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ARTICLE

Compositions of Forage and Seed from Second-Generation Glyphosate-Tolerant Soybean MON 89788 and Insect-Protected Soybean MON 87701 from Brazil Are Equivalent to Those of Conventional Soybean (*Glycine max*)

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Brazil has become one of the largest soybean producers. Two Monsanto Co. biotechnology-derived soybean products are designed to offer benefits in weed and pest management. These are second-generation glyphosate-tolerant soybean, MON 89788, and insect-protected soybean, MON 87701. The second-generation glyphosate-tolerant soybean product, MON 89788, contains the *5-enolpyruvylshikimate-3-phosphate synthase* gene derived from *Agrobacterium* sp. strain CP4 (cp4 epsps). MON 87701 contains the *cry1Ac* gene and expression of the Cry1Ac protein providing protection from feeding damage caused by certain lepidopteran insect pests. The purpose of this assessment was to determine whether the compositions of seed and forage of MON 89788 and MON 87701 are comparable to those of conventional soybean grown in two geographically and climatically distinct regions in multiple replicated sites in Brazil during the 2007−2008 growing season. Overall, results demonstrated that the seed and forage of MON 89788 and MON 87701 are compositionally equivalent to those of conventional soybean. Strikingly, the results also showed that differences in mean component values of forage and seed from the two controls grown in the different geographical regions were generally greater than that observed in test and control comparisons. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) of compositional data generated on MON 89788, MON 87701, and their respective region-specific controls provide a graphical illustration of how natural variation contributes more than biotechnology-driven genetic modification to compositional variability in soybean. Levels of isoflavones and fatty acids were particularly variable.

KEYWORDS: Soybean (*Glycine max*); glyphosate-tolerant; insect-protected; biotechnology; composition

INTRODUCTION

The ease and geographical range of its agricultural production make soybean an inexpensive source of oil and protein for use as food and as animal feed (*1*). Brazil has become one of the largest soybean producers; soybean production in Brazil in 2008/2009 is estimated at 58 million tonnes and exports at 25 million tonnes, according to the USDA Foreign Agricultural Service (*2*). Soybean production for 2009/2010 is expected to rise to 59.5 million tonnes (*2*). Two Monsanto Co. biotechnology-derived soybean products are designed to offer benefits in weed and pest management. The second-generation glyphosate-tolerant soybean product, MON 89788, contains the *5-enolpyruvylshikimate-3-phosphate synthase* gene derived from *Agrobacterium* sp. strain CP4 (cp4 epsps)(*3*). Expression of the gene product (CP4 EPSPS) renders the plant tolerant to glyphosate, which is the active ingredient in the Roundup family of herbicides. Insect-protected soybean product, MON 87701, contains the *cry1Ac* gene derived from *Bacillus thuringiensis* (*4*, *5*). Expression of the Cry1Ac protein renders MON 87701 resistant to targeted lepidopteran pests. MON 87701 is anticipated to be an important base trait for future breeding improvements with multitrait products for tropical and subtropical soybean production.

This paper reports results from a comprehensive compositional assessment of glyphosate-tolerant soybean MON 89788 and insect-protected soybean MON 87701 grown in multiple replicated fields in the northern and southern regions of Brazil during the 2007/2008 growing season. The purpose of this assessment was to determine whether the composition of seed and forage of

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these two important agronomic products were comparable to those of conventional soybean grown in two geographically and climatically distinct in Brazil. The northern and southern regions represent major growing areas but are distinguished by different climate and growing conditions. The MON 89788 and MON 87701 traits were each introgressed into the conventional Monsoy 8329 and A5547 varieties that are adapted to Brazil's northern and southern growing regions, respectively. Additionally, commercially available soybean varieties were grown concurrently with MON 89788, MON 87701, and its conventional controls at each field site. The National Technical Commission on Biotechnology (CTNBio) of Brazil has established a science-based regulatory framework for approval of all domestic and imported agricultural biotech products. Compositional assessments follow globally accepted guidelines outlined in Organization for Economic Cooperation and Development (OECD) consensus documents (*1*). The OECD consensus emphasizes direct comparisons between the new biotechnology-enhanced crop and a near-isogenic control to identify potential differences in the levels of key nutrient and antinutrient components (*6*). In this assessment, soybean forage samples were analyzed for levels of proximates (ash, fat, moisture, and protein), carbohydrates by calculation, and fiber. Seed samples were analyzed for proximates, carbohydrates by calculation, fiber, amino acids, fatty acids, antinutrients, and vitamin E.

MATERIALS AND METHODS

Soybean Samples for Compositional Analyses. Forage and harvested seed samples were collected from test (MON 89788 and MON 87701), conventional control, and reference materials grown in a 2007/2008 Brazil field production. The geographic regions selected for the Brazil field trials were representative of major commercial soybean production areas in both the northern and southern hemispheres. The field trials were conducted at four replicated sites: Cachoeira Dourada, Minas Gerais (CD), and Sorriso, Mato Grosso (SR) in the northern region of Brazil; and Não-Me-Toque, Rio Grande do Sul (NT), and Rolândia, Paraná (RO), in the southern region of Brazil.

