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Sucrose and Raffinose Family Oligosaccharides (RFOs) in Soybean Seeds As Influenced by Genotype and Growing Location

Vineet Kumar,*,† Anita Rani,† Lokesh Goyal,† Amit Kumar Dixit,†
J. G. Manjaya,§ Jai Dev,# and M. Swamy⊥

†National Research Centre for Soybean, Khandwa Road, Indore, Madhya Pradesh, India 452017, §Bhabha Atomic Research Centre, Mumbai, India, #Himachal Pradesh Krishi Vishvavidhalaya, Himachal Pradesh, India, and ⊥University of Agricultural Sciences, Bangalore, India

Sucrose content in soybean seeds is desired to be high because as a sweetness-imparting component, it helps in wider acceptance of soy-derived food products. Conversely, galactosyl derivatives of sucrose, that is, raffinose and stachyose, which are flatulence-inducing components, need to be in low concentration in soybean seeds not only for augmenting utilization of the crop in food uses but also for delivering soy meal with improved metabolizable energy for monogastric animals. In the present study, analysis of 148 soybean genotypes for sucrose and total raffinose family oligosaccharides (RFOs) contents revealed a higher variation (4.80-fold) for sucrose than for RFOs content (2.63-fold). High-performance liquid chromatography analyses revealed ranges of 0.64−2.53 and 2.09−7.1 mmol/100 g for raffinose and stachyose contents, respectively. As information concerning the environmental effects on the sucrose and RFOs content in soybean seeds is not available, we also investigated a set of seven genotypes raised at widely different geographical locations for these quality traits. Sucrose content was found to be significantly higher at cooler location (Palampur); however, differences observed for raffinose and stachyose contents across the growing locations were genotype-dependent. The results suggest that soybean genotypes grown at cooler locations may be better suited for processing soy food products with improved taste and flavor.

KEYWORDS: Soybean; sucrose; raffinose; stachyose; genotype variability; growing location

INTRODUCTION

Sucrose, raffinose, and stachyose are major soluble sugars present in soybean seeds. Raffinose and stachyose are the galactosyl derivatives of sucrose, the former with one and the latter with two moieties of galactose attached to sucrose via α1→6 glycosidic linkage. Collectively, they are referred to as raffinose family oligosaccharides (RFOs). Verbascose, a third type of RFO, which is found in legumes such as pea and lupin, is present in negligible traces in soybean. Sucrose in soybean seeds imparts sweetness to soy-based food products, namely, soy milk, tofu, and natto (*1*). RFOs, unlike sucrose, are not digested in the human gastrointestinal tract due to the absence of α1→6 galactosidase enzyme in the small intestine required to break down the α1→6 glycosidic linkage. As a result, RFOs pass to the lower gut, where they serve as ideal substrates for fermentation caused by intestinal microflora. In the process, flatulence-inducing gases, namely, CO_2, CH_4, and H_2S, are liberated, causing abdominal discomfort and diarrhea. The severity of the flatulence symptoms varies from individual to individual depending upon ethnic background, gastrointestinal microflora, and amount of the soy product ingested (*2*). The concentration of RFOs is very low in the fermented soy products, namely, tempeh, natto, and miso, due to the α-galactosidase enzyme secreted by the microbial population; however, these soy products are popular only in some of the Southeast Asian countries. In nonfermented soy products, namely, soy milk and soy nuts, prepared using conventional processing methods, RFOs are retained due to their heat resistance property. Besides, poor digestibility of RFOs results in reduction in metabolizable energy and increase in flatulence and diarrhea in monogastric animals fed soy meal (*3*). Therefore, soybean genotypes with genetically low levels of RFOs are sought to enhance the utilization of soybean in food as well as feed uses.

Madhya Pradesh state is the hub of soybean production in India; however, a dramatic increase in the area of the crop across the country in recent years has occurred due to the expansion of soybean cultivation in different agroclimatic zones. Changes in some of the quality attributes, namely, protein, oil, fatty acids, phytic acid, trypsin inhibitor, and lipoxygenases of soybean seeds, due to different environmental conditions prevailing during the cropping season at widely different locations have been studied

*Address correspondence to this author at the Biochemistry Laboratory, Department of Crop Improvement, National Research Centre for Soybean (Indian Council of Agricultural Research), Khandwa Road, Indore, Madhya Pradesh, India (fax 917312470520; e-mail vineetksahni@yahoo.com).

