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Communication

Investigation on the Genetic Variability of Soybean Seed Sucrose Content in Germplasm Accessions from Different Countries of Origin

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ABSTRACT

The palatability of soy-food products can be enhanced by increasing sucrose content in soybean grains which are used as raw material. Therefore, soybean genotypes with high sucrose content are desired for processing good quality soy-food products with higher organoleptic acceptance and sweetness. In this study, estimation of sucrose content was carried out through high performance liquid chromatography (HPLC) in 321 soybean accessions from 14 countries. Sucrose was resolved using a silica NH_2 column as stationary phase. The mobile phase (acetonitrile/water 75:25 v/v) was run isocratically at a flow rate of 1.0 ml/min. The elution was monitored by a refractive index detector. Wide genetic variability in sucrose content was observed, with a range of 1.2 -9.6 g/100g thereby exhibiting about 8-fold genetic variation. Twenty-six genotypes were identified which showed sucrose content >7.0 g/100g. However, nine genotypes were identified which showed sucrose content < 2.0 g/100g. The highest sucrose content was observed in two genotypes, namely, PP-45 (9.6 ± 0.84 g/100g) and P5-40-2 (9.4 ± 0.78 g/100g). Genotypes identified from diverse background and with contrasting levels of sucrose content may be used for developing mapping populations which can be used for tagging genomic regions underlying biosynthesis of this trait in soybean seeds. Further, these genotypes can be used for developing novel genotypes with sucrose content higher than observed in the germplasm lines evaluated in this study.

KEY WORDS: GENETIC VARIABILITY, HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY), SUCROSE CONTENT, SOYBEAN, SOY-FOOD.

INTRODUCTION

Soy-based food products are fast gaining the sobriquet of 'functional food of the century' due to the presence of several nutraceutical components that stave off atherosclerosis, diabetes, breast cancer, osteoporosis at bay (Kumar et al., 2010a). Apart from basic nutrients like protein (40%), oil (21%), vitamins, soybean has tocopherols and isoflavones as major nutraceutical components. Despite its nutrients-rich profile, utilisation of soybean in food products is very meagre. Presently, only 7-10 % of the total soybean produced in the country is utilized in processing soy-food products. The quality of these food products depends upon the seed attributes used as initial raw material. Apart from the presence of antinutritional factors, its astringent/bland taste is also the main culprit for poor acceptance of food products processed from soybean seeds (Taira et al., 1990; Kumar et al., 2011; Salari et al., 2020).

This taste related deterrent can be overcome by increasing sucrose content of soybean seeds which imparts sweetness and enhances organoleptic acceptance of food-grade soybean (Taira et al., 1990; Kumar et al., 2011). Sucrose constitutes 41.3–67.5% of the total soluble sugars in soybean seed. Of the dried seed, sucrose content is about 2.5-5.0%. Globally, high sucrose content soybean genotypes are desired by soy food industry for processing soy milk, tofu, natto and other soy food products as sucrose contributes to favourable taste (Escamilla et al., 2019). In soybean meal, high sucrose content is desirable as it contributes positively to the potential metabolizable energy and thereby leading to the weight gain among animals as per their genetic potential (Bilyeu and Wiebold 2016; Salari et al., 2020).

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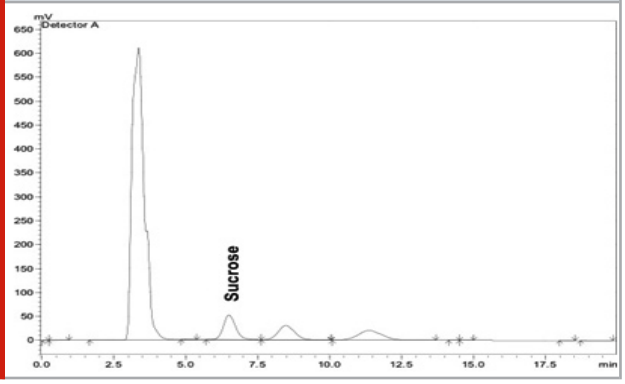
Therefore, development of high sucrose content soybean genotypes is important plant breeding objectives in soybean to meet the requirements of soy food and feed industry. The studies focusing on the screening of soybean germplasm for sucrose content are limited and were conducted only in selected fewer number of genotypes (Kumar et al., 2007; Kumar et al. 2010b). Hou et al., (2009) and Ficht (2018), investigated the genetic variability for sucrose content in 241 and 296 soybean genotypes. The high sucrose genotypes viz. PI 200508, V99-5089, PI 243545, and LD02-4485 mentioned in previous studies are not in the public domain due to the strict IPR regime (Skoneczka et al., 2009; Mozzoni et al., 2013; Zeng et al., 2014; Salari et al., 2020). Therefore, it is important to constantly screen large number of germplasm lines to identify and develop new genetic combinations with high sucrose content, along with focused crossing programme. In the present investigation, we screened 321 soybean germplasm accessions from different countries for the identification of genotypes with high sucrose content.

