# Verdeca Petition (17-223-01p) for Determination of Non-regulated Status of HB4 Soybean

## OECD Unique Identifier: IND-00410-5

## Plant Pest Risk Assessment

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## A. Introduction

Verdeca LLC (hereafter referred to as Verdeca) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) for a determination that the genetically engineered (GE) soybean (*Glycine max* (L.) Merr.) Event HB4 under OECD Unique Identifier IND-00410-05 (hereafter referred to as HB4 soybean) is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived and, therefore, should no longer be a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 17-223-01p, and is hereafter referenced as Verdeca 2017. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7702 *et seq.*)[1]. This plant pest risk assessment was conducted to determine if HB4 soybean is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of part 340 when APHIS determines that it is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived. A GE organism is considered a regulated article under part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest[2]. HB4 soybean was produced by the *Agrobacterium tumefaciens*-mediated transformation of pre-germinated soybean seeds (p. 26, Verdeca 2017), and four inserted construct components come from plant pest organisms listed in 7 CFR 340.2, including the non-coding pr2x35S promoter from *cauliflower mosaic caulimovirus* (Odell et al. 1985), terminator sequence from *A. tumefaciens* (Depicker et al. 1983), T-DNA border sequences from *A. tumefaciens* (Gelvin 2003), and leader sequence from tobacco etch virus (Gallie et al. 1995) (Table IV.A, pp. 28-30, Verdeca 2017). Therefore, the GE soybean Event HB4 is considered a regulated article under APHIS regulations at 7 CFR part 340. Verdeca has conducted introductions of HB4 soybean as a regulated article under APHIS-authorized notifications since 2011 (p. 21, Verdeca 2017), in part, to gather information to support that HB4 soybean is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived.

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[1] Plant Protection Act in 7 U.S.C. 7702 § 403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

[2] Limited exclusions or exemptions apply for certain engineered microorganisms and for interstate movement of some organisms, as in 7 CFR 340.1 and 340.2.(b).

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with HB4 soybean and its progeny and their use in the absence of confinement relative to the unmodified recipient and/or other appropriate comparators. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if HB4 soybean is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about HB4 soybean related to plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities and indirect plant pest effects on other agricultural products; effects of the regulated article on non-target organisms; weediness of the regulated article; impact on the weediness of any other plant with which it can interbreed; changes to agricultural or cultivation practices that may impact diseases and pests of plants; and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology'(51 FR 23302 1986; 57 FR 22984 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq)* the distribution, sale, use, and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. Chapter 9). Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 156. Other applicable EPA regulations include 40 CFR part 152 - Pesticide Registration and Classification Procedures, part 174 - Procedures and Requirements for Plant Incorporated Protectants (PIPs) and part 172 - Experimental Use Permits.  No EPA reviews are relevant to HB4 soybean.

The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from GE crops comply with their obligations, the FDA encourages them to participate in its voluntary early food safety evaluation for new non-pesticidal proteins produced by new plant varieties intended to be used as food (FDA 2006) and a more comprehensive voluntary

consultation process prior to commercial distribution of food or feed (57 FR 22984 1992). Verdeca (2017) has completed an Early Food Safety Evaluation (EFSE) for the HAHB4 protein expressed in HB4 soybean (FDA 2015) and a full safety consultation for HB4 soybeans (FDA 2017). FDA did not identify any safety or regulatory issues under the Federal Food, Drug, and Cosmetic Act that would require further evaluation at this time.

## B. Development of the HB4 Soybean

Soybean belongs to the genus *Glycine*, which includes two subgenera and at least 28-32 species (Chung and Singh 2008; Sherman-Broyles et al. 2014). The subgenus *Soja* consists of only two species, i.e., the cultivated soybean (*G. max*) and its annual progenitor wild soybean *(G. soj*a Sieb. & Zucc*)*. The subgenus *Glycine* includes at least 26 species, which are predominantly perennials and native to Australia and surrounding islands. Cultivated soybean was originated and domesticated in China or South-East Asia from its wild progenitor soybean (*G. soja* Sieb. & Zucc.) about 3000–9000 years ago (Hymowitz 1970; Hymowitz and Newell 1981; Sedivy et al. 2017).

Soybean is a self-pollinating species, propagated commercially by seed (OECD 2000a). Soybean seeds contain about 18% oil and 38% protein (Hartman et al. 2011). Soybean is ranked number one (55%) worldwide among all major oil crops (Chung and Singh 2008). Nearly all soybean meal (98%) are used in livestock and aquaculture feeds (Hartman et al. 2011).

Soybean is grown worldwide, and the United States is the world's leading soybean producer followed by Brazil, Argentina, China, India, and other countries (USDA-FAS 2017). In the United States, soybeans are grown mostly in the Midwest (Figure 1) on over total of 89.5 million acres in 2017 (Figure 2) (USDA-NASS 2016, 2017a). Total soybean production in the U.S. has been increased by both increasing the area under cultivation and the yield per unit area (Figures 2 and 3) (USDA-NASS 2017a, b). For example, in the past 20 years soybean acreage increased from 70 million to nearly 90 million acres (Figure 2), and in the past 30 years soybean yields have increased about 53% (Fig 3).
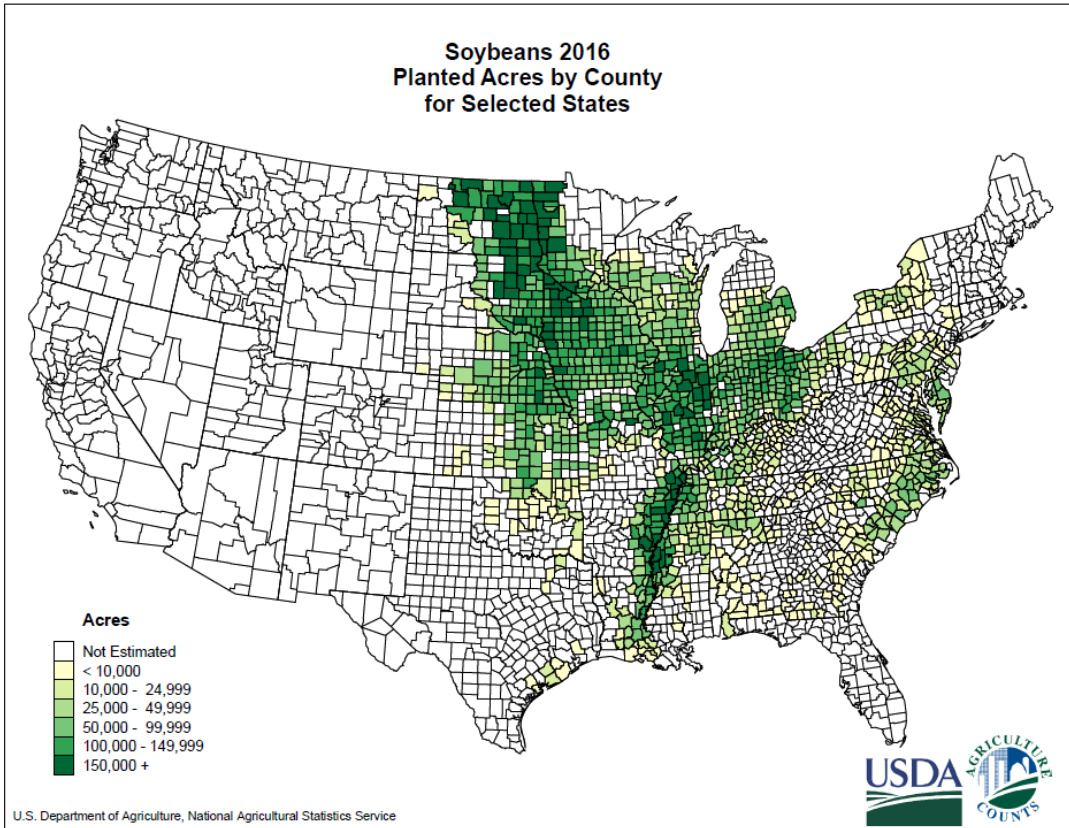
**Figure 1.** Soybean production areas in the U.S. (USDA NASS 2016).
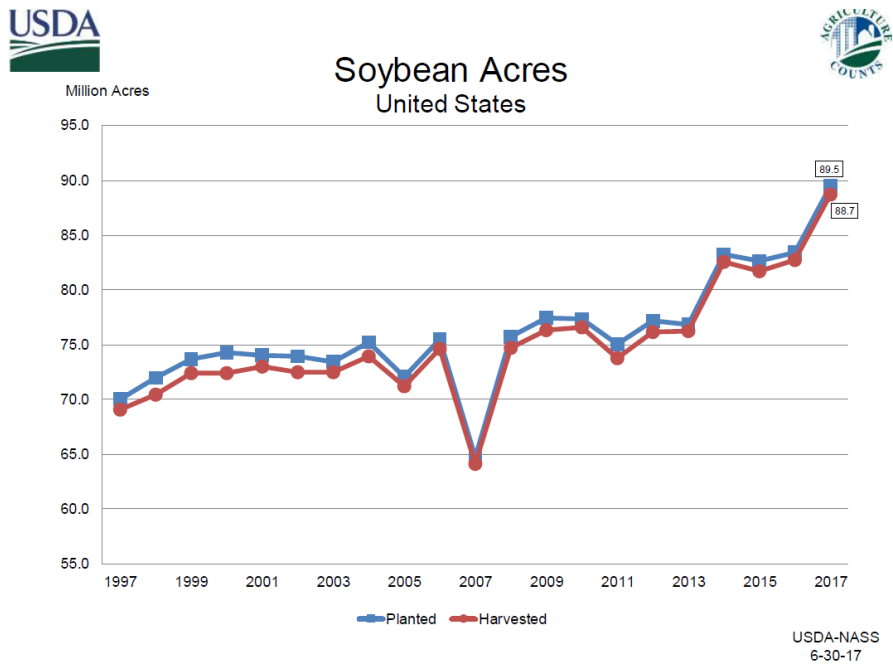


