GM Crop Database

Database Product Description

SYN-IR162-4 (MIR162)

Host Organism	*Zea mays* L. (Maize)
Trait	Resistance to lepidopteran pests
Trait Introduction	Agrobacterium tumefaciens-mediated plant transformation.
Proposed Use	Production of *Z. mays* for human consumption (wet mill or dry mill or seed oil), and meal and silage for livestock feed. These materials will not be grown outside the normal production area for corn.
Product Developer	Syngenta Seeds, Inc.

Summary of Regulatory Approvals

Country	Food	Feed	Env	Notes
Argentina	2010	2010	2011	
Australia	2009			
Brazil	2009	2009	2009	
Canada	2010	2010	2010	
Colombia	2012	2010		
Japan	2010	2010	2010	
Korea	2010	2010		
Mexico	2010	2010		
Philippines	2010	2010		
Taiwan	2009			Approval valid until April 20, 2014.
United States	2008	2008	2010	

Introduction

Maize is susceptible to attack by a variety of insects from the time it is planted until it is consumed as food or feed. The most economically significant insect pests of maize are: *Ostrinia nubilalis* (European corn borer), *Diatraea saccharalis* (sugarcane borer), *Diatraea grandiosella* (southwestern corn borer), *Diabrotica* spp. (corn rootworm complex), *Helicoverpa zea* (corn earworm/cotton bollworm), *Spodoptera frugiperda* (fall armyworm), *Agrotis ipsilon* (black cutworm), *Elasmopalpus lignosellus* (lesser cornstalk borer), *Rhopalosiphum maidis* (corn leaf aphids), and *Striacosta albicosta* (western bean cutworm). Pests of secondary economic importance in maize include both soil-dwelling insects that feed on roots (e.g., wireworms, billbugs, webworms, white grubs, corn root aphids, the seed corn maggot, grape colaspis and seedcorn beetles) and above-ground insects that attack the stalk, leaf, and ear (e.g., cutworms, chinch bugs, grasshoppers, corn fl ea beetles and Japanese beetles).

While crop losses attributable to *O. nubilalis* and Diabrotica infestations have been well characterized and are significant, there is not as much quantitative information available on the economic impacts of other major insect pests of maize, specifically *H. zea*, *S. frugiperda*

, *A. ipsilon*, and *S. albicosta*. These pests are not as widespread as corn borers and rootworms; however, crop infestations by these leaf and ear-feeding pests can be very costly to growers, as they have the potential to significantly lower grain yield and quality. Conventional insecticide applications are an option for reducing feeding damage caused by these insects; however, most growers do not treat their crops to control these pests because of cost and limited effectiveness of the chemical agents. Currently available novel varieties are not as efficacious against these lepidopteran insects as they are against *O. nubilalis*. For example, Bt11 maize containing the Cry1Ab toxin provides only limited or no protection against feeding damage caused by *H. zea*, *S. frugiperda*, *A. ipsilon*, and *S. albicosta*.

Transformation event MIR162 maize has been developed by Syngenta to provide growers with maize hybrids that are resistant to feeding damage caused by a number of lepidopteran insect pests. MIR162 maize contains a Vip3Aa protein from *Bacillus thuringiensis* that is highly toxic to *H. zea*, *S. frugiperda*, *A. ipsilon*, and *S. albicosta* larvae. In combination with an *O. nubilalis*-protected maize trait, the Vip3Aa protein in MIR162 can provide growers the means of protecting their maize crops from damage caused by a broad range of lepidopteran pests.

Summary of Introduced Genetic Elements

Code	Name	Type	Promoter, other	Terminator	Copies	Form
vip3Aa20	vegetative insecticidal protein	IR	ZmUbiInt (*Zea mays* polyubiquitin gene promoter and first intron).	CaMV 35S 3' polyadenylation signal	1	native
pmi	mannose-6-phosphate isomerase	SM	ZmUbiInt (*Zea mays* poly-ubiquitin gene promoter and first intron)	*A. tumefaciens* nopaline synthase (*nos*) 3'-untranslated region	1	

Characteristics of *Zea mays* L. (Maize)

Center of Origin	Reproduction	Toxins	Allergenicity
Mesoamerican region, now Mexico and Central America	Cross-pollination via wind-borne pollen is limited, pollen viability is about 30 minutes. Hybridization reported with teosinte species and rarely with members of the genus Tripsacum.	No endogenous toxins or significant levels of antinutritional factors.	Although some reported cases of maize allergy, protein(s) responsible have not been identified.