The MON 89788 and MON 87701 traits were introgressed into the Monsoy 8329 and A5547 varieties that are adapted to the northern and southern growing regions, respectively. Monsoy 8329 was the conventional control grown in the northern region, and A5547 was the conventional control for the southern region.

Four different commercially available soybean varieties were included as reference materials at each site of Brazilian field production to provide data for the establishment of a 99% tolerance interval for each compositional component analyzed. Monsoy 8360, Monsoy 8757, TMG 103, TMG 115, Monsoy 8352, BRS-Favorita, BRS Valiosa, and BRS Conquista were grown in the northern region, and CD 214, CD 213, V-Max, and CD 215 were grown in the southern region. Monsoy 8757, BRS Conquista, V-Max, and CD 215 were conventional soybean varieties, whereas the others were Roundup Ready (glyphosate-tolerant) soybean varieties. At each site, starting seeds were planted in a randomized complete block design with four replicates for each test, control, and reference material. Normal agronomic practices were followed for each growing region including the application of registered non-glyphosate-containing maintenance pesticides as required for optimal growth. Forage material was collected at approximately the R6 growth stage (full seed) from at least six plants from each plot by cutting at the base and compositing the six individual plants into one sample per plot. The forage samples were transferred to dry ice within 30 min after sampling. Seed was harvested at maturity and stored at ambient temperature. Forage samples were shipped frozen on dry ice, and seed samples were shipped at ambient temperature. At Monsanto Co., forage and seed samples were homogenized by grinding with dry ice to a fine powder and stored frozen at approximately −20 °C. The identity of the seed was confirmed by sample handling records and event-specific Polymerase Chain Reaction (PCR) analysis. The identity of the forage was confirmed by chain of custody and sample handling records.

Compositional Analyses. Components assessed in forage samples included proximates (ash, fat, moisture, and protein), carbohydrates by calculation, acid detergent fiber (ADF), and neutral detergent fiber (NDF). Components assessed in seed samples included proximates (ash, fat, moisture, and protein), carbohydrates by calculation, ADF, NDF, total amino acids, fatty acids, lectins, phytic acid, raffinose, stachyose, trypsin inhibitors, isoflavones (daidzein, genistein, and glycitein), and vitamin E. Compositional analyses were conducted at EPL-BAS Laboratories in Niantic, IL. Samples from each site were analyzed by tissue in a randomized order to minimize assay bias. Descriptions of the methods utilized for the analyses are described in Berman et al. (*7*).

Statistical Analysis of Composition Data. In all, 64 different analytical components were measured (7 in forage and 57 in seed). To conduct a statistical analysis for a compositional component, at least 50% of the values for any given analyte had to be greater than the assay limit of quantitation (LOQ). The following 11 analytes, which had $>50\%$ of observations below the LOQ for their respective assays, were excluded from statistical analysis of results on seed samples: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:1 heptadecenoic acid, 18:3 γ-linolenic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, and 22:1 erucic acid. For individual measurements below the LOQ, a value equal to half the LOQ was assigned prior to statistical analyses. For 20:2 eicosadienoic acid in seed, 29 of 129 (23%) of the observed values were assigned a value of half the LOQ value. A studentized PRESS residuals test was applied to the data set to identify outliers. A PRESS residual is the difference between any value and its predicted value from a statistical model that excludes the data point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be within ± 3 PRESS residuals. Extreme data points that were outside the ± 6 studentized PRESS residual range were considered for exclusion from the final statistical analysis. One seed sample's trypsin inhibitor value from the CD site was removed from further analysis. The outlier test procedure was reapplied to all remaining seed sample trypsin inhibitor data to detect potential outliers that may have been masked in the first analysis. No additional results had a PRESS residual value outside the ± 6 range.

All soybean compositional analysis components were statistically analyzed using a mixed model analysis of variance. The four replicated sites were statistically assessed individually (individual site data not presented) and as a combination of the two sites within each growing region. The combined sites were analyzed using the model

$$Y_{ijkl} = U + R_i + T(R)_{ij} + L(R)_{ik} + B(RL)_{ikl} + TL(R)_{ijk} + e_{ijkl}$$

where Y_{ijkl} = unique individual observation, U = overall mean, R_i = region effect, $T(R)_{ij}$ = material within region effect, $L(R)_{ik}$ = random location within region effect, $B(RL)_{ikl}$ = random block within location and region effect, $TL(R)_{ijk}$ = random material by location within region interaction effect, and e_{ijkl} = residual error. For each component analysis, mean comparison of the test material and the conventional control material were conducted. Significant differences were determined at the 5% level of significance ($p < 0.05$).

A range of observed values from all reference materials across both northern and southern regions was determined for each analytical component. The reference materials data were also used to develop population tolerance intervals. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional component, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial conventional materials. The data were first summarized by material within site and then by material across sites. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero.