**Figure 2.** Soybean acreage by year in the U.S. (USDA NASS 2017)
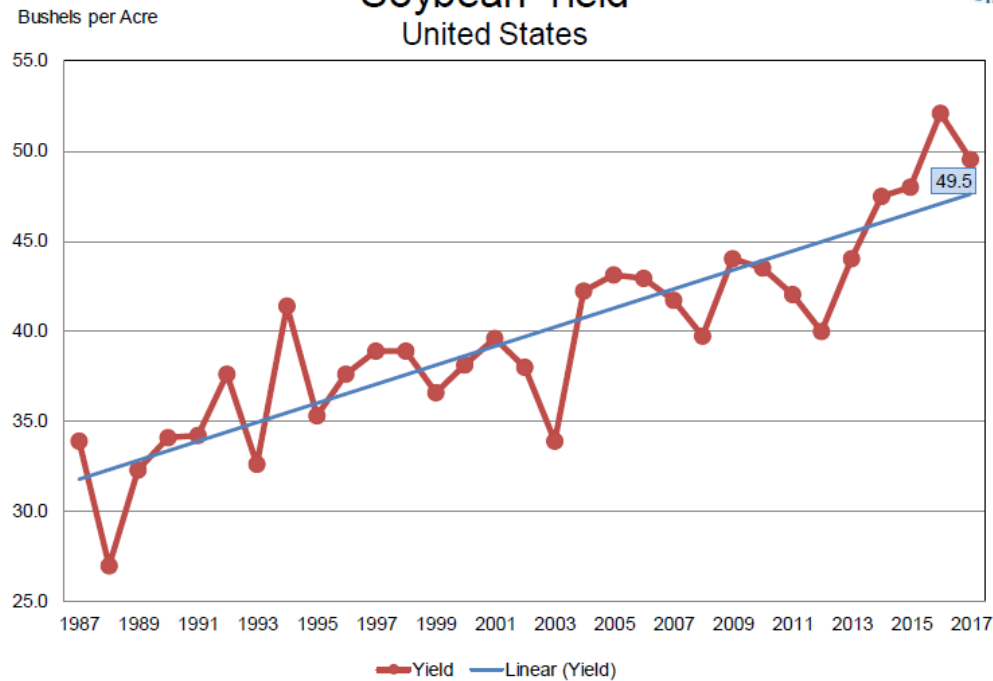
**Figure 3.** Soybean yield by year in the U.S. (USDA NASS 2017)

Despite the continuous soybean yield increase over the years, to maintain and/or further improve soybean yield is facing challenges by a variety of both biotic and abiotic stress factors. Typical abiotic stress factors include salinity, non-optimal temperatures, drought, flooding, and poor soil nutrition, etc. (Chung and Singh 2008). 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 the 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.

HB4 soybean was developed through genetic engineering to provide an increased yield opportunity across the differing soybean growing environments (Verdeca 2017). The variant isoform of a transcription factor gene from sunflower (*Helianthus annuus*)

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(*HaHB4v*) was inserted into the conventional soybean variety Williams 82 using *Agrobacterium*-mediated transformation. The *HaHB4v* gene encodes a DNA-binding protein with a leucine zipper homeodomain, and is shown to be activated by various environmental stimuli normally encountered throughout a growing season (Gago 2002 Manavella et al. 2006; Manavella et al. 2008). Based on the agronomic data from field trials, Verdeca expects that the *HaHB4v* gene in HB4 soybean might augment the plant's adaptability to the environment stress thereby enabling a greater grain yield than currently available commercial soybean varieties. In addition to *HaHB4v* gene, Verdeca also engineered HB4 soybean to express bialaphos resistance gene (*bar*) that confers resistance to glufosinate-ammonium herbicide. The herbicide resistance was not designed as part of the original product development concept for HB4 soybean but rather as a selectable marker for the selection of regenerated GE seedling plants during transformation process. The initial greenhouse dose response study showed HB4 soybean had low possibility to have field resistance to glufosinate. However, later, field trials for glufosinate resistance testing showed that the expression of the *bar* gene in HB4 soybean also confers field resistance to glufosinate-based herbicide (Product Supplemental Information, pp. 9-14, Verdeca 2018).

Verdeca evaluated the phenotypic and agronomic performance of HB4 soybean by comparing with its parental control variety, Williams 82, and multiple commercial soybean varieties at ten sites in the United States during the 2013 growing season and 15 field sites in Argentina during the 2012-2013 growing season (Appendix 12, pp.292-380, Verdeca 2017). Verdeca also conducted the composition analysis of HB4 soybean by comparing to its parental variety, Williams 82, and multiple commercial soybean varieties at five and six field sites in the U.S. and Argentina, respectively (Appendix 11, pp.207-291, Verdeca 2017). Field trial sites were selected within the major soybean production areas of both the U.S. and Argentina, representing the diverse environmental conditions in which soybean is typically cultivated in both countries. The reference varieties were adapted for each site and represented the range of natural variability among commercial soybean varieties.

Based on soybean biology (Hymowitz and Newell 1981; OECD 1999, 2000b; Chung and Singh 2008; Sedivy et al. 2017) and the data presented by Verdeca (2017), APHIS concludes that the HB4 soybean was developed in a manner common to other GE soybean and GE crops using *Agrobacterium*-mediated transformation (USDA-APHIS-BRS 2017). APHIS believes the use of the non-GE parental line Williams 82 and other reference varieties as comparators is sufficient to determine that HB4 soybean is not substantially different from its non-GE parental line and non-GE conventional soybean varieties (USDA-APHIS-BRS 2017).

## C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNAs and their expression products, APHIS assessed data and information presented in the petition related to: the transformation

process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction based on the location of the insertion (e.g. nucleus or organelle) and the number of loci inserted.

APHIS also assessed data presented in the petition on whether the genetic modification results in expression of new genes, proteins, or enzymes or changes in plant metabolism or composition in the Event HB4 soybean relative to the nontransgenic counterpart and other soybean comparator varieties. The assessment encompasses a consideration of the HAHB4v protein encoded by *HaHB4v* gene and phosphinothricin-N-acetyl transferase (PAT) protein encoded by *bar* gene and any observed or anticipated effects on plant metabolism, including, e.g. any relevant changes in levels of metabolites, antinutrients, or nutrients in harvested seeds derived from the GE crop event compared to those in the conventional counterpart and other comparators.

This information is used later in this risk assessment to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in the GE crop event; or for expression of inserted DNAs, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, non-target beneficial organisms, weediness, agricultural practices that impact pest or diseases or their management, or plant pest risks through horizontal gene flow.

### *Description of the genetic modification and inheritance of inserted DNA*

HB4 soybean was developed through *Agrobacterium*-mediated transformation of soybean variety Williams 82 using the disarmed *pIND2-HB4* binary vector (Sections 3 and 4, Appendix 2 and 3, Verdeca 2017). The disarmed binary vector does not have the native transfer DNA (T-DNA) region from tumor-inducing (Ti) plasmids normally responsible for the incitation of crown gall tumors upon *A. tumefaciens* infection (Gelvin 2003). Furthermore, following transformation the explant tissues were placed on selection medium supplemented with antibiotics cefotaxime, timentin, and vancomycin to kill or inhibit *Agrobacterium* growth (Appendix 2, Verdeca 2017).

Binary Plasmid Vector *pIND2-HB4*

The disarmed *pIND2-HB4* binary vector is approximately 11.1 kb. It contains two gene expression cassettes which are delineated by a right border (RB) and left border (LB) sequences of T-DNA as well as backbone vector sequences outside of the two T-DNA border sequences. Table IV.A (p.28, Verdeca 2017) lists all genetic elements in the binary plasmid vector pIND2-HB4. Transgene elements within the T-DNA regions are shown in Figure 4 below.
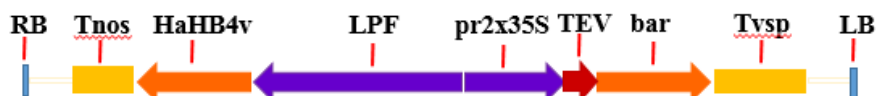
**Figure 4. Transgene elements within the T-DNA regions (adapted from Figure IV.A, p.28, Verdeca 2017).**

- The first expression cassette contains the gene of interest that is the *HaHB4v* gene from *H. annuus* (Gago et al. 2002), the large promoter fragment (LPF) of the *HaHB4* gene from *H. annuus* (Dezar et al. 2005)*, and the 3'-untranslated region (3'-UTR) of the nopaline synthase gene (Tnos) from *A. tumefaciens* (Depicker et al. 1983). The *HaHB4v* gene is a variant of native *HaHB4* gene from *H. annuus*, which contains a 12-nucleotide deletion near the N-terminus, without changing the translation frame, and five scattered substitutions (including two silent substitutions) outside highly conserved homeobox domain. According to the petitioner data, the changes of these nucleotides and their corresponding encoded amino acid residues did not expect to cause significant differences in the HAHB4v protein structure and its general properties, including its functional properties (Appendix 7, Verdeca 2017).