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Table 1. Indian and Exotic Soybean Genotypes Selected for the Study[a]

Indian genotypes	exotic genotypes
ADT1, Alankar, Ankur, Bhatt Yellow,[b] Bhatt Black,[b] Bhawali Bold, Birsa Soya 1, Bragg, Co-1, Co-2, Co-3, DS-228, Durga, Gujrat Soya 1, Gujrat Soya 2, Hara-Soya, Hardee, Improved Pelican, Indira Soya-9, JS93-05, JS97-51, JS2, JS-335, JS-71-05, JS 72-280, JS-72-44, JS75-46, JS76-205, JS79-81, JS80-21, JS90-41, JS97-52, Kalitur,[b] KB79, KHSb2, Lee, LSb1, MACS124, MACS13, MACS450, MACS57, MACS58, MAUS61, MAUS32, MAUS1, MAUS2, MAUS61, MAUS71, MAUS81, Monetta, NRC48, NRC67, NRC37, NRC64, NRC7, NRC12, NRC2, Palam Soya, PK1024, PK327, PK262, PK1092, PK416, PK-472, PK564, PK1029, PK308, PK471, PS1092, PS1042, PS1241, PS1347, Punjab1,[b] Pusa16, Pusa 20, Pusa 22, Pusa 24, Pusa37, Pusa 40, RAUS5, RKS18, RKS15, Samrat, Shilajeet, Shiwalik, SL295, SL96, SL459, SL525, T49, TAMS38, VLS1, VLS21, VLS59, IC210	vegetable-type genotypes: AGS191 (Taiwan), AGS2, Akiyoshi (Japan), Boiling- type (Japan), Cocker Stuart (Taiwan), Dada-cha 2000 (Taiwan), Dada-cha ma-me (Taiwan), Fukuyutaka (Japan), G76 (Taiwan), GC25-9-1-3X95024, Hakucho (Japan), Hatsataka (Japan), Hougyoku, Kegone (Japan), ASG Kocha (Taiwan), ASG328 Kohine (Taiwan), PI542044, NewTarpian2xGL981532ASG401, ASG Pedegra (Taiwan), ASG328 Sricanan (Taiwan), Table variety (Taiwan), Toyoshrizome (Japan), ASG328 Whydox (Taiwan), EC57043, EC100791, EC175322, EC175324, EC175330, EC210380, EC216374, EC216376, EC216378, EC216379, EC37656, EC39484, EC39486, EC39488, EC39491, EC468479, EC241811 high protein lines:[c] G11 (44.7), G15A (43.2), G205 (45.2), G2129 (43.1), G214 (42.4), G257 (43.4), G288 (47.3), G332 (43.0), G35 (45.2), G620 (43.7), G688 (45.4), G83 (45.2), G9 (42.4)

[a] Most of the Indian genotypes have at least one parent or grandparent from maturity group V−VII of American soybean. ADT1, Bragg, Hardee, and Lee are American genotypes introduced directly as varieties in India. [b] Purely indigenous. [c] Values given in parentheses are the numerical values (%) for protein content of the respective lines.

previously (*4*−*6*). Weather conditions, especially ambient temperature, during seed development has been suggested to influence the synthesis of oligosaccharides in lupin seeds (*7*). However, investigations focusing on changes in the contents of sucrose and RFOs in soybean seeds raised under different environmental conditions at widely different geographical locations have not yet been conducted.

In the present work, 148 soybean genotypes were analyzed for contents of sucrose and RFOs to identify genotypes with high sucrose and low total RFOs content, which may be useful in soybean breeding programs for the development of soybean genotypes that are suitable for processing soy products with improved taste and for developing soy-based feeding rations with enhanced metabolizable energy. Furthermore, a set of seven soybean genotypes was raised at three widely different growing locations, namely, Palampur (32° N), Indore (22° N), and Bangalore (12° N), to study the effect of various weather conditions on the accumulation of sucrose and RFOs in seeds.

