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A comparative study of oligosaccharides in immature and mature seeds of soybean genotypes

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Concentrations of oligosaccharides, raffinose, stachyose, and galactosyl derivatives of sucrose alongwith sucrose content were determined using HPLC in mature and immature soybean seeds shelled from the pods picked at a stage when they were completely filled but the pods and seeds were still green in colour. Among the seven genotypes ('Shilajeet', 'JS335', 'NRC7', 'LSb1', 'Hara Soya', 'Sappori Midori', 'Kegone') studied, sucrose content was higher than raffinose and stachyose in immature and mature seeds. Raffinose and stachyose contents were lower in immature seeds; however, genotypic differences were observed for percentage accumulation at this stage. Results indicated a significant ($p<0.01$) interaction between genotype and picking stage for sucrose, raffinose and stachyose contents suggesting the need for a separate breeding programme for reducing flatus causing factors in mature and immature seeds.

Keywords: Soybean, Genotypes, Raffinose, Stachyose, Immature seed, Mature seed

Soybean is one of the most economical and nutritious foods which can combat the diseases stemming from malnutrition and the nutraceutical ingredients present in it and can reduce the risk of major chronic diseases like cancer, atherosclerosis, osteoporosis and diabetes. Sucrose and the galactosyl derivatives of sucrose viz. raffinose and stachyose present in soybean play important role in the utilization of soybean for food uses. The former has been indicated to be closely related with quality and taste of many soy products (Taira 1990) while raffinose and stachyose, the heat stable oligosaccharides, have been implicated as causative factors for flatulence and abdominal discomforts often experienced after consumption of soy products (Staggerda 1968, Rackis et al 1970). This is ascribed to the human digestive tract lacks α 1→6 galactosidase required for breaking the α 1→6 galactosidic linkages present in these oligosaccharides. Consequently, both raffinose and stachyose pass unabsorbed to the lower intestinal tract where they are metabolized by intestinal microflora, leading to the production of carbon dioxide, hydrogen and methane (Liener 1994).

Apart from the traditional soy products like tofu and soy milk which are prepared from mature seeds of soybean, green seeds shelled from the immature pods bear great potential for human consumption for availing the nutritional and nutraceutical benefits of soybean as immature green seeds of soybean are soft in texture and can be cooked just like sweet pea (*Pisum sativum*), chickpea (*Cicer arietinum* L) or lima bean (*Phaseolus limensis* L). A few reports are available on the genotypic variation of sucrose, raffinose and stachyose in the mature seeds of soybean (Hymowitz et al 1972, Qui and Chang 2004). However, studies showing the comparative differences in the levels of sucrose, raffinose and stachyose among mature and immature green seeds are scarce (Liu and Markakis 1987). Among Indian genotypes, such type of studies have not yet been carried out although, genotypic variation for other biochemical constraints like trypsin inhibitor, phytic acid and lipoxygenase isozymes are available (Kumar et al 2001, 2002 a, b, c). Hence, the present investigation was undertaken to determine the levels of oligosaccharides in immature and mature seeds of 5 Indian and 2 Japanese cultivars of soybean.

Materials and methods

Five Indian genotypes ('Shilajeet', 'JS335', 'NRC7', 'LSb1', 'Hara Soya') and two Japanese genotypes ('Kegone', 'Sappori Midori') (procured from Japanese International Co-operative Agency) were grown in random block design with three replications at National Research Centre for Soybean, Indore. Each plot consisted of three rows of 3 m-long planted 45 cm apart. Plant to plant distance was 5 cm. The soil of experimental plot belonged to typic chromburst – very deep, dark-greyish, brown, clayey and with low levels of organic carbon (0.45%) and slightly alkaline pH (7.8). The seeds were grown under rain-fed conditions. Recommended agronomic practices were followed. Pods of all the cultivars were picked at R_6 stage when the pods were completely filled but still green in colour as described earlier (Fehr et al 1971, Shanmughamsundaram et al 1991). Mature seeds with pods of all the seven genotypes were harvested at the end.