- The second expression cassette contains the selectable marker gene, *bar,* from *S. hygroscopicus* (Thompson et al. 1987)*, duplicated 35S promoter from Cauliflower Mosaic Virus (pr2x35S) (Odell et al. 1985), the 5' leader sequence from Tobacco Etch Virus (TEV) (Gallie et al. 1995), and the 3' terminator of a soybean vegetative storage protein gene (Tvsp) (Rapp et al. 1990).

Among the above transgene elements inserted into the Event HB4 soybean, four elements, including the T-DNA border sequences, Tnos terminator, leader sequence from TEV and 35S promoter are derived from plant pathogens. However, none of them is known to cause plant diseases.

<u>Characteristics, Stability, and Inheritance of the Introduced DNA</u>

Verdeca has provided data to characterize the inserted transgene DNAs in HB4 soybean with a combination of techniques, including Southern blot analysis, PCR, and DNA sequencing (both conventional Sanger capillary sequencing and whole genome sequencing) (Appendix 4, pp.86-133, Verdeca 2017). Both the Southern blot analyses and sequence data demonstrate that HB4 soybean genome does not contain any binary vector backbone elements outside of the T-DNA region (Appendix 4, pp.97-106, Verdeca 2017). The sequence data analyses show that a single, intact copy of the T-DNA containing *HaHB4v* and *bar* gene expression cassettes is integrated into the soybean HB4 genome (Appendix 4, pp.100-106, Verdeca 2017). Verdeca also assessed T-DNA inheritance in an $F_2$ segregating population from a cross between HB4 soybean and a non-transgenic soybean variety and showed that the T-DNA is inherited as a single locus according to Mendel's principles of inheritance (Appendix 4, pp.131-133, Verdeca 2017). Through analyzing the DNA sequence of the T-DNA insertion junctions, Verdeca

demonstrated that the inserted T-DNA is localized to an intergenic region of chromosome 9, where a 142 base pairs of nucleotide sequence were deleted. However, this deletion would not result in disruption to any known or putative genes (Appendix 4, pp.109-130, Verdeca 2017). Furthermore, Verdeca analyzed the integrity and stability of the inserted T-DNA through sequencing and demonstrated that the DNA sequence of T-DNA and its flanking host genome regions is stable over six generations (Appendix 4, pp.109-133, Verdeca 2017).

### *Expression of inserted DNA, changes in gene expression, new proteins or metabolism, and toxicity and allergenicity*

HB4 soybean expresses two recombinant proteins, i.e., the sunflower HAHB4v protein and the bacterial PAT protein.

*HAHB4v Protein*

HAHB4v protein expressed in HB4 soybean contains 177 amino acids with a predicted molecular weight of 20.9 kDa. It is a variant of the native sunflower HAHB4 protein (GenBank Accession number AAA63768.2) with a four amino acid deletion near the amino-terminus end and three scattered substitutions in other positions (Appendix 7, pp.152-153, Verdeca 2017). Verdeca concludes that the amino acid changes in HAHB4v protein are not located in the functional domains and are not expected to have significant effects on the functional activities of the protein based on a detailed bioinformatics analysis (Appendix 7, pp.152-154, Verdeca 2017).

HAHB4 belongs to the HD-Zip family of transcription factors with a homeobox domain (HD) that binds DNA and a leucine zipper (LZ) motif that mediates protein-protein interaction (Ariel et al. 2007; Elhiti and Stasolla 2009). Various abiotic and biotic stresses, such as drought, salinity, darkness, insect feeding, and chemical exposure, are shown to induce the expression of HAHB4, which in turn regulates the ethylene signaling pathway and the expression of genes that are responsive to those stresses (Gago 2002; Dezar et al. 2005; Manavella et al. 2006; Manavella et al. 2008; Dezar et al. 2011).

The expression levels of HAHB4v protein in HB4 soybean seed and leaf were determined from field samples collected at multiple sites in Argentina (2012-2013 growing season) and the United States (2013 growing season). With a sensitive LC-MS/MS-based detection method (Bronsema et al. 2013), Verdeca showed that HAHB4v protein was detected only in two of the field samples at a low level of 0.005 µg/g (5ng/g) dry weight (DW) while the levels of HAHB4v in all the other field samples were below the lower limit of detection (LOD). Under a controlled growth chamber condition under which soybean plants are exposed to abiotic stresses to elicit HAHB4v expression, Verdeca also showed that the HAHB4v expression level is low with a range from below the limit of detection to up to 0.005 µg/g (5ng/g) DW in root and 0.004 µg/g (4 ng/g) DW in leaf (Appendix 9, pp.172-199). Therefore, the expression level of HAHB4v in HB4 soybean is very low compared to the native HAHB4 protein levels in sunflower ranging from 0.0252 to 0.0623 µg/g (25.2 to 62.3 ng/g) DW (Verdeca 2017).

Verdeca evaluated the potential toxicity and allergenicity of HAHB4v by comparing its sequence homology with known toxins and allergens, and showed that HAHB4v protein has no significant homology to known protein toxins and allergens (Appendix 5, pp. 134-136, Verdeca 2017). Furthermore, HAHB4 belongs to a large class of transcription factors that are present in many plant species including edible plants, suggesting HAHB4 protein has a history of prior exposure and a history of safe use (pp.37-38, Verdeca 2017). Also, as described above, the levels of HAHB4v in seed and forage tissues of HB4 soybean grown under field trial conditions were extremely low.

Recombinant HAHB4v protein was expressed in *Escherichia coli* to facilitate the safety characterization of HAHB4v protein. *E. coli*-produced HAHB4v protein was shown to be equivalent to the protein expressed in HB4 soybean based on LC-MS, MALDI-TOF and N-terminal sequence analysis (Verdeca, 2017). The *E. coli*-produced HAHB4v protein was degraded rapidly *in vitro* with simulated gastric fluid with no observed protein fragments after the first 30 seconds of digestion (Appendix 10, pp. 200-204, Verdeca 2017).

Verdeca concludes that HAHB4v lacks toxic and allergenic potential based on the broad weight of evidence.

*PAT Protein*

PAT protein expressed in HB4 soybean is 187 amino acids in length with an approximate molecular weight of 22 kDa. It is an enzyme that inactivates glufosinate ammonium herbicides to confer resistance (Thompson et al. 1987; Strauch et al. 1988).

With an enzyme-linked immunosorbent assay (ELISA) method, Verdeca showed that the average PAT levels ranged from 23 to 69 µg/g fresh weight (FW) in seeds and from 5 to 13 µg/g FW in leaves (Appendix 6, Table 6-1, p.151, Verdeca 2017). These PAT expression levels in HB4 soybean fall within the broad range of PAT protein expression levels found in existing glufosinate tolerant crops (Center for Environmental Risk Assessment 2011).

PAT protein has been used extensively to confer herbicide resistance to GE crops cultivated under field conditions as well as in research laboratory as a selectable marker for selection of transgenic plants during the transformation process. Verdeca demonstrated that PAT protein expressed in HB4 soybean does not have toxic and allergenic potential. The safety of the PAT proteins has been previously well established (OECD 1999; Herouet et al. 2005; ILSI 2011). In the United States, the USDA APHIS has issued 28 Determinations of Nonregulated Status for crops, including soybean expressing PAT protein (USDA-APHIS 2017a), and FDA has completed several food and feed consultations involving PAT proteins expressed in various crops including soybean. Also, in the United States residues of the PAT enzyme are exempt from the requirement of a tolerance when used as plant-incorporated protectant inert ingredients in all food commodities (40 CFR 174.522).

*Potential new ORFs*

In addition to HAHB4v and PAT proteins, Verdeca analyzed the potential new open reading frames (ORFs) that are likely to result from the insertion of T-DNA. Based on the bioinformatics analysis of the sequence data, Verdeca concluded that no new ORFs were created in the soybean genome because of the inserted DNAs (p.3 and Table 5-2 in Appendix 5, Verdeca 2017). Two pre-existing putative ORFs in the soybean genome, however, were modified by the insertion. Verdeca noted that there is no experimental evidence indicating that transcription of the above putative ORFs indeed occurs. Furthermore, Verdeca showed that even in the highly unlikely event that any of the above putative sequences were to be transcribed and eventually translated, the two putative peptides have no sequence similarity to known allergens and toxins and do not have the potential to be allergenic or toxic.