MATERIAL AND METHODS

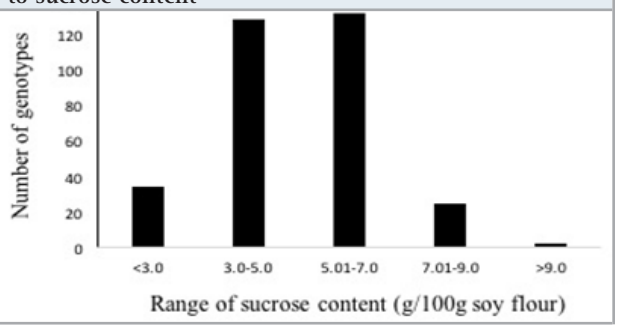
For the plant material, three hundred twenty-one soybean accessions comprising of exotic accessions, indigenous collections, advanced breeding lines developed in plant breeding programme for food grade characters were raised in the field of ICAR-Indian Institute of Soybean Research, Indore, India in single row plot of 3 m length with row-to-row spacing of 45 cm and plant-to-plant distance of 5 cm, in triplicate in the randomized block design. Standard agronomic practices recommended for soybean cultivation in Central India were followed from sowing to harvesting. Genotypes were harvested at maturity and freshly harvested seeds were subjected to sucrose estimation though HPLC.

Figure 1: Chromatogram showing the separation of sucrose standard (10mg/ml).



For the determination of sucrose content using HPLC, extraction of sucrose from mature seeds of soybean accessions from 14 countries viz. Australia (1), Brazil (3), China (2), Ghana (2), Hungary (1), India (221), Italy (1), Japan (1), Philippines (1), Russia (1), Shri lanka (2), Taiwan (11), USA (15) and 59 genotypes of unknown origin was carried out following the method of Liu and Markakis (1987). The extracted sugars were determined through HPLC as described elsewhere (Kumar et al. 2007). The extracted sugar sample obtained was filtered using syringe membrane filter (0.22µm, 13 mm diameter), and 20µl of this sample was injected in Shimadzu high performance liquid chromatography (LC 10 AT vp).

Figure 2: Distribution of 321 soybean genotypes according to sucrose content



Sucrose was resolved using a silica NH2 column (Phenomenex Luna 5µm, dimension 250mm×15mm), preceded by a guard column, maintained at 40 ^{0}C in 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). Peak of sucrose in the sample was identified using the retention time of the peak of the sucrose standard (10mg/ml), which was obtained at 6.5 mins (Figure 1). The concentration of sucrose (per gram of the flour) in the sample was computed by comparing its peak area with that of the known concentration of the standard, procured from Sigma Aldrich, using software CSW 1.7. Seeds of all the soybean accessions were analysed in triplicate samples for sucrose content. For the statistical analysis, phenotypic data and standard deviation was analysed and performed in Microsoft excel 2019.

RESULTS AND DISCUSSION

Three twenty-one soybean accessions were collected from 13 different countries. The concentration of major soluble sugar viz. sucrose was determined in seeds of the 321 soybean genotypes which exhibited a normal distribution ranging from 1.2 to 9.6 g/100g with the majority (260) of the genotypes containing 3.0 to 7.0 g/100g sucrose (Table 1, Figure 2), thereby exhibiting about 8-fold genetic variation. Twenty-six genotypes from four different origins had sucrose content above 7.0 g/100g (Table 2), whereas nine genotypes from Indian origin had sucrose content below 2.0 g/100g. The highest sucrose content was identified in PP-45 (9.6 g/100g) from unknown country of origin followed by P5-40-2 (9.4 g/100g), which was from India. The lowest sucrose content genotypes were P1-12-3, VP64-2, P1-11-2 and P4-5-3 (1.2, 1.21, 1.35 and 1.5 g/100g, respectively), and all these genotypes are from India.

Table 1. Average sucrose content (g/100g soy flour) in soybean genotypes from different countries.