Donor Organism Characteristics

Latin Name	Gene	Pathogenicity
Bacillus thuringiensis strain AB88	vip3A	While target lepidopteran insects are susceptible to oral doses of VIP proteins, there is no evidence of toxic effects in laboratory mammals, in birds, and in non-target arthropods, including beneficial insects.

Modification Method

MIR162 maize was produced by *Agrobacterium tumefaciens*-mediated transformation of immature embryos using the plasmid vector pNOV1300. The transformation vector is a disarmed, binary vector that contains both left and right transfer-DNA (T-DNA) border sequences to facilitate transformation. The vector region between the left and right border sequences, which included the *vip3Aa19* and *pmi* gene expression cassettes, was inserted into the maize genome during transformation. One expression cassette consists of the *vip3Aa20* (see below for explanation of name change) coding region regulated by the *Zea mays* polyubiquitin promoter (ZmUbilInt) and cauliflower mosaic virus 35S 3′ polyadenylation sequences. A second expression cassette consists of the *pmi* coding region regulated by the *Zea mays* polyubiquitin promoter (ZmUbilInt) and the nopaline synthase (NOS) polyadenylation sequence.

Characteristics of the Modification

The Introduced DNA

Southern blot analyses and nucleotide sequencing demonstrated that MIR162 maize contains a single intact T-DNA insert in the nuclear maize genome. Southern blot analyses further demonstrated that the T-DNA insert contains: i) single copies of a *vip3Aa* gene and a *pmi* gene; ii) two copies of the ZmUbiInt promoter; iii) one copy of the NOS terminator; and iv) no backbone sequences from transformation plasmid pNOV1300. Nucleotide sequencing of the T-DNA insert in MIR162 maize revealed two codon changes within the *vip3Aa* coding sequence relative to the intended *vip3Aa* sequence; one of these was a silent mutation and the other codon change resulted in an amino acid substitution. The *vip3Aa* gene variant present in MIR162 maize has been designated *vip3Aa20*. Nucleotide sequencing additionally determined that the MIR162 maize T-DNA insert did not locate within any known *Z. mays* gene. Further, no novel open reading frames were created that spanned either the 5′ or 3′ junctions between the T-DNA and *Z. mays* genomic sequences. These genetic characterization data demonstrate that, apart from the well-characterized change that resulted in a single altered amino acid in the *vip3Aa* coding sequence, there were no unintended changes in the MIR162 maize genome as a result of the T-DNA insertion. Observations of *vip3Aa20* and *pmi* segregation ratios over several generations of MIR162 maize were consistent with the genes being linked at a single locus in the maize genome, and indicate stable inheritance of the transgenes. These data also indicated that no novel proteins, other than Vip3Aa20 and PMI, would be produced in MIR162 maize.

Expressed Material

The levels of expression of the Vip3Aa20 and PMI proteins in maize plants derived from event MIR162 were determined in several plant tissues and whole plants at four growth stages (V9-V12, anthesis, kernel maturity and senescence) in two field maize hybrids that were hemizygous for both transgenes. Kernels from MIR162 maize are the most likely tissue to comprise foodstuffs, either as grain or grain by-products. The average Vip3Aa20 concentration measured in kernels from MIR162 maize (43.56 µg Vip3Aa20/g dry weight at senescence) represents less than 0.004% of the total protein. The average PMI concentration measured in kernels from MIR162 maize (1.93 µg PMI/g dry weight at senescence) represents less than 0.0002% of the total protein (these calculations are based on maize grain or kernels containing 10% total protein by weight). Given the low levels of Vip3Aa20 and PMI in MIR162 kernels, dietary exposure potential can be considered minimal. Since no health hazards were identified for either the Vip3Aa20 or PMI proteins (summarized below), specific dietary exposure estimates are not necessary to support a conclusion regarding the safety of MIR162 maize.

Field Testing

The genetic modification resulting in transgenic maize event MIR162 was not intended to affect a specific agronomic or phenotypic characteristic except to confer resistance to certain lepidopteran insect pests. Grain yield and agronomic performance of MIR162 maize (field corn) hybrids were evaluated in a series of trials across a total of 16 locations over two years. In addition to inspections for disease and insect damage, qualitative and quantitative comparisons for a number of morphological and agronomic traits were made between MIR162 hybrids and non-transgenic control hybrids. The traits chosen for agronomic comparison were those typically observed by professional maize breeders and agronomists, covering a broad range of characteristics that encompass the entire life cycle of the maize plant. The agronomic performance and phenotypic data generated for MIR162-derived hybrids and their corresponding near isogenic non-transgenic control hybrids suggest that the genetic modification resulting in event MIR162 did not have any unintended effect on plant growth habit and general morphology, lifespan, vegetative vigour, flowering and pollination, grain yield, or disease susceptibility.