### Metabolism composition Analysis

To assess any potential metabolite alteration as a result of the expression of the above inserted genes, Verdeca analyzed the metabolism composition of HB4 soybean grown at 11 field sites (five in the U.S. and six in Argentina), in comparison with the parental variety, Williams 82, and commercial reference varieties representing a range of the natural variability (pp.41-42 and Appendix 11, Verdeca 2017). The metabolic analysis included 1) soybean seed nutrient components, including proximates (moisture, protein, fat, ash, and carbohydrates), fiber ((acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude fiber)), minerals (phosphorus and calcium), fatty acids, amino acids, and vitamins E and K1; 2) seed anti-nutrient components, including  isoflavones (daidzein, genistein, and glycitein), stachyose, raffinose, phytic acid, lectin, and trypsin inhibitors; and 3) soybean forage nutrient components, including proximates (moisture, protein, fat, ash, and carbohydrates), fiber (ADF, NDF), and minerals (phosphorus and calcium) (Appendix 11, pp.207-291, Verdeca 2017).

Two seed nutrient components, i.e., cysteine and vitamin K1 showed a significant difference between HB4 soybean and Williams 82 parental variety (Tables 11-18 and 11-19, Verdeca 2017). The content of cysteine in HB4 soybean seed was significantly lower when compared to Williams 82. However, the cysteine level of HB4 soybean is within the range of cysteine levels found in the commercial reference varieties and the reported levels in the literature. The value of vitamin K1 in HB4 soybean was also significantly lower to that of Williams 82 control. However, Williams 82 was also lower than the levels observed among commercial reference varieties, suggesting a genotypic effect of the Williams 82 parental variety (Appendix 11, Verdeca 2017). Nevertheless, the vitamin K1 values in both HB4 soybean and Williams 82 were within the range of values found in soybean varieties (Verdeca 2017). All the other seed nutrients and the forage nutrients in HB4 soybean were similar to those found in the Williams 82 control and within the range of the commercial reference varieties.

With regard to the anti-nutrient composition, although some anti-nutrients showed significant differences between the HB4 soybean and Williams 82 control, the levels in

all cases were within the values obtained for commercial varieties and/or reported in the literature (Verdeca 2017). Based on these results, it can be concluded that HB4 soybean is compositionally and nutritionally equivalent to conventional soybean varieties. There are no observed or anticipated unintended metabolic composition changes in the HB4 soybean that could impart any new plant pest or disease risk than non-GE soybean varieties.

The expression of the inserted DNAs and the resulting phenotype in HB4 soybean are consistent with the stability/inheritance of the introduced genetic material. The ORF analysis showed no evidence of new ORFs or any unintended effects resulting from the insertion of the genetic materials (Verdeca 2017) (Section 5.6, p. 70; Bayer 2017). Based on the multi-location field test and evaluation of HB4 soybean as well as the previous citations and deregulated petitions for similar genes and gene products that have a history of safe use and have not been implicated in disease or pest issues, the gene products HAHB4v and PAT in HB4 soybean are not expected to incur any additional plant pest or increased disease risks.

## D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences, from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in HB4 soybean event that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses (as identified from the previous section). APHIS also assessed whether HB4 soybean is likely to have significantly increased disease and pest susceptibility compared to Williams 82 control based on data and observations from field trials on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes are assessed to determine if they would (1) affect the new GE crop and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility is evaluated with respect to the context of non-transgenic comparator (i.e. Williams 82 control), currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (OECD 2000a; University 2016; USDA-APHIS 2017b). Soybean rust as a serious disease causing crop losses in many parts of the world is regulated by PPQ. Soybean rust is caused by two fungal species,

*Phakopsora pachyrhizi* and *Phakopsora meibomiae*. It has been reported in many countries and has become a common problem in the southern USA (Hartman et al. 2016; USDA-APHIS 2017b).

In addition to soybean rust, there exist a number of other major soybean diseases and pests, including seed discoloration caused by *Soybean mosaic virus* (SMV) and Bean pod mottle virus (BPMV), Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum*, soybean aphid caused by *Aphis glycines*, soybean cyst nematode caused by *Heterodera glycines*, sudden death syndrome (SDS) caused by *Fusarium virguliformethe,* and Stink bugs (Hemiptera: Pentatomidae) (Hartman et al. 2016).

Soybean is not a plant pest in the United States (7 CFR 340(USDA-NRCS 2017). The genetic modifications of HB4 soybean, including genetic elements, expression of the gene products and their functions have been summarized above. The *Agrobacterium* strain *A. tumefaciens* used in the generation of HB4 soybean were disarmed and also were already killed with antibiotics during the transformation process (Appendix 2, Verdeca 2017). The inserted DNA elements derived from plant pests do not result in the production of infectious agents or disease symptoms in plants. Thus, it is unlikely that HB4 soybean could pose a plant pest risk.

Verdeca evaluated the disease susceptibility and insect interactions of HB4 soybean along with Williams 82 control variety and other commercial soybean varieties under field conditions at 10 locations in the United Sates during the 2013 growing season and at 15 locations in Argentina during the 2012-2013 season (Appendix 12, pp.301-305, Verdeca 2017). These 25 trial locations represent a diverse range of environmental and agronomic conditions where HB4 soybean is expected to be grown. The disease susceptibility and insect interaction data include eight soybean diseases (septoria brown spot, *Xanthomonas Campestris*, frogeye leaf spot, downy mildew, soybean vein necrosis virus, white mold, rust, and bacterial brown rot), 22 insect incidences (*Frankliniella occidentalis*, *Megascelis species*, *Diabrotica speciosa*, *Lagria hirta*, *Epinotia aporema*, *Loxostege bifidalis*, *Rachiplusia nu*, *Anticarsia Gemmatalis, Spilosoma virginica, Helicoverpa geltopoeon, Spodoptera frugiperda, Nezara viridula, Piezodorous guildinii, Edessa meditabunda, Dichelops furcatus, Chrysopidae, Coccinellidae,* Spiders*, Geocoris species, Orius species, Nabis Species, Forficula auricularia, and Chrysopidae*), and five insect damages (pod damage, aphid damage, leafhopper damage, defoliation damage, and damage by unknown insects). The soybean diseases, insect incidence and damage were observed for Event HB4 soybean, Williams 82 control, and five non-GE commercial soybean varieties. Observations were carried out at R1/R2, R3/R4, and R5/R6 developmental stages at all locations in the United States and at Vn, R1/R2, R3/R4, and R5/R6 developmental stages at all locations in Argentina. At all locations in both the United States and Argentina, there were no significant differences between HB4 soybean and the comparator varieties throughout the developmental stages for diseases, arthropod counts, and insect damages both at the individual site level as well as in the combined site analysis (Tables 12-25 to 12-51, Verdeca 2017).

The agronomic performance of HB4 soybean along with conventional control variety Williams 82 and multiple commercial varieties specific for the growing region were also

evaluated under field conditions in major soybean production regions in both the United States and Argentina.  Based on the collected data, HB4 soybeans showed no biologically meaningful difference from the control variety Williams 82 and the commercial comparator varieties, other than the intended effect of potential yield improvement. These observed agronomic performance data and the metabolic composition data as discussed earlier demonstrated that there were neither significantly altered agronomic traits nor metabolism compositions that would render HB4 soybean more susceptible to pests and diseases over control or reference soybean varieties. Thus, HB4 soybean is unlikely to be more susceptible to plant pathogens and insect pests than conventional soybean or unlikely to pose a greater plant pest risk than the unmodified organism (i.e. Williams 82) from which it was derived.

The introduced genes did not significantly alter the observed insect pest infestation and disease occurrence or resulting damage on HB4 soybean over the control line. The expression of HAHB4v and PAT proteins in HB4 soybean is not expected to cause plant disease or change susceptibility of HB4 soybean or its progeny to diseases or other pests. For this reason, soybean is unlikely to differ from conventional soybean in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products. Therefore, pest and disease control methods for HB4 soybean are expected to be similar to those for conventional variety soybean and no direct or indirect plant pest effect on raw or processed plant commodity is expected.

## E. Potential Impacts on Non-target Organisms Beneficial to Agriculture

The HB4 soybean is not engineered for pest resistance, thus there are no 'target' species, and no 'non-target' species either. APHIS assessed whether exposure or consumption of the HB4 soybean would have a direct or indirect adverse impact on species beneficial to agriculture. Organisms considered were representatives of the species associated with production of the regulated crop in the agricultural environment. The assessment includes an analysis of data and information on the HB4 soybean compared to the control variety Williams 82 and other comparator varieties for any biologically relevant changes in the phenotype or substances (e.g. proteins, nutrients, or anti-nutrients) produced which may be novel or expressed at significantly altered amounts that are associated with impacts on organisms beneficial to agriculture, and/or any observations of beneficial organisms associated with the plants.

As described above in Section C "*Expression of inserted DNA, changes in gene expression, new proteins or metabolism*", Verdeca showed that HB4 soybean contains the levels of nutrients, antinutrients, and isoflavones in seed and forage equivalent to those in the control Williams 82 variety and/or are within the natural variable range of the commercial reference varieties (Appendix 11, pp.222-232, Verdeca 2017). Also, as described above in Section C, both recombinant proteins HAHB4v and PAT in HB4 soybean do not possess toxicity and allergenicity potential. Furthermore, Verdeca demonstrated that HB4 soybean is similar to its parental variety in its disease susceptibility, interactions with insects and the symbiotic nitrogen-fixing bacterium *Bradyrhizobium japonicum*. Thus, the HB4 soybean does not show a detrimental effect on beneficial arthropods compared to the control variety Williams 82 and reference commercial varieties.

