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Classification of Distinct Seed Carbohydrate Profiles in Soybean

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ABSTRACT: Soybeans are an important source of protein-rich meal for livestock feed formulations. Recent changes in the cost of commodity-based sources of metabolizable energy (ME) inputs has put pressure on soybean meal to deliver both protein and ME in feed formulations. The non-oil fraction of soybean contains approximately 12% soluble carbohydrates, principally sucrose, raffinose, and stachyose. Of these carbohydrates, only sucrose is positive for ME. Both raffinose and stachyose, belonging to the raffinose family of oligosaccharides (RFOs), are considered antinutritional because of the negative consequences of their fermentation in the gut of monogastric animals when RFOs are consumed in the diet. Therefore, there is an interest in improving soybean seed composition so that it contains higher ME and fewer antinutritional components by increasing the sucrose content while lowering the RFOs. Several soybean lines have been discovered that contain altered levels of RFOs, and recent molecular genetic investigations have shown the phenotype to be caused by mutations in a raffinose synthase 2 (RS2) gene encoding the enzyme that is the committed step for RFO biosynthesis. The objective of this research was to determine the variation in carbohydrate profile for different soybean lines grown in a single location containing one of three different alleles of the RS2 gene. The results indicate that, although there is variation in the carbohydrate profiles for each line, different lines with the same RS2 genotype tend to produce a characteristic carbohydrate profile. Although the carbohydrate profile for each RS2 genotype class was consistent in different genetic backgrounds under two conditions grown at one location, more research will be necessary to determine the environmental stability of the carbohydrate profiles in multiple locations over different years.

KEYWORDS: *Soybean, carbohydrate, raffinose, stachyose, sucrose, galactinol*

INTRODUCTION

United States soybean growers planted a record of nearly 80 million acres of soybeans in 2010 and produced 3.3 billion bushels of soybeans, making 2010 the largest soybean harvest ever.[1] The value of soybean is in the vegetable oil and high protein soybean meal. The dry weight of a typical soybean can be divided into three fractions: 21% oil, 40% protein, and 11% soluble carbohydrates.[2] The smaller oil fraction is extracted first during processing, and the remaining soybean protein and carbohydrate is made into meal. In the U.S., three-quarters of soybean meal produced is used for poultry and swine feed.[1] The largest fraction of the carbohydrate portion of the meal is made up of sucrose, with lesser amounts of stachyose and raffinose and minimal amounts of other sugars. Both raffinose and stachyose are carbohydrates, belonging to the raffinose family of oligosaccharides (RFOs). RFOs are derived from sucrose, although they are not readily digested by humans and other monogastric animals because of their lack of enzymes capable of breaking the α-galactosidic linkages. Microbes present in the gut of monogastric animals do, however, contain α-galactosidases capable of metabolizing RFOs, which results in fermentation within the gut. The fermentation process results in the production of gastrointestinal gas.[3] Because of this effect, RFOs are considered to be antinutritional factors, and their presence in soybean meal causes flatulence, diarrhea, and other digestive distress.[4] Additionally, these carbohydrates sequester potential metabolic energy.

These properties pose problems when soybean meal is used in animal feeds, where RFOs decrease both metabolizable energy (ME) and protein efficiency ratio in roosters and broilers.[5,6] Smiricky et al.[7] examined the digestibilities of swine diets consisting of soy protein concentrate or soybean meal as the sole source of protein, when supplemented with additional raffinose and stachyose through the addition of soy solubles (3.5% raffinose and 11.5% stachyose, byproducts of meal processing). The authors found that adding soybean oligosaccharides reduced the nutrient digestibilities from 1.1 to 7.4% units.[7] Decreasing RFOs in soymeal has been shown to increase ME. In chickens, low RFO soymeal was found to have higher ME than conventional soymeal.[8] While standard processing of soybean meal does not completely remove RFOs, research has shown that an ethanol extraction of soybean meal can decrease the amount of stachyose and raffinose in soybean meal from about 5 and 1%, respectively, to below detectable levels in ethanol-extracted soybean meal.[9] This method, while effective in increasing total ME in soybean meal, is not suitable on a commercial scale for reducing RFOs in soybean meal; further, this method has also been shown to reduce the sucrose content from 10.4 to 0.2%, causing a drastic decrease in potential ME.[9] While soybeans have been identified that have increased sucrose along with decreased

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RFOs, the individual impacts of positive ME from increased sucrose content and negative issues from the presence of RFOs have not been fully elucidated. Producing a soybean with a decreased RFO content and increased ME through plant breeding is a seed compositional target, and understanding the genetic and environmental factors underlying the mechanism of RFO accumulation in soybean is essential to this goal.