Potential for Increased Weediness or Invasiveness

Maize has lost the ability to survive in the wild due to its long process of domestication, and needs human intervention to disseminate its seed. Although maize from the previous crop year can over-winter and germinate the following year, it cannot persist as a weed. In contrast to weedy plants, maize has a pistillate inflorescence (ear) with a cob enclosed with husks. Consequently seed dispersal of individual kernels does not occur naturally. The phenotypic comparison of event MIR162-derived hybrids and non-transgenic hybrids did not reveal any consistent biologically meaningful differences in vegetative vigour, time to maturity and seed production. These data support the conclusion that event MIR162-derived hybrids are unlikely to form feral persistent populations, or to be more invasive or weedy than conventional maize hybrids.

Altered Plant Pest Potential

The intended effects of expression of the Vip3Aa20 and PMI proteins are unrelated to plant pest potential, and maize itself is not a plant pest in the United States or Canada. In addition, agronomic characteristics of MIR162-derived hybrids were not consistently significantly different than near-isogenic non-transgenic hybrids and indicated that growth habit of maize had not been unintentionally altered as a result of the genetic modification. Field observations did not indicate modifications to disease and pest susceptibilities.

Potential Impacts on Non-target Organisms

An assessment of risk for nontarget organisms and endangered species that might be exposed to the Vip3Aa20 protein in MIR162 maize was performed. Extensive nontarget organism studies were performed with 12 different species representative of wild birds, wild mammals, pollinators, above ground arthropods, soil-swelling arthropods, aquatic organisms and farmed fish. No adverse effects were observed in any study that exposed representative nontarget organisms to Vip3Aa proteins. The concentration of Vip3Aa tested in the studies was sufficient to achieve margins of exposure of ? 1 for all but one species based on realistic expected environmental concentrations.

There is a weight of evidence that at concentrations in MIR162 maize, the toxicity of Vip3Aa20 will be limited to certain species of Lepidoptera. Its receptor-mediated mechanism of action and absence of activity in bioassays with multiple species outside of the order Lepidoptera support this conclusion. The comparison of hazard and exposure data corroborate the hypothesis that Vip3Aa20 is not harmful to nontarget organisms at concentrations likely to result from cultivation of MIR162, and provide a weight of evidence that Vip3Aa20 in MIR162 maize will have no harmful effects on populations of potentially exposed nontarget organisms.

The only endangered lepidopteran with potential for exposure to insecticidal proteins in maize is the Karner blue butterfly (*Lycaeides melissa samuelis*), which is listed as extirpated (a wildlife species that no longer exists in the wild in Canada, but exists elsewhere in the wild) in Schedule 1 of the Species at Risk Act. Even should Karner blue butterfly populations be recovered in the wild in Canada, exposure to maize pollen from MIR162 would be minimal because of the large separation between populations of its food plant (wild lupine; *Lupinus perennis*) and cultivated maize, and because maize anthesis usually occurs after the Karner blue has finished feeding.

Potential Impact on Biodiversity

Event MIR162 has no novel phenotypic characteristics which would extend its use beyond the current geographic range of maize production in the United States and Canada. Since maize does not out cross to wild relatives in the United States or Canada, there will be no transfer of novel traits to unmanaged environments. MIR162 maize provides excellent protection against feeding damage caused by *A. ipsilon, H. zea, S. albicosta*, and *S. frugiperda*. For this reason, its introduction will impact current maize insect control practices in a very positive way, having the potential to displace conventional insecticide applications for control of these pests. Therefore, the potential impact on biodiversity of MIR162 is equivalent to its unmodified counterparts.

Abstract

Food and Feed Use

Maize event MIR162 will be grown for the same uses as maize varieties currently commercially available in the United States. While maize grown in the United States is predominantly (roughly 60 percent) used to feed domestic animals, either as grain or silage, maize grain is also processed by wet or dry milling to yield food products such as high fructose corn syrup, starch, oil, grits, and flour. Non-food/feed purposes for grain include use for fuel ethanol production; however, the by-products of such industrial distilling processes may also be used in animal feeds. Maize event MIR162 is suitable for the same uses as conventional maize.