Therefore, based on the above analysis of the composition of seed and forage tissues, the interaction with symbiotic bacterium, the effect on beneficial arthropods, and the HAHB4v protein's toxicity potential of HB4 soybean, APHIS concludes that exposure to and/or consumption of the GE plant are unlikely to have any adverse impacts to organisms beneficial to agriculture.

## F. Potential for Enhanced Weediness of HB4 soybean

APHIS assessed whether the GE crop event is likely to become weedier (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the nontransgenic progenitor from which it was derived, or other varieties of the crop currently under cultivation. The assessment considers the basic biology of the crop, the situations in which crop volunteers or feral populations are considered weeds, and an evaluation of the GE crop event compared to the nontransgenic progenitor or the other reference varieties evaluated under field (and/or lab) conditions characteristic for the regions of the U.S. where the HB4 soybean is intended to be grown. The characteristics for the evaluation of the GE crop event are related to establishment, competitiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage the crop as a weed. For this crop, such characteristics include seed dormancy and germination, pollen fertility, agronomic and phenotypic traits, disease and pest susceptibility, abiotic stress tolerance, and plant-symbiont characteristics.

The assessment also considers whether the engineered trait affects methods of control for the crop in situations where it is managed as a weed or volunteer in subsequent crops or in feral populations.

In the United States, soybean is not listed as a noxious weed species by the federal government (USDA-NRCS 2017) nor is listed as a weed in the major weed references (Holm et al. 1979; Randall 2017). Soybean is not frost tolerant, does not survive freezing winter conditions (OECD 2000a), and does not reproduce vegetatively. After crop harvest, soybean may germinate as a volunteer in the succeeding crop due to lack of dormancy (OECD 2000a). If any seeds remain viable and germinate the following year, mechanical methods or herbicides can be used to control volunteers. In managed ecosystems, soybean does not effectively compete with other cultivated plants or primary colonizers (OECD 2000a).

Verdeca conducted field trials at 10 sites in the U.S. during 2013 growing season and at 15 sites in Argentina during 2012-2013 growing season to evaluate phenotypic, agronomic and ecological characteristics comparing HB4 soybean with the nontransgenic progenitor and other commercial soybean varieties lacking the *HaHB4v* gene (Verdeca 2017). HB4 soybean characteristics measured included: 1) seed germination and dormancy (Tables 12-2 and 3, Verdeca 2017); 2) pollen morphology and pollen fertility (Figure 12-1, Verdeca 2017); 3) 15 agronomic and phenotypic characteristics (Tables 12-14 to 12-20, Verdeca 2017); and 4) ecological characteristics, including disease

susceptibility, insect interactions, abiotic stress and plant-symbiont characteristics (Table 12-13, Verdeca 2017). There were no significant differences for majority of the measured characteristics between HB4 soybean and the control variety Williams 82 except days to 50% emergence, early plant stand, seedling vigor, days to 50% maturity, plant stand at R8 stage and 1000 count seed weight. However, while these six characteristics showed significant differences between HB4 soybean and Williams 82, all measured values were within the range of the commercial reference varieties except the 1000 seed weight in Argentina locations, which was slightly out of range (Table 12-14, Verdeca 2017). Another difference is that HB4 soybean is resistant to glufosinate herbicide whereas the control parental line is not. However, the herbicide resistance trait in HB4 soybean is similar to that found in other glufosinate resistance soybean varieties already cultivated for years. In those instances, however, there was little evidence of enhanced invasiveness or persistency. Therefore, these data support the conclusion that the inserted *HaHB4v* and *bar* genes do not confer phenotypic or ecological characteristics resulting in a selective advantage for HB4 soybean over the parental control Williams 82.

Based on the agronomic field data and literature survey concerning weediness potential of the crop, HB4 soybean is unlikely to persist as a troublesome weed or to have an impact on current weed management practices. Furthermore, extensive post-harvest monitoring of field trial plots planted with the HB4 soybean under USDA-APHIS notifications (Verdeca 2017) did not reveal any differences in survivability or persistence relative to other varieties of the same crop currently being grown. These data suggest that HB4 soybean is no more likely to become a weed than conventional varieties of the crop. GE crop event volunteers and feral populations can be managed using a variety of currently available methods and herbicides.

## G. Potential Impacts on the Weediness of Any Other Plants with which HB4 Soybean Can Interbreed

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant 1981; Rieseberg and Wendel 1993; Soltis et al. 1993; Hegde et al. 2006). Even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg and Wendel 1993; Preston et al. 2002). It has been a common practice by plant breeders to intentionally introgress traits from wild relatives into crop plants to develop new cultivars (Khoury et al. 2013). However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and a few other crops (Ellstrand et al. 1999). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from the GE crop event to sexually compatible relatives in the United States and its territories, and 2) if so, the risk potential with respect to weediness of those taxa based on the phenotypic changes that have been observed in the engineered plants.

***Potential for gene flow, hybridization and gene introgression***

The genus *Glycine* includes at least 28 species, which are grouped into two subgenera, *Soja* and *Glycine* (Chung and Singh 2008; Sherman-Broyles et al. 2014). The cultivated soybean (*G. max*) and its annual progenitor wild soybean (*G. soja* Sieb. & Zucc) are the only two species of the subgenus *Soja* while all the other at least 26 species, which are predominantly perennials and native to Australia and surrounding islands, belong to subgenus *Glycine*. The two subgenus *Soja* species are predominantly self-pollinating with less than 3% of outcrossing rate because of the stringent cleistogamy of soybean flowers (Ahrent and Caviness 1994; OECD 2000a; Ray et al. 2003). However, the two species are sexually compatible and can be reciprocally crossed resulting in fertile offspring without any genetic isolation (Wang and Li 2011).

Cultivated soybean is the only species of the genus *Glycine* that grows in the United States and its territories. Majority of soybeans are cultivated in the upper Midwest, and the leading soybean producing states include Illinois, Iowa, Minnesota, Nebraska, Indiana, Ohio, South Dakota, North Dakota, Missouri, and Arkansas (USDA-NASS 2016). Wild soybeans are distributed only in eastern Asia (OECD 2000a; Wang and Li 2011). While some subgenus *Soja* wild species are occasionally grown in research plots, there have been no reported escaping and naturalization of wild soybean species in North America (OECD 2000a). Therefore, it is highly unlikely that gene flow and introgression will occur between HB4 soybean and its wild relative species in the United States.

### *Potential for enhanced weediness of recipients after gene flow and/or introgression*

As discussed above in Section F "*Potential for Enhanced Weediness of HB4 Soybean*", the expression of the integrated genetic materials in HB4 soybean does not confer or enhance weedy characteristics of cultivated soybean other than enhancing yield potential and herbicide resistance. Should gene flow and/or introgression from HB4 soybean to its wild relatives occur, the introduced genetic materials are unlikely to cause enhanced weediness of the recipient plants. Furthermore, cultivated soybean is the only soybean species grown in the U.S. and its territories and there is no sexually compatible wild relative species reported in natural environments in North America. Thus, APHIS has determined that any adverse consequences of gene flow and/or introgression from HB4 soybean to wild relative or weedy species in the U.S. and its territories are highly unlikely.

Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions. The genetic modification in the HB4 soybean is not expected to increase the potential for gene flow, hybridization and/or introgression to sexually compatible taxa compared to the nontransgenic recipient or other varieties of the crop commonly grown. Gene flow, hybridization and/or introgression of genes from the HB4 soybean to other sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories is not likely to occur. Therefore, HB4 soybean is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. and its territories.

## H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from adoption of the HB4 soybean are likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

HB4 soybeans were field-studied in a wide range of environmental conditions in 2012-2013 growing season in Argentina and in 2013 growing season in the United States. In 25 different sites that represent most of the soybean growing environments, HB4 soybean was cultivated and compared with Williams 82 control and five commercial varieties under the same agronomic practices (pp. 74-76, Verdeca 2017). Most of the agronomic and ecological characteristics, including germination, dormancy, plant height, days to 50% flowering, flower color, pollen morphology and fertility, lodging, shattering, disease and pest susceptibility, plant-symbiont interaction, and grain moisture, show no significant differences between HB4 soybean and its parental line Williams 82 (Table 12-14, pp. 327-328, Verdeca 2017). A few characteristics, including days to 50% emergence, early plant stand, plant stand at R8, seedling vigor, days to 50% maturity, and 1000 count seed weight show significant difference between HB4 soybean and Williams 82, but all except one values were within the range of the commercial reference varieties. Therefore, HB4 soybean has similar agronomic and ecological characteristics to conventional soybean and currently commercial soybean varieties, no changes in cultivation or management practices such as planting times, row spacing, irrigation, crop residue management, tillage or pesticide use are anticipated with the introduction of HB4 soybean.

It is noteworthy that HB4 soybean contains an inserted *bar* gene that confers resistance to glufosinate herbicide. This glufosinate resistance trait in HB4 soybean is similar to the other deregulated commercialized glufosinate tolerant soybean varieties. Thus, the adoption of HB4 soybean is not expected to change weed management practice compared to other herbicide resistant soybean varieties.

APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of HB4 soybean; therefore, no impact on plant diseases or pests or their management is likely to occur.

