

# Compositional Variability in Conventional and Glyphosate-Tolerant Soybean (*Glycine max* L.) Varieties Grown in Different Regions in Brazil

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**S** Supporting Information

**ABSTRACT:** The compositions of a diverse range of commercially available conventional and genetically modified (GM; glyphosate-tolerant) soybean varieties from maturity groups 8 and 5, respectively, grown in the northern and southern soybean regions of Brazil during the 2007–2008 and 2008–2009 growing seasons were compared. Compositional analyses included measurement of essential macro- and micronutrients, antinutrients, and selected secondary metabolites in harvested seed as well as measurement of proximates in both forage and harvested seed. Statistical comparisons utilized a mixed analysis of variance model to evaluate the relative contributions of growing season, soybean growing region, production site, phenotype (GM or conventional), and variety. The study highlighted extensive variability in the overall data set particularly for components such as fatty acids, vitamin E, and isoflavones. There were few differences between the GM and non-GM populations, and most of the variability in the data set could be attributed to regional and variety differences. Overall, the results were consistent with the expanding literature on the lack of any meaningful impact of transgene insertion on crop composition.

**KEYWORDS:** soybean (*Glycine max*), genetically modified, glyphosate tolerant, composition, statistical analysis

## INTRODUCTION

Roundup Ready (Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC) soybean 40-3-2 was commercially introduced in 1996 and has been rapidly adopted due to cost efficiencies for weed control and simplicity of use. Glyphosate-tolerant soybeans including enhanced second-generation products such as MON 89788 are expected to provide excellent agronomic base traits for future breeding improvements and for the development of combined or “stacked” multi-trait transgenic (GM) products. New GM crops are subject to detailed characterization studies prior to regulatory approval, and comparative compositional studies are included as an integral component of these assessments. These studies have now yielded a large body of high-quality compositional data, generated according to principles outlined in Organisation for Economic Cooperation and Development consensus documents (www.oecd.org). A recent review of compositional data from different GM corn and GM soybean products concluded that the contribution of transgene insertion to compositional variation is minimal and that the composition of GM and conventional crops cannot be disaggregated.<sup>1</sup> These studies further confirmed a very broad range of naturally variable levels of key nutrients and antinutrients in both the GM and conventional crops.

Recently, the Monsanto soybean products MON 87701, MON 89788, and MON 87701 × MON 89788 were grown in multiple replicated fields in the northern and southern soybean regions of Brazil in two growing seasons (2007–2008 and 2008–2009). Compositional studies confirmed the equivalence of seed and forage of these products to those of their conventional control comparators

but also illustrated extensive variability in all nutrients and antinutrients measured.<sup>2–5</sup> This observation extended to the GM products, their conventional comparators, and a diverse range of commercially available conventional and GM (glyphosate-tolerant) reference soybeans from maturity groups 8 and 5 grown concurrently in the same field productions. The purpose of this study was to further evaluate the composition of seed and forage from the conventional comparators and the commercial variety soybeans and to determine the relative contributions of growing season, soybean growing region, phenotype (GM and conventional), and variety on variability within this data set.

## MATERIALS AND METHODS

**Soybean Samples for Compositional Analyses.** Forage (all above-ground material) and harvested seed samples were collected from soybeans grown in 2007–2008 and 2008–2009 Brazil field productions. Both trials were conducted at four replicated sites: Cachoeira Dourada, Minas Gerais (CD), and Sorriso, Mato Grosso (SR), in the northern soybean region (maturity group 8) of Brazil, and Nãome-Toque, Rio Grande do Sul (NT), and Rolândia, Paraná (RO), in the southern soybean region (maturity group 5). Each site included four different commercially available soybean varieties as references (see Table 1). All field sites were planted in a randomized complete block design with four blocks per substance. The conventional controls included in the compositional assessments of MON 87701, MON

