GM Crop Database

Database Product Description

ASR368 (SMG-368ØØ-2)

Host Organism	*Agrostis stolonifera* (Creeping Bentgrass)
Trait	Herbicide tolerant, glyphosate.
Trait Introduction	Microparticle bombardment of plant cells or tissue
Proposed Use	Production of turf in golf courses. Incidental usage in livestock feed.
Product Developer	Scotts Seeds

Summary of Regulatory Approvals

Country	Food	Feed	Env	Notes
United States	2003	2003	2017	

Introduction

ASR368 creeping bentgrass (Roundup Ready® Creeping bentgrass) was developed to allow the use of glyphosate, the active ingredient in the herbicide Roundup®, as a weed control option in turfgrass production (*e.g.*, putting greens). This genetically engineered wheat variety contains a glyphosate-tolerant form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), isolated from the soil bacterium *Agrobacterium tumefaciens* strain CP4. The novel form of this enzyme is termed hereafter CP4 EPSPS.

The EPSPS enzyme is part of the shikimate pathway that is involved in the production of aromatic amino acids and other aromatic compounds in plants (Steinrucken & Amrhein, 1980). When conventional plants are treated with glyphosate, the herbicide binds to EPSPS, thereby preventing the synthesis of aromatic amino acids needed for plant growth. The CP4 EPSPS enzyme in ASR368 creeping bentgrass has a reduced affinity for glyphosate; its enzymatic activity is therefore not hindered by the herbicide.

EPSPS is present in all plants, bacteria, fungi, but not in animals, which do not synthesize their own aromatic amino acids. Because the aromatic amino acid biosynthetic pathway is not present in mammalian, avian or aquatic life forms, glyphosate has little if any toxicity for these organisms (U.S. EPA, 1993; WHO, 1994; Williams et al. 2000). The EPSPS enzyme is normally present in food derived from plant and microbial sources.

ARS368 was developed by introducing the CP4 EPSPS coding sequences into the creeping bentgrass line B99061R using microprojectile bombardment.

Summary of Introduced Genetic Elements

Code	Name	Type	Promoter, other	Terminator	Copies	Form
CP4 epsps	5-enolpyruvyl shikimate-3-phosphate synthase	HT	enhanced CaMV 35S, HSP70 intron, gox cassette	*A. tumefaciens* nopaline synthase (*nos*) 3'-untranslated region		
CP4 epsps	5-enolpyruvyl shikimate-3-phosphate synthase	HT	rice actin I promoter and intron sequences	*A. tumefaciens* nopaline synthase (*nos*) 3'-untranslated region		

Characteristics of *Agrostis stolonifera* L. (Creeping Bentgrass)

Center of Origin	Reproduction	Toxins	Allergenicity
Populations native to North America and Eurasia.	Cross-pollination *via* wind-borne pollen. Completely outcrossing. Reproduces by seed and also vegetatively by stolons.	No endogenous toxins. An endophytic toxin, corynetoxin, is produced by a nematode-bacteria complex, *i.e.*, when *Clavibacter toxicus* is transmitted to the plant via the nematode vector *Anguina agrostis* (Edgar. 1994).	unknown

Donor Organism Characteristics

Latin Name	Gene	Pathogenicity
Agrobacterium tumefaciens strain CP4	CP4 epsps	*Agrobacterium tumefaciens* is a common soil bacterium that is responsible for causing crown gall disease in susceptible plants. There have been no reports of adverse effects on humans or animals.

Modification Method

ASR368 creeping bentgrass was produced by microprojectile bombardment of plant cells from the line B99061R. The plasmid vector PV-ASGT08 used for the transformation contained two *cp4 epsps* gene cassettes coding for glyphosate tolerance. The T-DNA portion of the plasmid (PV-ASGT08L) contained two *cp4 epsps* gene expression cassettes. Joined with each of the *cp4 epsps* gene sequences was a chloroplast transit peptide (*cpt2*) sequence, derived from the *Arabidopsis thaliana epsps* gene. Two unique promoters were used to regulate the expression of CP4 EPSPS in both vegetative and reproductive tissues. In the first cassette, gene expression was regulated by an enhanced 35S promoter from the cauliflower mosaic virus (CaMV) and the intron of the hsp70 (heat shock protein) gene from *Zea mays*. The expression of the second *cp4 epsps* gene was regulated by the 5′ region of the rice actin1 gene which contains the promoter, transcription start site, and first intron. The transcription termination signal sequences were derived from the 3′

nontranslated region of the nopaline synthase gene from *Agrobacterium tumefaciens*.