## I. Potential Impacts from Transfer of Genetic Information to Organisms with which HB4 Soybean Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into HB4 soybean to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. The horizontal gene transfer (HGT) between unrelated organisms is one of the

most intensively studied fields in the biosciences since the late 1940s (Soucy et al. 2015), and the issue gained extra attention with the release of transgenic plants into the environment (Droge et al. 1998). Potential risks from stable HGT from genetically engineered organisms to another organism without reproduction or human intervention were reviewed by Keese (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements (Keese 2008; Soucy et al. 2015). HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in the long run, to major transitions in evolution (Keese 2008).

### Potential for horizontal gene transfer to bacteria, fungi, or invertebrates

Soybean Event HB4 has two gene elements from bacteria, i.e., the *bar* gene from *S. hygroscopicus* and non-coding terminator sequence from *A. tumefaciens*.

Horizontal gene transfer and expression of DNA from a plant species to bacterial, fungal or invertebrate species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g., as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores) and with invertebrates as plant pests, there are almost no evolutionary examples of HGT from eukaryotes to bacteria or from plants to fungi or invertebrates (van den Eede et al. 2004; Keeling and Palmer 2008; Keese 2008). Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keeling and Palmer 2008; Keese 2008) and HGT between plants and fungi is extremely rare (Richards et al. 2009). Examples of HGT between plants and invertebrates are also extremely rare, and most examples of HGT in insects involve acquisition of genes from their pathogens or endosymbionts (Keese 2008; Zhu et al. 2011; Acuna et al. 2012).

Horizontal transfer from plants to bacteria and expression in bacteria of the foreign DNA inserted into the nuclear genome of the GE plant is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keeling and Palmer 2008; Keese 2008; Isaza et al. 2011). Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al. 2001; Brown 2003; EFSA 2009b). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, both the FDA (1998) and the European Food Safety Authority (2009a) have evaluated horizontal gene transfer from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes

from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is very rare or remote.

### *Potential for horizontal gene transfer to viruses*

HB4 soybean contains the following sequences from plant viruses, non-coding regulatory sequences from cauliflower mosaic virus and tobacco etch virus. APHIS also considered whether horizontal transfer of DNA from the GE plant to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. This issue has been considered before by other science review panels and government regulatory bodies (EPA-FIFRA-SAP 2006; Keese 2008). HGT is not unusual among plant viruses; however this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the point of crossover (Keese 2008). HGT from virus sequences engineered into plants has been demonstrated with infecting or challenge viruses, including both DNA viruses (e.g. geminiviruses which replicate in the nucleus) (Frischmuth and Stanley 1998) and RNA viruses which typically replicate in the cytoplasm; however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves 2007; Keese 2008; Thompson and Tepfer 2010). Populations of recombinants between virus transgenes expressed in transgenic plants and infected related viruses are similar to recombinants found in mixed infections of the same viruses in nontransgenic plants, indicating that there was no novel recombination mechanism in the transgenic plants and no increased risk is expected over what is expected from mixed infections (Keese 2008; Turturo et al. 2008). Nonhomologous recombination in HGT among viruses or between virus transgenes and infecting viruses can occur, but frequently results in gene deletions which can result in nonviable viruses (Morroni et al. 2013). Depending on the particular virus and sequences involved, various hot-spots for recombination have been found in both coding and noncoding regions, and strategies implemented in design of transgenes to avoid recombination have been suggested. No recombinant or undesirable viruses with new properties have been detected for over at least 8-10 years in field tests or during commercial growth of deregulated virus resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the U.S. (Fuchs and Gonsalves 2007).

### *Potential for horizontal gene transfer to parasitic plants*

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants (Barr et al. 2005), to other mitochondria genomes, and mostly involve parasitic plants and their hosts (Richardson and Palmer 2007). Recently, a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*) from its monocot host plant (Yoshida et al. 2010). According to this study, the incorporation of the specific genetic

sequence (with an unknown function) occurred between sorghum and purple witchweed. However, this HGT occurred before speciation of purple witchweed and related cowpea witchweed (*S. gesnerioides*) from their common ancestor. Furthermore, *S. hermonthica* is not found in the U.S. and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA-APHIS 2017c). More recent studies demonstrated that in a few parasitic species of the Rafflesiaceae family, out of several genetic sequences examined, about 2.1% of nuclear (Xi et al. 2012) and 24 –41% of mitochondrial (Xi et al. 2013) gene transcripts appeared to be acquired from their obligate host species. However, all the above-mentioned instances of HGT between parasitic plants and their hosts were reported to be of ancient origins, on an evolutionary time scale spanning thousands to millions of years ago. Furthermore in HB4 soybean, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome (Verdeca 2017).

If HB4 soybean becomes infected by a parasitic plant or is naturally grafted to another plant, there is a very low probability that HGT could result in the other plant acquiring DNA from HB4 soybean. However, in both scenarios this newly introduced DNA would likely reside in somatic cells with little chance of reaching the germ cells, and could not persist in subsequent generations unless the recipient plant reproduced asexually from the affected cells.

Based on the above analysis APHIS therefore concludes that HGT of the new genetic material inserted into HB4 soybean to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

## J.  Conclusion

APHIS has reviewed the information submitted in the petition, supporting documents, and other relevant information to assess the plant pest risk of the HB4 soybean compared to the unmodified variety from which it was derived. APHIS concludes that the HB4 soybean is unlikely to pose a greater plant pest risk than the unmodified organism (i.e., Williams 82) from which it was derived based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in the HB4 soybean because the *Agrobacterium* (a plant pest) used as a vector to transfer the genetic material was disarmed and was shown not to be present in the final Event HB4 soybean . The only plant pest sequences in the inserted genetic material are five noncoding sequences, including the noncoding T-DNA 25 base pairs left- and right-border sequences as well as the nopaline synthase (*nos*) gene terminator from *Agrobacterium,* the leader sequence from Tobacco Etch Virus (TEV), and 35S promoter sequence from Cauliflower Mosaic Virus. The addition of these genetic material did not confer any plant pest characteristics to HB4 soybean.
- No increase in plant pest risk was identified in the HB4 soybean from the expression of the inserted genetic material (HAHB4v or PAT proteins), or changes in

metabolism composition because there were no significant changes in agronomic, ecological and compositional characteristics that would render HB4 soybean more susceptible to pests and diseases over its control or reference soybean varieties.

- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in the HB4 soybean compared to the control variety or other comparators in field trials conducted in growing regions representative of where the HB4 soybean is expected to be grown. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that the HB4 soybean is more susceptible to pests or diseases. Therefore, no plant pest effects are expected on these or other agricultural products and no impacts are expected to APHIS pest control programs.
- Exposure to and/or consumption of the HB4 soybean are unlikely to have any adverse impacts on organisms beneficial to agriculture based on the analysis of compositional, phenotypic and agronomic data.
- The HB4 soybean is no more likely to become a weed than conventional soybean varieties based on its observed agronomic characteristics, weediness potential of the crop and current management practices available to control the HB4 soybean as a weed. Volunteers and feral populations of the HB4 soybean can be managed using a variety of currently available methods and herbicides.
- The HB4 soybean is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. or its territories. Gene flow, hybridization and/or introgression of inserted genes from the HB4 soybean to other sexually compatible relatives with which it can interbreed is not likely to occur. HB4 soybean does not confer or enhance weedy characteristics of cultivated soybean. Furthermore, there is no sexually compatible wild relative or weedy species reported in natural environments in North America.
- Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of the HB4 soybean were not identified.
- Horizontal gene transfer of the new genetic material inserted into the HB4 soybean to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

## K. References

51 FR 23302. 1986. *Coordinated Framework for Regulation of Biotechnology*.

57 FR 22984. 1992. *Statement of Policy: Foods Derived from New Plant Varieties*.

Acuna R, Padilla BE, Florez-Ramos CP, Rubio JD, Herrera JC, Benavides P, Lee SJ, Yeats TH, Egan AN, Doyle JJ, and Rose JK. 2012. *Adaptive horizontal transfer of a bacterial gene to an invasive insect pest of coffee*. PNAS 109, pp. 4197-4202. Retrieved from http://www.pnas.org/content/early/2012/02/17/1121190109.abstract

Ahrent DK and Caviness CE. 1994. *Natural Cross-Pollination of Twelve Soybean Cultivars in Arkansas*. Crop Science 34, pp. 376-378. Retrieved from

https://dl.sciencesocieties.org/publications/cs/pdfs/34/2/CS0340020376  Last accessed 09/22/2017.

Ariel FD, Manavella PA, Dezar CA, and Chan RL. 2007. *The true story of the HD-Zip family*. Trends in plant science 12, pp. 419-426. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17698401

Barr CM, Neiman M, and Taylor DR. 2005. *Inheritance and recombination of mitochondrial genomes in plants, fungi and animals*. New Phytologist 168, pp. 39-50; DOI: 10.1111/j.1469-8137.2005.01492.x. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2005.01492.x/pdf  Last accessed Aug 2014.

Bayer CropScience. 2017. *Petition for a Determination of Nonregulated Status for Herbicide Tolerance Cotton Transformation Event GHB811*. Submitted by M. Weeks, M.Sc. Bayer CropScience. Research Triangle Park, NC.  Last accessed Original petition recieved May 16, 2017. Final amended petition recieved July 21, 2017.