Investigating soybean lines with observed low raffinose and stachyose phenotypes (reduced RFO traits) at a molecular genetic level has proven successful in determining the genetic basis of some of the available low RFO traits. A class of mutants has been found to contain reduced levels of RFOs accompanied by significant decreases in phytic acid in seeds. Hitz et al.[10] characterized LR33, which the authors demonstrate had lower raffinose, stachyose, *myo*-inositol, and phytic acid than the commercial average. The authors determined that the mutation responsible for this phenotype is an amino acid change in a highly conserved region of the *myo*-inositol-1-phosphate synthase 1 gene (MIPS1), resulting in reduced enzyme-specific activity.[10] Additional MIPS1 mutants have been identified that were characterized to have seed phenotypes similar to the missense MIPS1 mutant line. However, all of the MIPS1 mutant lines have been reported to have issues with germination and emergence,[11−15] and whether defects in phytic acid production, *myo*-inositol metabolism, including a decreased accumulation of raffinose and stachyose, or other factors are causative for problems with field emergence have yet to be fully elucidated. In pea seeds, Blochl et al.[16] showed that blocking the breakdown of RFOs drastically decreased germination rates. In soybean, however, a reduction in the RFO content did not delay germination.[17]

A soybean plant introduction line, PI 200508, was identified by Kerr and Sebastian[18] with lower raffinose and stachyose and increased sucrose compared to wild-type plants. Hitz et al.[10] characterized the line further and showed that the reduced RFO mutant line had increased levels of galactinol and sucrose. In addition, the authors reported a decrease in the raffinose synthase enzyme activity in maturing seeds of the soybean line. Neus et al.[19] studied the agronomics of lines containing the reduced RFO trait derived from PI 200508 and determined that there were no negative agronomic characteristics associated with the trait.

The genetic basis of the reduced RFO trait derived from PI 200508 was reported by Dierking and Bilyeu,[20] where the authors found that a novel allele (the deletion of a codon encoding a conserved tryptophan residue at amino acid position 331, W331−) of a raffinose synthase gene, raffinose synthase 2 (RS2, Glyma06g18890), was responsible for the improved seed composition phenotype of PI 200508 through complete association of the PI 200508 rs2 W331− allele with the increased sucrose and decreased raffinose and stachyose phenotype. A reverse genetics screen was successful in identifying a soybean line with an independent mutant allele of the RS2 gene: a missense mutation leading to the incorporation of isoleucine at amino acid position 117 rather than a threonine (rs2 T107I).[21] Soybean lines that inherited the rs2 T107I alleles also exhibited reductions in raffinose and stachyose along with an increased sucrose content. Additionally, recurrent selection and plant breeding have led to the development of new soybean lines containing more severe alterations in sucrose, raffinose, and stachyose contents, and these lines are currently being studied to determine the molecular genetic basis for the trait.

Previous studies have characterized soybean lines with either modest variation in carbohydrate profiles, lines containing defects in RS2 with MIPS mutants, or comparisons of lines with one mutant RS2 allele with functional RS2 lines for their carbohydrate profile.[8,10,17,19−24] However, these studies failed to address the impact that the genetic background plays on the carbohydrate phenotype and do not report on growing a diverse array of genetic backgrounds with low RFO phenotypes together in one location. In this work, we address these questions by examining the carbohydrate profile of 19 different soybean lines with varying alleles (functional and missense mutations) of the soybean RS2 gene grown at one location. We evaluated sampling variation and describe the most consistent representation of the phenotypic data to differentiate among four different categories of soybean genotypes.

MATERIALS AND METHODS

Soybean Genotypes. Soybean lines were selected from available germplasm based on their RS2 genotype and broadly fit into one of four categories: functional RS2 (WT-RFO, no known mutations in the RS2 gene), rs2 T107I (weak RFO[21]), rs2 W331− (low RFO[20]), and rs2 W331−+ (ultralow RFO, inbred line provided by a private company as well as two lines containing the rs2 W331− alleles, which were originally developed and selected independently by carbohydrate phenotype for extreme reductions in stachyose content).

Growth Conditions. Soybean lines (approximately 20 seeds) were hand-planted into 3 ft plots with 1 ft spacing between plots in random line order on May 21 (date 1) and June 19 (date 2), 2009 at the Bradford Research and Extension Center (BREC) near Columbia, MO. Commodity soybeans were grown on both sides of the experiment (30 in. rows).

Tissue Collection. Three single plants were tagged within each line, and four pods were collected from the middle position of each plant. One seed was used from each of the four pods. Seeds were lyophilized prior to powdering in liquid nitrogen. Extraction of soluble carbohydrates was performed on individual seed samples by ion-exchange chromatography as previously described,[20] except that extracted samples were dried under vacuum and redissolved in the same volume of water prior to separation by ion-exchange chromatography and electrochemical detection. Briefly, 12.5 mg of sample was extracted with 1 mL of 50% ethanol for 30 min at 70 °C. The samples were allowed to settle overnight at 4 °C prior to careful removal of 400 μL of the supernatant for filtration through a Millipore 0.45 μM filter plate. Following filtration, 100 μL of each sample was dried and resuspended in an equal volume of water, with 10 μL of injection. Samples were arrayed in one dimension on a 96-well sample plate and analyzed in order of the second dimension.

