Plant Pest Risk Assessment for DP-32138-1 Corn

Pioneer Hi-Bred International, has petitioned APHIS (APHIS number 08-338-01p) for a determination that genetically engineered (GE) corn (*Zea mays* L. subsp. *mays*) event DP-32138-1 is unlikely to pose a plant pest risk (Pioneer 2009) and, therefore, should no longer be a regulated article under APHIS' 7 Code of Federal Regulations (CFR) part 340. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2000[1]. This plant pest risk assessment was conducted to determine if DP-32138-1 is unlikely to pose a plant pest risk.

DP-32138-1 was produced by transformation of a developing corn embryo of line H-II using *A. tumefaciens.* Because *A. tumefaciens* is a plant pest and some of the regulatory sequences (Cauliflower Mosaic Virus enhancer) used to facilitate expression of these genes in corn were derived from plant pests, this corn has been considered a regulated article under APHIS regulations at 7 CFR part 340.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of DP-32138-1 and its progeny in the absence of confinement. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if DP-32138-1 is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, then APHIS has no regulatory authority over that organism.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk characteristics. APHIS regulation 7 CFR 340.6(c) specifies the information needed for consideration in a petition for nonregulated status. APHIS will evaluate information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, any impacts on the weediness of any other plant with which it can interbreed, and transfer of genetic information to organisms with which it cannot interbreed.

The Environmental Assessment (EA) for this petition considered whether agricultural or cultivation practices for DP-32138-1 may result in impacts on the environment. A thorough assessment of the effects of the determination on non-target and beneficial organisms, and threatened and endangered species is included in the Environmental Assessment.

Development of Male Fertility/ Male Sterility DP-32138-1 Corn

[1] Section 403 (14) of the Plant Protection Act (7USC Sec 7702(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."

Since the 1930s, corn productivity in the U.S. has been greatly enhanced by the use of hybrid corn seed (Sleper 2006). Corn hybrids are characterized by increased resistance to diseases and enhanced agronomic characteristics compared with the parental lines (Brewbaker 1964). The production of hybrid corn seed involves a cross between two inbred lines[2], where the pollen from the tassel/male parent is used to fertilize the ear/female parent. Because corn is mostly self pollinated (OECD 2003), hybrid corn seed is typically produced by the removal of male flowers (tassels) from the female parent either mechanically or by hand. However, mechanical detasseling can result in up to 40 percent reduction in seed yield when compared to that of hand detassel treatments (Wych 1988). In addition, female plants may be blown over by a storm and escape detasseling, allowing secondary tassels to develop after completion of the manual detasseling. In either case, some of the female plants will be self-pollinated resulting in seed of the female inbred being harvested along with the hybrid seed.

As an alternative to tassel removal, numerous genetic strategies have been attempted to achieve male sterility[3] (Skibbe 2005). Over 40 genetic elements are associated with male sterility. Some of these elements are located on the nuclear chromosomes while others are located on the mitochondrial chromosomes (Skibbe 2005). A major drawback to male sterile genetic approaches is the difficulty in generating male inbred lines because no functional pollen is produced in a male sterile plant line.

Pioneer has developed a novel method (Pioneer 2009, Appendices 1 through 7) that uses DP-32138-1 to produce male sterile/female inbred plants for the generation of hybrid corn seed that is non-transgenic (known as "Seed Production Technology", Pioneer 2009, pp. 23- 26, figures 3, 4 & 5). As detailed in the petition, the carefully controlled expression of a seed color marker gene and pollen fertility and sterility genes facilitates the generation of red transgenic seed for seed increase of male sterile-female inbred lines, and for the production of non-transgenic fertile pollen for use in non-transgenic hybrid seed production (Pioneer 2009, figure 3, 4 & 5).

DP-32138-1 lines are used to produce male sterile female inbred lines and are not used for commercial production of commodity corn (Pioneer 2009, pp. 23, 101, 122-123). The total acreage in the U.S. planted each year with DP-32138-1 is expected to be less than 5,000 acres (Pioneer 2009, table 20). If the DP-32138-1 is licensed to third parties and adopted across the entire U.S. seed industry, the total acreage is not expected to exceed 20,000 acres (Pioneer 2009, table 20).

FDA has completed review of the new protein consultations for DsRed2 and ZM-AA1 on January 29, 2010 and has no further questions.