Sample preparation and extraction of sugars: Immature green seeds and mature seeds of all the genotypes were oven-dried at 60°C till they became moisture free. Dried seeds were ground using metallic pestle and mortar and passed through 100 mesh size. The flour was defatted with hexane for 4 h in Soxhlet extractor and air-dried. The defatting step was necessary to remove interfering lipids present in large amounts in oilseed crops. Sugars were extracted from the defatted flour as described by Liu and Markakis (1987). One gram of defatted soy flour was extracted with 10 ml of 80% ethanol in a water bath for 4 h at 80°C with occasional shaking. The extract was clarified by adding 1 ml of 10% lead acetate twice and thereafter centrifuged at 10000×**g** for 10 min. The supernatant was filtered through a membrane filter (0.22 µ, 13 mm dia).

HPLC analysis: A 20 µl of sample was injected into HPLC system (Shimadzu, LC10AT *vp*). The separation of sucrose, raffinose and stachyose was achieved using a Phenomenex Luna 5u NH_2 column maintained at 40°C in a column oven,

Shimadzu CTO 10AT *vp*. The elution was monitored by a refractive index detector (Shimadzu, RID10A) and the mobile phase, acetonitrile/water (75/25 v/v), was used isocratically at a flow rate of 1.0 ml/min. The sugars of interest (sucrose, raffinose and stachyose) were quantified on the basis of their peak area relative to the external standards (procured from Sigma-Aldrich).

Statistical analysis: All analyses were performed in triplicate and statistical analysis was carried out using MSTAT-C programme.

Results and discussion

Mean data of sucrose, stachyose and raffinose content in seven genotypes harvested at immature stage and at maturity are presented in Table 1. Significant differences were observed for all the 3 saccharides at immature as well as mature stage ($p<.01$). Among immature green seeds, sucrose content ranged from 1.12 for 'Sappori Midori' to 2.02% for 'Shilajeet' with a mean value of 1.46%. Among the flatulence-causing factors, raffinose ranged from 0.00 (not detectable) for 'Kegone' to 0.37% for 'Sappori Midori' with a mean value of 0.23% while stachyose content ranged from 0.31 for 'Sappori Midori' to 0.83% for 'NRC7' with a mean value of 0.42%. The content of total oligosaccahrides in immature green seeds ranged from 0.40 for 'Kegone' to 1.11% for 'NRC7'. In mature seeds, sucrose content ranged from 2.58 for 'NRC7' to 4.25% for 'JS335%'. Raffinose content ranged from 0.6 for 'Kegone' and 'NRC7' to 1.55% for 'Sappori Midori' with a mean value of 0.86% while stachyose ranged from 1.55% for 'Hara soya' to 2.76% for 'JS335' with a mean value of 2.1%. With regard to total oligosaccharides content in mature seeds, it ranged from 2.33 for 'NRC7' to 4.30% for 'Sappori Midori'. Our results indicated a significant ($p<.01$) interaction between genotype and picking stage for sucrose, raffinose and stachyose contents.

Among all the genotypes studied, sucrose content was higher than raffinose and stachyose in immature green seeds and at maturity stage. This is in agreement with earlier two reports (Dornobos and Mc Donald 1986, Liu and Markakis 1987). Table 1 also indicates that genotypes also differed in levels of sucrose, raffinose and stachyose accumulated in immature green seeds when compared to mature seeds. Compared to the level of sucrose in mature seeds, accumulation of sucrose in immature seeds ranged from 30.0 for genotype 'Kegone' to 48.4% for genotype 'NRC7' and 'Shilajeet'. Compared to the levels of raffinose and stachyose in mature seeds, accumulation of raffinose content ranged from 0.00 (not detectable) for variety 'Kegone' to 51.5% for variety 'LSb1' while stachyose content ranged from 11.2 for variety 'Sappori Midori' to 48.0% for variety 'NRC7'. The much lower content of flatus-causing sugars (raffinose and stachyose) in immature seeds as compared to mature seeds is important from the point view of consumption of green seeds.