**Received:** July 12, 2011

**Revised:** August 19, 2011

**Accepted:** August 31, 2011

**Published:** August 31, 2011

Table 1. Conventional and Transgenic Soybean

year	northern soybean region		southern soybean region	
	site CD	site SR	site NT	site RO
2007–2008	Monsoy 8352 <sup>a</sup>	Monsoy 8360 <sup>a</sup>	CD-214 <sup>a</sup>	CD-214 <sup>a</sup>
	BRS-Favorita <sup>a</sup>	TMG 103 <sup>a</sup>	CD-213 <sup>a</sup>	CD-213 <sup>a</sup>
	BRS Valiosa <sup>a</sup>	TMG 115 <sup>a</sup>	V-Max <sup>b</sup>	V-Max <sup>b</sup>
	BRS Conquista <sup>b</sup>	Monsoy 8757 <sup>b</sup>	CD 215 <sup>b</sup>	CD 215 <sup>b</sup>
	Monsoy 8329 <sup>b,c</sup>	Monsoy 8329 <sup>b,c</sup>	A5547 <sup>b,c</sup>	A5547 <sup>b,c</sup>
2008–2009	Monsoy 7908 <sup>a</sup>	Monsoy 8360 <sup>a</sup>	Apollo <sup>a</sup>	Magna <sup>a</sup>
	Monsoy 8360 <sup>a</sup>	TMG 103 <sup>a</sup>	Impacto <sup>a</sup>	CD 225 <sup>a</sup>
	BRS Valiosa <sup>a</sup>	TMG 115 <sup>a</sup>	CD-226 <sup>a</sup>	CD-215 <sup>b</sup>
	BRS GO Luziânia <sup>b</sup>	Monsoy 8757 <sup>b</sup>	Fundacep 53 <sup>a</sup>	V-Max <sup>b</sup>
	Monsoy 8329 <sup>b,c</sup>	Monsoy 8329 <sup>b,c</sup>	A5547 <sup>b,c</sup>	A5547 <sup>b,c</sup>

<sup>a</sup> Transgenic (glyphosate-tolerant). <sup>b</sup> Conventional. <sup>c</sup> Used as a near-isogenic control for MON 87701 and MON 89788 in refs 2 and 3.

89788, and MON 87701 × MON 89788 were also included in this study.<sup>2–5</sup> The control for the northern soybean region studies was Monsoy 8329, and that for the southern soybean region studies was A5547. Overall, the study contained 22 GM samples representing 15 different varieties and 18 conventional samples representing 7 different varieties. Eight of the conventional samples included Monsoy 8328 and A5547, which were planted at four sites each and which were used as conventional controls in earlier studies; details of the field trial design and material harvested [seed and forage (all above-ground material at time of harvest)] are presented in refs 2, 3, and 5.

**Compositional Analyses.** Brief descriptions of the methods utilized for the analyses are described in refs 2 and 3. Compositional analyses were conducted according to Good Laboratory Practices (GLP) requirements at EPL-BAS Laboratories, in Niantic, IL.

**Statistical Analysis of Composition Data.** In addition to the analytes discussed below, 11 fatty acids were assayed but found to be below the assay limit of quantitation in at least 50% of the samples in the data set; these included caprylic acid, capric acid, lauric acid, myristoleic acid, pentadecanoic acid, pentadecenoic acid, heptadecenoic acid,  $\gamma$ -linolenic acid, eicosatrienoic acid, arachidonic acid, and erucic acid. These were not included in the statistical analysis. Residuals outside  $\pm 6$  studentized PRESS residual ranges were found in acid detergent fiber, carbohydrates by calculation, and neutral detergent fiber for forage; daidzein, genistein, glycitein, and trypsin inhibitor outliers were found for seed. Natural log transformation was applied to the analytes with outliers; no more outliers were observed after log transformation.

Means, ranges, and standard errors were determined for each analyte across the entire data set and are presented in the Supporting Information (Supplementary Table 1).

All soybean compositional components were statistically analyzed using the SAS PROC MIXED procedure [SAS software release 9.2 (TS1M0), copyright 2002–2008, SAS Institute Inc., Cary NC]. Data were analyzed using the following model; as the three-way interactions between study, soybean region, and phenotype (GM or non-GM) were never found to be significant, they were excluded from the model.

$$Y_{ijkl} = U + S_i + R_j + P_l + S_i \times R_j + S_i \times P_l + R_j \times P_l + L_k(\text{SR})_{ij} + \text{Sub}(\text{PSR})_{ijl} + P_l \times L_k(\text{SR})_{ij} + \text{Rep}(\text{LSR})_{ijk} + e_{ijkl}$$

$Y_{ijkl}$  = unique individual observation;  $U$  = overall mean;  $S_i$  = study effect;  $R_j$  = region effect;  $P_l$  = phenotype effect;  $L_k(\text{SR})_{ij}$  = random location within study and region effect;  $\text{Sub}(\text{PSR})_{ijl}$  = random substance within