Description of the Modification

[2] Inbred lines are populations of identical or nearly identical plants used as stocks for the creation of hybrid lines.
[3] Male sterility - the inability to produce functional pollen.

DP-32138-1 corn was produced by transformation using disarmed *Agrobacterium tumefaciens* (Pioneer 2009, pp. 35 through 37, figures 11 and 12). Immature embryos of Hi-II (*ms45/ms45*) (Cigan 2001) were infected with *Agrobacterium* strain LBA4404 containing plasmid PHP24597. Plants containing the introduced DNA were selected based on the production of a red color marker and then screened for male fertility (Pioneer 2009, p. 35, figure 11).

The plasmid PHP24597 contained three gene expression cassettes flanked by the right and left border of T-DNA from the *Agrobacterium* Ti-plasmid (Pioneer 2009, figure 13 & 14, table 2):

Pollen fertility restorer *Ms45* - expression cassette consists of two genetic elements (Pioneer 2009, p. 43):

- The 5126 anther-preferred[4] promoter from *Z. mays* localizes the expression to the anther (Cigan 1997).

- The pollen fertility restoration gene (*Ms45*) from *Z. mays* restores development of the microspore cell wall that gives rise to pollen[5] (Cigan 2001). The presence of the transgene *Ms45* allows for production of viable pollen.

α-Amylase *zm-aa1* - expression cassette consists of four genetic elements (Pioneer 2009, p. 43):

- The polygalacturonase promoter (*PG47*) from *Z. mays* localizes expression to pollen (Allen 1993).

- Transit peptide *brittle*-1 (*zm-bt1*) from *Z. mays* targets expression to the amyloplast[6] (Sullivan 1991). The *zm-aa1* gene was truncated to remove the native transit peptide sequence and replaced by the *zm-bt1* amyloplast-targeting sequence.

- The alpha-amylase (α-amylase)[7] *zm-aa1* gene from *Z. mays* (Pioneer 2009, figure 43) encodes the ZM-AA1 protein which breaks down starch (Janeček 1994). When the ZM-AA1 protein is expressed in immature pollen, the depletion of starch reserves renders the pollen infertile.

- The *In2-1* terminator from *Z. mays* from the *In2-1* gene (Hershey 1991).

[4] Pollen development takes place within the anther (Bedinger 1992). The 5126 promoter localizes expression to an inner cell layer of the anthers called the tapetum (petition figures 8A & 8B).
[5] Mutations in the *Ms45* gene causes male sterility because there is no functional pollen produced. A mutation in the Ms45 gene is designated as ms45.
[6] Amyloplast is an organelle in the cytoplasm of plant cells where starch is stored.
[7] Catalyze the hydrolysis of α-1,4- D-glucosidic bonds in polysaccharides such as starch.

Red color marker *DsRed2(AltI)* - expression cassette consists of four genetic elements (Pioneer 2009, p. 43):

- A *35S* enhancer from the cauliflower mosaic virus (CaMV) (Odell 1985, Odell 1988).

- The lipid transfer protein (*Ltp2*) promoter from barley localizes expression to the aleurone layer of the seed (Kalla 1994).

- The *DsRed2* gene from a marine coral-like anemone *Discosoma* sp. (Matz 1999, Wasson-Blader 2001) was modified by a single base pair substitution (in which an internal *Bst*EII restriction site was removed without changing the amino acid sequence in the DsRed2 protein) to produce *DsRed2(AltI*).

  DP-32138-1 seeds expressing the DsRed2 protein are colored pinkish-red (Pioneer 2009, figure 10, Wasson-Blader 2001) and can be sorted from yellow seed (that does not express DsRed2) using a color sorting machine (Pioneer 2009, figures 4, 5 and 10, Appendices 2 through 7). The DsRed2 protein enables detection and sorting of transgenic red corn seeds from non-transgenic yellow seeds.

- The proteinase inhibitor II (*pinII*) terminator from *Solanum tuberosum* (An 1989, Keil 1986).

Data from Southern blot analyses demonstrate that DP-32138-1 contains: (1) a single copy of the 5126 promoter and *Ms45* gene (Pioneer 2009, table 4, figure 19); (2) a single copy of the PG47 promoter, *zm-bt1* transit peptide, *zm-aa1* gene and *In2-1* terminator (petition table 4, figures 20 and 21); and (3) a single copy of the 35S enhancer, the *Ltp2* promoter, *DsRed2(AltI)* and *pinII* terminator (Pioneer 2009, table 4, figures 22 and 23).