Data Analysis. Peak areas were integrated for galactinol, sucrose, raffinose, and stachyose. Carbohydrates were quantified on the basis of standard curves generated for each carbohydrate. Previous experiments revealed technical variability in extraction efficiency but consistent relative quantification among the carbohydrate components extracted. We report here the content of galactinol, sucrose, raffinose, and stachyose as a percent of the total carbohydrate detected.

Statistical Analysis. Analysis of variance (ANOVA) was used in a completely randomized design (CRD) to analyze and compare the percentage of four different sugars between 19 different plant lines (1−19). There were three plant replicates per line consisting of the mean percent sugar values of four seeds per plant. The percentage of the four different sugars measured sum to 100%; therefore, four mixed model single-factor ANOVAs were performed. Pairwise plant line comparisons were made at $p \leq 0.05$ using differences of least-squares means when significant *F*-test values from the ANOVA were obtained. If the number of pairwise comparisons was large, a Bonferroni adjustment was implemented to control for type I error. SAS 9.2 (TS2M3) software was used for all analyses.

ANOVA was used in a CRD to analyze and compare the percentage of four different sugars between four groups consisting of different plant lines grouped by the RS2 genotype. The RS2 group classes were

comprised of the following lines: WT-RFO (1−4), weak RFO (5−7), low RFO (8−16), and ultralow RFO (17−19).

ANOVA was used in a CRD to analyze and compare the percentage of four different sugars between eight groups consisting of different plant lines based on their RS2 genotype and date of planting. The four RS2 group classes (all from planting date 1, listed above) were compared to four RS2 group classes from planting date 2 that contained a subset of the lines used in planting date 1. The planting date 2 group classes were comprised of the following lines: WT-RFO (1−3), weak RFO (6), low RFO (8, 9, 10, and 15), and ultralow RFO (17 and 18).

Discriminant analysis was performed using quantitative variables stachyose, sucrose, galactinol, and raffinose, measured on a set of 19 plant lines (all from date 1) with three plant replications/line (means of four seeds/plant) for determining grouping of the plant lines. Because the four carbohydrates are not independent (they sum to 100%), backward stepwise selection was used to obtain the best subset of the carbohydrate variables. The variable that was the least useful in separating the groups was raffinose; hence, it was removed. The best subset of all possible variables for determining plant line group composition was stachyose, sucrose, and galactinol, which were all significant contributing variables ($p \leq 0.0001$) for predicting plant line group composition.

Wilks' λ, the same test used in multivariate ANOVA, was used to test multivariate differences among plant line groups based on stachyose, sucrose, and galactinol. Wilks' λ was significant at $p < 0.0001$ for this discriminant analysis. The first linear combination of the remaining carbohydrate variables, canonical variable 1, has the most discriminatory power in separating the plant line groups (88%). Adding canonical variable 2 boosted the discriminatory power to 95%. These canonical variables were both significant group discriminators at $p < 0.0001$ (the plot of canonical variable 1 versus canonical variable 2 shows group multivariate means at the center of each circle, with the size of the circle corresponding to the 95% confidence limit for the mean).

RESULTS

Soybean lines were previously developed that contained different alleles of the RS2 gene controlling the accumulation of raffinose and stachyose (Table 1). Some of these lines were related to each other, and the most common genetic background was 'Williams 82'.[20,21,25] The lines that are not listed as inbred or mutation were chosen based on their RS2 genotype from our genetics studies, and they were likely still segregating for a number of agronomic traits. For a subset of the lines, a second planting date was used to increase the range of plant maturities generated. The maturity date is listed for all of the lines. We determined the amounts of galactinol, sucrose, raffinose, and stachyose extracted from single mature seeds from these soybean lines grown in the same field environment. To reduce the technical error associated with variations in extraction efficiency, the RFO phenotype was expressed as the relative amount of galactinol, sucrose, raffinose, and stachyose present in each extract and is herein presented as the percent of each carbohydrate from the total amount of carbohydrate extracted. This representation of the data highlights the overall distribution of the four carbohydrates and the relationships among them for each sample rather than including the additional variation arising from the extraction efficiency from each sample and the potential variation in total carbohydrate content.

We divided the soybean lines based on their raffinose synthase allele status into four different genotypic categories: those containing wild-type versions of the RS2 gene (WT-RFO), those with the T107I missense alleles of the RS2 gene (rs2 T107I,[21] weak RFO), those with the W331− alleles of the RS2 gene (rs2 W331−,[20] low RFO), and those with the W331− alleles of the RS2 gene plus additional genetic factors (rs2 W331−+, ultralow RFO). The rs2 T107I and rs2 W331− mutant lines were developed by genotypic selection for those mutant alleles, while the rs2 W331−+ lines were developed by conventional plant breeding combined with phenotypic selection for altered RFO content.

