Food

Biotechnology Consultation Note to the File BNF No. 000116

Return to inventory: Submissions on Bioengineered New Plant Varieties[14]

See also Biotechnology: Genetically Engineered Plants for Food and Feed[15] and about Submissions on Bioengineered New Plant Varieties[16]

Biotechnology Consultation - Note to the File
Biotechnology Notification File BNF No. 000116

DATE

December 9, 2010

Subject

MON 87460, drought tolerant corn

Keywords

Maize; corn; *Zea mays* L.; drought tolerance; MON 87460; OECD unique identifier MON-8746Ø-4; cold shock protein B (CSPB) from *Bacillus subtilis*; neomycin phosphotransferase II (NPTII) from *Escherichia coli*, Monsanto

Purpose

This document summarizes our evaluation of Biotechnology Notification File (BNF) No. 000116. In a submission dated December 19, 2008, the Monsanto Company (Monsanto) submitted a safety and nutritional assessment of the bioengineered corn MON 87460. Monsanto provided additional information in submissions dated April 9, May 1, June 1, June 29, July 7, August 10, September 10, November 2, 2009, and August 3, 2010. Monsanto is voluntarily consulting with the agency as discussed in the agency's 1997 Guidance on Consultation Procedures for Foods Derived from New Plant Varieties. Here we discuss the outcome of the consultation, but do not intend to restate the information provided in the final consultation in its entirety.

Intended Effects

The intended effect of the modification in corn event MON 87460 is to reduce yield loss under water-limited conditions compared to conventional corn. To accomplish this objective, Monsanto introduced the gene (*cspB*) for cold shock protein B (CSPB) from *B. subtilis*. CSPB confers tolerance to water-limited conditions. Monsanto also introduced the gene (*nptII*) for neomycin phosphotransferase II (NPTII) protein from *E. coli*. NPTII was used as a selectable marker in the development of corn event MON 87460. The NPTII protein does not confer tolerance to water-limited conditions.

Regulatory Considerations

The purpose of this evaluation is to assess whether the developer has introduced a substance requiring premarket approval as a food additive or has unintentionally adulterated the food with respect to the Federal Food, Drug and Cosmetic Act (FFDCA).

Genetic Modification and Characterization

Parental Variety

Monsanto transformed the recipient LH59 (a non-transgenic conventional corn variety) callus tissue to obtain MON 87460.

Transformation Plasmid and Method

Monsanto described the development of corn event MON 87460 using *Agrobacterium tumefaciens*-mediate transformation of corn embryo-derived tissue. The *A. tumefaciens* strain harbored the transformation vector PV-ZMAP595. The transforming vector carried a transfer DNA sequence comprised of both *cspB* and *nptII* expression cassettes. Following transformation, the corn tissue was transferred to medium containing the antibiotics carbenicillin to eliminate *A. tumefaciens*, and paromomycin (related to neomycin) to eliminate cells that were not transformed.

Characteristics, Inheritance, and Stability of the Introduced DNA

Monsanto provided genomic DNA blot (Southern) analyses to demonstrate that corn event MON 87460 has one intact copy of the transfer DNA region comprised of tandem *cspB* and *nptII* expression cassettes inserted in its genome. Monsanto provided genomic DNA blot analyses and polymerase chain reaction analyses in conjunction with DNA sequencing to verify the integrity of the integrated *cspB* and *nptII* genes along with their associated regulatory elements. Monsanto used genomic DNA blot analyses to demonstrate that MON 87460 does not contain any detectable DNA from the transformation vector, other than the transfer DNA region.

Monsanto presented genomic DNA blot analyses to demonstrate the stability of the inserted *cspB* and *nptI* expression cassettes across multiple generations of corn event MON 87460. Monsanto presented Chi-square analyses of the segregation patterns across multiple generations of corn event MON 87460 to demonstrate the stable Mendelian inheritance of the transfer DNA region containing both the *cspB* and the *nptII* cassettes.

Monsanto assessed the inserted DNA sequence for any potential unintended open reading frames (ORFs) that might encode proteins. Monsanto identified several putative polypeptides of at least eight amino acids that could be expressed. Monsanto performed bioinformatic analyses to compare sequences within these putative polypeptides to those of known toxins and allergens in standard toxin, allergen and protein databases. Monsanto found no similarity to any known allergen or toxin.

Protein Characterization

Function and expression levels of introduced proteins

Monsanto describes the CSPB protein produced in corn event MON 87460 as identical to the native CSPB protein produced in *B. subtilis* except for one amino acid introduced for cloning purposes. Bacterial cold shock proteins (CSP) are hypothesized to function by binding to RNA secondary structures, thus reducing the free energy required for unfolding misfolded RNA. CSPs are classified as RNA chaperones. Monsanto notes that similar cold shock domain-containing proteins are also present in plants where they appear to play a role in conferring stress tolerance.