Potential of DP-32138-1 to Become Invasive and/or a Weed

Corn possesses few of the characteristics of those plants that are notably successful as weeds (Baker 1965, Keeler 1989). In the U.S., corn is not listed as a weed (Crockett 1977, Holm 1979, Muenscher 1980), nor is it present in the Federal noxious weed list (7 CFR part 360;[8]. Furthermore, corn is grown throughout the world without any report that it is a serious weed or that it forms persistent feral populations (Gould 1968). Like many domesticated crops, corn seed from a previous year's crop can overwinter and germinate the following year. For instance, the appearance of corn seedlings in soybean fields following a corn crop is a common occurrence. Manual or chemical measures are often applied to remove these volunteers, but the plants that are not removed do not typically result in feral populations in following years.

APHIS assessed whether DP-32138-1 is any more likely to become a weed than the

[8] http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist2006.pdf

isogenic nontransgenic corn line, or other corn varieties currently under cultivation. The assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of the unique characteristics of DP-32138-1 under field conditions. Pioneer has been conducting agronomic field trials of DP-32138-1 since 2005 in the U.S. corn belt at over 43 locations (Pioneer 2009, Appendix 12). Agronomic data were collected from DP-32138-1 and its control counterparts in six locations that provide a range of environmental conditions representative of where the DP-32138-1 is expected to be grown for seed production (Pioneer 2009, figure 47). Field trial data (Pioneer 2009, pp. 101- 107, tables 11 and 12) indicated that DP-32138-1 does not exhibit characteristics that would cause it to be weedier than the parental corn line. No differences in phenotypic characteristics that might contribute to enhanced weediness were observed between DP-32138-1 and control lines for the wide range of phenotypic endpoints assessed in these field trials or in greenhouse or laboratory experiments (Pioneer 2009, table 11). There was no increase in weediness potential as measured by differences observed in the field for germination, seedling vigor, plant height, ear height, stalk lodging, root lodging, final population, plant health, time to silking, time to pollen, pollen viability, and seed germination (Pioneer 2009, tables 10 and 12). None of the measured attributes showed any differences relative to its comparator, suggesting that DP-32138-1 is not weedier than current corn cultivars.

These results on growth characteristics, seed production and germination indicate that the DP-32138-1 is not significantly different from its comparators. There is no indication that DP-32138-1 possesses a selective advantage that would result in increased weediness. DP-32138-1 lacks the ability to persist as a troublesome weed, and there would be no significant impact on current weed management practices for corn cultivation.

Potential for Gene Flow and Gene Introgression from DP-32138-1 into Sexually-Compatible Relatives

Gene flow from crops to wild relatives may have the potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower, and a few other crops (Ellstrand 1999). 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 (Soltis 1993, Rieseberg 1997) and even in existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987, Rieseberg 1993). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars.

APHIS evaluated the potential for gene introgression to occur from DP-32138-1 to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Cultivated corn is sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum* (OECD 2003). The closest wild relatives of corn, various *Zea* species referred to as teosinte, are normally confined to the tropical and subtropical regions of Mexico,

Guatemala, and Nicaragua. In the U.S. a fairly rare, sparsely dispersed feral population of teosinte has been reported in Florida (USDA Plant database[9]).

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America. Three of these species (*T. dactyloides, T. floridatum*, and *T. lanceolatum*) exist as wild and/or cultivated species in the continental U.S.; and two taxa (*T. fasciculatum* and *T. latifolium*) also occur in Puerto Rico (USDA PLANTS Database, accessed 11/9/2009). Though many of these species occur where corn might be cultivated, gene introgression from DP-32138-1 under natural conditions is highly unlikely. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility and none of them typically withstand even the mildest winters (Galinat 1988, Magelsdorf 1939). Furthermore, none of the sexually compatible relatives of corn in the U.S. are considered to be weeds in the U.S. (Holm 1979). Therefore, even in those instances of incidental gene flow between DP-32138-1 and wild relatives, the transgenes of DP-32138-1 are unlikely to transform corn wild relatives into more weedy species.

