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# Glyphosate-Tolerant Soybeans Remain Compositionally Equivalent to Conventional Soybeans (*Glycine max* L.) during Three Years of Field Testing

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Previous studies have shown that the composition of glyphosate-tolerant soybeans (GTS) and selected processed fractions was substantially equivalent to that of conventional soybeans over a wide range of analytes. This study was designed to determine if the composition of GTS remains substantially equivalent to conventional soybeans over the course of several years and when introduced into multiple genetic backgrounds. Soybean seed samples of both GTS and conventional varieties were harvested during 2000, 2001, and 2002 and analyzed for the levels of proximates, lectin, trypsin inhibitor, and isoflavones. The measured analytes are representative of the basic nutritional and biologically active components in soybeans. Results show a similar range of natural variability for the GTS soybeans as well as conventional soybeans. It was concluded that the composition of commercial GTS over the three years of breeding into multiple varieties remains equivalent to that of conventional soybeans.

**KEYWORDS: Soybean (*Glycine max* L.); glyphosate-tolerant; composition; genetically modified; natural variability**

## INTRODUCTION

The first agricultural biotechnology crops were commercially introduced in the early 1990s. Among the first of these products was glyphosate-tolerant soybeans (GTS, marketed under the trade name Roundup Ready (Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC) soybean) that were available in the U.S. in 1995. Since then, numerous agronomically and nutritionally enhanced traits have been introduced in more than 70 different crop species, and 50 products have been approved in numerous countries around the world (*1*−*3*). Before commercialization, a new biotechnology crop undergoes a thorough safety assessment (*4*, *5*). The comparative safety assessment process of a food or feed derived from biotechnology crops is based upon the concept that there is a “reasonable certainty of no harm” from its intended use (*5*−*8*). This process compares the phenotypic characteristics and composition of the biotechnology-derived crop to that of its conventional counterpart crop that has a history of safe use (*9*). The process is known as substantial equivalence, which is utilized by many regulatory and food safety authorities worldwide as a key part of assessing the safety of food or feed derived from biotechnology crops. Beyond this compositional comparison, additional safety assessments are made including the agronomic performance of the crop, the safety of the source gene, toxic and allergenic potential of the protein, unintended effects due to the insertion of the gene, and nutritional equivalence (*5*, *7*, *10*).

GTS was genetically engineered to produce a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein as encoded by the *Agrobacterium* sp. strain *cp4 epsps* gene. The CP4 EPSPS protein is functionally similar to plant EPSPS enzymes but has a much reduced affinity for glyphosate, a selective EPSPS inhibitor and the active ingredient in Roundup agricultural herbicides (*11*). The continued activity of CP4 EPSPS, even when treated with glyphosate, allows the plant to continue to produce aromatic amino acids and other secondary metabolites that are required for normal growth and development (*12*). The safety of the CP4 EPSPS protein in GTS has been well documented (*12*−*14*).

The composition of GTS was determined in seed and various processed fractions from a multi-location trial over two years in previous studies (*15*, *16*). In these studies, a comprehensive assessment of the components measured in seed included proximates, amino acids, fatty acids, antinutrients, and isoflavones. The conclusion was that the composition of GTS and selected processed fractions was substantially equivalent to that of conventional soybeans over a wide range of analytes. Furthermore, the nutritional equivalence was also determined by growth and performance studies in chicken, dairy cattle, and swine (*17*, *18*). These additional studies demonstrated that

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animals fed GTS had growth performance and feed efficiency similar to animals fed conventional soybeans.

The benefit of GTS is that it allows the soybean farmer to control a broad spectrum of weeds using glyphosate herbicides with minimal impact on the plant and environment (*19*). GTS was grown in eight countries with 102 million acres (41.4 million hectares) cultivated in 2003 (*20*), indicating the widespread adoption of this technology. An important consideration for any new technology is to demonstrate consistent and reliable performance over time. Performance can be defined for agronomic traits as the stability of the intended trait in diverse genetic backgrounds.

