q1 | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 | |
| R1 | | | 1.00 (0.41) | 0.25 (0.25) | ns | 0.5 | 2.00 | R1 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| W1 | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.50 | R3 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.75 | R5 | | | 0.25 (0.25) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | Vn | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | R1 | | 0.50 (0.29) | 0.75 (0.48) | ns | 0.25 | 0.75 | R1 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | R3 | | 0.25 (0.25) | 0.00 (0.00) | ns | 0.00 | 0.25 | R3 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 | |
| | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | R5 | | | 0.89 (0.25) | 0.98 (0.34) | ns | 0.33 | 6.50 | |
| | AR Combined | all | 2.13 (0.42) | 2.06 (0.49) | ns | 0.75 | 3.67 | AR Combined | all | | 2.06 (0.42) | 1.81 (0.38) | ns | 1.58 | 6.50 | |
| | AR Combined | R5 | 0.02 (0.02) | 0.04 (0.03) | ns | 0.00 | 0.23 | AR Combined | R5 | | 1.75 (0.63) | 2.75 (0.75) | ns | 2.50 | 6.50 | |
| | Global Combined | R5 | 0.01 (0.01) | 0.02 (0.01) | ns | 0.00 | 0.23 | Global Combined | R5 | | 0.39 (0.13) | 0.44 (0.17) | ns | 0.00 | 6.50 | |

Table 12-43. Data results and analysis of the incidence of *Spilosoma virginica* and *Helicoverpa geltopoeon* in HB4 and Williams 82 control in the US and Argentina (AR).

| Country | | Site | Stage | Entries | | Ref. range | | Country | | Site | Stage | Entries | | Ref. range | | | |
|----------------------------|-----|-------|-------|-------------|-------------|------------|------|---------|-----------------|------|-------|----------|-------------|-------------|-----|------|------|
| | | | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | | | | HB4 (SE) | W82 (SE) | Sig. | Min | Max | |
| Helicoverpa geltopoeon | | | | | | | | | | | | | | | | | |
| <i>Spilosoma virginica</i> | | | | | | | | | | | | | | | | | |
| US sites | IA | R5-R6 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US sites | IA | R5-R6 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | NE | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH1 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH1 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH2 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH2 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OK | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OK | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| US Combined | | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US Combined | | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR sites | D1 | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | AR sites | D1 | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1 | | | 0.42 (0.42) | 0.17 (0.17) | ns | 0.00 | 0.17 | | | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.08 |
| | R3 | | | 0.25 (0.16) | 0.33 (0.19) | ns | 0.08 | 0.42 | | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5 | | | 0.08 (0.08) | 0.08 (0.08) | ns | 0.00 | 0.21 | | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | D2 | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | D2 | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.08 |
| | R1 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | | 0.17 (0.17) | 0.17 (0.10) | ns | 0.00 | 0.33 |
| | R3 | | | 0.00 (0.00) | 0.08 (0.08) | ns | 0.00 | 0.17 | | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | Q1 | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | Q1 | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | R5 | | | 0.25 (0.25) | 0.00 (0.00) | ns | 0.00 | 0.75 | | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | W1 | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | W1 | Vn | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | | 0.25 (0.25) | 0.75 (0.48) | ns | 0.00 | 0.75 |
| | R3 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | R5 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| AR Combined | all | | | 0.31 (0.15) | 0.25 (0.14) | ns | 0.00 | 0.00 | AR Combined | all | | | 0.13 (0.09) | 0.31 (0.15) | ns | 0.06 | 0.50 |
| AR Combined | R5 | | | 0.08 (0.06) | 0.02 (0.02) | ns | 0.00 | 0.00 | AR Combined | R5 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.06 |
| Global Combined | R5 | | | 0.04 (0.03) | 0.01 (0.01) | ns | 0.00 | 0.00 | Global Combined | R5 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.06 |

Table 12-44. Data results and analysis of the incidence of *Spodoptera frugiperda* and *Nezara viridula* in HB4 and Williams 82 control in the US and Argentina (AR).