Introgression of genes from corn into teosinte or *Tripsacum* species has not been described to occur in nature in the U.S. While some teosinte may be considered weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez 1997 and references therein). Teosinte is described as being susceptible to many of the same pests and diseases that attack cultivated corn (Sánchez 1997). In the wild, introgressive hybridization from corn to teosinte is currently limited, in part, by several factors including geographic isolation, differing degrees of genetic incompatibility, differences in flowering time in some cases, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Doebley 1990a and 1990b, Galinat 1988, Ellstrand 2007). First-generation hybrids are generally less fit for survival and dissemination in the wild and show substantially reduced reproductive capacity, which thus acts as a significant constraint to introgression. Even if gene flow to a wild relative of corn did occur, an engineered trait that does not offer any adaptive advantage will probably not persist in the weed population (Ellstrand 1990). DP-32138-1 contains a male fertility gene that is normally present in corn, a male sterility gene, and a color selectable marker gene. None of these genes would be expected to confer a selective advantage if gene flow were to occur. Data included in the petition demonstrated that there were no significant differences in viability and morphology (as measured by size shape and color) of pollen collected from DP-32138-1 plants and near-isoline reference plants (Pioneer 2009, figure 9, table 12); therefore, the outcrossing rate of DP-32138-1 is not expected to be any different from other corn. Moreover, its potential impact due to the extremely limited potential for gene introgression into teosinte is not expected to be any different than that of other cultivated corn varieties. Therefore, USDA has determined that any adverse consequences of gene flow from DP-32138-1 to wild or weedy species in the U.S. are highly unlikely.

[9] http://plants.usda.gov/java/county?state_name=Florida&statefips=12&symbol=ZEME

Potential for Transfer of Genetic Information to Organisms with which DP32138-1 Cannot Interbreed

APHIS assessed whether horizontal gene transfer might occur between DP-32138 corn and inserted genes with other organisms. However, such transfer and expression of DNA from a plant species to bacteria is unlikely to occur (Keese 2008). First, many bacteria (or parts thereof) that are closely associated with plants have been sequenced, including *Agrobacterium* and *Rhizobium* (Kaneko 2000, Kaneko 2002, Wood 2001). There is no evidence that these organisms contain genes derived from plants. Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events were inferred to have occurred on an evolutionary time scale on the order of millions of years (Brown 2003, Koonin 2001). Third, FDA has evaluated horizontal gene transfer following plant transformation with 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 remote (FDA 1998). Therefore, APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk.

Potential for DP32138-1 to have Altered Disease and Pest Susceptibilities

APHIS assessed whether DP-32138-1 is likely to have significantly altered disease and pest susceptibility. This assessment encompassed a thorough consideration of the introduced trait and disease and pest susceptibility data from DP-32138-1 field trials.

DP-32138-1 has been field tested in the U.S. since 2005 (Pioneer 2009, p. 101, Appendix 12). Agronomic data was collected at six different locations in 2007 that provided a range of environmental conditions representative of where DP-32138-1 is expected to be grown (Pioneer 2009,figure 47). Pioneer routinely monitors their corn field trials for the following disease agents: *Aspergillus* sp., *Aureobasidium zeae, Cercospora zeae-maydis, Colletotrichum graminicola, Exserohilum turcicum, Fusarium* spp., *Gibberella zeae, Pantoea stewartii, Phytopthora* spp., *Puccinia polysora, Puccinia sorghi* and *Ustilago zeae* (Pioneer 2009, Appendix 13, table 2). The corn insects monitored include Aphididae, *Adoretus sinicus, Chaetocnema pulicaria, Chrysoperla carnea Cicadellidae,* Coleoptera, *Diabrotica* spp., *Glischrochilus quadrisignatus, Helicoverpa zea,* Lepidoptera, *Ostrinia nubilalis, Popillia japonica, Richia albicosta, Spodoptera frugiperda, Syrphidae, Tetranychidae, Thripidae* (petition Appendix 13, table 1). The data submitted by Pioneer indicated no meaningful differences between DP-32138-1 and non-transgenic counterparts for disease or insect damage (Pioneer 2009, p. 107, Appendix 13, tables 1 and 2).

Furthermore, DNA sequences derived from plant pests that were incorporated in DP-32138-1 did not result in the production of infectious agents or disease symptoms in field trials with the plants, and so it is unlikely that DP-32138-1 could pose a plant pest risk. The description of the genetic modifications, including genetic elements, expression of the gene product and their functions for DP-32138-1 has been summarized above.

The use of *Agrobacterium* for transforming DP-32138-1 is unlikely to propagate infectious agents. Transformed plants used in the generation of DP-32138-1 were treated with an antibiotic to eliminate the *Agrobacterium* from plant culture using well known protocols (Pioneer 2009).