In this paper, consistent performance of GTS was assessed by comparing the composition of representative GTS seed in diverse genetic backgrounds harvested during three different years in the United States and Canada to conventional soybean varieties. The composition of GTS seed that are commercially available in each year tested was compared to the composition of seed from conventional soybean varieties. The GTS and conventional soybean varieties were not matched for similar genetic backgrounds as in previous composition studies (*15*, *16*). Analyses measured the levels of proximates, lectin, trypsin inhibitor, and isoflavones in soybean seed. These selected analytes represent basic nutritional and biologically active components in soybeans. Comparisons of the values of the GTS and conventional seed were then made to those values published in the literature and the International Life Science Institute (ILSI) Crop Composition Database (*21*).

## MATERIALS AND METHODS

**Soybean Seed Samples.** Samples were collected from Monsanto soybean seed stations during the 2000, 2001, and 2002 field seasons in the United States and Canada. All of the soybean varieties were commercially available or near commercial germplasms reflective of a broad range of maturity groups (I−IX). The GTS varieties were treated in-season with Roundup agricultural herbicide applications according to the labeled rates and timing. The soybean seed samples were stored at ambient temperature until ground. The ground samples were stored in a −20 °C freezer until analysis, and appropriate chain-of-custody accompanied all shipments.

**2000 Field Season.** Seed from 25 different varieties of GTS and 25 different varieties of conventional soybeans were harvested in 2000. The GTS varieties were: AG0801, AG2101, AG2301, AG3503, AG3702, AG3903, AG4901, AG5401, AG5501, CSR3623, CSR3922N, CSR4003, CSR4122N, CSR4812, CSR2523, CSR2900, CSR3112N, CSR6212, CSRX124, CSRX58, H4998RR, H5999RR, H6686RR, H7550RR, and H8001RR. The conventional soybean varieties were: A1900, A2069, A2104, A2247, A2553, A2704, A2804, A2869, A3469, A3904, A4341, A4604, A4922, A5547, A5959, A6297, A6961, CX284C, CX393C, CX400, CX420C, CX470C, H4994, H6255, and H6686.

**2001 Field Season.** Seed from 25 different varieties of GTS and 25 different varieties of conventional soybeans were harvested in 2001. The GTS varieties were: AG1401, AG1701, AG1902, AG2102-14, AG2403, AG2705, AG3303, AG3401, AG3702, AG3703, AG4201, AG4603, AG5001, AG5301, AG5903, AG6202, AG6701, CSR5952N, DKB17-51, DKB25-51, DKB27-51, DKB46-51, H5110RR, H5223RR, and H5887RR. The conventional soybean varieties were: A2824, A3244, A3834, A3836, A5427, A5547, A6297, CBN2001D0C, CCP4301A1C, CEI3900E0C, CGL5200B0C, CJW1901B1C, CMA5801B0C, CMA5901C0C, COX3501B0C, COX3501C0C, CRM2401H1C, CST321N, CST341, CX229, CX232, FP24960, H518, QR5282B, and WP25920.

**2002 Field Season.** Seed from 16 different varieties of GTS and 16 different varieties of conventional soybeans were harvested in 2002. The GTS varieties were: H5333RR, AG5402, AG5605, DKB64-51, AG1401, DKB19-52, DKB22-51, AG2403, DKB37-51, AG2403, DKB44-52, AG3801, DKB009-51, AG0101, FLS2602R, and AG0601. The conventional soybean varieties were: H922, A4922, A5547, CMA5801B0C, A2225, CST231N, DJW2501B0C, FP29930, A3525, COX3501B0C, CEI3900E0C, ACP4301A1C, Opal, Exeter, FL0062, and BJB2100D0C.

**Compositional Analysis.** Soybean seed samples harvested during 2000, 2001, and 2002 were ground and analyzed for the levels of proximates (ash, carbohydrates, moisture, protein, and total fat), lectin, trypsin inhibitor, and isoflavones (expressed as aglycones: daidzein, genistein, and glycitein). Covance Laboratories Inc. (Madison, WI) generated the analytical data for these samples.

Each method conducted utilized a standard or a quality control sample with known analyte content, and each sample was analyzed once. Brief descriptions of the analytical methods utilized are below. All laboratory activities followed Good Laboratory Practices (*22*).