In addition, multivariate analyses (cluster analysis and principal component analysis) were conducted to identify any differences among the two test and control materials across the sites within the two regions. The multivariate analysis included the measurements from all seed analytes.

Table 1. Fiber and Proximate Composition of Forage from Insect-Protected Soybean MON 87701 and Glyphosate-Tolerant Soybean MON 89788

	northern			southern				
component[a]	MON 87701 mean[b] (range)[b]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	MON 87701 mean[b] (range)[b]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][d]	literature range[e]
fiber								
ADF	34.68[f] (26.27−45.40)	37.61 (30.64−44.64)	40.27 (31.23−47.27)	32.49 (24.58−37.11)	32.70 (25.91−39.68)	34.60 (27.65−40.64)	(27.62−59.03) [24.55, 52.92]	32−38
NDF	42.15 (36.99−50.32)	49.64 (38.23−68.64)	49.83 (41.61−73.05)	40.24 (32.11−47.09)	42.11 (31.42−56.22)	42.03 (36.47−50.77)	(34.95−68.32) [36.77, 59.62]	34−40
proximate								
ash	5.74 (5.06−6.18)	5.30 (4.91−5.74)	5.62 (5.11−6.18)	6.19 (5.76−7.58)	6.12 (5.33−6.78)	6.39 (5.60−7.02)	(4.87−7.51) [4.41, 7.47]	6.72−10.78
carbohydrates	71.00 (68.79−73.04)	72.72 (68.69−75.62)	73.25 (68.05−75.53)	71.27[f] (68.45−76.14)	69.89[f] (65.05−75.66)	66.97 (64.06−72.73)	(67.16−80.18) [68.70, 77.23)	59.8−74.7
moisture	75.43 (73.78−77.11)	74.39 (73.09−75.76)	75.83 (74.91−76.71)	75.23 (73.74−78.26)	75.26 (72.66−77.38)	76.57 (73.35−78.64)	(70.06−84.60) [72.43, 80.09]	73.5−81.6
protein	20.08 (18.82−21.54)	19.26 (16.69−22.12)	18.54 (16.96−23.03)	18.88[f] (14.27−21.66)	20.97[f] (15.39−24.70)	23.65 (18.87−27.00)	(11.77−23.94) [14.49, 22.00]	14.38−24.71
total fat	3.18 (2.29−5.10)	2.72 (2.28−3.44)	2.59 (1.77−3.35)	3.66 (2.20−5.69)	3.02 (2.38−4.31)	2.95 (1.67−4.15)	(1.01−4.68) [1.37, 4.32]	1.30−5.13

[a] Percent dry weight (moisture = % fresh weight, carbohydrates by calculation). [b] The least-squares mean and range of 16 values (four replicates from each of two field sites within a growing region). [c] The range of sample values for all of the commercial soybean varieties grown at the same field sites. [d] TI = tolerance interval, specified to contain 99% of the commercial soybean variety population with 95% confidence; negative limits set to zero. [e] Reference *9*. [f] Statistically different ($p < 0.05$) from control.

Table 2. Fiber and Proximate Composition of Seed from Insect-Protected Soybean MON 87701 and Glyphosate-Tolerant Soybean MON 89788

	northern			southern				
component[a]	MON 87701 mean[b] (range)[b]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	MON 87701 mean[b] (range)[b]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][d]	literature range[e]
fiber								
ADF	15.62 (14.02−18.13)	15.46 (12.90−17.95)	15.50 (12.71−18.34)	14.60 (13.02−16.79)	13.95 (12.48−15.26)	13.86 (11.72−16.91)	(12.36−18.89) [12.36, 18.08]	7.81−18.61
NDF	16.79 (15.96−17.36)	16.38 (14.75−17.88)	16.80 (15.34−17.96)	15.33 (14.92−15.85)	14.69 (13.81−15.85)	15.11 (14.11−16.02)	(13.41−18.12) [13.55, 18.77]	8.53−21.25
proximate								
ash	4.61 (4.29−4.77)	4.68 (4.54−4.79)	4.58 (4.43−4.80)	4.76 (4.39−5.05)	4.79 (4.49−4.93)	4.72 (4.53−4.92)	(4.35−5.16) [4.40, 5.35]	3.89−6.99
carbohydrates	35.17 (34.79−35.71)	34.97 (33.91−36.76)	36.18 (34.56−37.20)	38.41 (36.62−39.91)	39.09 (37.44−40.43)	38.28 (36.94−39.87)	(31.13−40.55) [26.78, 43.77]	29.6−50.2
moisture	8.70 (7.83−9.26)	9.09 (7.97−9.65)	8.68 (7.93−9.74)	9.27 (8.39−9.82)	8.91 (7.83−10.06)	8.77 (7.73−9.97)	(6.64−11.59) [5.92, 11.46]	4.7−34.4
protein	39.67[f] (38.99−40.49)	40.10[f] (39.15−41.53)	38.59 (36.86−40.42)	37.33 (36.12−38.39)	37.78 (36.22−38.71)	37.72 (35.50−39.36)	(35.42−43.51) [31.47, 46.26]	33.2−45.5
total fat	20.56 (20.07−21.17)	20.26 (18.69−21.23)	20.65 (19.35−21.55)	19.50 (17.59−21.28)	18.34 (17.40−19.12)	19.29 (17.77−20.74)	(17.59−23.67) [16.05, 25.93]	8.10−23.6

