GM Crop Database

Database Product Description

GA21 (MON-ØØ021-9)

Host Organism *Zea mays* (Maize)

Trade Name Roundup Ready®

Trait Glyphosate herbicide tolerance.

Trait Introduction Microparticle bombardment of plant cells or tissue

Proposed Use Production for human consumption and livestock feed.

Product Developer Syngenta Seeds, Inc. (formerly Zeneca Seeds)

Summary of Regulatory Approvals

Country	Food	Feed	Env	Notes
Argentina	2005	2005	1998	
Australia	2000			
Brazil	2008	2008	2008	
Canada	1999	1998	1998	
China	2004	2004		
Colombia	2012			
European Union	2006	2005		
Japan	1999	1999	1998	
Korea	2002	2005		
Mexico	2002	2002		
Philippines	2003	2003	2009	
Russia	2007	2007		
South Africa	2002	2002		
Taiwan	2003			
United States	1996	1996	1997	
Uruguay	2011	2011	2011	

Introduction

The GA21 line of maize (*Zea mays* L.) was genetically engineered, by particle acceleration (biolistic) transformation, to be tolerant of glyphosate-containing herbicides. The isolated endogenous maize 5-enolpyruvylshikimate-3-phosphate synthase (EPSSP) gene was modified through site-directed mutagenesis, such that its encoded enzyme was insensitive to inactivation by glyphosate, and inserted into the inbred AT maize variety.

Glyphosate specifically binds to and inactivates EPSPS, which is involved in the biosynthesis of the aromatic amino acids tyrosine, phenylalanine and tryptophan. This enzyme is present in all plants, bacteria and fungi, but not in animals, which do not synthesize their own aromatic amino acids. Thus, EPSPS is normally present in food derived from plant and microbial sources. The modified maize line permits farmers to use glyphosate-containing herbicides, such as Roundup herbicide, for weed control in the cultivation of maize.

Summary of Introduced Genetic Elements

Code	Name	Type	Promoter, other	Terminator	Copies	Form
epsps	5-enolpyruvyl shikimate-3-phosphate synthase	HT	rice actin I promoter and intron sequences	A. tumefaciens nopaline synthase (nos) 3\\\'-untranslated region	3*	Modified by in vitro mutagenesis; single insertion site with 3 complete copies of EPSPS cassette plus 3 incomplete copies

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.

Modification Method

The GA21 maize line was created through biolistic transformation of embryogenic maize cells with DNA-coated gold particles and regeneration of plants by tissue culture on selective medium. The 3.4 kb DNA restriction fragment (*Not*I digested pDPG434 plasmid DNA) used for transformation contained a copy of the EPSPS encoding gene originally isolated from maize and subsequently modified by *in vitro* site-directed mutagenesis to be insensitive to inactivation by glyphosate. The expression of the modified (m)-EPSPS encoding gene was controlled in part by the rice actin promoter intron sequences and the NOS 3' termination sequence derived from the Ti plasmid of the plant pathogen *Agrobacterium tumefaciens*.

The mEPSPS encoding gene was fused to an optimized chloroplast transit peptide (OTP) to allow subcellular targeting of mEPSPS protein into the chloroplast, the site of both the shikimate pathway and glyphosate mode-of-action. The chloroplast transit peptide sequences were derived from ribulose l,5-bisphosphate carboxylase oxygenase (RuBisCo) genes isolated from maize and sunflower.
Because a purified fragment of DNA was used in the transformation, no extraneous bacterial genes, including antibiotic resistance marker genes, were transferred. Genes present in the original pDPG434 plasmid backbone but not in the *Not*I restriction fragment used for transformation included *lacZ*, *Col*E1(ori) and the beta-lactamase encoding *bla* gene from *E. coli* plasmid pBR322.

Characteristics of the Modification

The Introduced DNA
Southern blot analysis of genomic DNA from GA21 demonstrated the integration at a single site on an 18.5 kb DNA restriction fragment containing three complete copies in tandem of the plasmid fragment used in the transformation, plus three incomplete copies. The copy at the 5' end of the insert was shown to include a partial rice actin promoter and a full length mEPSPS encoding gene with the NOS 3' untranslated region. Following the three complete copies is a full length rice actin promoter

element followed by a partial mEPSPS coding sequence. A rice actin promoter element is located at the 3' end of the insert. Transcription of the intact mEPSPS encoding gene(s) was determined by Northern blot analysis of mRNA isolated from GA21.

Because the 3'-terminal region of the inserted DNA was truncated after a rice actin promoter, there was the potential of creating a chimeric open reading frame (ORF) that included plant DNA sequences. Based on DNA sequence data, two putative overlapping open reading frames, ORF-1 (97 amino acids) and ORF-2 (19 amino acids) were identified. However, Northern blot analysis using poly (A+) RNA prepared from leaf tissue of maize line GA21, probed with maize genomic DNA sequence flanking the insert, demonstrated that there was no detectable RNA transcript of this region.