MATERIALS AND METHODS

Materials and Reagents. One hundred and forty-eight soybean genotypes, which included 95 Indian cultivars and 53 exotic genotypes, were raised in the research fields of the National Research Centre for Soybean, Indore (22° N), Madhya Pradesh, India, under isoclimatic conditions in the cropping season 2007. Most of the 95 Indian cultivars listed in **Table 1** have at least one parent or grandparent from maturity group V−VII of American soybean. Of the 53 exotic genotypes, 40 are vegetable types and 13 are high-protein genotypes. All of the genotypes were raised in triplicate in complete random block design in single row plots of 5 m length and 0.45 m distance between rows. A set of 7 genotypes ('NRC7', 'JS335', 'LSb1', 'VLS59', 'EPS472', 'IC210', and 'EC241811') was raised at two more locations, namely, Palampur (32° N) and Bangalore (12° N), in triplicate in complete random block design to study the effects of planting location on sucrose and RFO contents. Crops were raised at different locations following recommended agronomic practices of the respective region. Genotypes were harvested at their respective maturity at all three locations. Seeds harvested from all three locations were analyzed in the Biochemistry Laboratory at the National Research Centre for Soybean, Indore.

Rapid screening for sucrose and RFO contents in 148 soybean genotypes was carried out using a raffinose/D-glucose assay enzymatic kit procured from Megazyme International Ltd., Ireland. It consists of α-galactosidase (*Aspergillus niger*), invertase (yeast), and glucose determination reagent (glucose oxidase + peroxidase, i.e., GOPOD) for colorimetric estimation of sucrose and RFO content. HPLC-grade solvents (acetonitrile) and standards of sucrose, raffinose, and stachyose were procured from Sigma-Aldrich.

Estimation of Sucrose and RFOs Using Enzymatic Kit. Finely ground soy flour (0.5 g) was treated with 95% ethanol (to digest the endogenous enzymes completely) at 88 °C for 20 min, and the final volume was made up to 50 mL using sodium acetate buffer (50 mM, pH 4.5). Digested mixture so obtained was incubated at the same temperature for 20 min and vortexed to obtain uniform slurry. Subsequently, 2 mL of chloroform was added to 5 mL of slurry and vortexed for 15 s followed by centrifugation at 1000*g* for 10 min. A volume of 0.2 mL from the aqueous phase of the supernatant so obtained was taken in three tubes (namely, A, B, and C). A volume of 0.2 mL of sodium acetate buffer, invertase, and a mixture of α-galactosidase + invertase was poured into tubes A, B, and C, respectively. All three tubes were incubated at 50 °C for 20 min. Reagent blank (0.4 mL of sodium acetate buffer) and glucose control (0.1 mL of standard glucose solution, which contained 0.556 μmol of glucose + 0.3 mL of sodium acetate buffer) were also taken simultaneously. Subsequently, 3 mL of GOPOD reagent was added in all of the tubes and incubated again at 50 °C for 20 min. Change in absorbance for tubes A, B, and C and glucose control was measured at 510 nm against the reagent blank. The concentration of sucrose and RFOs was calculated as

$$\text{sucrose (mmol/100 g)} = (\Delta B - \Delta A) \times F \times 50$$

The exact equations are

$$\text{sucrose (mmol/100 g)} = (\Delta B - \Delta A) \times F \times 250 \times 200 \times 1/1000$$

$$\text{RFOs (mmol/100 g)} = (\Delta C - \Delta B) \times F \times 50$$

$$\text{RFOs(mmol/100 g)} = (\Delta C - \Delta B) \times F \times 250 \times 200 \times 1/1000$$

where F = factor to convert from absorbance to micromoles of glucose

$$\frac{0.556\ (\mu\text{mol of glucose})}{\text{GOPOD absorbance for } 0.556\ \mu\text{mol of glucose}}$$

250 = conversion to 50 mL of extract, 200 = conversion from 0.5 to 100 g of sample, and 1/1000 = conversion from micromoles to millimoles.

HPLC Determination of Sucrose, Raffinose, and Stachyose. Oil from ground seeds was extracted with 180 mL of hexane in an automated Soxhlet unit (Pelican Equipments, Chennai, India) for 3 h, and oil content (percent) was determined by weight difference (data not given). The defatted flour so obtained was used for extraction of sugars as given by Liu and Markakis (*8*). One gram of the sample was extracted with 10 mL of 80% ethanol in a water bath for 4 h at 80 °C with occasional shaking. The extract was cleaned by adding 1 mL of 10% lead acetate solution followed by centrifugation at 10000*g* for 10 min. The step was repeated again. The supernatant so obtained was filtered through a syringe membrane filter (0.22 μm, 13 mm diameter), and a sample volume of 20 μL was injected into a Shimadzu high-performance liquid chromatograph (LC10AT *vp*). The separation of sucrose (disaccharide), raffinose, and stachyose was achieved using a silica NH_2 column (Phenomenex Luna 5 μm, dimension 250 mm × 15 mm), preceded by a guard column, maintained at 40 °C in a