[a] Percent dry weight (moisture = % fresh weight, carbohydrates by calculation). [b] The least-squares mean and range of 16 values (four replicates from each of two field sites within a growing region). [c] The range of sample values for all of the commercial soybean varieties grown at the same field sites. [d] TI = tolerance interval, specified to contain 99% of the commercial soybean variety population with 95% confidence; negative limits set to zero. [e] Reference *10*. [f] Statistically different ($p < 0.05$) from control.

RESULTS AND DISCUSSION

The compositional analysis data and the statistical assessment are presented in **Tables 1−5**. Results of the combined-site analyses of forage and harvested seed showed that there were few significant differences ($p < 0.05$) between the test materials and the conventional control, regardless of growing region. Furthermore, mean test (MON 89788 and MON 87701) values for all 53 components statistically assessed fell within the 99% tolerance interval established from soybean varieties representative of the current market place and grown across both northern and southern regions. They were therefore considered to be within that of the commercial soybean population.

Strikingly, the results also showed that differences in mean component values of forage and seed from the two controls grown in the different geographical regions were generally greater than that observed in test and control comparisons. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) of compositional data generated on MON 89788, MON 87701, and their respective region-specific controls provide a graphical illustration of how location (site and region) and/or germplasm effects can contribute more to compositional variability than genetic modification (**Figure 1**).

Proximate and Fiber Composition in Forage. As presented in **Table 1**, there were a total of only five significant differences ($p < 0.05$) between mean values of test and control components. In those instances, relative magnitudes of differences, with respect to the control, were small: ∼16.2% for MON 87701 ADF (northern), ∼4.2 and ∼6.0% for MON 89788 and MON 87701 carbohydrates by calculation, respectively (both southern), and ∼11.3 and ∼20.2% for MON 89788 and MON 87701 and

Table 3. Amino Acid Composition of Seed from Insect-Protected Soybean MON 87701 and Glyphosate-Tolerant Soybean MON 89788