Genetic Stability of the Introduced Trait
The stability of the inserted DNA was assessed from Southern blots of genomic DNA obtained from 5 generations of progeny from successive backcrossing of the transgenic line. The introduced trait segregated as a single locus according to Mendelian rules of inheritance.

Expressed Material

The deduced amino acid sequence of the mEPSPS (445 amino acids) was 99.3% identical to that of the wild-type enzyme from maize. Western blot analysis of GA21 tissue extracts confirmed the expression of a 47.4 kDa protein, which was the size predicted for full-length mEPSPS protein and also corresponded to the size of this protein when expressed in bacterial cells transformed with a plasmid containing the mEPSPS encoding gene.

The expression of the mEPSPS gene was quantitated by enzyme linked immunosorbent assay (ELISA) of samples of forage and grain from plants at 5 field locations. Pooled grain samples from 9-16 ears were analyzed. The mEPSPS protein was expressed in all tissues of the modified plant (roots, stem, leaves and pollen). Levels of mEPSPS protein in grain averaged 3.2 + 1.7 µ/ fresh weight (fwt). Expression of the wild-type EPSPS in grain was undetectable at all sites. Expression of mEPSPS protein in forage (entire plant minus the roots) averaged 118.7 µ/g fwt, ranging from 46.6-210.4 µ/g fwt. In forage, the wild type EPSPS was detected at 4 out of 5 sites but not at levels high enough to quantify.

Studies to evaluate the activity of the mEPSPS and potential toxicity were performed on the bacterial expressed form of the enzyme. *Escherichia coli* were transformed with a plasmid containing the same mEPSPS encoding gene that was introduced into GA21 maize. Bridging data on immunological cross-reactivity and physiochemical properties were provided to demonstrate equivalence between the bacterial and plant expressed forms of the mEPSPS enzyme. Enzyme kinetics and substrate specificity analysis demonstrated that the bacterial expressed mEPSPS had the same substrate specificity as the wild-type maize enzyme.

Environmental Safety Considerations

Field Testing

Maize line GA21 was field tested from 1994 to 1996 in four U.S. states, Puerto Rico and Canada. Field trial reports indicated that transgenic GA21 maize did not display any abnormal characteristics, did not display any increased tendancy to weediness, and had no observable effect on non-target organisms or the general environment. Maize line GA21 retained the agronomic characteristics of its parental inbred line and differed

only in its tolerance to glyphosate.

Outcrossing

Since pollen production and viability were unchanged by the genetic modification resulting in maize line GA21, pollen dispersal by wind and outcrossing frequency should be no different than for other maize varieties. Gene exchange between GA21 and other cultivated maize varieties will be similar to that which occurs naturally between cultivated maize varieties at the present time. In Canada and the United States, where there are few plant species closely-related to maize in the wild, the risk of gene flow to other species is remote. Cultivated maize, *Zea mays* L. subsp. *mays*, is sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum*. None of the sexually compatible relatives of maize in Canada or the United States are considered to be weeds in these countries and it is therefore unlikely that introgression of the mEPSPS gene would provide a selective advantage to these populations as they would not be routinely subject to herbicide treatments.

Weediness Potential

No competitive advantage was conferred to maize line GA21, other than that conferred by resistance to glyphosate herbicide. Resistance to glyphosate containing herbicides will not, in itself, render maize weedy or invasive of natural habitats since none of the reproductive or growth characteristics were modified.
Cultivated maize is unlikely to establish in non-cropped habitats and there have been no reports of maize surviving as a weed. In agriculture, maize volunteers are not uncommon but are easily controlled by mechanical means or by using herbicides that are not based on glyphosate as appropriate. *Zea mays* is not invasive and is a weak competitor with very limited seed dispersal.

Secondary and Non-Target Adverse Effects

Analysis of GA21 maize determined that no toxic components were present in concentrations significantly different from the levels in non-transgenic maize. The expression of mEPSPS in maize line GA21 was not expected to pose a risk since this protein was nearly (99.3%) identical to the endogenous maize EPSPS. Field observations of maize line GA21 revealed no negative effects on nontarget organisms. These results combined with the lack of known toxicity of EPSPS suggested that the mEPSPS protein had no deleterious effects on organisms recognized as beneficial to agriculture (*e.g.*, earthworms, honey bees) or on other organisms, including any species recognized as threatened or endangered in Canada and the United States.