**Proximate Analysis.** The moisture content was estimated by loss of weight upon drying the sample in an oven to a constant weight (*23*, *24*). Protein concentration was estimated by determining the total nitrogen using the Kjeldahl method, previously described (*25*, *26*). The total fat content was estimated using a Soxhlet extraction method (*27*, *28*). The ash content was estimated by the ignition of a sample with a furnace and determining the percent ash gravimetrically (*29*). The carbohydrate content was calculated using the following equation (*30*):

$$\%\ \text{carbohydrates} = 100\% - (\%\ \text{protein} + \%\ \text{fat} + \%\ \text{ash} + \%\ \text{moisture})$$

**Antinutrient and Isoflavone Analysis.** The lectin content was measured by suspending the sample in a phosphate buffered saline solution, adding lyophilized rabbit blood (Sigma, St. Louis, MO), and reading the absorbance at 650 nm (*31*, *32*). Total trypsin inhibitor activity was measured by suspending the sample in a sodium hydroxide solution, adding trypsin (Sigma, St. Louis, MO) and benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (Sigma, St. Louis, MO), and reading the absorbance at 410 nm (*33*). The isoflavone content was measured as the three aglycones daidzein, genistein, and glycitein. The sample was extracted with a hydrochloric acid/alcohol solution. Isoflavone aglycones were purified by passage through a C18 solid-phase extraction column. Analysis was conducted on a high-performance liquid chromatography system with ultraviolet spectrophotometric quantitation as previously described (*34*, *35*).

**Data Reduction and Statistical Analysis.** Data generated on the samples were measured on a fresh weight basis. Using the moisture content that was determined for each sample, the data were converted to dry weight. Simple means and ranges for the GTS and conventional soybean seed were determined on the dry weight values of each analyte. Standard error (SE) of the mean was calculated for each simple mean as the standard deviation divided by the square root of the number of values. The means and ranges were compared to published values and those values in the ILSI Crop Composition Database (*21*) to place the values in the perspective of natural variability.

## RESULTS AND DISCUSSION

**Proximate Analysis of Soybean Seed from 2000, 2001, and 2002.** The data for the proximate analysis of GTS and conventional soybean seed for the 2000, 2001, and 2002 field seasons are found in **Table 1**. The means and ranges of the levels of proximates measured in GTS seed are similar to the conventional soybean seed means and ranges. Across all three years, the moisture content ranged from 5.1% to 7.5% fresh weight (fw) for GTS seed and 5.1−8.8% fw for conventional soybean seed. The protein content ranged from 33.4% to 43.0% dry weight (dw) for GTS seed and 34.4−45.3% dw for conventional soybean seed. The total fat content ranged from 14.6% to 21.2% dw in GTS seed and 14.4−22.3% dw for conventional soybean seed. The ash content ranged from 4.9% to 6.0% dw for GTS seed and 5.0−6.0% dw for conventional soybean seed. The carbohydrate content ranged from 31.5 to 42.9% dw for GTS seed and 31.0−42.1% dw for conventional

**Table 1.** Proximate Analysis of Seed from GTS and Conventional Soybeans Harvested in 2000, 2001, and 2002

| | 2000 field season | | 2001 field season | | 2002 field season | | | |
|---|---|---|---|---|---|---|---|---|
| component[a] | GTS mean[c] ± SE[d] (range)[f] | conven.[b] mean[c] ± SE[d] (range)[f] | GTS mean[c] ± SE[d] (range)[f] | conven.[b] mean[c] ± SE[d] (range)[f] | GTS mean[e] ± SE[d] (range)[f] | conven.[b] mean[e] ± SE[d] (range)[f] | literature range[a] | ILSI[g] range[a] |
| moisture | 5.4 ± 0.03 (5.1–5.7) | 5.3 ± 0.03 (5.1–5.6) | 6.6 ± 0.03 (6.3–6.9) | 6.6 ± 0.03 (6.3–6.9) | 6.0 ± 0.21 (5.2–7.5) | 6.3 ± 0.28 (5.3–8.8) | 5.2–14.3[h] | 5.1–14.9 |
| protein | 39.7 ± 0.32 (37.0–43.0) | 39.8 ± 0.30 (36.4–42.6) | 37.8 ± 0.38 (33.4–41.5) | 38.3 ± 0.31 (34.4–40.6) | 40.2 ± 0.25 (38.0–42.4) | 41.2 ± 0.55 (37.7–45.3) | 32.9–43.6[i] 36.0–48.4[j] | 33.2–45.5 |
| total fat | 18.5 ± 0.27 (15.8–21.2) | 18.9 ± 0.31 (15.5–22.3) | 18.2 ± 0.23 (14.6–20.0) | 17.9 ± 0.31 (14.4–20.9) | 20.4 ± 0.13 (19.5–21.2) | 19.7 ± 0.31 (17.8–22.1) | 12.0–24.0[k] 19.8–27.7[i] | 8.1–23.6 |
| ash | 5.2 ± 0.04 (4.9–5.5) | 5.4 ± 0.05 (5.0–5.8) | 5.6 ± 0.05 (4.9–6.0) | 5.5 ± 0.06 (5.0–6.0) | 5.6 ± 0.07 (5.0–6.0) | 5.6 ± 0.06 (5.0–6.0) | 4.3–5.9[l] | 3.9–6.5 |
| carbohydrates | 36.6 ± 0.30 (33.7–39.1) | 36.0 ± 0.32 (32.6–39.7) | 38.4 ± 0.40 (35.3–42.9) | 38.3 ± 0.36 (34.8–42.1) | 33.8 ± 0.27 (31.5–35.9) | 33.5 ± 0.46 (31.0–36.4) | 29.3–41.3[l] | 29.6–50.2 |