	northern			southern				
component[a]	MON 87701 mean[b] (range)[b]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	MON 87701 mean[b] (range)[b]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][d]	literature range[e]
alanine	1.75	1.79[g]	1.72	1.66	1.67	1.68	(1.59−1.90)	1.51−2.10
	(1.70−1.81)	(1.74−1.86)	(1.66−1.85)	(1.62−1.71)	(1.60−1.73)	(1.59−1.73)	[1.50, 1.98]	
arginine	2.64	2.67	2.57	2.52	2.53	2.52	(2.29−2.96)	2.29−3.40
	(2.60−2.72)	(2.59−2.74)	(2.44−2.68)	(2.36−2.62)	(2.40−2.79)	(2.22−2.73)	[2.01, 3.20]	
aspartic acid	4.99[g]	5.05[g]	4.80	4.59	4.66	4.71	(4.39−6.04)	3.81−5.12
	(4.83−5.15)	(4.90−5.19)	(4.57−5.24)	(4.51−4.73)	(4.40−4.86)	(4.50−4.85)	[3.98, 6.33]	
cystine/cysteine	0.58	0.57	0.56	0.61	0.61	0.59	(0.44−0.71)	0.37−0.81
	(0.43−0.74)	(0.50−0.62)	(0.52−0.61)	(0.53−0.66)	(0.54−0.67)	(0.52−0.66)	[0.48, 0.72]	
glutamic acid	8.48[g]	8.54[g]	8.15	7.79	7.84	7.98	(7.20−9.15)	7.53[f]−8.72[f]
	(8.29−8.65)	(8.28−8.78)	(7.90−8.83)	(7.53−8.12)	(7.40−8.26)	(7.40−8.40)	[6.38, 9.94]	
glycine	1.75	1.77	1.75	1.68	1.66	1.65	(1.59−1.91)	1.46−2.00
	(1.70−1.78)	(1.72−1.81)	(1.66−1.78)	(1.62−1.72)	(1.60−1.80)	(1.54−1.75)	[1.52, 1.99]	
histidine	1.13	1.14	1.12	1.10	1.05	1.06	(1.00−1.22)	0.88−1.18
	(1.09−1.15)	(1.09−1.19)	(1.06−1.17)	(1.06−1.13)	(0.98−1.13)	(1.00−1.11)	[0.93, 1.30]	
isoleucine	1.85	1.87	1.84	1.78	1.77	1.76	(1.61−1.99)	1.54−2.08
	(1.76−1.93)	(1.80−1.93)	(1.74−1.92)	(1.74−1.82)	(1.67−1.84)	(1.68−1.85)	[1.58, 2.10]	
leucine	3.05	3.08[g]	2.99	2.93	2.95	2.93	(2.75−3.28)	2.59−3.62
	(3.01−3.11)	(3.03−3.14)	(2.90−3.09)	(2.83−3.00)	(2.83−3.12)	(2.74−3.08)	[2.58, 3.45]	
lysine	2.84	2.92	2.96	2.76	2.83	2.77	(2.40−3.28)	2.29−2.84
	(2.71−3.06)	(2.74−3.18)	(2.62−3.32)	(2.64−3.03)	(2.51−3.08)	(2.59−3.13)	[2.26, 3.18]	
methionine	0.57	0.55	0.55	0.54	0.54	0.52	(0.41−0.64)	0.43−0.68
	(0.51−0.61)	(0.50−0.61)	(0.52−0.59)	(0.49−0.57)	(0.48−0.59)	(0.47−0.59)	[0.43, 0.66]	
phenylalanine	2.00	2.03	2.01	1.97	1.97	1.93	(1.76−2.18)	1.63−2.35
	(1.92−2.07)	(1.97−2.12)	(1.90−2.14)	(1.86−2.07)	(1.91−2.14)	(1.78−2.05)	[1.77, 2.26]	
proline	2.05[g]	2.07[g]	1.99	1.98	2.00	1.99	(1.88−2.22)	1.69−2.28
	(2.02−2.08)	(2.03−2.12)	(1.94−2.09)	(1.92−2.03)	(1.92−2.11)	(1.85−2.08)	[1.76, 2.32]	
serine	2.06	2.08[g]	2.02	1.99	1.96	1.96	(1.85−2.21)	1.11−2.48
	(2.02−2.08)	(2.04−2.13)	(1.93−2.10)	(1.91−2.06)	(1.87−2.11)	(1.84−2.08)	[1.76, 2.31]	
threonine	1.59	1.61	1.58	1.56	1.55	1.54	(1.47−1.69)	1.14−1.86
	(1.56−1.61)	(1.59−1.64)	(1.54−1.62)	(1.50−1.60)	(1.48−1.66)	(1.46−1.63)	[1.43, 1.76]	
tryptophan	0.49	0.49	0.47	0.49	0.48	0.48	(0.43−0.54)	0.36−0.50
	(0.48−0.50)	(0.48−0.50)	(0.45−0.49)	(0.48−0.51)	(0.45−0.53)	(0.39−0.51)	[0.41, 0.58]	
tyrosine	1.11	1.11	1.11	1.03	1.02	1.02	(0.95−1.34)	1.02−1.61
	(1.03−1.20)	(1.05−1.16)	(1.03−1.17)	(0.96−1.08)	(0.97−1.10)	(0.92−1.12)	[0.98, 1.36]	
valine	1.92	1.94	1.90	1.89	1.88	1.87	(1.81−2.09)	1.60−2.20
	(1.87−1.97)	(1.90−1.99)	(1.85−1.97)	(1.83−1.92)	(1.77−1.99)	(1.75−1.96)	[1.71, 2.16]	

[a] Percent dry weight. [b] The least-squares mean and range of 16 values (four replicates from each of two field sites within a growing region). [c] The range of sample values for all of the commercial soybean varieties grown at the same field sites. [d] TI = tolerance interval, specified to contain 99% of the commercial soybean variety population with 95% confidence; negative limits set to zero. [e] Reference *10*. [f] Reference *7*. [g] Statistically different ($p < 0.05$) from control.

protein, respectively (both southern). In most cases (**Table 1**) differences between the two controls appeared to be greater than that observed between test and control. Thus, for example, the relative magnitude of difference between protein levels in the two region-specific controls is ∼21.6% and between ADF levels is ∼16.4%, values that are greater, albeit modestly so, than that observed for the corresponding test versus control comparisons noted above.

Proximate and Fiber Composition in Seed. Results for proximates and fiber in seed are presented in **Table 2**. There were a total of only two significant differences ($p < 0.05$) between mean values of test and control components, both attributable to protein levels in the northern region. In both instances, the relative magnitude of difference, with respect to the control, was small: ∼3.8% for MON 89788 (northern) and ∼2.7% for MON 87701 (northern).

Amino Acid Composition. Results for amino acids in seed are presented in **Table 3**. For MON 89788 grown in the northern region there were, with the exception of alanine, aspartic acid, glutamic acid, leucine, proline, and serine, no significant differences ($p > 0.05$) between test and control. For MON 87701 grown in the northern region there were, with the exception of aspartic acid, glutamic acid, and proline, no significant differences ($p > 0.05$) between test and control. There were no significant differences ($p > 0.05$) between both tests and the control from the southern region. In those instances when a significant difference ($p < 0.05$) in mean values of amino acids was observed, the relative magnitude of difference, with respect to the control, was small: <5.2% for both MON 89788 (northern) and MON 87701 (northern).