Table 2. Ranges of Values for Protein and Fat from Soybean Seed

production	no. of samples <sup>a</sup>	protein (% dw)	fat (% dw)
southern 2007/2008	6	35.49–39.61	17.77–22.69
southern 2007/2008 (GM)	4	35.42–38.94	17.59–21.84
southern 2008/2009	5	34.78–38.46	18.92–22.29
southern 2008/2009 (GM)	6	35.46–38.94	17.46–22.05
northern 2007/2008	4	36.72–41.44	19.35–23.33
northern 2007/2008 (GM)	6	37.74–43.51	19.28–23.67
northern 2008/2009	4	36.56–39.21	16.60–21.43
northern 2008/2009 (GM)	6	36.72–39.70	19.70–22.83
all conventional	18	34.78–41.44	16.60–23.33
all GM	22	35.42–43.51	17.46–23.67

<sup>a</sup> Overall, the study contained 22 GM samples representing 15 different varieties, and 18 conventional samples representing 8 different varieties. Eight of the conventional samples included the two control substances, Monsoy 8329 and A5547, which were planted at four sites each. For each sample,  $n = 4$ .

phenotype, study, and location effect;  $P_l \times L_k(\text{SR})_{ij}$  = phenotype across random location within study and region effect;  $\text{Rep}(\text{LSR})_{ijk}$  = random block within location, study, and region effect; and  $e_{ijkl}$  = residual error.

## RESULTS AND DISCUSSION

Overall, the study contained 22 GM samples representing 15 different varieties and 18 conventional samples representing 7 different varieties. Eight of the conventional samples included Monsoy 8329 (northern) and A5547 (southern), which were planted at four sites each and used as controls in earlier studies.<sup>2,3,5</sup> All field sites were planted in a randomized complete block design with four blocks per substance. Means, ranges, and standard errors determined for each analyte across the entire data set are presented in the Supporting Information (Supplementary Table 1). The results of the mixed-model analysis of variance for all analytes in seed and forage are presented in Supplementary Tables 2 and 3 of the Supporting Information. The model estimated the contribution of experimental factors [region, growing season, phenotype (GM versus conventional), and variety, as well as interaction terms] to compositional variability within the overall data set. Results demonstrated a high degree of variability in each category of compositional components discussed below but also confirmed that the impact of differences between the GM and conventional samples was low when compared to the impact of other experimental factors. This is discussed in more detail below.

**Protein and Amino Acids in Seed.** No statistically significant differences ( $p > 0.05$ ) in protein levels could be associated with the different growing seasons or soybean regions, although a growing season (year) effect for the amino acids alanine, aspartic acid, glutamic acid, lysine, phenylalanine, and tryptophan and a soybean region effect for lysine were observed (Supplementary Table 2 of the Supporting Information). The random effects in the mixed model showed that most variation in protein and amino acid levels was due to individual substance variation within the entire experiment; the parameter substance (region × year × phenotype), which represents this variation, was different ( $p < 0.05$ ) for protein and most amino acids assessed (Supplementary Table 3 of the Supporting Information). Table 2 shows that the

Table 3. Ranges of Values for Selected Components from Soybean

production	no. of samples <sup>a</sup>	oleic <sup>b</sup>	linoleic <sup>b</sup>	linolenic <sup>b</sup>	vitamin E <sup>c</sup>	daidzein <sup>d</sup>	stachyose <sup>e</sup>
southern 2007/2008	6	19.33–25.94	49.98–54.97	6.47–9.04	2.12–6.29	555.2–2099.7	3.30–4.74
southern 2007/2008 (GM)	4	19.54–24.91	51.58–54.84	6.65–8.58	1.08–4.69	368.6–1023.9	3.89–4.69
southern 2008/2009	5	18.91–29.54	47.63–55.01	5.64–8.35	3.81–8.49	266.6–935.8	3.80–4.87
southern 2008/2009 (GM)	6	18.69–22.76	48.18–57.10	5.81–8.72	1.74–5.52	326.4–1324.4	3.38–4.99
northern 2007/2008	4	26.08–45.68	35.36–51.60	4.87–6.19	3.56–5.12	197.9–411.9	3.47–4.55
northern 2007/2008 (GM)	6	20.65–37.65	42.00–56.48	5.37–7.17	2.43–4.57	197.3–420.5	2.09–5.02
northern 2008/2009	4	23.36–30.57	43.37–54.02	4.96–6.35	1.57–6.39	171.7–446.6	3.47–4.32
northern 2008/2009 (GM)	6	16.67–33.84	45.99–59.66	5.17–6.74	2.91–4.35	198.5–367.5	2.88–5.55
all conventional	18	18.91–45.68	35.36–55.01	4.87–9.04	1.57–8.49	171.7–2099.7	3.30–4.87
all GM	22	16.67–37.65	42.00–59.66	5.17–8.72	1.08–5.52	181.0–1349.6	2.09–5.55