| Spodoptera frugiperda | | Nezara viridula | | | | | | | | | | | | | | | |
|-----------------------|------|-----------------|-------------|-------------|-------------|------|------------|------|-----------------|------|-------|-------------|-------------|-------------|------|------------|------|
| Country | Site | Stage | Entries | | W82 (SE) | Sig. | Ref. range | | Country | Site | Stage | Entries | | W82 (SE) | Sig. | Ref. range | |
| | | | HB4 (SE) | Max | | | Min | Max | | | | HB4 (SE) | Max | | | Min | Max |
| US sites | IA | R5-R6 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US sites | IA | R5-R6 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | NE | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH1 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH1 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH2 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH2 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OK | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OK | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| US Combined | | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US Combined | | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR sites | D1 | Vn | 0.50 (0.50) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1 | | 0.33 (0.24) | 0.58 (0.21) | 0.00 (0.00) | ns | 0.08 | 0.67 | | R1 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.33 |
| | R3 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R3 | | 1.17 (0.22) | 1.00 (0.14) | 0.00 (0.00) | ns | 0.92 | 1.33 |
| | R5 | | 0.08 (0.08) | 0.17 (0.10) | 0.00 (0.00) | ns | 0.17 | 0.33 | | R5 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | D2 | Vn | 0.00 (0.00) | 0.08 (0.08) | 0.00 (0.00) | ns | 0.00 | 0.25 | | D2 | Vn | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R1 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3 | | 0.08 (0.08) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.17 | | R3 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R5 | | 0.17 (0.10) | 0.08 (0.08) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.17 |
| | R1 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R1 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R3 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R5 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | W1 | Vn | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | W1 | Vn | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R1 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R1 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R3 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5 | | 0.02 (0.02) | 0.04 (0.03) | 0.00 (0.00) | ns | 0.00 | 0.17 | | R5 | | 0.04 (0.03) | 0.02 (0.02) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR Combined | all | | 0.38 (0.26) | 0.31 (0.12) | 0.00 (0.00) | ns | 0.00 | 0.75 | AR Combined | all | | 0.44 (0.16) | 0.44 (0.16) | 0.00 (0.00) | ns | 0.00 | 1.00 |
| AR Combined | R5 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | AR Combined | R5 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| Global Combined | R5 | | 0.01 (0.01) | 0.02 (0.01) | 0.00 (0.00) | ns | 0.00 | 0.17 | Global Combined | R5 | | 0.02 (0.01) | 0.01 (0.01) | 0.00 (0.00) | ns | 0.00 | 0.10 |

Table 12-45. Data results and analysis of the incidence of *Piezodorus guildinii* and *Edessa mediotabunda* in HB4 and Williams 82 control in the US and Argentina (AR).

| Piezodorus guildinii | | Edessa mediotabunda | | | | | | | | | | | | | | | | |
|----------------------|----------|---------------------|-------------|-------------|-------------|-------------|------------|--------------------|-------------|----------|-------------|-------------|-------------|-------------|-------------|------------|------|------|
| Country | Site | Stage | Entries | | W82 (SE) | Sig. | Ref. range | | Country | Site | Stage | Entries | | W82 (SE) | Sig. | Ref. range | | |
| | | | HB4 (SE) | Max | | | Min | Max | | | | HB4 (SE) | Max | | | Min | Max | |
| US sites | IA | R5- R6 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | US sites | IA | R5- R6 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | NE | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | NE | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | OH1 | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH1 | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | OH2 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH2 | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | OK | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | OK | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | US Combined | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | US Combined | | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | AR sites | D1 | Vn | 0.00 (0.00) | ns | 0.50 (0.50) | ns | 0.00 | 0.25 | AR sites | D1 | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | | | R1 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.08 (0.08) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | | R3 | | 0.17 (0.10) | ns | 0.00 (0.00) | ns | 0.08 | 0.25 | | | R3 | 0.00 (0.00) | ns | 0.25 (0.08) | ns | 0.08 | 0.58 |
| | | | R5 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.08 (0.08) | ns | 0.00 (0.00) | ns | 0.00 | 0.08 |
| D2 | | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | D2 | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R1 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| R3 | | | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.17 | | | R3 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R5 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.08 | | | R5 | 0.08 (0.08) | ns | 0.00 (0.00) | ns | 0.08 | 0.33 | |
| Q1 | | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | Q1 | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R1 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.75 (0.25) | ns | 0.25 (0.25) | ns | 0.5 | 2.25 | |
| R3 | | 0.25 (0.25) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | 0.25 (0.25) | ns | 0.00 (0.00) | ns | 0.25 | 0.75 | | |
| | R5 | 0.25 (0.25) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 | | | R5 | 0.50 (0.29) | ns | 0.00 (0.00) | ns | 0.00 | 1.00 | | |
| W1 | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | W1 | Vn | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 | | |
| | R1 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 | | |
| R3 | | 0.25 (0.25) | ns | 0.00 (0.00) | ns | 0.00 | 0.25 | | | R3 | 1.75 (1.11) | ns | 0.25 (0.25) | ns | 0.00 | 0.25 | | |
| | R5 | 0.19 (0.14) | ns | 0.00 (0.00) | ns | 0.00 | 0.75 | | | R5 | 0.29 (0.14) | ns | 0.06 (0.06) | ns | 0.00 | 0.47 | | |
| AR Combined | all | 0.44 (0.22) | ns | 0.13 (0.13) | ns | 0.13 | 1.00 | AR Combined | | all | 1.06 (0.44) | ns | 0.38 (0.13) | ns | 0.25 | 1.67 | | |
| AR Combined | R5 | 0.50 (0.50) | ns | 0.00 (0.00) | ns | 0.00 | 0.75 | AR Combined | | R5 | 0.50 (0.50) | ns | 0.25 (0.25) | ns | 0.00 | 0.50 | | |
| Global Combined | R5 | 0.08 (0.06) | ns | 0.00 (0.00) | ns | 0.00 | 0.75 | Global Combined | | R5 | 0.13 (0.07) | ns | 0.03 (0.03) | ns | 0.00 | 0.47 | | |