Fatty Acid Composition. Table 4 contains the data for the fatty acid composition in seed. For MON 89788 grown in the northern region there were, with the exception of 16:1 palmitoleic acid, 17:0 heptadecanoic acid, and 18:0 stearic acid, no significant differences ($p > 0.05$) between test and control. There were, with the exception of 16:0 palmitic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 18:0 stearic acid, and 20:0 arachidic acid, no significant differences ($p > 0.05$) between test and control from the southern region. For MON 87701 grown in the northern region there were, with the exception of 16:1 palmitoleic acid, 17:0 heptadecanoic acid, and 20:1 eicosenoic acid, no significant differences ($p > 0.05$) between test and control and, with the exception of 20:2 eicosadienoic acid, no significant differences ($p > 0.05$) between test and control from the southern region.

Table 4. Fatty Acid Composition of Seed from Insect-Protected Soybean MON 87701 and Glyphosate-Tolerant Soybean MON 89788

	northern			southern				
component[a]	MON 87701 mean[b] (range)[b]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	MON 87701 mean[b] (range)[b]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][d]	literature range[e]
14:0 myristic	0.070	0.069	0.069	0.089	0.094	0.091	(0.068−0.091)	0.071−0.238
	(0.064−0.076)	(0.061−0.078)	(0.065−0.077)	(0.084−0.095)	(0.082−0.099)	(0.087−0.093)	[0.063, 0.10]	
16:0 palmitic	10.34	10.17	10.28	11.52	12.13[g]	11.66	(10.27−12.47)	9.55−15.77
	(9.92−10.77)	(9.74−10.62)	(9.87−10.62)	(11.25−11.74)	(11.27−12.39)	(11.23−12.08)	[9.27, 13.42]	
16:1 palmitoleic acid	0.11[g]	0.11[g]	0.12	0.093	0.10[g]	0.095	(0.078−0.13)	0.086−0.194
	(0.10−0.11)	(0.10−0.11)	(0.11−0.12)	(0.086−0.10)	(0.091−0.11)	(0.089−0.10)	[0.049, 0.16]	
17:0 heptadecanoic	0.084[g]	0.084[g]	0.080	0.098	0.095[g]	0.097	(0.071−0.11)	0.076[f]−0.10[f]
	(0.080−0.087)	(0.081−0.086)	(0.076−0.084)	(0.095−0.10)	(0.090−0.099)	(0.093−0.11)	[0.045, 0.13]	
18:0 stearic	2.99	3.05[g]	2.92	4.28	4.12[g]	4.25	(2.59−4.96)	2.70−5.88
	(2.81−3.23)	(2.79−3.35)	(2.68−3.21)	(4.09−4.50)	(3.90−4.50)	(4.07−4.58)	[0.83, 6.00]	
18:1 oleic	38.70	41.11	40.43	22.48	20.21	22.60	(19.54−37.65)	14.3−32.2
	(32.18−44.56)	(32.72−47.23)	(36.87−45.68)	(20.81−23.91)	(19.33−22.98)	(19.33−25.94)	[7.12, 45.77]	
18:2 linoleic	41.24	39.07	39.73	52.25	53.75	52.23	(42.00−56.48)	42.3−58.8
	(36.28−46.63)	(34.06−46.12)	(35.36−42.48)	(51.53−53.20)	(51.60−54.58)	(49.98−54.09)	[37.23, 64.46]	
18:3 linolenic	5.34	5.13	5.20	8.06	8.45	7.91	(5.37−8.58)	3.00−12.52
	(4.81−5.89)	(4.57−5.99)	(4.87−5.41)	(7.36−8.82)	(7.91−9.02)	(6.98−9.04)	[3.37, 9.77]	
20:0 arachidic	0.36	0.37	0.35	0.42	0.40[g]	0.42	(0.32−0.45)	0.163−0.482
	(0.34−0.38)	(0.35−0.41)	(0.33−0.39)	(0.42−0.43)	(0.38−0.43)	(0.39−0.43)	[0.24, 0.52]	
20:1 eicosenoic	0.29[g]	0.31	0.31	0.20	0.19	0.18	(0.14−0.31)	0.140−0.350
	(0.25−0.34)	(0.25−0.35)	(0.26−0.35)	(0.16−0.23)	(0.16−0.21)	(0.16−0.20)	[0.049, 0.40]	
20:2 eicosadienoic	0.023	0.025	0.031	0.044[g]	0.038	0.026	(0.017−0.065)	0.017[f]−0.064[f]
	(0.015−0.042)	(0.016−0.040)	(0.016−0.046)	(0.034−0.053)	(0.019−0.056)	(0.017−0.053)	[0.019, 0.069]	
22:0 behenic	0.48	0.50	0.48	0.46	0.42	0.44	(0.40−0.54)	0.277−0.595
	(0.45−0.50)	(0.47−0.53)	(0.46−0.50)	(0.45−0.47)	(0.40−0.44)	(0.41−0.47)	[0.30, 0.62]	

[a] Percent total fatty acid. [b] The least-squares mean and range of 16 values (four replicates from each of two field sites within a growing region). [c] The range of sample values for all of the commercial soybean varieties grown at the same field sites. [d] TI = tolerance interval, specified to contain 99% of the commercial soybean variety population with 95% confidence; negative limits set to zero. [e] Reference *10*. [f] Reference *7*. [g] Statistically different ($p < 0.05$) from control.