<sup>a</sup> Overall, the study contained 22 GM samples representing 15 different varieties and 18 conventional samples representing 8 different varieties. Eight of the conventional samples included the two control substances, Monsoy 8329 and A5547, which were planted at four sites each. For each sample,  $n = 4$ .

<sup>b</sup> Total FA (%). <sup>c</sup> mg/100g dw. <sup>d</sup>  $\mu$ g/g dw. <sup>e</sup> % dw.

protein values from each individual production overlapped extensively and that this observation extended to comparisons of the GM and conventional samples within and across productions. For example, the mean and range of values for the GM samples across all soybean regions and growing seasons were 38.39 and 35.42–43.51 % dw, and corresponding values for the conventional samples were 37.90 and 34.78–41.44 % dw (Table 2; Supplementary Table 1 of the Supporting Information). In addition, the fixed effect, phenotype, in the analysis of variance model showed no statistically significant difference ( $p = 0.3096$ ) between the GM and conventional samples when assessed across the entire data set (Supporting Information, Supplementary Table 2).

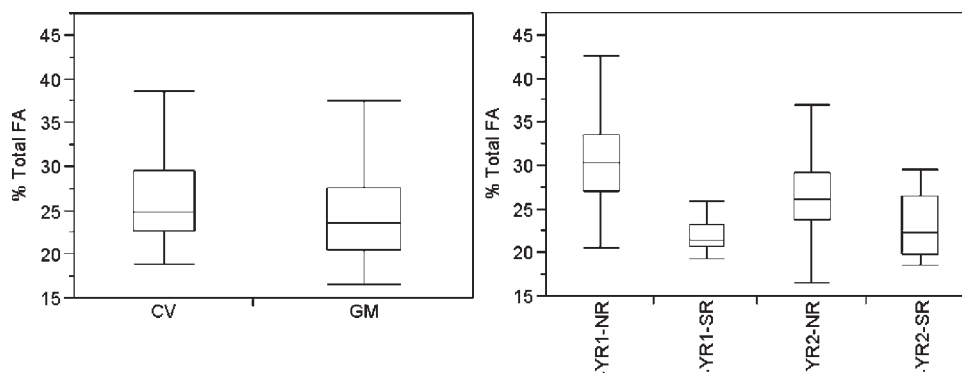
Previous studies have demonstrated that the protein and amino acid content of soybean can vary depending on growing conditions<sup>6</sup> and country.<sup>7</sup> Grieshop and Fahey<sup>7</sup> found extensive variation in soybean quality *within* each of the three countries they assessed (Brazil, China, and the United States). Goldflus et al.<sup>8</sup> also confirmed differences in protein and amino acid content between soybeans harvested from different Brazilian states. The results of our study confirm that protein and amino acid content in soybean seed varied significantly but that any difference between the GM and conventional populations was not a major contributor to that variation.

**Fat, Fatty Acids, and Vitamin E in Seed.** There were no consistent differences in fat content associated with the experimental factors in this study. As for protein, Table 2 shows that fat (storage oil) values from each individual production overlapped extensively and that this observation extended to comparisons of the GM and conventional samples. The mean and range of values for the GM samples across all soybean regions and growing seasons were 20.31 and 17.46–23.67 % dw, and corresponding values for the conventional samples were 20.53 and 16.60–23.33 % dw. The fixed effect, phenotype, in the analysis of variance model showed no statistically significant difference ( $p = 0.5198$ ) between the GM and conventional samples when assessed across the entire data set (Supporting Information, Supplementary Table 2).