[a] All data expressed as percent dry weight of sample, except moisture, which is percent fresh weight of sample. [b] Conventional soybean seed samples. [c] The simple mean of 25 values. [d] The standard error (SE) of the mean. [e] The simple mean of 16 values. [f] Range denotes the lowest and highest individual values across samples.[g] ILSI Crop Composition Database, ref 21. [h] Reference 16. [i] Reference 36. [j] Reference 37. [k] Reference 41. [l] Reference 15.

**Table 2.** Lectin, Trypsin Inhibitor, and Isoflavone Analysis of Seed from GTS and Conventional Soybeans Harvested in 2000, 2001, and 2002

| | 2000 field season | | 2001 field season | | 2002 field season | | | |
|---|---|---|---|---|---|---|---|---|
| component[a] (unit) | GTS mean[b] ± SE[d] (range)[f] | conven.[c] mean[b] ± SE[d] (range)[f] | GTS mean[b] ± SE[d] (range)[f] | conven.[c] mean[b] ± SE[d] (range)[f] | GTS mean[e] ± SE[d] (range)[f] | conven.[c] mean[e] ± SE[d] (range)[f] | literature range[a] | ILSI[g] range[a] |
| lectin (HU/mg) | 1.05 ± 0.06 (0.46–1.62) | 1.07 ± 0.09 (0.55–2.34) | 2.8 ± 0.24 (1.1–6.5) | 3.4 ± 0.41 (1.1–9.0) | 0.79 ± 0.12 (0.15–1.69) | 1.82 ± 0.38 (0.57–6.13) | 0.8–2.4[h,i] 37–426[j,k] | 0.11–9.0 |
| total trypsin inhibitor (TIU/mg) | 45.9[l] ± 1.8 (24.6–64.6)[l] | 44.4[l] ± 1.9 (30.1–65.8)[l] | 41.8 ± 2.6 (23.7–72.9) | 41.7 ± 1.9 (27.9–75.5) | 51.0 ± 3.4 (36.1–90.6) | 50.5 ± 2.8 (34.7–75.0) | 33.2–54.5[h] | 19.6–119 |
| | | | | Isoflavones ($\mu$g/g) | | | | |
| daidzein | 397 ± 32 (145–680) | 377 ± 40 (25–946) | 677 ± 66 (238–1797) | 600 ± 69 (218–1530) | 542 ± 52 (189–928) | 538 ± 64 (175–1236) | 98.8–1242[m,n] | 60–2453 |
| genistein | 544 ± 35 (255–838) | 562 ± 48 (28–1033) | 875 ± 52 (399–1572) | 805 ± 64 (390–1477) | 630 ± 36 (414–998) | 640 ± 48 (323–919) | 130–1501[m,n] | 144–2837 |
| glycitein | 148 ± 9.4 (75–243) | 142[o] ± 8.1[o] (73–229)[o] | 156 ± 13 (85–363) | 154 ± 11 (90–309) | 137 ± 11 (69–204) | 130 ± 12 (45–228) | 42.2–204[m,n] | 15–310 |