Table 1. Soybean Lines, RS2 Allelic Status, Maturity Information, Genetic Background, and Nomenclature

line	RS2 genotype	maturity date 1	maturity date 2	pedigree	name
WT-RFO-1	RS2[a]	40[b]	48	inbred[c]	Deuel
WT-RFO-2	RS2	52	73	inbred	Maverick
WT-RFO-3	RS2	54	68	inbred	Williams 82
WT-RFO-4	RS2+	73		inbred	TN05-5109[d]
weak RFO-5	rs2 T107I	56		W82 mutation	397#25
weak RFO-6	rs2 T107I	56	73	W82 mutation	397#8
weak RFO-7	rs2 T107I	57		W82 mutation	397#16
low RFO-8	rs2 W331−	40	59	inbred	PI 200508
low RFO-9	rs2 W331−	47	73	not available	247F
low RFO-10	rs2 W331−	48	51	[W82 × (PI 200508 × W82)]	KB07-15C
low RFO-11	rs2 W331−	51		[W82 × (PI 200508 × W82)]	KB07-15F
low RFO-12	rs2 W331−	51		PI 200508 × W82	PW#84-37
low RFO-13	rs2 W331−	51		PI 200508 × W82	PP#84-27
low RFO-14	rs2 W331−	52		[W82 × (PI 200508 × W82)]	KB07-15H
low RFO-15	rs2 W331−	56	73	[W82 × (PI 200508 × W82)]	KB07-15B
low RFO-16	rs2 W331−	56		[W82 × (PI 200508 × W82)]	KB07-15D
ultralow RFO-17	rs2 W331−+	48	55	not available	SGUL
ultralow RFO-18	rs2 W331−+	81	81	[TN05-5109 × (PI 200508 × W82)]	KB07-9BT
ultralow RFO-19	rs2 W331−+	frost[e]		[TN05-5109 × (PI 200508 × W82)]	KB07-9BCB1

[a]RS2 indicates functional raffinose synthase 2 gene similar to the Williams 82 reference sequence for Glyma06g18890. rs2T107I and rs2 W331− indicate the mutant alleles described in refs 21 and20, respectively. The + symbol indicates that an additional uncharacterized modifying gene is present. [b]Maturity date is indicated by the number of days after August 1 when plants reached full maturity (R8). [c]Inbred indicates that the line is a cultivar or landrace; the pedigree or mutated cultivar is otherwise indicated. W82 is an abbreviation for the cultivar Williams 82. [d]Inbred line TN05-5109 is a late maturity group 4 line of pedigree S97-1688/CX1834-1-2. The female parent is a higher protein, multiple SCN− race resistant line, and the male parent was a low-phytate donor; however, TN05-5109 was not a low-phytate line. [e]One line (ultralow RFO-19) was stopped by frost before reaching full maturity, but seeds had reached physiological maturity.

The overall distribution of galactinol, sucrose, raffinose, and stachyose in the four seeds harvested from a single plant as well as the four carbohydrate distributions from the three plants of one genotype were highly similar. The results from the 12 seeds (four seeds from each of three different plants from that line) for one example soybean line from each raffinose synthase genotypic