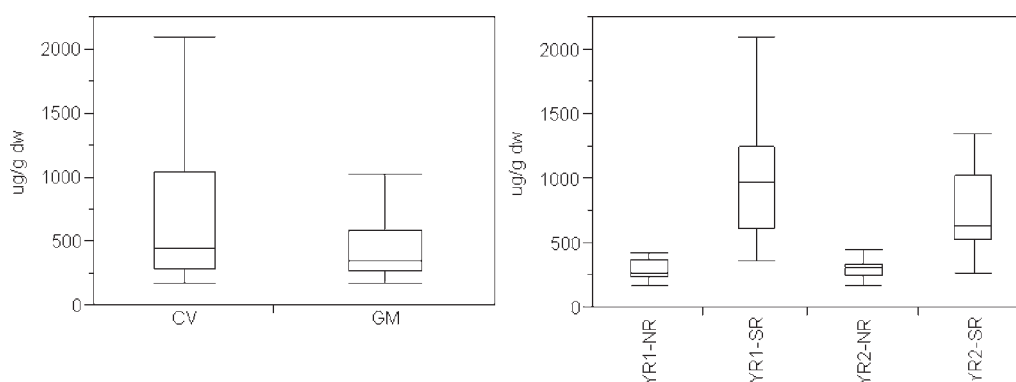
The mixed model demonstrated profound soybean regional differences in the fatty acid profiles (Supporting Information, Supplementary Table 2). Six different fatty acids were significantly different ( $p < 0.05$ ) when compared on a regional basis; these included heptadecanoic acid, stearic acid, oleic acid, linolenic acid, arachidic acid, and eicosenoic acid. Both the northern and

southern soybean regions require specifically adapted germplasm (maturity groups), and no soybean variety was cultivated in both regions. The observed regional differences in fatty acid composition were therefore attributable to varietal and/or geographic differences and/or varietal/geographic interactions. Table 3 and Figure 1 show, as examples, that levels of oleic acid were higher in the northern soybean region, whereas levels of linolenic acid were higher in the southern soybean region. This was true for both growing seasons but most pronounced in 2007–2008. In this season, oleic acid and linolenic acid from conventional soybean grown in the northern soybean region ranged from 26.08 to 45.68% and from 4.87 to 6.19% total FA, respectively, whereas the corresponding southern region samples ranged from 19.33 to 25.94% and from 6.47 to 9.04% total FA. Although there were pronounced regional effects, there was also extensive variation in fatty acid levels attributable to individual substance variation within the entire experiment; the parameter substance (region  $\times$  year  $\times$  phenotype), which represents this variation, was different ( $p < 0.05$ ) for all fatty acids assessed (Supplementary Table 3 of the Supporting Information). An illustration of this type of variability is exemplified in differences in oleic acid levels in seed from Monsoy 8329, the conventional control used in comparative assessments of MON 87701, MON 89788, and MON 87701  $\times$  MON 89788,<sup>2,3,5</sup> and levels in that of other conventional commercial varieties grown in the northern soybean region. In 2007–2008, Monsoy 8329 values ranged from 36.87 to 45.68% total FA, whereas the conventional commercial varieties ranged from 20.65 to 37.65% total FA; in 2008–2009, the corresponding values were 29.4–33.7 and 23.8–24.1% total FA, again emphasizing variability within the conventional soybean population (Supplementary Tables 4 and 5 of the Supporting Information). Despite these differences it is worth noting that no statistically significant differences in oleic acid levels of the three GM products listed above and their conventional comparators in either the northern or southern soybean region were observed.<sup>2,3,5</sup> This is consistent with the results from this study, in which the mixed model showed no difference between the GM and conventional samples in levels of the major fatty acids (Supporting Information, Supplementary Table 2).

For vitamin E, the analysis of variance model also showed no statistically significant differences ( $p > 0.05$ ) for any of the fixed effect experimental factors. Rather, an extensive range of variation in vitamin E levels, consistent with results from an earlier study of conventional soybean grown in Brazil,<sup>9</sup> was observed. For vitamin



**Figure 1.** Levels of oleic acid according to phenotype [conventional (CV) and transgenic (GM)] and according to production (YR1, 2007–2008; YR2, 2008–2009; NR, northern region; SR, southern region).



**Figure 2.** Levels of daidzein according to phenotype [conventional (CV) and transgenic (GM)] and according to production (YR1, 2007–2008; YR2, 2008–2009; NR, northern region; SR, southern region).