Table 2. Range and Mean Values for Sucrose and Total RFOs Content[a] of 148 Soybean Genotypes

character	sucrose	total RFOs	ratio of sucrose/ total RFOs
range	3.45−16.55	3.5−9.22	0.60−2.62
mean	8.90	6.64	1.35
ratio between maximum and minimum	1:4.80	1:2.63	1:4.36

[a] Expressed as mmol/100 g of db.

Table 3. Soybean Genotypes Identified with Higher Sucrose Content with Their Respective Total RFOs Values[a]

genotype	sucrose	total RFOs
ASG328 Whydox	16.55 ± 0.92	6.31 ± 0.23
Dada-cha 2000	15.32 ± 0.86	7.83 ± 0.31
Dada-cha ma-me	14.86 ± 0.77	8.50 ± 0.44
EC39488	14.63 ± 0.68	7.76 ± 0.51
ASG 328 Kohine	14.43 ± 0.63	6.64 ± 0.47
Alankar	14.39 ± 0.79	8.25 ± 0.73
Monetta	14.27 ± 0.87	6.82 ± 0.65
SL96	14.04 ± 0.92	8.78 ± 0.30
Kegone	14.03 ± 0.34	7.16 ± 0.25
EC39484	13.59 ± 0.45	6.86 ± 0.35

[a] Expressed as mmol/100 g; values given are mean of triplicate samples.

Shimadzu CTO 10AT *vp* oven. The mobile phase, acetonitrile/water (75:25 v/v), was run isocratically at a flow rate of 1.0 mL/min, and the elution was monitored by means of a refractive index detector (Shimadzu, RID10A). Identification and assignment of the peaks in the sample were done using the retention time of the peaks of different sugars, which were 7.09, 9.7, and 13.7 min for sucrose, raffinose, and stachyose, respectively. Quantification of sucrose, raffinose, and stachyose in the sample was done by comparing the area of the peaks of the respective sugars in the sample chromatogram with that of the standards using software CSW 1.7. The concentrations of sucrose, raffinose, and stachyose were determined in the defatted flour; therefore, to express the concentration per gram of ground flour, the value of the oil content (percent) was employed to convert the sucrose and total RFOs estimated per gram of the defatted flour into per gram of soy flour.

Statistical Analyses. All of the statistical analyses were carried out using SPSS (evaluation version 18) for two-way analysis of variance and discriminant analysis.

RESULTS AND DISCUSSION

Table 2 exhibits the ranges of sucrose and total RFO content in 148 soybean genotypes screened using enzymatic rapid assay method. Sucrose content ranged from 3.45 ('VLS1') to 16.55 mmol/100 g ('ASG328 Whydox') with a mean value of 8.90 mmol/100 g. Total RFO content varied from 3.5 ('SL525') to 9.22 ('EC216379') with a mean value of 6.64 mmol/100 g. Our results showed comparatively wider variation (4.80-fold) for sucrose content than for total RFOs content (2.63-fold). The proportion of sucrose to total RFOs content ranged from 0.60 ('EC39491') to 2.62 ('ASG328-Whydox') with a mean value of 1.35. Genotypes 'ASG328 Whydox', 'Dada-cha 2000′, and 'Dada-cha ma-me' were the top-ranking genotypes, exhibiting sucrose content in the range of 14.86−16.55 mmol/100 g. 'SL525' and 'PK1042' were the only two genotypes that showed very low levels of RFOs content (< 4.00 mmol/100 g). Hou et al. (*9*) investigated 18 specialty soybean genotypes for sucrose and individual raffinosaccharides. As the authors expressed the sucrose and RFOs in milligrams per gram, we converted the values reported in their study to millimoles per 100 g so that a comparison could be drawn with our results. Contrary to our observation that genotypes with high sucrose content (13.59−16.55 mmol/100 g) also showed comparatively high levels of total RFOs (6.31−8.78 mmol/100 g) (**Table 3**), Hou et al. (*9*) reported low levels of RFOs content (1.25 mmol/100 g) in genotype '03CB-14 line' with a high sucrose content (25.71 mmol/100 g), although in another genotype ('PI243545') with high sucrose content (30.77 mmol/100 g) in the same study, the authors reported RFOs of 6.57 mmol/100 g. In our study, genotypes 'Pusa24' and 'AGS191' exhibited comparatively low levels of sucrose as well as RFOs. Furthermore, genotypes with high protein content ('G11', 'G15A', 'G205', 'G2129', 'G214', 'G257', 'G288', 'G332', 'G35', 'G620', 'G688', 'G83', 'G9') showed moderate levels of sucrose and RFOs, which matches with the results for the high-protein line 'R95-1705' in the study of Hou et al. (*9*). Furthermore, most of the genotypes with low levels of RFOs content in our study (**Table 4**) are Indian cultivars. The correlation coefficient computed between sucrose and total RFOs content was positive and significant (0.470; $p < 0.000$).