The PV-ASGT08 vector was replicated in *E. coli* and digested with the restriction enzyme HindIII to remove the backbone from the DNA segment PV-ASGT08L containing the gene construct. PV-ASGT08L was precipitated onto gold particles and introduced into the line B9961R by microprojectile bombardment. Transformed callus was selected *in vitro* using culture medium to which glyphosate had been incorporated. Plants were regenerated from tolerant callus using tissue culture techniques. ASR368 was isolated from these regenerated plants.

Characteristics of the Modification

The Introduced DNA

Southern blot analysis and Polymerase Chain Reaction (PCR) amplification of the genomic DNA of ASR368 creeping bentgrass demonstrated that there was one site of integration of a single copy of the DNA insert PV-ASGT08L. The stability of the insertion was assessed over three generations was confirmed using Southern blot analysis and segregation studies.

The *cp4 epsps* gene was isolated from *Agrobacterium tumefaciens* strain CP4. The EPSPS protein is a chloroplast-localized enzyme that is transported from the cytosol to the chloroplast by the chlorophyll transit peptide. In ASR368, the inserted *ctp2* gene codes for a chlorophyll transit peptide that binds to the CP4 EPSPS enzyme to enable transportation to the chloroplast.

Genetic Stability of the Introduced Trait

Mendelian segregation studies were conducted to investigate the stability of inheritance of the glyphosate-tolerance trait. Segregation data were derived from reciprocal crosses between F1 hemizygous plants and nonmodified parental plants. The progeny of the reciprocal crosses segregated in a 1:1 ratio of glyphosate-tolerant to non-tolerant plants. This indicated that the glyphosate-tolerance trait is inherited as a single locus, and is stable across several generations.

Expressed material

An enzyme-linked immunosorbent assay (ELISA) analysis and Western blot analysis was used to quantify the levels of the CP4 EPSPS proteins in tissues from ASR368. Samples for analysis were obtained from four field sites. The mean expression level of CP4 EPSPS in ASR368 tissues was 68.6 ?g/g fresh weight.

Food and/or Feed Safety Considerations

Dietary exposure

ASR368 creeping bentgrass is intended solely for the production of turfgrass in golf courses, and is not expected to be grown as forage. Some of the vegetation left over from seed production could be used as animal feed. The use of this forage would be very limited, however, since the nutritional value is very poor at such an advanced growth stage.

Nutritional and Compositional Data

The nutritional components of ASR368 forage were determined analytically

and compared to those of the parental line B99061R, and seven commercial creeping bentgrass cultivars, grown at four locations. Forage samples were analyzed for proximates (crude protein, crude fat, crude fibre, ash, and moisture), acid detergent fibre, neutral detergent fibre, and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc). No significant differences in any of the nutritional parameters were found between ASR368, the parental line, and the conventional cultivars, when averaged over all locations. There were significantly higher levels of moisture and phosphorus at two sites, and significantly lower levels neutral detergent fibre at another site, for ASR368 compared to its parental line. These differences would be attributable to location rather than genotype effects. Overall, the values of the nutritional components in ASR368 were within the range determined for the commercial cultivars

Toxicity and Allergenicity

The potential for toxicity and allergenicity of the novel protein CP4 EPSPS in ASR368 was investigated using the following data and information: the history of safe consumption of this protein by humans and animals; results from the determination of amino acid sequence similarity between the CP4 EPSPS protein and known toxins and allergens; analysis of the stability the novel protein in simulated gastric fluids; results from an acute oral toxicity study in mice fed CP4 EPSPS protein.

The results of database searches for amino acid sequence similarity between CP4 EPSPS and known toxins and allergens were negative; CP4EPSPS was rapidly digested under simulated gastric fluid conditions; and no adverse effects were observed when mice were fed the highest dose in acute oral toxicity studies. The results of these studies, along with history of safe consumption of CP4 EPSPS, led to the conclusion that ASR368 is not more toxic or allergenic than conventional creeping bentgrass cultivars.

Abstract

Creeping bentgrass (*Agrostis stolonifera* L.) is a perennial grass (Familiy Poaceae) grown in pastures, as a turfgrass in golf courses, and also for revegetation and soil stabilization. It is a cool-season grass that has become naturalized in many temperate and cold regions of the world. It is considered a native grass in the United States and Canada; however, some populations have become naturalized from sources native to Eurasia.