Table 12-46. Data results and analysis of the incidence of *Dichelops furcatus* and Chrysopidae (unspecified) in HB4 and Williams 82 control in the US and Argentina (AR).

| Dichelops furcatus | | Chrysopidae (unspecified) | | | | | | | | | | | | | |
|--------------------|-------------|---------------------------|-------------|-------------|-------------|------|---------|-----------------|-------|-------------|-------------|-------------|------|------|------|
| Country | Site | Stage | Entries | | Ref. range | | Country | Site | Stage | Entries | | Ref. range | | | |
| | | | HB4 (SE) | W82 (SE) | Sig. | Min | | | | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| US sites | IA | R5- R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH2 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | US Combined | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR sites | D1 | Vn | 0.50 (0.50) | 0.25 (0.25) | ns | 0.00 | 0.25 | AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.08 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.33 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | D2 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.17 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.08 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 | | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| W1 | | R5 | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.25 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.50 |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | W1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.50 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.75 (0.48) | 0.25 (0.25) | ns | 0.25 | 1.25 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.19 (0.10) | ns | 0.03 | 0.75 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR Combined | all | | 0.31 (0.18) | 0.31 (0.15) | ns | 0.17 | 1.25 | AR Combined | all | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| AR Combined | R5 | | 0.00 (0.00) | 0.50 (0.29) | ns | 0.25 | 1.50 | AR Combined | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.17 | |
| Global Combined | R5 | | 0.00 (0.00) | 0.08 (0.05) | ns | 0.00 | 0.75 | Global Combined | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.17 | |

Table 12-47. Data results and analysis of the incidence of Coccinellidae (larvae) and Coccinellidae (adult) in HB4 and Williams 82 control in the US and Argentina (AR).

| Coccinellidae (larvae) | | Coccinellidae (adult) | | | | | | | | | | | | | |
|------------------------|-------------|-----------------------|-------------|-------------|-------------|------|---------|-----------------|-------------|-------------|-------------|-------------|-------------|------|------|
| Country | Site | Stage | Entries | | Ref. range | | Country | Site | Stage | Entries | | Ref. range | | | |
| | | | HB4 (SE) | W82 (SE) | Sig. | Min | | | | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| US sites | IA | R5- R6 | 0.75 (0.48) | 1.25 (0.25) | ns | 0.25 | 4.00 | US sites | IA | R5- R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | | 0.25 (0.25) | 1.00 (0.58) | ns | 0.00 | 0.50 | NE | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH1 | | 1.50 (0.65) | 1.00 (0.71) | ns | 0.25 | 1.25 | OH1 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH2 | | 0.25 (0.25) | 0.00 (0.00) | ns | 0.00 | 0.50 | OH2 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | OK | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | US Combined | | | 0.55 (0.20) | 0.65 (0.21) | ns | 0.00 | 0.00 | US Combined | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 |
| AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | D2 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | D2 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | W1 | Vn | 0.75 (0.48) | 0.00 (0.00) | ns | 0.00 | 0.25 | | W1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.25 | | R1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R3 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| AR Combined | all | | 0.25 (0.14) | 0.06 (0.06) | ns | 0.00 | 0.25 | AR Combined | all | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR Combined | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | AR Combined | R5 | | 0.06 (0.06) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| Global Combined | R5 | | 0.31 (0.12) | 0.36 (0.13) | ns | 0.00 | 4.00 | Global Combined | R5 | | 0.03 (0.03) | 0.00 (0.00) | ns | 0.00 | 0.00 |