Table 4. HPLC Analysis of Individual Members of RFOs in 10 High-Ranking and 10 Low-Ranking Soybean Genotypes for Total RFOs Content Identified Using Enzymatic Kit[a]

sample	genotype	method	sucrose	raffinose	stachyose	total RFOs
1	EC216379	enzymatic	7.23			9.2
		HPLC	6.64	1.6	7.1	8.7
2	Dada-cha ma-me	enzymatic	14.86			8.5
		HPLC	11.90	2.53	4.80	7.33
3	Alankar	enzymatic	14.39			8.25
		HPLC	11.29	1.65	5.24	6.89
4	NRC67	enzymatic	9.97			8.19
		HPLC	6.95	1.82	5.09	6.91
5	G2129	enzymatic	10.39			8.15
		HPLC	8.27	1.78	5.31	7.09
6	EC100791	enzymatic	6.12			8.15
		HPLC	5.57	1.30	6.02	7.32
7	G205	enzymatic	11.21			8.14
		HPLC	9.06	1.38	5.85	7.23
8	Durga	enzymatic	10.41			8.13
		HPLC	9.26	1.38	5.97	7.35
9	Punjab 1	enzymatic	7.75			8.06
		HPLC	6.15	1.19	6.03	7.22
10	VLS 21	enzymatic	12.51			7.92
		HPLC	9.97	1.36	5.70	7.06
11	SL 525	enzymatic	5.52			3.50
		HPLC	4.98	1.17	2.09	3.26
12	PK 1042	enzymatic	7.71			4.10
		HPLC	6.73	0.65	3.27	3.92
13	RKS15	enzymatic	7.51			4.38
		HPLC	6.58	0.67	2.76	3.43
14	AGS191	enzymatic	5.77			4.50
		HPLC	5.17	0.91	2.99	3.90
15	NRC64	enzymatic	8.74			4.52
		HPLC	7.37	1.43	2.69	4.12
16	PUSA24	enzymatic	5.83			4.81
		HPLC	5.08	1.29	2.73	4.02
17	TAMS 38	enzymatic	9.23			4.82
		HPLC	8.11	0.7	3.53	4.23
18	NRC 37	enzymatic	7.25			4.90
		HPLC	6.61	0.90	3.59	4.49
19	JS 75-46	enzymatic	7.43			4.98
		HPLC	6.49	1.32	2.89	4.21
20	MAUS 71	enzymatic	7.10			5.01
		HPLC	6.71	0.64	3.89	4.53

[a] Samples 1−10 are high-ranking, whereas samples 11−20 are low-ranking genotypes for total RFOs content. Values (mmol/100 g) given are mean of triplicate samples.

The enzymatic method employed to screen the genotypes for sucrose and total RFOs content does not quantify raffinose and stachyose contents individually. Therefore, of the 148 genotypes, 10 high-ranking and 10 low-ranking genotypes for RFOs content