Bronsema KJ, Bischoff R, and van de Merbel NC. 2013. *High-sensitivity LC-MS/MS quantification of peptides and proteins in complex biological samples: the impact of enzymatic digestion and internal standard selection on method performance*. Analytical chemistry 85, pp. 9528-9535. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24010948

Brown JR. 2003. *Ancient horizontal gene transfer*. Nature Reviews Genetics 4, pp. 121-132; doi:110.1038/nrg1000. Retrieved from http://www.nature.com/nrg/journal/v4/n2/full/nrg1000.html  Last accessed Aug 2014.

Center for Environmental Risk Assessment IRF. 2011. *A review of the environmental safety of the PAT protein*. Environ Biosafety Res 10, pp. 73-101. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22781085

Chung G and Singh RJ. 2008. *Broadening the Genetic Base of Soybean: A Multidisciplinary Approach*. Critical Reviews in Plant Sciences 27, pp. 295-341.

Depicker A, Stachel S, Dhaese P, Zambryski P, and Goodman HM. 1983. *Nopaline synthase: transcript mapping and DNA sequence*. Journal of Molecular and Applied Genetics 1, pp. 561-573.

Dezar CA, Gago GM, González DH, and Chan RL. 2005. *Hahb-4, a sunflower homeobox-leucine zipper gene, is a developmental regulator and confers drought tolerance to Arabidopsis thaliana plants*. Transgenic Research 14, pp. 429-440. Retrieved from https://doi.org/10.1007/s11248-005-5076-0

Dezar CA, Giacomelli JI, Manavella PA, Re DA, Alves-Ferreira M, Baldwin IT, Bonaventure G, and Chan RL. 2011. *HAHB10, a sunflower HD-Zip II transcription factor, participates in the induction of flowering and in the control of phytohormone-mediated responses to biotic stress*. J Exp Bot 62, pp. 1061-1076. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21030388

Droge M, Puhler A, and Selbitschka W. 1998. *Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern*. Journal of Biotechnology 64, pp. 75-90.

EFSA. 2009a. *Statement of EFSA on the consolidated presentation of opinions on the use of antibiotic resistance genes as marker genes in genetically modified plants*.

EFSA Journal 1108. Retrieved from
http://www.efsa.europa.eu/en/efsajournal/pub/1108.htm

EFSA. 2009b. *Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants*. The European Food Safety Authority Journal 7, pp. 1-107. Retrieved from http://www.efsa.europa.eu/en/efsajournal/doc/1108.pdf  Last accessed Aug 2014.

Elhiti M and Stasolla C. 2009. *Structure nad function of homodomain-leucine zipper (HD-Zip) proteins*. Plant Signaling & Behaviour 4, pp. 86-88.

Ellstrand NC, Prentice HC, and Hancock JF. 1999. *Gene flow and introgression from domesticated plants into their wild relatives*. Annu. Rev. Ecol. Syst 30, pp. 539-563.

EPA-FIFRA-SAP. 2006. *Plant Incorporated Protectants Based on Virus Coat Protein Genes: Science Issues Asosciated with the Proposed Rule*. Retrieved from https://archive.epa.gov/scipoly/sap/meetings/web/pdf/minutes1205.pdf  Last accessed 09/28/2017.

FDA. 1998. *Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants*. Retrieved from http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096135.htm

FDA. 2006. *Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use*. U.S. Food and Drug Administration. Retrieved from http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Biotechnology/ucm096156.htm

FDA. 2015. *Agenecy Response Letter NPC 000016*. Retrieved from https://www.fda.gov/Food/IngredientsPackagingLabeling/GEPlants/Submissions/ucm458598.htm

FDA. 2017. *Agenecy Response Letter BNF 000155*. Retrieved from https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GEPlants/Submissions/ucm572731.pdf

Frischmuth T and Stanley J. 1998. *Recombination between viral DNA and the transgenic coat protein gene of African cassava mosaic geminivirus*. Journal of General Virology 79, pp. 1265-1271. Retrieved from http://vir.sgmjournals.org/content/79/5/1265.full.pdf+html  Last accessed Aug 2014.

Fuchs M and Gonsalves D. 2007. *Safety of virus-resistant transgenic plants two decades after their introduction: Lessons from realistic field risk assessment studies*. Annual Review of Phytopathology 45:, pp. 173-202; doi:110.1146/annurev.phyto.1145.062806.094434. Retrieved from http://www.annualreviews.org/doi/full/10.1146/annurev.phyto.45.062806.094434?url_ver=Z39.88-2003  Last accessed Aug 2014.

Gago GM, Almoguera C, Jordano J, Gonzalez DH, and Chan RL. 2002. *Hahb-4, a homeobox-leucine zipper gene potentially involved in abscisic acid-dependent responses to water stress in sunflower*. Plant, Cell and Environment 25, pp. 633-640.  Last accessed 9/28/2017.

Gago GMA, C.; Jordano, J.; Gonzalez, D.H.; Chan, R.L. 2002. *Hahb-4, a homeobox-leucine zipper gene potentially involved in abscisic acid-dependent responses to water stress in sunflower*. Plant, Cell and Environment 25, pp. 633-640.

Gallie DR, Tanguay RL, and Leahters V. 1995. *The tobacco etch viral 5' leader and poly(A) tail are functionally synergistic regulators of translation*. Gene 165, pp. 233-238.

Gelvin SB. 2003. *Agrobacterium-Mediated Plant Transformation: the Biology behind the "Gene-Jockeying" Tool*. Microbiology and Molecular Biology Reviews 67, pp. 16-37.

Grant V. 1981. *Plant Speciation, 2nd Edition*. New York: Columbia University Press, 563 pp.

Hartman G, Pawlowski M, Herman T, and Eastburn D. 2016. *Organically Grown Soybean Production in the USA: Constraints and Management of Pathogens and Insect Pests*. Agronomy 6, pp. 16.

Hartman GL, West ED, and Herman TK. 2011. *Crops that feed the World 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests*. Food Security 3, pp. 5-17.

Hegde SG, Nason JD, Clegg JM, and Ellstrand NC. 2006. *The evolution of California's wild radish has resulted in the extinction of its progenitors*. Evolution 60, pp. 1187-1197. Retrieved from http://dx.doi.org/10.1554/05-634.1  Last accessed Dec 2014.

Herouet C, Esdaile DJ, Mallyon BA, Debruyne E, Schulz A, Currier T, Hendrickx K, van der Klis RJ, and Rouan D. 2005. *Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the pat and bar sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants*. Regulatory toxicology and pharmacology : RTP 41, pp. 134-149. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15698537

Holm L, Pancho JV, Herberger JP, and Plucknett DL. 1979. *A Geographical Atlas of World Weeds*.

Hymowitz T. 1970. *On the domestication of the soybean*. Economic Botany 24, pp. 408-421. Retrieved from https://doi.org/10.1007/BF02860745

Hymowitz T and Newell CA. 1981. *Taxonomy of the Genus Glycine, Domestication and Uses of Soybeans*. Economic Botany 35, pp. 272-288. Retrieved from http://www.jstor.org/stable/4254296

ILSI. 2011. *A Review of the Environmental Safety of the PAT protein*.

Isaza L, Opelt K, Wagner T, Mattes E, Bieber E, Hatley E, Roth G, Sanjuán J, Fischer H, Sandermann H, Hartmann A, and D. E. 2011. *Lack of glyphosate resistance gene transfer from Roundup Ready ® soybean to Bradyrhizobium japonicum under field*.  Last accessed 9/26/2017.

Kaneko T, Nakamura Y, Sato S, Minamisawa K, Uchiumi T, Sasamoto S, Watanabe A, Idesawa K, Iriguchi M, Kawashima K, Kohara M, Matsumoto M, Shimpo S, Tsuruoka H, Wada T, Yamada M, and Tabata S. 2002. *Complete Genomic*

*Sequence of Nitrogen-fixing Symbiotic Bacterium Bradyrhizobium japonicum USDA110*. DNA Research 9, pp. 189 - 197.

Keeling PJ and Palmer JD. 2008. *Horizontal gene transfer in eukaryotic evolution*. Nature Reviews Genetics 9, pp. 605-618; doi:610.1038/nrg2386. Retrieved from http://www.nature.com/nrg/journal/v9/n8/full/nrg2386.html  Last accessed Aug 2014.

Keese P. 2008. *Risks from GMOs due to horizontal gene transfer*. Environmental Biosafety Research 7, pp. 123-149; DOI: 110.1051/ebr:2008014. Retrieved from http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=8208895&fileId=S1635792208000146  Last accessed Dec 2014.

Khoury CK, Greene S, Wiersema J, Maxted N, Jarvis A, and Struik PC. 2013. *An inventory of crop wild relatives of the United States*. Crop Science 53, pp. 1496; doi:1410.2135/cropsci2012.1410.0585. Retrieved from https://www.crops.org/publications/cs/abstracts/53/4/1496  Last accessed Aug 2014.

Koonin EV, Makarova KS, and Aravind L. 2001. *Horizonal gene transfer in prokaryotes: Quantification and classification*. Annual Review of Microbiology 55:, pp. 709-742. Retrieved from http://www.annualreviews.org/doi/full/10.1146/annurev.micro.55.1.709?url_ver=Z39.88-2003  Last accessed Aug 2014.