Monsanto describes the function of the NPTII protein as being a selectable marker used in the development of corn event MON 87460. NPTII phosphorylates neomycin and related antibiotics, thus inactivating them.

Monsanto estimated the levels of CSPB and NPTII proteins in corn event MON 87460 from both well-watered (i.e., received adequate water throughout the growing season) and water-limited plants grown in the field. Monsanto noted that given the genetic construct of the transferred DNA, both CSPB and NPTII proteins would be expected to be present in all tissues. Using standard biochemical techniques, Monsanto reports CSPB protein being present in all tissues tested. Monsanto reports NPTII protein also being present in all tissues tested, with the exception of mature grain where it was below the detection method's limit of quantitation (where the limit of quantitation is 0.0047 micrograms per gram of tissue on a fresh weight basis).

Potential Toxicity of the Introduced Proteins

Monsanto assessed the potential for toxicity for both CSPB and NPTII proteins. Regarding CSPB, Monsanto noted that the source of the *cspB* gene is *B. subtilis*, a microorganism used as the donor organism for several enzyme preparations used in food manufacturing applications. In support of the safety of *B. subtilis* used in such food applications, Monsanto cited several published studies as well as the opinions of expert scientific bodies. In support of the safety of the CSPB protein, Monsanto noted that it is homologous to several proteins from microbial and plant sources present in the human diet. Using the CSPB protein sequence, Monsanto performed bioinformatic analyses to look for similarities between CSPB and proteins

known to be toxic or bioactive and found no such similarities. Monsanto also conducted an acute oral toxicity study in mice (single dose of 4.7 milligrams/kilogram) using *E. coli*-produced CSPB protein. Monsanto noted no treatment-related adverse effects. Given the totality of the evidence, Monsanto concluded that the CSPB protein from corn event MON 87460 is unlikely to exhibit toxic effects when incorporated into food or feed.

Regarding NPTII, Monsanto stated that the source of the *nptII* gene is *E. coli* K-12. NPTII, also referred to as APH(3')II, is regulated as a food additive under 21 CFR 173.170 and 21 CFR 573.130 for use as a processing aid in the development of new varieties of tomato, oilseed rape and cotton. Scientific studies and evaluations regarding the use of NPTII in new plant development have been performed since FDA file the food additive petition for these uses. In reviewing NPTII, FDA concluded that NPTII does not have any properties that would distinguish it toxicologically from any other phosphorylating enzymes in the food supply. Using the NPTII protein sequence, Monsanto performed bioinformatic analyses, which found no similarities between NPTII and proteins known to be toxic or bioactive and provided additional citations supporting its safety. Given the totality of the evidence, Monsanto concluded that the NPTII protein from corn event MON 87460 is unlikely to exhibit toxic effects when consumed in food or feed.

Assessment of the Potential for Allergenicity of the Introduced Proteins

Monsanto evaluated the potential for allergenicity of CSPB and NPTII proteins. For CSPB and NPTII proteins, bioinformatics analyses of sequence similarities using standard methods, including the allergen database in conjunction with the FASTA sequence alignment tool, and an eight-amino acid sliding window search (ALLERGENSEARCH) revealed no significant homology to known allergens. For the CSPB protein, *in vitro* gastric and intestinal digestibility studies using standard methods did not identify significant resistance to proteolysis. For the NPTII protein, Monsanto cites an FDA draft guidance document to support its conclusion that the NPTII protein poses no allergenic risk [Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants (Draft Guidance, issued September 4, 1998)]. In this draft guidance, FDA states that NPTII protein is known to be rapidly degraded under simulated gastric conditions, is neither glycosylated nor heat-resistant and lacks homology to known food allergens using several databases. FDA concludes that there are no allergenicity concerns.

Food & Feed Use

Corn (*Zea mays* L.) originated in Mexico and was grown as a food crop as early as 2700 B.C. Today, corn is grown worldwide for food, feed, and industrial uses. Corn grain is used in food primarily in the form of processed products, such as high fructose corn syrup, cereals, oil, meal, flour, starch, and grits. Corn is a source of nutritionally important amino acids (methionine and cystine), carotenoids, and vitamin E. Corn oil is rich in polyunsaturated fatty acids and is used mainly as a salad and cooking oil and in margarine production.

Corn is also used in animal feed. Corn grain is primarily fed to cattle, poultry, and swine either as intact or processed grain or as dry or wet milling byproducts, but may be a component of most animal feeds. Corn silage (entire above ground portion of the corn plant that is harvested prior to maturation) is primarily fed to ruminants.