identified using the enzymatic kit (**Table 4**) were subjected to HPLC for separation and quantification of raffinose and stachyose. The results of HPLC analyses showed that raffinose content ranged from 0.64 to 2.53 mmol/100 g, whereas stachyose content ranged from 2.09 to 7.1 mmol/100 g. Trugo et al. (*10*) analyzed 20 Brazilian cultivars and repatorted ranges of 0.4−1.4 and 4.8−6.90 mmol/100 g for raffinose and stachyose contents, respectively. Hartwig et al. (*11*) analyzed 40 soybean genotypes and reported ranges for contents of raffinose and stachyose similar to those in our results. Hou et al. (*9*) reported a range of 0.14−3.31 mmol/100 g for raffinose content and 0.60−6.86 mmol/100 g for stachyose content, which showed much lower values for raffinose and stachyose than observed in the present study. Hou et al. (*12*) analyzed soybean germplasm lines from three different maturity groups for various sugars and reported the ranges of 0.46−27.85, 0.01−3.34, and 0.03−10.44 mmol/100 g for sucrose, raffinose, and stachyose, respectively. The maximum value for sucrose content observed in their study is significantly higher than observed in our study. Furthermore, our results showed that total RFOs content computed by summing up the HPLC values for the raffinose and stachyose is less than the values obtained using the rapid assay enzymatic kit (**Table 4**). These differences may be because of the trace amounts of verbascose, free cyclitols (pinitol, myoinositol, and chiroinositol), and galactosyl derivatives of cyclitols present in soybean seeds.

Mean maximum temperature was higher at Indore (31.6 °C) than at Palampur (25.4 °C) and Bangalore (25.5 °C). Mean minimum temperature was lower at Palampur (14.8 °C) than at Bangalore (19.8 °C) and Indore (23.8 °C). **Table 5** indicates that across the three locations and seven genotypes, sucrose content ranged from 5.53 mmol/100 g for 'VLS59' at Indore to 14.80 mmol/100 g for 'JS335' at Palampur. Averaged over the three locations, 'LSb1' showed the highest value (10.92 mmol/100 g), whereas 'IC210' showed the lowest value (7.43 mmol/100 g) for sucrose content. A highly significant effect of the location and location × genotype interaction was observed for sucrose content, as indicated by ANOVA (**Table 5**). All seven genotypes grown at Palampur showed higher concentration of sucrose compared to those grown at Indore and Bangalore. Conversely, the effect of location on raffinose and stachyose contents was nonsignificant. However, genotype × location interaction was found to be significant ($p < 0.05$) for these traits. Bertrand et al. (*13*) tested the possibility to discriminate the growing locations of coffee (*Coffea arabica* L.) varieties based upon chemical constituents such as fatty acids, chlorogenic acids, and elements. In the present study, discriminant analysis of the locations (**Table 6**) shows significant classification for sucrose only, as estimated by the *P* value associated with the Wilk's λ coefficient. The proportion of correctly classified samples with sucrose content was 71.4%, which is an acceptable value. However, locations could not be discriminated on the basis of the rafffinose and stachyose contents. The nonsignificance of the discriminant analysis performed with raffinose and stachyose as shown in **Table 6** is congruent with the nonsignificance of the effect of the location on these traits as analyzed by ANOVA (**Table 5**). The authors did not find reports in the literature concerning the effect of planting location on sucrose and RFO content in soybean seeds to compare our results with the previous works. However, the investigation carried out by Wolf et al. (*14*) in the controlled conditions of a phytotron does support our results. The authors reported 56% reduction for sucrose content in soybean seeds when maximum/minimum temperature increased from 18/13 to 33/28 °C. In our study, the mean maximum/minimum temperatures at Palampur (25.4/14.8 °C) were significantly lower than those at Indore (31.6/23.8 °C). Consequently, we observed approximately 35% lower average sucrose content in soybean genotypes grown at Indore compared to Palampur. Similarly, significantly higher sucrose content in soybean genotypes grown at Bangalore than in those grown at Indore may be attributed to the lower mean maximum/minimum temperature at the former location. The difference in the sucrose content between soybean genotypes grown at Palampur and Indore was higher than between Bangalore and Indore. This may be because of mean minimum temperature, which is much lower at Palampur compared to Bangalore. These results are