Our results indicated a genotypic variation for sucrose, raffinose, stachyose in the immature green as well as mature seeds. Hymowitz et al (1972) also reported a considerable variation in the content of sucrose, raffinose and stachyose among 60 selected soybean lines of different maturity groups. The ranges for sucrose, raffinose and stachyose were 2 - 8.2%, 0.1 - 0.9% and 1.4 - 4.7%, respectively. Qui and Chang (2004) reported a range of 2.7-6.4% for total oligosaccharides in mature seeds of 181 Chinese soybean accessions. Liu and Markakis (1987) studied the effect of maturity on sucrose, raffinose and stachyose in three collections of soybean pods. Their picking stage (II) wherein the pods picked were yellow-green and seeds were also green coincided with our sampling stage. They observed that in genotype 'Beeson', sucrose, raffinose and stachyose contents were 4.44, 0.35 and 0.52%, respectively while in genotype 'Pella' sucrose, raffinose, stachyose contents were 4.85, 0.27 and 0.59%, respectively; the contents increased on maturity in both the genotypes as observed in our study. However, our study is in contrast to Dornobos and McDonald (1986) who observed a decline in sucrose, raffinose and stachyose content towards maturity in genotype 'William 79'.

Table 1. Oligosaccharides and sucrose contents (% dry weight) in immature and mature seeds of selected soybean genotypes

Genotype	Picking stage*	Sucrose	Raffinose	Stachyose	Total oligosaccharides
'Shilajeet'	I	2.02 (48.4)	0.23 (24.7)	0.42 (26.3)	0.65 (21.7)
	II	4.17	0.93	2.07	3.0
'NRC7'	I	1.25 (48.4)	0.28 (46.7)	0.83 (48.0)	1.11 (47.6)
	II	2.58	0.60	1.73	2.33
'JS335'	I	1.65 (38.8)	0.10 (11.6)	0.46 (16.7)	0.56 (15.5)
	II	4.25	0.86	2.76	3.62
'Hara soya'	I	1.29 (34.8)	0.27 (32.9)	0.39 (25.2)	0.66 (22.0)
	II	3.71	0.82	1.55	2.37
'LSb1'	I	1.65 (40.6)	0.35 (51.5)	0.63 (32.1)	0.98 (38.6)
	II	4.06	0.68	1.96	2.64
'Sappori Midori'	I	1.12 (40.4)	0.37 (23.9)	0.31 (11.2)	0.68 (15.8)
	II	2.77	1.55	2.75	4.30
'Kegone'	I	1.24 (30.0)	0.00	0.40 (21.3)	0.40 (16.1)
	II	4.13	0.60	1.88	2.48
Mean	I	1.46	0.23	0.42	0.65
	II	3.67	0.86	2.1	2.96
CD at 1%					
Genotype (G)	I	0.19	0.06	0.08	0.14
	II	0.23	0.12	0.26	0.38
Picking stage (PS)		0.28	0.15	0.37	0.52
G×PS		0.16	0.05	0.12	0.17

Values in the parentheses indicate % of particular oligosaccaride accumulated in immature seed when compared to the mature seed (n=3); *I – immature green stage when pods were completely filled and still green in colour (R_6 stage); II – mature seeds

In conclusion, our studies indicated significant differences ($p<0.01$) for sucrose, raffinose and stachyose contents among the genotypes evaluated in immature and mature seeds and these genotypic variations provide scope for further selection and improvement. Furthermore, significant interaction was observed between genotype and picking stage suggesting that separate breeding programme may be undertaken to reduce oligosaccharides and to increase sucrose content in mature and immature green seeds as the concentration of these components in mature seeds may not be the true indicator of their concentration levels at immature stage.

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