Composition

Scope of Analysis

Monsanto analyzed the composition of forage and grain from the MON 87460 corn and compared it with its near isogenic parental line (a non-transgenic corn variety with a similar genetic background to MON 87460, hereafter referred to as the comparator control line). Two field studies were summarized in Monsanto's submission (United States (U.S.) and Chilean studies).

- In the U.S. study, Monsanto evaluated the composition of forage and grain from MON 87460, its comparator control line, and from a total of eighteen commercial non-transgenic corn varieties.
- In the Chilean study, Monsanto evaluated the composition of forage and grain from MON 87460, its comparator control line, and sixteen commercial non-transgenic corn varieties grown under water-limited or irrigation conditions.

Monsanto used the data derived from the reference varieties to generate a 99% tolerance[1] interval for each component. Monsanto states that these data illustrate the natural variability in commercially grown corn varieties grown under similar field conditions.

Study Design - Compositional Analyses

As described by Monsanto, corn event MON 87460 and the comparator control line were grown at six replicated field sites in corn production regions in the U.S. during the 2006 growing season. At the same sites, three unique conventional commercial hybrid lines were also grown at each of the six sites, thus providing a total of 18 commercial references for compositional analyses of forage and grain tissue samples. Plants in all six sites were grown under commercially acceptable agronomic practices typical for the area, with four sites being rain-fed and two receiving supplemental irrigation. At each field site, seed was planted in a randomized block design with three replicates per block. Forage and grain samples were collected from corn event MON 87460 and the comparator control line from all three blocks. Forage and grain samples were collected from the three conventional commercial hybrid lines from a single block. Samples of forage at the early dent plant growth state and grain at physiological maturity were collected from all plots and analyzed for nutritional components, toxicants, and anti-nutrients. Monsanto measured and evaluated seven components in forage and 68 in grain. Compositional analyses of the forage samples included measurement of moisture, crude fat, crude protein, ash, carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium, and phosphorus. Compositional analyses of the grain samples included measurement of moisture, crude fat, crude protein, ash, carbohydrates by calculation, ADF, NDF, total dietary fiber (TDF), amino acids (18), fatty acids (C8-C22), minerals (calcium copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamins (vitamin B1, vitamin B2, vitamin B6, vitamin E, niacin, and folic acid), and key secondary metabolites (furfural, p-coumaric acid, and ferulic acid), anti-nutrients (raffinose and phytic acid).

Monsanto also described field trials from the 2006-2007 growing season in commercial corn production regions of Chile. According to Monsanto, these four sites are well-suited to growing corn, but typically do not receive any rainfall so crops receive water only through controlled irrigation. Monsanto planted corn event MON 87460, the comparator control line, and four unique commercial conventional hybrid corn lines per site, providing a total of 16 commercial references for compositional analyses of forage and grain samples. At the Chilean sites, Monsanto used a strip plot design with three replicates per site with the water level treatment (either well-watered for optimal growth using irrigation or water-limited to impose a drought stress during a critical stage of growth) as the whole plot and substance type as the subplot to allow for a comparison of plants grown under the two different water level treatments. Monsanto described the whole plot factor as arranged as a randomized complete block design and the split-plot factor consisting of corn event MON 87460, the comparator control line, and the conventional commercial hybrid lines. Monsanto collected forage (early dent stage) and grain samples (at physiological maturity) from corn event MON 87460 and the comparator control line from all three blocks for each treatment and forage and grain samples from the four conventional commercial hybrid lines from a single block for each irrigation treatment.

For the U.S. study, statistical assessments of the compositional data were conducted using mixed model analysis of variance on each individual site and a comparison across all sites, referred to as the combined site analysis. For the Chilean field trials, Monsanto described statistical considerations to assess the effects of the two irrigation treatments. As described by Monsanto, for a site to be considered in the combined-site analysis, the commercial conventional hybrid lines must have exhibited a phenotypic response indicative of the treatment. For the water-limited plants, this response was defined as a minimum of a 15 percent reduction in yield. In summary, for the Chilean field trials, there were four sets of statistical analyses made for each treatment, three used data from each of the field sites and the fourth used data from a combined-site analysis

Statistical differences at the 5 percent level ($P<0.05$) were declared to be significant. Where statistically significant differences between corn event MON 87460 and the comparator control line were observed, Monsanto notes that all mean component values of the test and control substances were within the 99 percent tolerance interval established from the commercial references.

Results of analyses:

Monsanto made the following observations and conclusions regarding the results of the levels of components measured in forage and grain from corn event MON 87460. For the U.S. field trials, 77 different analytical components were measured, 15 had more than 50 percent of the observations below the assay limit of quantitation. These components are known to be present at low levels in corn grain. Noted below are statistically significant differences detected for the combined-site analysis only. Similarly for the Chilean well-watered field trials, of the 77 different analytical components measured, 16 had more than 50 percent of the observations below the assay limit of quantitation.