Table 12-48. Data results and analysis of the incidence of Spiders and Geocoris species in HB4 and Williams 82 control in the US and Argentina (AR).

| Spiders | | Geocoris species | | | | | | | | | | | | | |
|-----------------|-------------|------------------|-------------|-------------|-------------|------|---------|-----------------|-------------|-----------|-------------|-------------|-------------|------|------|
| Country | Site | Stage | Entries | | Ref. range | | Country | Site | Stage | Entries | | Ref. range | | | |
| | | | HB4 (SE) | W82 (SE) | Sig. | Min | | | | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| US sites | IA | R5- R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US sites | IA | R5- R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | NE | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.25 | 2.00 |
| | OH1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH1 | | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.25 |
| | OH2 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH2 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | US Combined | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US Combined | | | 0.00 (0.00) | 0.05 (0.05) | ns | 0.00 |
| AR sites | D1 | Vn | 0.50 (0.29) | 0.50 (0.29) | ns | 0.25 | 1.00 | AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | | R1 | 0.50 (0.22) | 0.50 (0.32) | ns | 0.17 | 0.67 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.83 (0.32) | 1.00 (0.27) | ns | 0.58 | 1.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.33 (0.24) | 0.75 (0.08) | ns | 0.25 | 0.67 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | D2 | Vn | 0.00 (0.00) | 0.08 (0.08) | ns | 0.00 | 0.00 | | D2 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 |
| Q1 | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.33 (0.14) | 0.17 (0.17) | ns | 0.17 | 0.25 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.25 (0.25) | 0.33 (0.14) | ns | 0.25 | 0.42 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.75 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| W1 | | R3 | 0.00 (0.00) | 0.50 (0.50) | ns | 0.00 | 0.75 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.25 (0.25) | 0.25 (0.25) | ns | 0.00 | 0.50 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Vn | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.50 | | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.75 (0.48) | 0.25 (0.25) | ns | 0.25 | 0.50 | | W1 | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.25 (0.25) | 0.75 (0.48) | ns | 0.00 | 0.50 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR Combined | all | | 1.25 (0.25) | 1.75 (0.31) | ns | 1.38 | 2.25 | AR Combined | all | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.08 |
| AR Combined | R5 | | 0.27 (0.11) | 0.58 (0.10) | ns | 0.22 | 0.75 | AR Combined | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| Global Combined | R5 | | 0.12 (0.05) | 0.26 (0.07) | ns | 0.00 | 0.75 | Global Combined | R5 | | 0.00 (0.00) | 0.03 (0.03) | ns | 0.00 | 2.00 |

Table 12-49. Data results and analysis of the incidence of Orius species and Nabis species in HB4 and Williams 82 control in the US and Argentina (AR).