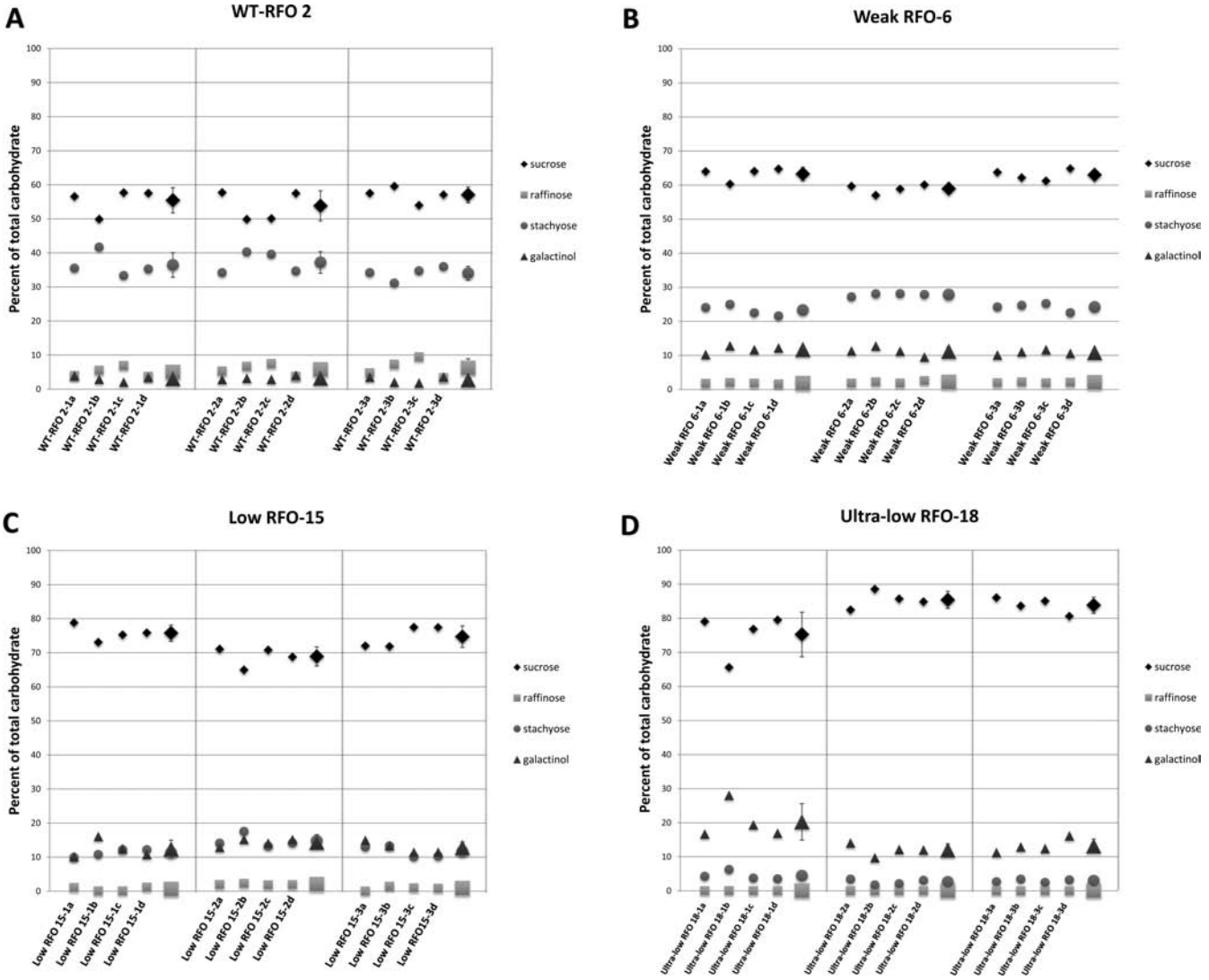

Figure 1. Examples of relative carbohydrate components of 12 individual seeds from soybean lines: (A) functional RS2 line 'WT-RFO-2', (B) rs2 T107I line 'weak RFO-6', (C) rs2 W331− line 'low RFO-15', and (D) rs2 W331−+ line 'ultralow RFO-18'. The relative content of galactinol, sucrose, raffinose, and stachyose are indicated for 12 individual seed samples from each line. Four seeds from three individual plants were sampled, and the results for each seed are plotted as four values from one plant, followed by the mean and standard deviation as indicated by larger symbols in the fifth position. Vertical lines in the figure separate the seeds from the three different plants sampled.

category are plotted with the averages of the percentages of total carbohydrate for each individual component as the final point of the series (Figure 1). These examples illustrate that there was little variation in the percentage of galactinol, sucrose, raffinose, and stachyose of the total carbohydrate extracted from seeds collected from one plant. These data also show that there is little difference in the variation of the four carbohydrate components among the three plants of a single soybean line when plants are grown together at one location. Subsequent analyses were performed on plant lines, with the plant line mean representing the average of the three means from the individual plants as collected from the subset of four individual seeds per plant.

The rank order of the individual lines was different for each of the four carbohydrate components (Figure 2). However, significant differences were observed across the range of values for all four carbohydrate components for the lines. Stachyose means ranged from above 40 to less than 5% of the carbohydrate extracted. For stachyose, the WT-RFO lines were not significantly different from each other but were higher than all other lines. The weak RFO lines had the next highest stachyose values, but there was some overlap with two of the low RFO lines. Five of the low RFO lines were significantly lower in stachyose than the weak RFO lines, and seven were significantly higher in stachyose than the ultralow RFO lines. The two low RFO lines with the smallest stachyose values were not significantly different from the ultralow RFO lines. The ultralow RFO lines contained the least amounts of stachyose. The rank order of stachyose values separated the lines by RS2 category.

Sucrose means ranged from about 50 to more than 85% of the carbohydrate extracted. The WT-RFO lines contained the lowest sucrose values, but they were not significantly different from each other, the weak RFO lines, or one low RFO line. There was significant overlap in sucrose means for the weak RFO and low RFO lines. Two of the three ultralow RFO lines produced the highest sucrose values, but there were overlaps in sucrose values among the low and ultralow RFO categories. Only one ultralow RFO line (17) prevented the rank order from separating the sucrose values by RS2 category.