The success of creeping bentgrass as a pasture grass, turfgrass, and soil stabilizer is due to its growth habit. Creeping bentgrass is a prostrate grass, forming numerous stolons that spread along the ground. Creeping bentgrass reproduces by seed, but also vegetatively by the formation of stolons. New tillers grow from meristematic points on the nodes of the stolons; from each new tiller there is the formation of new stolons. This vegetative growth results in the formation of sod, *i.e.*, a fairly continuous mat of vegetation. Creeping bentgrass is resistant to grazing and mowing since the growing points of the tillers and stolons are near the ground. Grazing and mowing suppresses the apical dominance of the main culm (stem), which stimulates the formation of new stolons at the base. Fibrous roots grow from each new tiller; these roots stabilize and add biomass to the soil.

Grasses grown for turf, especially in golf courses, are intensively managed. Part of this management includes the use of herbicides for the establishment and maintenance of the turf. Creeping bentgrass is used in

putting greens, tees, and fairways. Roundup Ready® creeping bentgrass (ASR368) was developed to allow the use of glyphosate, the active ingredient in the herbicide Roundup®, as a weed control option in the production of creeping bentgrass in golf courses. This genetically engineered grass contains a novel form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). This enzyme allows ASR368 to survive an otherwise lethal application of glyphosate. The EPSPS gene introduced into ASR368 was isolated from a strain of the common soil bacterium *Agrobacterium tumefaciens* strain CP4; the novel form of the EPSPS enzyme produced by this gene is tolerant to glyphosate.

The EPSPS enzyme is part of the shikimate pathway, an important biochemical pathway in plants involved in the production of aromatic amino acids and other aromatic compounds. When conventional plants are treated with glyphosate, the plants cannot produce the aromatic amino acids needed for growth and survival. EPSPS is present in all plants, bacteria, and fungi. It is not present in animals, since these organisms are unable to synthesize their own aromatic amino acids. Because the aromatic amino acid pathway is not present in mammals, birds, or aquatic life forms, glyphosate has little, if any, toxicity for these organisms. The EPSPS enzyme is naturally present in foods derived from plant and microbial sources. ASR368 creeping bentgrass was developed by introducing two CP4 EPSPS genes into the *A. stolonifera* line B9906R using microprojectile bombardment (*i.e.* , particle acceleration, biolistics transformation).

The livestock feed safety of ASR368 creeping bentgrass was based on: a history of safe consumption of the CP4 EPSPS enzyme in previously approved glyphosate tolerant crops; the lack of toxicity or allergenicity of CP4 EPSPS; and by direct laboratory and safety studies of the CP4 EPSPS protein. The nutritional equivalence and wholesomeness of ASR368 compared to the nonmodified parental line was demonstrated by the compositional analysis of the forage, including proximates (crude protein, crude fat, crude fibre, ash, and moisture), acid detergent fibre, neutral detergent fibre, and minerals.

Links to Further Information

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

USDA-APHIS Petition for Determination of Nonregulated Status: Roundup Ready® Creeping Bentgrass (Agrostis stolonifera L.) Event ASR368 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/05-209-011.pdf) [PDF Size: 7.33M bytes]

U.S. Food and Drug Administration

Biotechnology Consultation Note to the File BNF No. 000079 (http://cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/04-300-007.pdf) [PDF Size: 134.71K bytes]

United States, USDA

Determination of Nonregulated Status for the Scotts Company and Monsanto Company ASR368 Creeping Bentgrass (http://cera-gmc.org/files/cera/GmCropDatabase/decdocs/ASR368/ARS368_creepingbentgrass_

%20Determination.pdf)
Petition for the Determination of Nonregulated Status for Glyphosate Tolerant Creeping Bentgrass Event ASR368 (http://cera-gmc.org/files/cera/GmCropDatabase/decdocs/ASR368/ARS368_creepingbentgrass_%20Petition.pdf)
Record of Decision The Scotts Company and Monsanto Company Petition (15-300-0lp) for Determination of Nonregulated Status for ASR368 Creeping Bentgrass (http://cera-gmc.org/files/cera/GmCropDatabase/decdocs/ASR368/ARS368_creepingbentgrass_%20Record%20of%20Decision.pdf)
The Scotts Company and Monsanto Company Petition (15-300-01p) for Determination of Non-regulated Status of Glyphosate Resistant ASR368 Creeping Bentgrass (http://cera-gmc.org/files/cera/GmCropDatabase/decdocs/ASR368/ARS368_creepingbentgrass_%20PPRA.pdf)
The Scotts Company and Monsanto Company Petition (15-300-01p) for Determination of Nonregulated Status for ASR368 Creeping Bentgrass (http://cera-gmc.org/files/cera/GmCropDatabase/decdocs/ASR368/ARS368_creepingbentgrass_%20EIA.pdf)