| Orius species | | Nabis species | | | | | | | | | | | | | |
|-----------------|-------------|---------------|-------------|-------------|-------------|------|---------|-----------------|-------------|-------------|-------------|-------------|-------------|------|------|
| Country | Site | Stage | Entries | | Ref. range | | Country | Site | Stage | Entries | | Ref. range | | | |
| | | | HB4 (SE) | W82 (SE) | Sig. | Min | | | | Max | HB4 (SE) | W82 (SE) | Sig. | Min | Max |
| US sites | IA | R5- R6 | 6.00 (0.91) | 7.25 (3.71) | ns | 3.50 | 28.8 | US sites | IA | R5- R6 | 3.00 (0.41) | 5.00 (2.38) | ns | 2.50 | 8.00 |
| | NE | | 0.25 (0.25) | 0.75 (0.48) | ns | 0.00 | 0.50 | | NE | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | OH1 | | 1.75 (0.48) | 1.25 (0.25) | ns | 1.25 | 3.25 | | OH1 | | 0.25 (0.25) | 0.25 (0.25) | ns | 0.00 | 0.50 |
| | OH2 | | 2.75 (1.38) | 0.25 (0.25) | ns | 0.25 | 3.50 | | OH2 | | 0.25 (0.25) | 0.00 (0.00) | ns | 0.00 | 0.50 |
| | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.50 | | OK | | 0.25 (0.25) | 0.25 (0.25) | ns | 0.00 | 1.50 |
| | US Combined | | | 2.15 (0.59) | 1.90 (0.91) | ns | 0.00 | 28.8 | US Combined | | | 0.75 (0.28) | 1.10 (0.62) | ns | 0.00 |
| AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.08 (0.08) | ns | 0.00 | 0.17 |
| | | R3 | 0.50 (0.10) | 0.42 (0.08) | ns | 0.42 | 0.75 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | D2 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | 0.08 (0.08) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.75 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| W1 | | R5 | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | W1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR Combined | all | | 0.25 (0.11) | 0.31 (0.12) | ns | 0.00 | 0.58 | AR Combined | all | | 0.06 (0.06) | 0.13 (0.13) | ns | 0.00 | 0.38 |
| AR Combined | R5 | | 0.00 (0.00) | 0.06 (0.06) | ns | 0.00 | 0.00 | AR Combined | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.06 |
| Global Combined | R5 | | 1.19 (0.37) | 1.08 (0.52) | ns | 0.00 | 28.8 | Global Combined | R5 | | 0.42 (0.17) | 0.61 (0.35) | ns | 0.00 | 8.00 |

Table 12-50. Data results and analysis of the incidence of *Forficula auricularia* and Chrysopidae (larvae) in HB4 and Williams 82 control in the US and Argentina (AR).

| Forficula auricularia | | Chrysopidae (larvae) | | | | | | | | | | | | | | | |
|-----------------------|-------------|----------------------|-------------|-------------|-------------|-------------|------------|------|-----------------|-------------|-----------|-------------|-------------|-------------|------|------------|------|
| Country | Site | Stage | Entries | | W82 (SE) | Sig. | Ref. range | | Country | Site | Stage | Entries | | W82 (SE) | Sig. | Ref. range | |
| | | | HB4 (SE) | Max | | | Min | Max | | | | HB4 (SE) | Max | | | Min | Max |
| US sites | IA | R5- R6 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US sites | IA | R5- R6 | 0.25 (0.25) | 0.25 (0.25) | ns | 0.25 | 3.00 | |
| | NE | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | NE | | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.50 | |
| | OH1 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH1 | | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.25 | |
| | OH2 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OH2 | | 0.25 (0.25) | 0.00 (0.00) | ns | 0.00 | 0.25 | |
| | OK | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | OK | | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.25 | |
| | US Combined | | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | US Combined | | | 0.10 (0.07) | 0.20 (0.09) | ns | 0.00 | 3.00 |
| AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | 0.08 (0.08) | ns | 0.00 | 0.08 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.17 | |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | 0.17 (0.10) | 0.25 (0.08) | ns | 0.00 | 0.17 | |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | D2 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.08 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 | |
| W1 | | R3 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.25 | | | R3 | 0.00 (0.00) | 0.25 (0.25) | ns | 0.00 | 0.50 | |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.50 | |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | W1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| AR Combined | all | | 0.00 (0.00) | 0.00 (0.00) | 0.06 (0.06) | ns | 0.00 | 0.08 | AR Combined | all | | 0.13 (0.09) | 0.25 (0.11) | ns | 0.00 | 0.33 | |
| | R5 | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.03 | AR Combined | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 | |
| Global Combined | | | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.03 | Global Combined | | | 0.06 (0.04) | 0.11 (0.05) | ns | 0.00 | 3.00 | |

Table 12-51. Data results and analysis of the incidence of Chrysopidae (egg) and Chrysopidae (adult) in HB4 and Williams 82 control in the US and Argentina (AR).