E, the mean and range of values for the GM samples across all soybean regions and growing seasons were 3.22 and 1.08–5.52 mg/100 g dw, and the corresponding values for the conventional samples were 4.32 and 1.57–8.49 mg/100 g dw ( $p = 0.0608$ ) (Table 3; Supplementary Table 1 of the Supporting Information). In other words, a  $\sim 5$ -fold difference between minimum and maximum values was observed in both the GM and conventional populations.

The mixed model showed extensive variation due to individual substance effects (Supporting Information, Supplementary Table 3). As for the fatty acids, there were extensive differences between vitamin E levels in seed from Monsoy 8329 and those of other conventional commercial varieties grown in the northern soybean region (Supplementary Tables 4 and 5 of the Supporting Information). An analogous observation could be extended to vitamin E data from the southern soybean region, where A5547, the conventional control used in earlier studies of MON 87701, MON 89788, and MON 87701  $\times$  MON 89788,<sup>2,3,5</sup> ranged from 3.40 to 6.29 mg/100 g dw (2007–2008) and from 5.68 to 8.49 mg/100 g dw (2008–2009), whereas the corresponding values from the conventional commercial varieties grown in the southern soybean region ranged from 2.12 to 3.26 mg/100 g dw and from 3.81 to 4.43 mg/100 g dw, respectively (Supplementary Tables 4 and 5 of the Supporting Information).

**Isoflavones in Seed.** The mixed model showed regional differences ( $p < 0.05$ ) for levels of the isoflavones daidzein, genistein, and glycitein. Levels of all isoflavones were higher in the southern soybean region than in the northern soybean region

(Table 3; Figure 2). This effect as well as the extensive variability in values is illustrated for daidzein in Table 3. Values from the northern soybean region (conventional and GM) ranged from 171.7 to 446.6  $\mu\text{g/g dw}$ , whereas corresponding values from the southern region ranged from 266.6 to 2099.7  $\mu\text{g/g dw}$  (Supplementary Table 1 of the Supporting Information). The mixed model showed that most variation in isoflavone composition due to individual substance variation within the entire experiment and the parameter substance (region  $\times$  year  $\times$  phenotype), which represents this variation, was statistically significantly different ( $p < 0.05$ ) for all isoflavones assessed (Supplementary Table 3 of the Supporting Information). These results are consistent with recent publications that have established that isoflavone accumulation is affected by many biotic and abiotic factors. The extent of variation within a single variety can be up to 3-fold between samples from the same environment.<sup>10,11</sup> The data generated in this study confirm that multiple factors are associated with isoflavone variation, including environment (inclusive of region, temperature, and growing season) and genetic background.<sup>12–14</sup> However, the mixed model showed no statistically significant differences ( $p > 0.05$ ) between the GM and conventional samples in this study when assessed across the entire data set (Supporting Information, Supplementary Table 3) and is consistent with a lack of any effect that can be attributed to transgene insertion.

**Other Components.** Values for antinutrients in seed (lectin, trypsin inhibitors, raffinose, stachyose, and phytic acid) and proximates and fiber in seed and proximates in forage were

characterized by considerable variation in levels across the entire data set (Supplementary Table 1 of the Supporting Information). A brief review of results is presented in the Supporting Information.

**Concluding Remarks.** Compositional data from GM and conventional soybeans grown in two different growing seasons and in different growing regions of Brazil were statistically assessed to determine contributing factors to variability within the overall data set. Results highlighted extensive compositional differences in seed from soybean harvested from the different regions, particularly with respect to fatty acids, vitamin E, and isoflavones. This variation is due to differences in the regional varieties grown this study as well as environmental differences and is also consistent with the extensive literature on the variability of these metabolites. There were few differences in the composition of the GM and conventional samples in this study when assessed across the entire data set, a finding consistent with an expanding literature on the lack of any effect of transgene insertion on crop composition.

## ■ ASSOCIATED CONTENT

**S Supporting Information.** Variance analysis and additional tables. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## ■ ACKNOWLEDGMENT

We thank the Monsanto Agronomy and Sample Management group from Brazil (Daniel Soares, Wagner Motomiya, Patrick Dourado, Hallison Vertuan, and Glauucia Malvestiti) for generation and preparation of the samples used in this study and the Monsanto Product Characterization group for molecular analysis.

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