Compositional analysis of corn forage

Monsanto reported no statistically significant differences in moisture, crude fat, crude protein, ash, carbohydrates by calculation, ADF, NDF, calcium, and phosphorus levels between MON 87460 and the comparator control line in the U.S study. Similarly, there were no statistically significant differences in moisture, crude fat, crude protein, ash, ADF, NDF, calcium, and phosphorus levels in the water-supplemented treatment in Chile. Carbohydrates by calculation was higher in forage obtained from MON 87460 when compared to the comparator control line in corn grown under water-supplemented treatment, but the values for MON 87460 and the comparator control line fell within the 99 percent tolerance interval for conventional varieties grown under similar conditions. Total fat was higher in forage obtained from MON 87460 when compared to the comparator control line in corn grown under water-limited conditions, but the values for MON 87460 and the comparator control line fell within the 99 percent tolerance interval for conventional varieties grown under similar conditions.

Compositional analysis of corn grain

U.S. Study:
Monsanto reported no statistically significant differences in moisture, crude fat, crude protein, carbohydrates by calculation, ADF, NDF, TDF, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, and zinc), all 18 amino acids, 7 fatty acids (palmitic, palmitoleic, oleic, linoleic, linolenic, arachidic, and behenic acids), 6 vitamins, phytic acid, raffinose, ferulic acid, and p-coumaric aci between MON 87460 and the comparator control line. Ash and stearic fatty acid contents were statistically higher in MON 87460 than the comparator control line, but the MON 87460 and comparator control line values fell within the 99% tolerance interval for conventional varieties grown under similar conditions. Eicosenoic fatty acid content was statistically lower in MON 87460 when compared to the comparator control line and both of these values were slightly lower than the lowest value for the 99% tolerance interval.

Chilean Water-Supplemented Sub-plot:
Monsanto reported no statistically significant differences in moisture, crude protein, ash, carbohydrates by calculation, ADF, NDF, TDF, minerals (except for magnesium), all 18 amino acids, the 8 detectable fatty acids, 6 vitamins, phytic acid, raffinose, ferulic acid, and p-coumaric acid between MON 87460 and comparator control line. Ash and magnesium contents were statistically higher in MON 87460 than the comparator control line, but the values for MON 87460 and the comparator control line fell within the 99% tolerance interval for conventional varieties grown under similar conditions.

Chilean Water-Limited Sub-plot:
Monsanto reported no statistically significant differences in moisture, crude fat, crude protein, ash, carbohydrates by calculation, ADF, NDF, TDF, 8 minerals, 18 amino acids, fatty acids (except eicosenoic acid), 6 vitamins, phytic acid, raffinose, ferulic acid, and p-coumaric acid between MON 87460 and comparator control line. Although the values for eicosenoic acids were statistically different, the values were numerically (at two decimal points) the same and these values fell within the 99 percent tolerance interval for conventional varieties grown under similar conditions.

Summary of Compositional Analyses

As noted above, in Monsanto's combined site analyses, a few statistically significant differences were found in the comparisons of corn event MON 87460 and the comparator control line. Of the statistically significant differences detected, all mean component values were within the 99 percent tolerance level established from the commercial references. In addition, the mean levels for each of these components were within the range of values reported in either the International Life Science Institute (ILSI) Crop Composition Database (ILSI 2006), the OECD consensus document (OECD, 2002), or both. Monsanto concluded that the differences were within the natural variability of corn.

Conclusion

FDA evaluated Monsanto's submission to determine whether the developer's product raises any safety issues with respect to the intended modification or with respect to the food itself, as discussed in the agency's 1992 Policy Statement on New Plant Varieties. Based on the information provided by the company and other information available to the agency, FDA did not identify any issues under Sections 402 and 409 of the Federal Food, Drug and Cosmetic Act that would require further evaluation at this time

Monsanto has concluded that its drought tolerant corn variety, corn event MON 87460 (MON-8746Ø-4) and the foods and feeds derived from it are as safe as conventional corn varieties and with the exception of

the drought tolerance trait, are not materially different in composition or any other relevant parameter from other corn varieties now grown, marketed, and consumed in the U.S. At this time, based on Monsanto's data and information, the agency considers Monsanto's consultation on MON 87460 corn to be complete

Susan Carlson

[1]A 99 percent tolerance interval represents, with 95 percent confidence, 99 percent of the values contained in the population of commercial conventional corn varieties.

Page Last Updated: 10/28/2013
Note: If you need help accessing information in different file formats, see Instructions for Downloading Viewers and Players.

15. /Food/FoodScienceResearch/Biotechnology/default.htm
16. /Food/FoodScienceResearch/Biotechnology/Submissions/default.htm