| Chrysopidae (egg) | | Chrysopidae (adult) | | | | | | | | | | | | |
|-------------------|-------------|---------------------|-------------|-------------|-------------|------|-----------------|-------------|-----------|-------------|-------------|-------------|------|------|
| Country | Site | Stage | Entries | | Ref. range | | Country | Site | Stage | Entries | | Ref. range | | |
| | | | HB4 (SE) | W82 (SE) | Sig. | Min | | | | Max | HB4 (SE) | W82 (SE) | Sig. | Min |
| US sites | IA | R5- R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | US sites | IA | R5- R6 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | NE | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | NE | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | OH1 | | 0.25 (0.25) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OH2 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | OH2 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | OK | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | US Combined | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | US Combined | | 0.05 (0.05) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | AR sites | D1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | D2 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | Q1 | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | | R1 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | | R3 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | | R5 | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | | Vn | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | W1 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | | W1 | | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | AR Combined | all | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | AR Combined | all | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 |
| R5 | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | AR Combined | R5 | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| Global Combined | | | 0.00 (0.00) | 0.00 (0.00) | ns | 0.00 | Global Combined | | | 0.03 (0.03) | 0.00 (0.00) | ns | 0.00 | 0.00 |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |

HB4 Ecological Evaluations: Plant-Symbiont Characteristics

Introduction

A component of the ecological evaluation assessment is the analysis of the effects of the introduction of the *HaHB4v* gene on the soybean plant-symbiont association (Hirsch *et al.* 2003 and Gage 2004). Soybean seed inoculation with appropriate symbiont bacteria is a common agricultural practice (Rodriguez-Navarro *et al.* 2011).

Plants are not able to assimilate atmospheric nitrogen directly. However, a wide diversity of nitrogen-fixing bacterial species has the capacity to colonize the rhizosphere and by their association with plants provide nitrogen to support plant growth. Leguminous plants, in particular, can form root nodules colonized by particular co-evolved soil bacteria that will fix atmospheric nitrogen and supply ammonium ions to plant root tissue in a symbiotic relationship (Franche *et al.* 2009). *Bradyrhizobia* grow as free-living organisms as well as becoming nitrogen-fixing symbionts within root nodules of legume plants (Gage 2004). This process is greatly relevant to agronomic practices, as it reduces a crop's needs for nitrogen fertilizer inputs.

Here a study was performed to assess the impact of the *HaHB4v* gene expression in HB4 soybean, on the symbiotic interaction with members of the *Bradyrhizobiaceae* family of symbiotic bacteria. The rationale behind this experiment was the hypothesis that any differences in the symbiotic relationship of HB4 soybean with *Bradyrhizobiaceae* rhizosphere-inhabiting N-fixing bacteria could produce a change on regular production practices and the environment. The objective of this analysis was to evaluate if the inclusion of the *HaHB4v* gene modified the symbiotic interaction of soybean with *Bradyrhizobium japonicum* in comparison to the parental control counterpart.

The establishment of a productive, N-fixing relationship between the plant and its rhizobial symbionts is a complex process that can be assessed by a variety of techniques. As this process includes the formation of root nodules (in which the bacteria undergo a complex differentiation stage leading to the deployment of the N-fixing ability), both nodule number and mass, together with some measurements of plant's growth and nitrogen status can provide a picture of the overall efficiency of the symbiotic process (Israel *et al.* 1986, Appunu and Dhar 2006).

Materials and Methods

Transgenic soybean plants with HB4 event and the non-transgenic control line (Williams 82) were grown in a growth chamber from seeds planted in pots with nitrogen-deficient soil. Among the N-fixing symbiotic rhizobacteria, we have selected a *B. japonicum* strain as a bacterial inoculum, which is commercially available and in general use for soybean production. Equivalences were evaluated for all the parameters measured. Five commercial reference varieties were also included in the experiment.

Soybean accessions for the symbiotic interactions experiment included event HB4, the non-transgenic parental control line (Williams 82), and five commercial varieties. The latter were included to provide reference values for the natural variability range of the parameters evaluated (Table 12-52). Number and weight of nodules, shoot dry weight and total dry biomass, from HB4, the control and the reference varieties were measured.

Table 12-52. Description of seeds from HB4, Control, and Commercial Reference Varieties used in the symbiotic interaction experiment.

| Material Name | Material Type | Phenotype |
|---------------|---------------|---------------------|
| HB4 | Test | HB4 |
| Williams 82 | Control | Conventional |
| SRM 3970 | Reference | Glyphosate Tolerant |
| SPS 3900 | Reference | Glyphosate Tolerant |
| DM 3810 | Reference | Glyphosate Tolerant |
| DM 4210 | Reference | Glyphosate Tolerant |
| NS 4009 | Reference | Glyphosate Tolerant |

Seeds from the HB4 and the non-transgenic parental control soybean were tested by event-specific polymerase chain reaction to confirm the presence or absence of the *HaHB4v* gene.