Table 5. Isoflavone, Antinutrient, and Vitamin Composition of Seed from Insect-Protected Soybean MON 87701 and Glyphosate-Tolerant Soybean MON 89788

	northern			southern				
component[a]	MON 87701 mean[b] (range)[b]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	MON 87701 mean[b] (range)[b]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][d]	literature range[e]
antinutrient								
lectin	2.44	3.23	2.32	3.30	2.84	3.68	(0.60−8.59)	0.09−8.46
	(1.36−4.98)	(0.59−7.05)	(0.17−7.67)	(1.49−5.65)	(1.04−6.72)	(0.28−5.67)	[0, 7.67]	
phytic acid	2.25	2.36	2.21	2.06	1.86	2.04	(1.17−2.46)	0.63−1.96
	(1.72−2.81)	(1.80−3.03)	(1.84−2.68)	(1.76−2.35)	(1.41−2.23)	(1.40−2.60)	[1.23, 2.18]	
raffinose	0.97	1.05[g]	0.84	1.01	1.04	0.97	(0.65−1.28)	0.52[f]−1.62[f]
	(0.81−1.17)	(0.90−1.28)	(0.70−1.00)	(0.92−1.16)	(0.89−1.18)	(0.81−1.21)	[0.47, 1.45]	
stachyose	3.91	3.82	4.17	4.06	4.20	3.93	(2.09−5.02)	1.21−3.50
	(3.38−4.28)	(3.26−4.33)	(3.75−4.55)	(3.45−4.34)	(3.86−4.57)	(3.65−4.20)	[1.83, 6.06]	
trypsin inhibitor	35.24	37.87	35.46	30.24	29.31	30.09	(23.99−41.03)	19.59−118.68
	(32.32−39.87)	(31.87−42.28)	(32.42−39.56)	(26.44−38.02)	(23.08−32.42)	(27.16−35.96)	[24.36, 46.20]	
isoflavone								
daidzein	235.22	235.94	234.30	1036.71	1165.32[g]	1013.77	(181.03−2099.75)	60.0−2453.5
	(194.71−288.79)	(214.42−271.57)	(197.89−265.32)	(627.45−1387.29)	(886.50−1420.52)	(605.39−1543.60)	[0, 2161.86]	
genistein	334.18	343.59	352.81	914.77	942.19	889.35	(176.48−1649.26)	144.3−2837.2
	(285.99−402.56)	(274.04−398.48)	(276.38−423.19)	(668.87−1155.06)	(772.55−1102.68)	(608.59−1190.42)	[0, 1924.15]	
glycitein	98.91	99.54	90.24	188.57	198.27	177.54	(32.88−260.51)	15.3−310.4
	(66.90−140.33)	(78.31−136.66)	(70.75−113.92)	(161.76−226.91)	(142.94−261.21)	(127.22−219.56)	[0, 406.94]	
vitamin								
E	4.72	4.93	4.84	6.51[g]	4.37	4.93	(1.08−4.69)	1.65[f]−8.08[f]
	(4.43−5.02)	(4.46−5.58)	(4.59−5.12)	(4.87−8.47)	(3.52−5.50)	(3.40−6.29)	[0, 6.52]	

[a] Isoflavones in mg/kg dry weight; lectin in HU/mg of fresh weight; phytic acid, raffinose, and stachyose in percent dry weight; trypsin inhibitor in TIU/mg of dry weight; and vitamin E in mg/100 g of dry weight. [b] The least-squares mean and range of 16 values (four replicates from each of two field sites within a growing region). [c] The range of sample values for all of the commercial soybean varieties grown at the same field sites. [d] TI = tolerance interval, specified to contain 99% of the commercial soybean variety population with 95% confidence; negative limits set to zero. [e] Reference *10*. [f] Reference *7*. [g] Statistically different ($p < 0.05$) from control.

In those instances when a significant difference ($p < 0.05$) in mean values of fatty acids was observed, the relative magnitude difference, with respect to the control, was small: < 10% for both MON 89788 and MON 87701.