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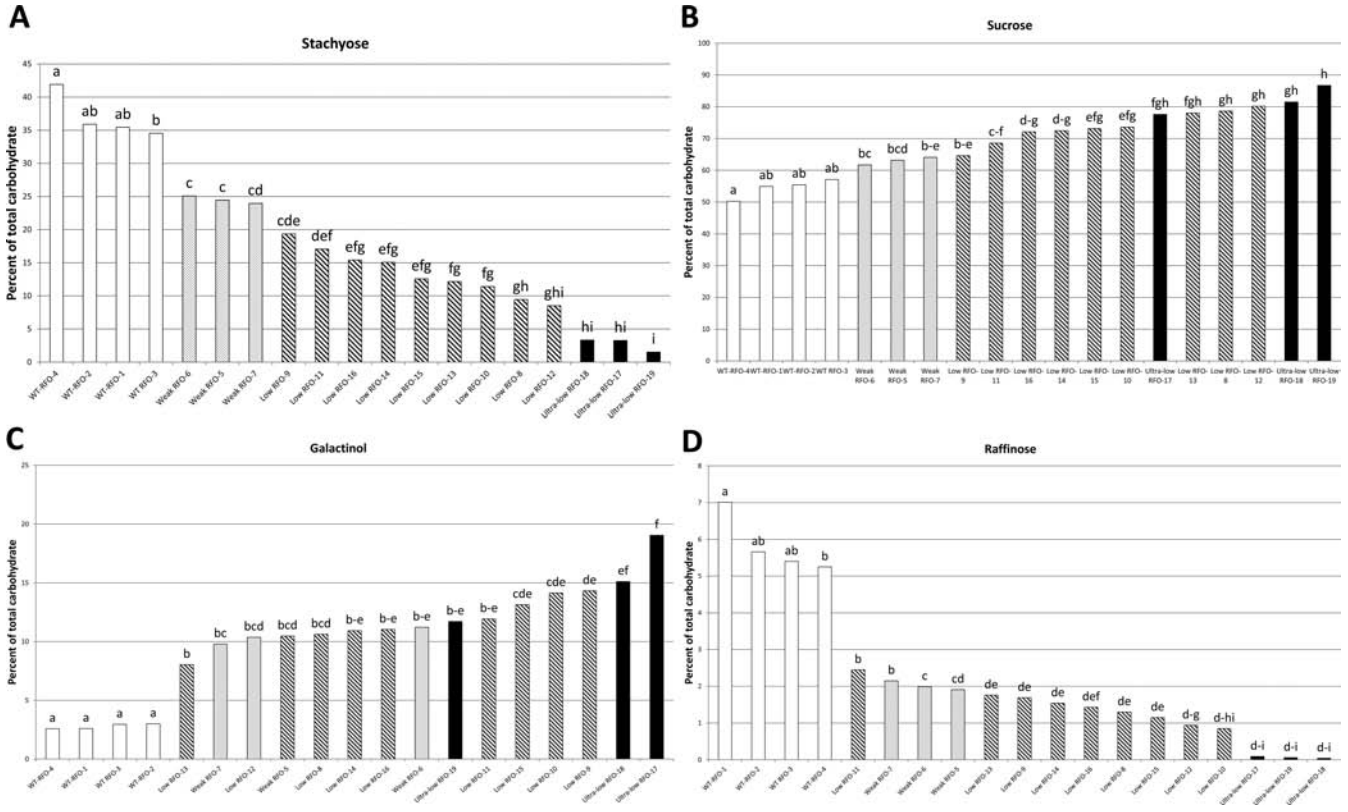

Figure 2. Relative carbohydrate components of soybean lines from planting date 1. The amounts of each carbohydrate component were measured as a percentage of total carbohydrate extracted. Each bar represents the mean from one soybean line, and the lines are in rank order based on means. Bars with the same letter are not significantly different based on the differences of least-squares means at $p \leq 0.05$ using a Bonferroni adjustment: (A) stachyose, (B) sucrose, (C) galactinol, and (D) raffinose.

Galactinol means ranged from about 3 to more than 15% of the carbohydrate extracted. The WT-RFO lines were not significantly different from each other and contained the lowest galactinol values; they were significantly different from all other lines. There were significant differences among the remaining lines, but they did not segregate by rank mean order into their respective categories as was the case for stachyose and, to a lesser degree, sucrose.

Raffinose means ranged from 7 to less than 1% of the carbohydrate extracted. The WT-RFO lines contained the highest raffinose values, but there were overlaps in means for lines across the range of raffinose values. The three ultralow RFO lines contained the lowest raffinose values. With the exception of line low RFO-11, the rank order of raffinose values separated the lines by RS2 category. The close range of raffinose values and the lack of significant differences beyond the WT-RFO category are presumably due to raffinose values near the lower limit of detection.