Growth Chamber Phase and Experimental Design

Seven replicates of two small pots of 300 ml were arranged for each soybean material (HB4, the non-transgenic parental control and the five reference varieties). Each pot contained nitrogen deficient soil from Alpachiri, La Pampa, Argentina. At the beginning of the experiment, each seed was inoculated with a solution containing 1×10^{10} cells of *B. japonicum* (RIZO-LIQ TOP®, *Rizobacter Argentina* S.A.). Three seeds were planted in each pot. Soybean plants were grown in a growth chamber with temperatures varying from 26 to 30°C. At emergence, pots were reduced to a single plant per pot. The experiment was arranged in seven replicated blocks under a randomized complete block design.

Demineralized water was added, three times a week, to ensure field capacity, after plant emergence.

Plant Harvesting and Data Collection

Five weeks after planting, plants were taken from pots, soil was removed from the roots and shoots, and root and nodules were separated. All nodules were removed from roots, counted and weighted to measure their fresh weight. Roots and shoots fresh weight was also measured.

Nodules, root and shoot fresh biomass materials were dried for approximately 72 hours at 65°C for dry weight measurements.

Statistical Analysis

Analysis of Variance was developed with SAS® (version 9), with a randomized complete block design. Dunnett's t-test was performed for post-hoc comparisons between the transgenic event and the parental control Williams 82. The level of statistical significance was set to 5% ($\alpha=0.05$). Soybean event HB4 was compared to the non-transgenic parental control for nodule number, nodule dry weight (g), shoot dry weight (g) and total dry biomass (g). No comparison was evaluated between HB4 and the commercial reference varieties. A minimum and maximum mean value among the five commercial varieties was

used to build a reference range accounting for the natural variability of each variable measured.

Results

Comparison between HB4 and the non-transgenic parental control did not show statistically significant differences (5% level of significance) for the parameters evaluated: nodule number, nodule dry weight, shoot dry weight and total dry biomass (Table 12-53).

Table 12-53. Symbiotic interaction parameters of HB4 and non-transgenic soybean parental control (Williams 82).

| Characteristics measured (units) | HB4 | | Mean difference (HB4-WT) | 95% Confidence Limits | | Sig. ⁴ | Williams 82 | | Reference Range ³ | |
|--|-------------------|-----------------|--------------------------|-----------------------|--------|-------------------|-------------------|-------------------|------------------------------|--------------|
| | Mean ¹ | SE ² | | Mean | SE | | Minimum Mean (SE) | Maximum Mean (SE) | | |
| Nodule number (per 2 plants) | 30.86 | 1.7 | -3.571 | -19.435 | 12.292 | NS ⁵ | 34.43 | 5.22 | 15.29 (2.71) | 27.43 (4.74) |
| Nodule Dry Weight (g/2 plants) | 0.16 | 0.01 | -0.011 | -0.057 | 0.035 | NS | 0.17 | 0.02 | 0.08 (0.02) | 0.13 (0.01) |
| Shoot Dry Weight (g/2 plants) | 3.74 | 0.13 | -0.007 | -0.456 | 0.443 | NS | 3.74 | 0.12 | 2.69 (0.06) | 3.16 (0.08) |
| Total Plant Biomass Dry Biomass (g/2 plants) | 4.8 | 0.17 | 0.321 | -0.213 | 0.855 | NS | 4.48 | 0.14 | 3.20 (0.08) | 3.83 (0.09) |

¹Mean based on n=7.

²SE= Standard Error.

³Reference Range. Reference ranges were obtained from the maximum and minimum mean values from the five commercial varieties.

⁴Sig. Significance differences between the transgenic event and the non-transgenic soybean parental control.

⁵NS. Indicates no significant differences between the transgenic event (HB4) and the non-transgenic parental control (Williams 82).

Conclusion

The results of the above experiments support the conclusion that the introduction of the *HaHB4v* gene in HB4 soybean does not introduce a statistically significant change in the symbiotic interaction between HB4 soybeans and *B. japonicum*. Therefore, we can also conclude that cultivation practices related to nitrogen inputs will not be modified by the introduction of *HaHB4v* gene into soybean HB4. Environmental effects resulting from the symbiotic interactions of soybean with *rhizosphere* nitrogen fixing bacteria in soybean event HB4 will not be different from the corresponding effects of the parental control cultivar Williams 82.