Impact on Biodiversity

Maize line GA21 has no novel phenotypic characteristics that would extend its use beyond the current geographic range of maize production. Since the risk of outcrossing with wild relatives in Canada and the United States is remote, it was determined that the risk of transferring genetic traits from GA21 to species in unmanaged environments was not a significant concern.

Other Considerations

Consideration was made as to whether the introduction of crops tolerant to glyphosate would result in a significant increase in the use of the

herbicide, and lead to the evolution of glyphosate resistant weeds. It was determined that the risk of increasing the selection of glyphosate tolerant weeds was low and could be mitigated through the use of other approved herbicides with a mode of action dissimilar to glyphosate. It was concluded that there was unlikely to be any significant adverse impact on agricultural practices associated with the use of the GA21 maize line.

Food and/or Feed Safety Considerations

Dietary Exposure

As GA21 maize was intended primarily for use in livestock feed, the level of dietary exposure to the mEPSPS protein was predicted to be very low. The major human food uses for maize are extensively processed starch and oil fractions prepared by wet or dry milling procedures and products include corn syrup and corn oil, neither containing protein. Human exposure to the modified protein from whole grain corn in the diet was considered to be very low due both to its low abundance in the protein fraction of the grain and to the proportionately low percentage of protein in the kernel, compared with the major starch component. Overall, the dietary exposure of consumers in Canada and the United States to grain from GA21 maize was anticipated to be the same as for other lines of commercially available field corn.

Nutritional Data

Maize line GA21 and non-transformed control lines were grown at five different locations in 1996. Several samples of both forage material and grains were collected and analyzed. Forage was analyzed for proximate composition: ash, calcium, carbohydrates, fibre [acid detergent fibre (ADF) and neutral detergent fibre (NDF)] moisture, and phosphorus, protein, and fat. Grains were analyzed for protein, fat, ash, carbohydrate (calculated), fibre (ADF and NDF), amino acid and fatty acid composition, calcium and phosphorus. Complementary analyses were conducted on trace elements, trypsin inhibitors, phytic acid and Vitamin E. There were no significant differences in nutrient composition between GA21 and commercial maize varieties in either the grain or forage material.
The data assessed from animal feeding studies clearly demonstrated the nutritional equivalence of grain from GA21 to isogenic material, on the basis of growth performance and body composition of broiler chickens receiving GA21 maize compared to isogenic grain for 40 days.

Toxicity

Because the amino acid sequence of the mEPSPS was 99.3% identical with the sequence of the wild-type enzyme the amino acid substitutions were not predicted to result in an increased potential for toxicity. Furthermore, the amino acid sequence of the inserted mEPSPS enzyme was compared to that of known protein toxins listed in the PIR, SwissProt, EMBL and GenBank genetic databases. Based on these computer searches the mEPSPS protein did not show homologies with known toxins and was judged not to have any potential for human toxicity.
The two putative open reading frames (ORFs) identified proximal to the mEPSPS insert were both derived from maize sequences and assessed for potential toxicity. Northern blot analysis of poly(A+) RNA from GA21 and control lines demonstrated that transcription of these ORFs did not occur in maize line GA21. Sequence analysis indicated no homologies with known toxins or allergens.

Bacterial-expressed mEPSPS was further evaluated for acute oral toxicity in a laboratory animal feeding trial. The test protein was administered by a single oral gavage to ten male and ten female CD-1 mice at doses corresponding to 3.7, 11.8 and 45.6 mg/kg. The highest dose of 45.6 mg mEPSPS/kg body weight was claimed to be at least 500-fold higher than the likely human exposure. A control group of ten mice/sex was administered only the carrier substance, at the same delivery volume as the test substance. An additional control group of ten mice/sex was administered bovine serum albumin (BSA) in the same carrier substance at the highest target dose (45.6 mg/kg). At defined stages throughout the duration of the study, clinical observations were performed for mortality and signs of toxicity, and body weights and food consumption measured. At the termination of the study (day 13-14), animals were sacrificed, examined for gross pathology and numerous tissues were collected and saved.

It was concluded that there was no evidence of toxicity in mice following a single oral dose of 45.6 mg/kg mEPSPS protein. The results of the study showed no statistically significant differences in group mean body weights, cumulative weight gains or food consumption in either males or females at any level of either the BSA control or test material, when compared with the respective carrier control group. All animals survived to the end of the study, and there were no clinical signs observed that could be related to the test material.