Manavella PA, Dezar CA, Bonaventure G, Baldwin IT, and Chan RL. 2008. *HAHB4, a sunflower HD-Zip protein, integrates signals from the jasmonic acid and ethylene pathways during wounding and biotic stress responses*. The Plant journal : for cell and molecular biology 56, pp. 376-388. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18643970

Manavella PA, Arce AL, Dezar CA, Bitton F, Renou JP, Crespi M, and Chan RL. 2006. *Cross-talk between ethylene and drought signalling pathways is mediated by the sunflower Hahb-4 transcription factor*. The Plant journal : for cell and molecular biology 48, pp. 125-137. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16972869

Morroni M, Jacquemond M, and Tepfer M. 2013. *Deep sequencing of recombinant virus populations in transgenic and nontransgenic plants infected with Cucumber mosaic virus*. Molecular Plant-Microbe Interactions 26, pp. 801-811. Retrieved from http://dx.doi.org/10.1094/MPMI-02-13-0057-R  Last accessed Aug 2014.

Odell JT, Nagy F, and Chua NH. 1985. *Identification of DNA sequences required for activity of the Cauliflower mosaic virus 35S promoter*. Nature 313, pp. 810-812.

OECD. 1999. *Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide*. Paris, France. Organisation for Economic Co-operation and Development. Retrieved from http://www.oecd.org/env/ehs/biotrack/46815628.pdf

OECD. 2000a. *Consensus Document on the Biology of Glycine max (L.). Merr. (Soybean).* Retrieved from http://www.oecd.org/dataoecd/16/56/46815668.pdf

OECD. 2000b. *Consensus Document on the Biology of Glycine max (L.) Merr. (Soybean) ENV/JM/MONO(2000)9*. Retrieved from http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2000)9  Last accessed Aug 2014.

Preston CD, Pearman DA, and Dines TD. 2002. *New Atlas of the British flora*. Oxford University Press.

Randall RP. 2017. *A Global Compendium of Weeds. Third Edition.* Perth, Western Australia. Retrieved from https://www.researchgate.net/profile/Roderick_Randall

Rapp WD, Lilley GG, and Nielsen NC. 1990. *Characterization of soybean vegetative storage proteins and genes*. Theoretical and Applied Genetics 79, pp. 785-792.

Ray JD, Kilen TC, Abel CA, and Paris RL. 2003. *Soybean natural cross-pollination rates under field conditions*. Environmental Biosafety Research 22, pp. 133-138.

Richards TA, Soanes DM, Foster PG, Leonard G, Thornton CR, and Talbot NJ. 2009. *Phylogenomic analysis demonstrates a pattern of rare and ancient horizontal gene transfer between plants and fungi*. Plant Cell 21, pp. 1897-1911. Retrieved from http://www.plantcell.org/content/21/7/1897.long Last accessed Aug 2014.

Richardson AO and Palmer JD. 2007. *Horizontal gene transfer in plants*. Journal of Experimental Botany 58, pp. 1-9; doi:10.1093/jxb/erl1148. Retrieved from http://jxb.oxfordjournals.org/content/58/1/1.full.pdf+html Last accessed Aug 2014.

Rieseberg LH and Wendel JF. 1993. *Introgression and Its Consequences in Plants*.

Sedivy EJ, Wu F, and Hanzawa Y. 2017. *Soybean domestication: the origin, genetic architecture and molecular bases*. The New phytologist 214, pp. 539-553. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/28134435

Sherman-Broyles S, Bombarely A, Powell AF, Doyle JL, Egan AN, Coate JE, and Doyle JJ. 2014. *The wild side of a major crop: soybean's perennial cousins from Down Under*. American journal of botany 101, pp. 1651-1665. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25326613

Soltis DE, Soltis PS, and Rieseberg LH. 1993. *Molecular data and the dynamic nature of polyploidy*. Critical Reviews in Plant Sciences 12, pp. 243-273; 210.1080/07352689309701903. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/07352689309701903#.UhekuRtwq-w Last accessed Dec 2014.

Soucy SM, Huang J, and Gogarten JP. 2015. *Horizontal gene transfer: building the web of life*. Nature reviews. Genetics 16, pp. 472-482. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26184597

Stace CA. 1987. *Hybridization and the plant species*.

Strauch E, Whohlleben W, and Puhler A. 1988. *Cloning of a phosphinothricin N-acetyltransferase gene from Steptomyces viridochromogenes Tu494 and its expression in Streptomyees lividans and Escherichia coli*. Gene 63, pp. 65-74.

Thompson CJ, Movva NV, Tizard R, Crameri R, Davies JE, Lauwereys M, and Botterman J. 1987. *Characterization of the herbicide-resistance gene bar from Streptomyces hygroscopicus*. The EMBO Journal 6, pp. 2519-2523.

Thompson JR and Tepfer M. 2010. *Assessment of the benefits and risks for engineered virus resistance*. Retrieved from http://www.sciencedirect.com/science/article/pii/S0065352710760024 Last accessed Dec 2014.

Turturo C, Friscina A, Gaubert S, Jacquemond M, Thompson JR, and Tepfer M. 2008. *Evaluation of potential risks associated with recombination in transgenic plants expressing viral sequences*. Journal of General Virology 89:, pp. 327-335; DOI

310.1099/vir.1090.83339-83330. Retrieved from http://vir.sgmjournals.org/content/89/1/327.full.pdf+html  Last accessed Aug 2014.

University KS. 2016. *Soybean Production Handbook*. Kansas State University.

USDA-APHIS-BRS. 2017. *Petitions for Determination of Nonregulated Status*. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status

USDA-APHIS. 2017a. *<List of Deregulated GE crops*. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status

USDA-APHIS. 2017b. *Plant Pests and Diseases*. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/

USDA-APHIS. 2017c. *Plant Pest Program Information*. Retrieved from http://www.aphis.usda.gov/plant_health/plant_pest_info/index.shtml  Last accessed 3 November, 2017.

USDA-FAS. 2017. *World Agricultural Porduction*. Retrieved from http://usda.mannlib.cornell.edu/usda/current/worldag-production/worldag-production-10-12-2017.pdf

USDA-NASS. 2016. *Soybean Production Acres by County*. Retrieved from https://www.nass.usda.gov/Charts_and_Maps/graphics/SB-PL-RGBChor.pdf

USDA-NASS. 2017a. *Soybean Production Acreage by Year*. Retrieved from https://www.nass.usda.gov/Charts_and_Maps/graphics/soyac.pdf

USDA-NASS. 2017b. *Soybean Yield by Year*. Retrieved from https://www.nass.usda.gov/Charts_and_Maps/graphics/soyyld.pdf

USDA-NRCS. 2017. *Plant Database - Soybean*. Retrieved from https://plants.usda.gov/core/profile?symbol=GLMA4

van den Eede G, Aarts H, Buhk HJ, Corthier G, Flint HJ, Hammes W, Jacobsen B, Midtvedt T, van der Vossen J, von Wright A, Wackernagel W, and Wilcks A. 2004. *The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants*. Food Chem Toxicol 42, pp. 1127-1156. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15123384

Wang KJ and Li XH. 2011. *Interspecific gene flow and the origin of semi-wild soybean revealed by capturing the natural occurrence of introgression between wild and cultivated soybean populations*. Plant Breeding 130, pp. 117-127.

Wood DW, Setubal JC, Kaul R, Monks DE, Kitajima JP, Okura VK, Zhou Y, Chen L, Wood GE, Almeida NF, Woo L, Chen YC, Paulsen IT, Eisen JA, Karp PD, Bovee D, Chapman P, Clendenning J, Deatherage G, Gillet W, Grant C, Kutyavin T, Levy R, Li MJ, McClelland E, Palmieri A, Raymond C, Rouse G, Saenphimmachak C, Wu ZN, Romero P, Gordon D, Zhang SP, Yoo HY, Tao YM, Biddle P, Jung M, Krespan W, Perry M, Gordon-Kamm B, Liao L, Kim S, Hendrick C, Zhao ZY, Dolan M, Chumley F, Tingey SV, Tomb JF, Gordon MP, Olson MV, and Nester EW. 2001. *The genome of the natural genetic engineer Agrobacterium tumefaciens C58*. Science 294, pp. 2317-2323. Retrieved from ISI:000172817200037

Xi Z, Wang Y, Bradley RK, Sugumaran M, Marx CJ, Rest JS, and Davis CC. 2013. *Massive Mitochondrial Gene Transfer in a Parasitic Flowering Plant Clade*. PLOS Genetics 9, pp. e1003265. Retrieved from https://doi.org/10.1371/journal.pgen.1003265

Xi ZX, Bradley RK, Wurdack KJ, Wong KM, Sugumaran M, Bomblies K, Rest JS, and Davis CC. 2012. *Horizontal transfer of expressed genes in a parasitic flowering plant*. BMC Genomics 13, pp. 227. Retrieved from http://www.biomedcentral.com/1471-2164/13/227

Yoshida S, Maruyama S, Nozaki H, and Shirasu K. 2010. *Horizontal gene transfer by the parasitic plant Striga hermonthica*. Science 328, pp. 1128; DOI:1110.1126/science.1187145. Retrieved from http://www.sciencemag.org/content/328/5982/1128.full  Last accessed September 26, 2017.

Zhu B, Lou MM, Xie GL, Zhang GQ, Zhou XP, Li B, and Jin GL. 2011. *Horizontal gene transfer in silkworm, Bombyx mori*. BMC Genomics 12, pp. 248.