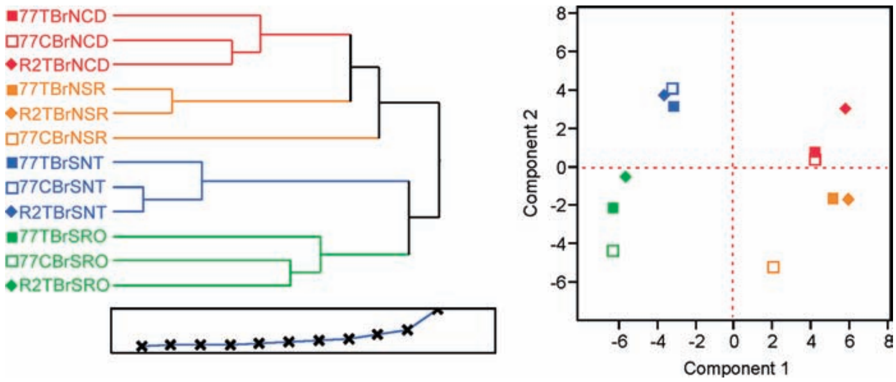

Figure 1. Hierarchical cluster analysis and principal component analysis of compositional data generated on the harvested seed of glyphosate-tolerant MON 89788 and insect-protected MON 87701 grown in the northern and southern regions of Brazil during the 2007/2008 season. The sample codes are as follows: the first three digits indicate the sample (R2T = MON 89788, 77T = MON 87701, 77C = conventional control for both MON 89788 and MON 87701); the remaining digits indicate the sites; [Cachoeira Dourada, Minas Gerais (BrNCD), Sorriso, Mato Grosso (BrNSR), Nao-Me-Toque, Rio Grande do Sul (BrSNT), Rolandia, Parana (BrSRO)].

Whereas differences in mean values of test and control fatty acids were generally statistically insignificant ($p > 0.05$) or of small relative magnitude, there was a remarkable difference in the fatty acids profiles of the two region-specific controls. This was particularly true of the most abundant fatty acids. Thus, for the northern region control (Monsoy 8329) the mean values for 18:1 oleic acid and 18:2 linoleic acid were 40.43 and 39.73% total FA, respectively, whereas corresponding values for A5547 in the southern region were 22.60 and 52.23% total FA, respectively.

Antinutrient, Isoflavone, and Vitamin E Composition. The results for antinutrients (lectin, phytic acid, raffinose, stachyose, and trypsin inhibitor), isoflavones (daidzein, genistein, and glycitein), and vitamin E are presented in **Table 5**. For MON 89788 there were, with the exception of raffinose in the northern region and daidzein in the southern region, no significant differences ($p > 0.05$) between test and control. For MON 87701 there were, with the exception of vitamin E in the southern region, no significant differences ($p > 0.05$). In those instances, relative magnitudes of differences, with respect to the control, were as follows: ~25.0% for MON 89788 raffinose (northern), ~13.0% for MON 89788 daidzein (southern), and ~24.3% for MON 87701 vitamin E (southern).

There were striking differences in values of the isoflavones from the two region-specific controls (see **Table 5**). There were 1.9-, 2.7-, and 4.4-fold differences in glycitein, genistein, and daidzein, respectively, between the conventional controls. Levels were higher in the southern region samples.

Multivariate Analyses. Figure 1 presents HCA and PCA of compositional data generated on MON 89788, MON 87701, and their respective region-specific controls grown at two replicated field sites in each of the northern and southern growing regions. It is apparent that location (site and region) and/or germplasm effects contribute more to compositional differences than genetic modification. This is consistent with the observations above that although differences in mean values of test and control components were generally statistically insignificant ($p > 0.05$) or of small relative magnitude, there were often remarkable differences in the compositional profiles of the two region-specific controls. This was particularly true for levels of fatty acids and isoflavones; for example, for the northern region control (Monsoy 8329) the mean values for oleic acid and linoleic acid were 40.43 and 39.73% of total FA, respectively, whereas corresponding values for the southern region were 22.60 and 52.23% total FA, respectively. Mean values for the major isoflavone daidzein were >4 times higher (1014 versus 234 ppm) in A5547 relative to that of Monsoy 8329.

Natural variation in oleic acid, linoleic acid, and daidzein levels was also confirmed by previous compositional studies (*7*) on A5547 seed harvested from U.S. and Argentinean field trials. Mean values for oleic acid, linoleic acid, and daidzein levels from the U.S. production were 21.71% total FA, 51.76% total FA, and 604 ppm for daidzein, respectively; corresponding figures from Argentinean field trials were 18.73% total FA, 54.51% total FA, and 934 ppm, respectively.

At least one other study by Carrao-Panizzi and Kitamura (*8*) has highlighted variability in isoflavone levels in Brazilian soybean cultivars. Thus, Carrao-Panizzi and Kitamura (*8*) showed, in an evaluation of 22 different conventional soybean varieties grown in two different years, that isoflavone content was significantly different among varieties and between years and varied widely (3−8-fold).

Conclusion. The forage and seed of MON 89788, MON 87701, corresponding conventional soybean control comparators, and multiple reference soybean varieties were grown at replicated fields in the northern and southern regions of Brazil during the 2007/2008 growing season. Any statistical differences between a test material and its corresponding soybean control were generally small in magnitude, and all test mean values for these components were within the 99% tolerance interval determined from commercial soybean varieties. It was observed that there were, overall, greater differences in the levels of components in the two controls used in this assessment than between the respective test and control materials. It is concluded that forage and seed of the second-generation glyphosate-tolerant soybean MON 89788 and insect-protected soybean MON 87701 are compositionally equivalent to that of conventional soybean. It is further concluded that geographical factors contribute extensively to compositional variation in soybean.

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