Allergenicity

The wild-type maize EPSPS enzyme has not been associated with any allergic effects, nor is its amino acid sequence homologous with any known protein allergens. The near complete identity (99.3%) between the amino acid sequences of the mEPSPS and wild-type EPSPS enzymes was expected to ensure a lack of allergenicity of the novel protein. The amino acid sequence of the inserted mEPSPS enzyme was compared to a database of 219 known allergens and gliadin sequences constructed from public domain databases. There were no significant sequence similarities discovered between mEPSPS and known allergens and gliadins. Data presented also showed that the protein was not glycosylated, a property common to many allergens. Also, unlike common allergens the mEPSPS protein was present at very low levels (Unlike known protein allergens, the mEPSPS was rapidly degraded by acid and/or enzymatic hydrolysis when exposed to simulated gastric or intestinal fluids. EPSPS isolated from leaves of GA21 maize, including mEPSPS (70% of activity), was rapidly degraded *in vitro* in artificial human gastric and intestinal fluids. The results of these experiments demonstrated that the mEPSPS protein was no longer detectable after 15 seconds in the gastric system and within one

minute in the intestinal system.

Together these results strongly supported the conclusion that maize line GA21 expressing mEPSPS did not pose any greater risk than conventional maize, with respect to potential allergenicity.

Links to Further Information

Australia New Zealand Food Authority

Draft Risk Analysis Report: Application A362, Food Derived From Glyphosate Tolerant Corn, GA21 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/01-290-058.pdf) [PDF Size: 238.81K bytes]
Final Risk Analysis Report: Application A362, food from glyphosate tolerant corn line GA21. (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/01-290-059.pdf) [PDF Size: 263.47K bytes]

Comiss? T?nica Nacional de Biosseguran? - CTNBio (Brazil)

Risk Assessment of Herbicide Tolerant Maize (GA21) (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/09-060-004.pdf) [PDF Size: 509.80K bytes]

European Commission

COMMISSION DECISION of 13 January 2006 authorising the placing on the market of foods and food ingredients produced from genetically modified Roundup Ready maize line GA21 as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council. (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/06-286-011.pdf) [PDF Size: 44.16K bytes]
COMMISSION DECISION of 28 March 2008 authorising the placing on the market of products containing, consisting of, or produced from genetically modified maize GA21 MON-Ø1-9) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/08-094-001.pdf)
[PDF Size: 48.43K bytes]

European Commission Scientific Committee on Food

Opinion of the Scientific Committee on Food on the safety assessment of the genetically modified maize line GA21, with tolerance to the herbicide glyphosate (expressed on 27 February 2002) (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/06-031-001.pdf) [PDF Size: 231.79K bytes]

European Commission Scientific Committee on Plants

Opinion of the Scientific Committee on Plants on the submission for placing on the market of genetically modified maize (Zea mays) line GA21 with tolerance to glyphosate herbicide notified by Monsanto (Notification C/ES/98/01) (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/2001093-a.pdf)

[PDF Size: 38.85K bytes]

European Commission: Community Register of GM Food and Feed

Notification of the placing on the Community Register of MON-Ø1-9. (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/06-286-012.pdf)
[PDF Size: 17.59K bytes]

Japanese Biosafety Clearing House, Ministry of Environment

Outline of the biological diversity risk assessment report: Type 1 use approval for GA21 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/06-291-009.pdf)
[PDF Size: 102.84K bytes]

Ministerio de Salud Proteccion Social

Resolución Número 0001692 de 2012 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/12-217-004.pdf)
[PDF Size: 423.59K bytes]

Monsanto Company

Product safety description (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/02-269-006.pdf)
[PDF Size: 221.65K bytes]

Office of Food Biotechnology, Health Canada

NOVEL FOOD INFORMATION - FOOD BIOTECHNOLOGY GLYPHOSATE TOLERANT CORN, GA21 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/ofb-099-133-a.pdf)
[PDF Size: 47.93K bytes]

Philippines Department of Agriculture, Bureau of Plant Industry

Determination of the Safety of Monsanto's Corn GA 21 (Herbicide Tolerant Corn) for Direct Use as Food, Feed and for Processing (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/09-131-004.pdf)
[PDF Size: 29.63K bytes]

Secretaria de Agricultura, Ganaderia, Pesca y Alimentos: Republica Argentina

Noticias de la SAGPyA: La SAGPyA autorizo el uso de maiz GA21 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/05-291-003.pdf)
[PDF Size: 79.56K bytes]

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

Petition for Determination of Nonregulated Status for Roundup Ready Corn Line GA21 (CBI-deleted) (http://cera-

gmc.org/files/cera/GmCropDatabase/docs/decdocs/04-225-008.pdf)
[PDF Size: 3.91M bytes]

US Food and Drug Administration

Memorandum to file concerning glyphosate herbicide-tolerant maize line GA21. (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/bnfM051.pdf)
[PDF Size: 194.59K bytes]

USDA-APHIS Environmental Assessment

DETERMINATION OF NONREGULATED STATUS FOR GLYPHOSATE-TOLERANT CORN LINE GA21 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/01-290-061.pdf)
[PDF Size: 55.97K bytes]