APHIS considered whether corn constituents that differed between DP-32138-1 corn and other corn varieties might be sufficient to lead to increased susceptibility to pathogens or pests. The data presented in the petition indicate no difference in compositional and nutritional quality of DP-32138-1 compared to conventional corn, apart from the presence of the MS45, ZM-AA1 and DsRed2 proteins (Pioneer 2009). None of the values for seed composition characteristics were outside the range of natural variability of conventional corn (Pioneer 2009, tables 13, 14, 15, 16, 17, 18 and 19). Therefore, the composition of DP-32138-1 is not biologically different than conventional corn (with the exception of the MS45, ZM-AA1 and DsRed2 proteins). Based on the known functions and mechanisms of actions of these proteins (summarized in Pioneer 2009), none of these proteins are expected to directly alter susceptibility to plant pathogens. Thus DP-32138-1 is expected to be susceptible to the same plant pathogens as conventional corn.

Corn is not a plant pest in the United States[10], and the introduced DNA in DP-32138 is unlikely to pose a plant pest risk. Based on the analysis of genetic modifications and their functions and field testing data submitted by petitioner, APHIS concludes that there are no significant differences between DP-32138-1 corn and the non-transgenic counterparts relative to pest and disease susceptibility.

Conclusion

APHIS has prepared this plant pest risk assessment in order to determine if event DP-32138-1 is unlikely to pose a plant pest risk. Based on the information provided by the applicant and the lack of plant pest risk from the inserted genetic material, weedy characteristics, atypical responses to disease or plant pests in the field, effects on non-targets or beneficial organisms in the agro-ecosystem, and horizontal gene transfer, APHIS has concluded that event DP-32138-1 is unlikely to pose a plant pest risk.

References

Allen RL, Lonsdale DM (1993) Molecular characterization of one of the maize polygalacturonase gene family members which are expressed during late pollen germination. Plant Journal 3:261-271.

An G, Mitra A, Choi HK, Costa MA, An K, Thornburg RW, Ryan CA (1989) Functional analysis of the 3' control region of the potato wound-inducible proteinase inhibitor II gene. Plant Cell 1:115-122.

Bedinger P (1992) The remarkable biology of pollen. Plant Cell 4: 879-887.

[10] http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist2006.pdf

Brewbaker JL (1964) Agricultural Genetics. Prentice-Hall, Englewood Cliffs, NJ.

Brown JR (2003) Ancient horizontal gene transfer. Nat. Rev. Genet. 4:121-132.

Cigan AM, Unger E, Xu R-J, Kendall T, Fox T (2001) Phenotypic complementation of *ms45* maize requires tapetal expression of MS45. Sex. Plant Reprod. 14:135-142.

Cigan AM, Albertsen MC (1997) Transgenic Plants and DNA Comprising Anther Specific Promoter 5126 and Gene to Achieve Male-Sterility. November 18, 1997. United States Patent No. 5,689,051.

Crockett L (1977) Wildly Successful Plants: North American Weeds. University of Hawaii Press, Hawaii.

Doebley J (1990a) Molecular evidence for gene flow among *Zea species*. BioScience 40:443-448.

Doebley J (1990b) Molecular systematics of *Zea* (Gramineae). Maydica 35:143-50.

Ellstrand NC, Hoffman CA (1990) Hybridization as an avenue of escape for engineered genes. BioScience 40:438-442.

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

Ellstrand NC, Garner L, Hegde S, Suadagnuolo R, Blancas L (2007) Spontaneous hybridization between maize and teosinte. Journal of Heredity 98:183-187.

FDA (1998) Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants. pp. 28.

Galinat WC (1988) The origin of corn. Pp. 1-31 *In* G. F. Sprague and J. W. Dudley, (eds.). Corn and corn improvement. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Gould FW (1968) Grass Systematics. McGraw-Hill, New York.

Hershey HP, Stoner TD (1991) Isolation and characterization of cDNA clones for RNA species induced by substituted benzenesulfonamides in corn. Plant Molecular Biology 17:679-690.

Holm L, Doll J, Holm E, Pancho JV, Herberger JP (1997) World Weeds: Natural Histories and Distribution. John Wiley and Sons, New York.

Janeček Š (1994) Sequence similarities and evolutionary relationships of microbial, plant and animal α-amylases. Eur. J. Biochem. 224:519-524.