Table 5. Effects of Genotype, Location, and Their Interaction on Sucrose, Raffinose, and Stachyose and the Probability of Significance As Computed by Two-Way Analysis of Variance over Three Different Locations (Palampur, Indore, and Bangalore)[a]

genotype/location		sucrose	raffinose	stachyose
NRC 7		8.39b	1.62c	4.98c
JS335		10.27d	1.46b	4.86c
LSb1		10.92e	1.45b	4.43b
VLS 59		8.70b	1.47b	4.22a
EPS472		9.28c	1.21a	4.56b
IC 210		7.43a	1.10a	4.90c
EC 241811		8.70b	1.21a	4.14a
F probability		0.000	0.016	0.018
Palampur		11.65c	1.42a	4.60a
Indore		7.39a	1.29a	4.94a
Bangalore		8.24b	1.37a	4.21a
F probability		0.000	0.144	0.243
Palampur	× NRC 7	10.93	1.42	4.99
	× JS335	14.80	1.56	5.35
	× LSb1	14.10	1.51	3.63
	× VLS 59	11.87	1.76	4.90
	× EPS472	10.25	1.09	5.12
	× IC 210	9.46	1.16	5.32
	× EC 241811	10.19	1.47	2.91
Indore	× NRC 7	6.37	1.58	5.23
	× JS335	8.00	1.43	4.51
	× LSb1	9.25	1.33	5.87
	× VLS 59	5.53	1.05	3.35
	× EPS472	7.84	1.08	6.07
	× IC 210	6.82	1.40	4.47
	× EC 241811	8.00	1.13	5.05
Bangalore	× NRC 7	7.89	1.86	4.71
	× JS335	8.01	1.40	4.72
	× LSb1	9.41	1.51	3.78
	× VLS 59	8.71	1.59	4.40
	× EPS472	9.76	1.47	2.48
	× IC 210	6.03	0.75	4.91
	× EC 241811	7.93	1.02	4.47
F probability		0.000	0.033	0.036

[a] Full-factorial experimental design employed was 3 locations × 7 genotypes × 3 field replications. Means followed by different letters are significantly different at $P = 0.05$.

Table 6. Discriminant Analysis of the Three Location Studies (Palampur, Indore, and Banglore) Based on Sucrose, Raffinose, and Stachyose Contents: Probability of Significance As Assessed by Wilks's λ and Corresponding *F* Value and Classification Efficiency, As Determined by Percent of Correct Classification[a]

				% correct classification			
compound	Wilk's λ	*F*	*P*	total	Palampur	Indore	Bangalore
sucrose	0.381	14.64	0.000	71.4	85.7	42.9	85.7
raffinose	0.953	0.442	0.649	47.6	0.0	57.1	28.6
stachyose	0.889	1.120	0.348	38.1	0.0	57.1	57.1

[a] Full-factorial experimental design employed was: 3 locations × 7 genotypes × 3 field replications.

also supported by the study of Guo and Oosterhuis (*15*), who observed that low-temperature stress caused an increase in the accumulation of seed sucrose content in hydroponically grown soybean plants. Similarly, higher mean values (16.22, 13.0, and 15.87 mmol/100 g) for sucrose content reported in the investigations conducted in the United States (*9*, *11*, *16*) compared to the mean sucrose content (8.9 mmol/100 g) in the present study may because of the higher daily mean temperature during crop growth in our experimental conditions of central India (Indore). With regard to the influence of ambient temperature on the accumulation of RFOs in legumes, Gorecki et al. (*7*) reported a 2-fold increase in stachyose when lupin seeds matured at comparatively low temperature; however, in the present investigation, we did not observe significantly high levels of raffinose and stachyose contents at the cooler geographical location, that is, Palampur.

Among all of the genotypes analyzed, we did not find any genotype with the most desirable combination of high sucrose and low RFOs, which would be suitable for both soy food and feed purpose. 'ASG328 Whydox', 'Dada-cha 2000', and 'Dada-cha ma-me' were identified with comparatively high sucrose content and may be utilized for processing soy products. 'SL525' was identified with the lowest value of RFOs and may be preferred in the formulation of soy-based feed rations with improved metabolizable energy. The genotypes identified for high sucrose and low RFOs content may be useful in soybean breeding programs for the development of specialty soybean genotypes for soy food and feed purposes, respectively. The present study suggested that the geographical locations experiencing low temperatures during the cropping season might be better suited for the accumulation of enhanced levels of sucrose content in soybean seeds, a desirable trait for processing soy products of improved quality. The results also showed that the influence of environmental conditions on the levels of flatulence-inducing components, raffinose and stachyose, in soybean seeds was genotype-dependent.

ABBREVIATIONS USED

RFOs, raffinose family oligosaccharides; HPLC, high-performance liquid chromatography.

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