Overall, lines within the same RS2 category tended to be not significantly different from each other for each of the carbohydrate components tested. An exception was the low RFO category, which consisted of more lines than the other categories, had a broad range of means, and had one line for raffinose that was a true outlier for the category (low RFO-11). For all of the carbohydrate components, the rank order of means revealed that the WT-RFO and weak RFO lines had the least desirable profiles, while the low RFO and ultralow RFO lines had the most desirable carbohydrate profiles.

Lines from each genotypic category were grouped together into four classes based on RS2 allelic status. Unique carbohydrate signatures resulted with nearly each carbohydrate component significantly different for each class compared to the same carbohydrate component in the other classes (Figure 3). The

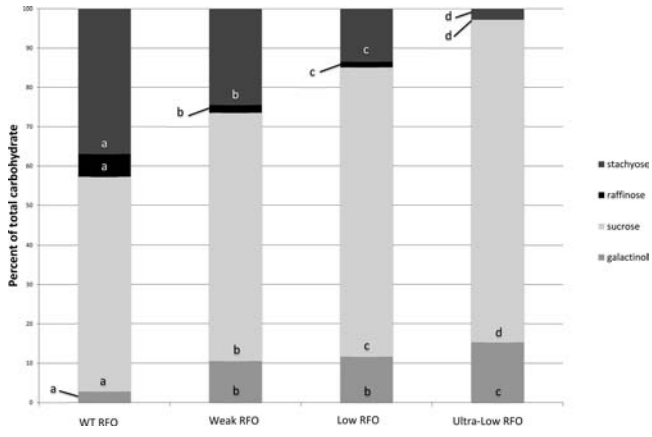

Figure 3. Comparison of overall relative carbohydrate profiles for soybean lines organized by RS2 alleles into different classes. The RS2 groups were comprised of the following lines: WT-RFO (1–4), weak RFO (5–7), low RFO (8–16), and ultralow RFO (17–19). The mean relative content of galactinol, sucrose, raffinose, and stachyose were combined from the lines in each category, generating a unique phenotypic signature; the values are indicated additively on a single scale. Letters within each carbohydrate component of each bar followed by the same letter are not significantly different based on the differences of least-squares means at $p \leq 0.05$. Each carbohydrate component may only be compared to the same component of the four allelic classes.

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only exception was that there were no significant differences for galactinol between the weak RFO class and the low RFO class. As functional RS2 alleles are replaced by different, potentially more deleterious alleles of RS2 and then combined with additional modifying alleles, the carbohydrate profile shifts to reflect more desirable contents of sucrose, raffinose, and stachyose.

Discriminant analysis was performed using quantitative variables stachyose, sucrose, galactinol, and raffinose, measured on a set of all 19 plant lines from date 1. The discriminant analysis on this set of lines resulted in the four classes clustering into different groups (Figure 4). The WT-RFO class is distanced from the others, and while the weak RFO, low RFO, and ultralow RFO classes group closer together, they still fall into distinct clusters.

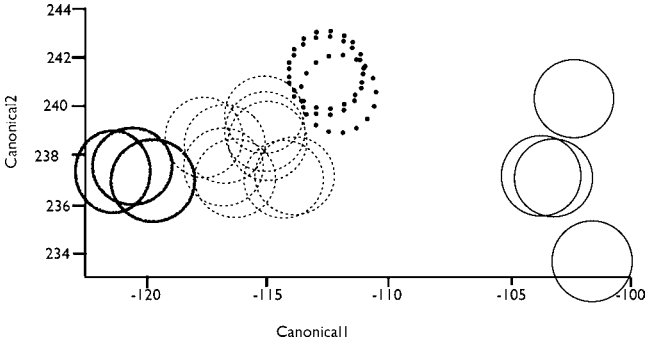

Figure 4. Discriminant analysis of all soybean lines in planting date 1. The plot of canonical variable 1 versus canonical variable 2 shows group multivariate means at the center of each circle, with the size of the circle corresponding to the 95% confidence limit for the mean. Although 19 lines were used for the analysis, for visual clarity, the labeling only indicates the class to which each line belongs. The WT-RFO class is indicated by thin circles; the dotted circles are weak RFO lines; the dashed circles are the low RFO lines; and the thick circles are ultralow RFO lines.

A number of environmental factors can potentially influence the seed carbohydrate profile. For this study, the carbohydrate profiles remained consistent when lines were planted at a later planting date and matured later than the same lines planted at the earlier planting date (Table 2). No consistent significant overall trends were observed in carbohydrate components between the two planting dates. An additional test of the environmental influence on carbohydrate profiles is to compare the carbohydrate content in lines from the same genotypic category that had differences in maturity. Similar to the result of two separate planting dates for any individual line, there were no consistent trends for carbohydrate profile changes for lines within the same raffinose synthase genotypic category that had wide differences in maturity (data not shown).