Kalla R, Shimamoto K, Potter R, Nielsen PS, Linnestad C, Olsen OA (1994) The promoter of the barley aleurone-specific gene encoding a putative 7 kDa lipid transfer protein confers aleurone cell-specific expression in transgenic rice. Plant Journal 6(6): 849-860

Kaneko T *et al.* (2000) Complete genome structure of the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*. DNA Research. 7:331-338.

Kaneko T *et al.* (2002) Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Research 9:189-197

Keeler K (1989) Can genetically engineered crops become weeds? Bio/Technology 7:1134-1139.

Keese P (2008) Risks from GMOs due to horizontal gene transfer. Environmental Biosafety Research 7:123-149.

Keil M, Sanches-Serrano J, Schell J, Willmitzer L (1986) Primary structure of a proteinase inhibitor II gene from potato. Nucleic Acids Research 14:5641-5650.

Koonin EV, Makarova KS, Aravind L (2001) Horizontal gene transfer in prokaryotes: quantification and classification. Annual Review of Microbiology. 55:709-742.

Mangelsdorf PC, Reeves RG (1939) The Origin of Indian Corn and its Relatives. Texas Agricultural Experiment Station. Bulletin No. 574.

Matz MV, Fradkov AF, Labas YA, Savitsky AP, Zaraisky AG, Markelov ML, Lukyanov SA (1999) Fluorescent proteins from non-bioluminescent *Anthozoa* species. Nature Biotechnology 17:969–973.

Muenscher WC (1980) Weeds. 2nd edition. Cornell University Press, Ithaca and London.

Odell JT, Knowlton S, Lin W, Mauvais, CJ (1988) Properties of an isolated transcription stimulating sequence derived from the cauliflower mosaic virus 35S promoter. Plant Molecular Biology 10:263-273.

Odell JT, Nagy F, Chua, NH (1985) Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature 313:810-812.

OECD (2003) Consensus Document on the Biology of *Zea mays* subsp. *mays* (Maize). pp 1-49.

Pioneer (2009). Petition for the Determination of Nonregulated Status for Maize 32138 SPT Maintainer Used in the Pioneer Seed Production Technology (SPT) Process.

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Rieseberg LH, Wendel JF (1993) Introgression and its Consequences in Plants. Pp. 70–109 *In* RG Harrison (ed.) Hybrid Zones and the Evolutionary Process. Oxford University Press, Oxford.

Rieseberg LH (1997) Hybrid origins of plant species. Annual Review of Ecology and Systematics 28:359-389.

Sánchez GJJ, Ruiz CJA (1997) Teosinte distribution in Mexico. Pp 18-39 *In* JA Serratos, MC Willcox, F Castillo-Gonzalez, (eds.) Gene flow among maize landraces, improved maize varieties, and teosinte: implications for transgenic maize. CIMMYT, Mexico, D.F.

Skibbe DS, Schnable PS (2005) Male sterility in maize. Maydica 50:367-367.

Sleper DA, Poehlman JM (2006) Breeding Corn (Maize). Chapter 17. Pp. 277-296 *In* Breeding Field Crops, 5th Edition. Blackwell Publishing.

Soltis DE, Soltis PS (1993) Molecular data and the dynamic nature of polyploidy. Critical Reviews in Plant Sciences 12:243–273.

Stace CA (1987) Hybridization and the plant species. Pp. 115–127 *In* KM Urbanska (ed.) Differentiation Patterns in Higher Plants. Academic Press, New York.

Sullivan TD, Strelow LI, Illingworth CA, Phillips RL, Nelson Jr OE (1991) Analysis of Maize Brittle-1 Alleles and a Defective Suppressor-Mutator-Induced Mutable Allele. Plant Cell 3:1337-1348.

USDA-APHIS (2009) Petitions of Nonregulated Status http://www.aphis.usda.gov/brs/not_reg.html Accessed January 11, 2010.

Wasson-Blader T (2001) Living Colors™ DsRed2: Improved red fluorescent protein for use in living cells. Clontechniques 16:2-3

Wood DW, *et al.* (2001) The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. Science. 294:2317-2323.

Wych RD (1988) Production of hybrid seed corn. Pp 565-607 *In* GF Sprague (ed.). Corn and Corn Improvement, American Society of Agronomy, Inc., Crop Science Society of American Inc., and Soil Science Society of America, Inc. Madison, Wisconsin,.