Compositional Analysis

Key nutritional components in maize grain and forage derived from event MIR162 and near-isogenic non-transgenic control plants were compared. Replicate trials of transgenic and corresponding near-isogenic non-transgenic control hybrids were planted in multiple locations selected to be representative of the range of environmental conditions under which the hybrid varieties would typically be grown. Samples of grain and forage were harvested from six of these trial locations and compositional data for the MIR162 and control hybrids were subjected to analysis of variance across all locations and compared to compositional analysis data for grain and forage published in the literature and in compositional analysis databases.

Among the numerous compositional analyses that were carried out, most showed no consistent statistically significant differences. Statistically significant differences were noted for levels of the proximates ash, NDF and starch; the minerals calcium, iron and phosphorus; vitamins A (b-carotene), B6 and E (a-tocopherol); linoleic and linolenic fatty acids; the secondary metabolites ferulic acid and p-coumeric acid; and the phytosterol, b-sitosterol. However, the magnitudes of the differences were small and in every case the average values (when quantifiable) were all within the ranges of natural variation as reported in the literature. Overall, no consistent patterns emerged to suggest that biologically significant changes in composition or nutritive value of the grain or forage had occurred as an unintended result of the transformation process or expression of the Vip3Aa20 or PMI proteins.

The conclusion based on these data was that grain and forage from MIR162 maize were substantially equivalent in composition to both the non-transgenic control hybrid included in this study, and to other commercial maize hybrids.

Potential Toxicity and Allergenicity

The potential toxicity and allergenicity of the Vip3Aa20 and PMI proteins expressed in event MIR162 maize was evaluated by acute oral toxicity testing, digestive fate, and heat stability studies. In addition, amino acid sequence homology searches were performed against known toxins and allergens. Neither the Vip3Aa20 protein nor the PMI protein produced any adverse effects in laboratory mice challenged with a single oral dose corresponding to 1250 mg/kg body weight or 3080 mg/kg body weight, respectively. Both the Vip3Aa20 and PMI proteins were rapidly degraded following exposure to pepsincontaining simulated gastric fluid, indicating that any Vip3Aa20 or PMI protein in the diet would be readily digested as conventional dietary protein. In addition, the Vip3Aa20 and PMI proteins were both shown to lose biological (Vip3Aa20) or enzymatic (PMI) activity following incubation at 65ºC for 30 minutes. Neither the Vip3Aa20 protein nor the PMI protein shows significant amino acid sequence similarity to known or putative mammalian toxins or to allergenic protein sequences that are biologically relevant or have implications for allergenic potential. Based on this weight-of-evidence approach, it was concluded that the Vip3Aa20 and PMI proteins expressed in event MIR162 were extremely unlikely to exhibit mammalian toxicity or allergenicity.

Links to Further Information

Comissão Técnica Nacional de Biossegurança - CTNBio (Brazil)

Technical Opinion No. 2042/2009 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/09-276-001.pdf)
[PDF Size: 96.78K bytes]

Food Standards Australia New Zealand

Application A1001 Food Derived from Insect-Protected Corn Line MIR162: Assessment Report (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/09-050-003.pdf)
[PDF Size: 323.56K bytes]
Australia New Zealand Food Standards Code – Amendment No. 106 – 2009 (Commonwealth of Australia Gazette No. FSC 48 Thursday, 12 February 2009). (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/09-050-004.pdf)
[PDF Size: 51.97K bytes]

Ministerio de Salud Proteccion Social

Resoluciòn 0001693 de 2012 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/12-217-005.pdf)
[PDF Size: 410.40K bytes]

U.S. Department of Agriculture, Animal and Plant Health Inspection Service

Petition for Determination of Nonregulated Status for Insect-Resistant MIR162 Maize (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/10-024-001.pdf)
[PDF Size: 6.52M bytes]

United States Environmental Protection Agency

Bacillus thuringiensis Vip3Aa20 Insecticidal Protein and the Genetic Material Necessary for Its Production (via Elements of Vector pNOV1300) in Event MIR162 Maize (OECD Unique Identifier: SYN-IR162-4) (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/09-131-023.pdf)
[PDF Size: 827.12K bytes]

United States Food and Drug Administration

Biotechnology Consultation Agency Response Letter BNF No. 000113 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/09-050-001.pdf)
[PDF Size: 20.58K bytes]
Biotechnology Consultation Note to the File BNF No. 000113

(http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/09-050-002.pdf)
[PDF Size: 102.24K bytes]