[a] All data expressed as dry weight of sample. [b] The simple mean of 25 values. [c] Conventional soybean seed samples. [d] The standard error (SE) of the mean. [e] The simple mean of 16 values. [f] Range denotes the lowest and highest individual values across samples. [g] ILSI Crop Composition Database, ref 21. [h] Reference 15. [i] Data expressed as fresh weight of sample. [j] Data expressed as hemagglutinating unit per milligam of protein. [k] Reference 42. [l] Data expressed as defatted weight of sample. [m] Data expressed as edible portion. [n] Reference 38. [o] One data point that was below the limit of quantitation of the assay was removed.

soybean seed. The SE for the above assays was very low ranging from 0.03% to 0.55% of the values. The ranges of the values from GTS and conventional soybean seed across all three years are similar to ranges in the published literature data (*15*, *16*, *36*, *37*) and the ILSI Crop Composition Database (*21*). These data show that the proximate values for GTS are within the range of natural variability observed in conventional soybean seed.

**Lectin, Trypsin Inhibitor, and Isoflavone Composition in Soybean Seed from 2000, 2001, and 2002.** The lectin, trypsin inhibitor, and isoflavone data are presented in **Table 2**. The ranges of values for lectin, trypsin inhibitor, and isoflavones show significant variability across all three years (i.e., large range of values). The mean and range of the GTS seed values, however, are similar to the mean and range of conventional soybean seed values for the levels of trypsin inhibitor and isoflavones (daidzein, genistein, glycitein) within each given year. Across all three years, the lectin values show a significant variability for both GTS and conventional soybean seed comparing year to year. However, the lectin range across all three years is similar between GTS seed (0.15−6.5 hemagglutinating unit (HU)/mg dw), conventional soybean seed (0.55−9.0 HU/mg dw), and the ILSI Crop Composition Database (0.11−9.0 HU/mg dw) (*21*).

The trypsin inhibitor content across all three years ranged from 23.7 to 90.6 trypsin inhibitor unit (TIU)/mg dw for GTS seed and 27.9−75.5 TIU/mg dw for conventional soybean seed with a SE ranging from 1.8 to 3.4. The trypsin inhibitor values for the GTS and conventional soybean seed are also within or similar to the range reported in the literature (33.2-54.5 TIU/mg dw) (*15*) and ILSI Crop Composition Database range (19.6−119 TIU/mg dw) (*15*, *21*).

The daidzein content ranged from 145 to 1797 $\mu$g/g dw for GTS seed and 25−1530 $\mu$g/g dw for conventional soybean seed with a SE ranging from 32 to 69. The genistein content ranged from 255 to 1572 $\mu$g/g dw for GTS seed and 28−1477 $\mu$g/g dw for conventional soybean seed with a SE ranging from 35 to 64. The glycitein content ranged from 69 to 363 $\mu$g/g dw for GTS seed and 45−309 $\mu$g/g dw for conventional soybean seed with a SE ranging from 8.1 to 13. The isoflavone values for the GTS and conventional soybean seed are also within or similar to the range reported in the literature (*38*) and the ILSI Crop Composition Database (*21*).

It is typical for the measured levels of all nutrients and antinutrients in crops to vary depending on the environmental conditions, cultivar grown, and method used (*39*, *40*). Because the GTS and conventional soybean seed were not matched for genetic backgrounds and were not grown at the same field site, during the same field season, and under the same environmental conditions, the variation observed for the isoflavone values of GTS and conventional soybeans was expected.

The comparative safety assessment process of a food or feed derived from biotechnology crops is based upon the concept that there is a "reasonable certainty of no harm" from its intended use. This process compares the phenotypic characteristics and composition of the biotechnology-derived crop to that of conventional crops with a known history of safe use. Previous studies have shown that the composition of GTS is substantially equivalent to that of conventional soybeans in previous studies. The results of this study further indicate that the composition of commercialized GTS over three years of breeding into multiple genetic backgrounds remains substantially equivalent to that of conventional soybeans. In conclusion, the nutritional and biologically active levels of GTS reported here are similar to the natural variability of nutritional and biologically active levels in soybean seed of conventional varieties and those values reported in the ILSI Crop Composition Database.

## ABBREVIATIONS USED

dw, dry weight; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; fw, fresh weight; HU, hemagglutinating unit; ILSI, International Life Science Institute; GTS, glyphosate-tolerant soybeans; SE, standard error; TIU, trypsin inhibitor unit.

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