DISCUSSION

This study is the first work addressing carbohydrate profiles of lines representing a range of characterized RS2 genotypes grown together in one location. Regardless of the underlying genetic background, lines in the same RS2 genotypic category produced characteristic carbohydrate profiles, as represented by the percent of galactinol, sucrose, raffinose, and stachyose from the total carbohydrate. This work demonstrates that seeds harvested from one plant and seed from plants within one genotype have very comparable carbohydrate profiles and can be compiled into one average. Also, the carbohydrate profiles of plants from within one RS2 genotypic class can be distilled into one value. Through this work, we have shown that these RS2 genotypic classes produce distinct phenotypic carbohydrate profiles, illustrating that RS2 genotype appears to be the single largest determinant of carbohydrate profile for lines with functional or either of two mutant versions of the RS2 gene.

We speculate that the absence of the highly conserved tryptophan residue in the RS2 protein sequence (rs2 W331−) is more deleterious to enzyme function than the rs2 T107I missense mutation in an amino acid position with less evolutionary conservation. The minor accumulation of galactinol and sucrose at the expense of raffinose and stachyose for the rs2 T107I lines compared to the functional RS2 category lines is less dramatic than the differences in carbohydrates between the rs2 W331− category lines and the functional RS2 category lines. The phenotypic category with the most severe carbohydrate content alterations contains soybean lines that have the rs2 W331− allele plus at least one additional factor, and the very low percentages of raffinose and stachyose in seeds from these lines suggests that the total raffinose synthase enzyme activity in developing seeds of those lines is nearly completely abolished. The raffinose synthase enzyme activity has been tested in developing seeds for lines containing the rs2 W331− alleles and compared to lines containing functional RS2, and the results demonstrated a 25-

Table 2. Comparison of the Mean Relative Content of Galactinol, Sucrose, Raffinose, and Stachyose from Soybean Lines Grouped into Classes Grown in the Same Location but at Two Different Planting Dates

		galactinol			sucrose			raffinose			stachyose		
group	*N*	mean	standard deviation		mean	standard deviation		mean	standard deviation		mean	standard deviation	
WT-RFO D1	12[a]	2.79	0.35	c[b]	54.44	3.94	d	5.83	0.91	a	36.95	3.85	a
WT-RFO D2	11	2.40	0.29	c	54.95	2.16	d	5.13	1.01	a	37.52	1.56	a
weak RFO D1	9	10.49	0.86	b	62.99	1.68	c	2.02	0.14	b	24.50	1.33	b
weak RFO D2	3	7.61	0.99	b	72.47	1.88	abc	1.38	0.05	bcd	18.55	1.01	bc
low RFO D1	27	11.61	2.15	b	73.48	5.43	b	1.46	0.58	bc	13.45	3.97	c
low RFO D2	11	11.59	2.02	b	75.57	8.73	ab	0.76	0.69	cd	12.08	8.11	c
ultralow RFO D1	9	15.29	3.92	a	81.94	4.87	a	0.06	0.03	d	2.70	1.08	d
ultralow RFO D2	6	15.55	4.60	a	80.84	6.57	ab	0.18	0.27	d	3.43	1.87	d

[a]Number of plants (three per line) in the group. [b]Mean estimates within a column followed by the same letter are not significantly different based on the differences of least squares means at $p \leq 0.05$ using a Bonferroni adjustment. D1 and D2 designations indicate planting date 1 and planting date 2, respectively.

fold decrease in raffinose synthase activity for the rs2 W331− lines.[10] The observed additional accumulation of galactinol in the rs2 W331−+ lines compared to lines in the rs2 W331− category also supports further restriction of the raffinose synthase step in the pathway.[10]

Seeds from each genotypic class produced a carbohydrate profile that was somewhat variable for each individual component but was overall distinguishable from the carbohydrate profile of seeds from a different genotypic class. Although we have observed heritable carbohydrate profiles in general after growing the same lines multiple years, those preliminary results indicate more research is needed to fully understand the environmental impacts on carbohydrate profiles.

Through the comparison of carbohydrate profiles of selected lines from two planting dates, we demonstrate that there was no consistent impact of maturity date on carbohydrate profile of these selected lines within one genotype or within each genotypic class. This study, however, includes individuals from only one location in a single environment. Another group recently reported the carbohydrate profile of seven soybean genotypes grown at three very different geographical locations.[23] The results demonstrated that the sucrose content increased with cooler growing conditions; however, raffinose and stachyose values were variable.[23] This area of research needs to be pursued further to better understand the role of the environment in the carbohydrate profile in soybean seeds from plant lines containing contrasting alleles of the RS2 gene.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ME, metabolizable energy; RFO, raffinose family of oligosaccharides

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