Country of origin	Number of genotypes	Range of Sucrose content	Average Sucrose content
Australia	1	5.0	5.0 ± 0.40
Brazil	3	4.8-6.9	5.6 ± 1.14
China	2	5.4-5.8	5.6 ± 0.63
Ghana	2	5.9-6.1	6.0 ± 0.83
Hungary	1	3.7	3.7 ± 0.35
India	221	0.89-9.4	4.7 ± 0.84
Italy	1	3.9	3.9 ± 0.00
Japan	1	7.5	7.5 ± 0.64
Philippines	1	4.9	4.9 ± 0.00
Russia	1	6.0	6.0 ± 0.46
Shri Lanka	2	4.2-6.4	5.3 ± 0.22
Taiwan	11	3.6-7.6	5.4 ± 0.75
USA	15	3.8-8.1	5.9 ± 1.06
Unknown	59	2.5-9.6	5.4 ± 0.75
	321	4.43-7.48	5.31 ± 0.84

Table 2. Soybean genotypes exhibiting sucrose content higher than 7 g/100g soy flour or lower than 10 g/100g soy flour.

Genotype	Sucrose content(g/100g)	Country of origin
PP-45	9.60 ± 0.84	Unknown
P 5-40-2	9.40 ± 0.78	India
IC 567316	8.88 ± 0.62	India
P 2-19-3	8.13 ± 0.50	India
EC 457201	8.12 ± 0.58	USA
P 3-8	8.10 ± 0.52	India
EC 457286	8.06 ± 0.43	Unknown
JS 20-82	8.03 ± 0.37	India
EC 685250	7.94 ± 0.29	Unknown
ECP-125-738	7.85 ± 0.27	Unknown
SKY/AK-1403	7.81 ± 0.21	India
IC 574378	7.71 ± 0.19	India
EC 170267	7.70 ± 0.22	Unknown
CAT-19	7.63 ± 0.23	Taiwan
JSM-226	7.63 ± 0.17	India
P 2-2-1	7.60 ± 0.19	India
EC 65772	7.56 ± 0.21	USA
CAT-842	7.50 ± 0.17	Japan
EC 963805	7.44 ± 0.12	Unknown
CAT-135A	7.40 ± 0.10	Taiwan
CAT-1099	7.35 ± 0.18	India
EC 95289	7.20 ± 0.16	USA
EC 458346	7.15 ± 0.10	Unknown
VP 96-2-2	7.10 ± 0.10	India
IC 263278	7.03 ± 0.11	India
NRC105	7.01 ± 0.01	India

Table 1 shows the country of origin of 321 soybean genotypes, their range and average of sucrose content investigated in this study. Genetic variability for sucrose content was the highest in soybean accessions from India (1.2-9.4 mg/g soy flour-7.8 fold) followed by USA (3.8-8.1 g/100g soy flour-2.2 fold) and Taiwan (3.6-7.6 g/100g soy flour-2.1fold), respectively. With regard to average sucrose content of soybean accessions from different country of origin, soybean accessions from Japan, Ghana and Russia exhibited average sucrose content of 7.5, 6.0 and 6.0 g/100g soy flour, respectively. Average sucrose content of soybean accessions from USA was 5.9 g/100g soy flour. Soybean accessions from Brazil and China both showed average sucrose content of 5.6 g/100g soy flour followed by Taiwan (5.4 g/100g flour), Sri Lanka (5.3 g/100g soy flour) and Australia (5.0 g/100g soy flour), respectively.

Maximum number of 221 soybean accessions were from India with average sucrose content of 4.7 g/100g soy flour. Hou et al., (2009) reported genetic variation of 59.6-fold for sucrose content in 241 plant introductions (PI). In an earlier study, Kumar et al., (2010b) investigated sucrose content in 148 soybean genotypes and reported 4.80-fold genetic variation. Moreover, Ficht (2018) investigated sucrose content of 296 soybean lines obtained from University of Guelph germplasm panel and reported only 2.3-fold of genetic variation. In comparison to the results by Hou et al., (2009), the genetic variation (8-fold) revealed in the present study for sucrose content was much lower but significantly higher than that reported in other related studies (Hou et al.,2009; Kumar et al., 2010b; Ficht 2018).

CONCLUSION

Wide genetic variability (8.0-fold) existed for sucrose

content in 321 soybean germplasm accessions of different country of origin screened in the present study. Germplasm accessions identified with high sucrose content were from India, USA and unknown country of origin, while accessions for low sucrose content were from India. The diverse genetic background of these high and low sucrose content genotypes can be exploited in developing mapping population to identify the genomic regions underlying sucrose biosynthesis. High sucrose content genotypes from diverse background can be crossed to develop soybean genotypes with higher sucrose content value than the maximum value observed for this trait in this investigation.

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Conflict of Interests: The authors declare no conflict of interests among themselves.

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