Physical characteristics and nutritional composition of some new soybean (*Glycine max* (L.) Merrill) genotypes

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Abstract Soybean is a major source of high quality protein and oil and soybean seed quality is often determined by seed nutritional and antinutritional parameters. The objective of this study was to investigate the physical characteristics and nutritional composition of some new soybean genotypes. Hundred seed weight and volume of soybean genotypes ranged from 8.7 to 11.1 g and 8.1 to 12.0 ml respectively, whereas, percent water absorption and percent volume expansion values ranged from 94.3 to 119.5% and 70.8 to 159.5% respectively. The genotypes contained % crude protein (39.4–44.4), oil (14.0–18.7), starch (4.3–6.7), total soluble sugars (5.6–7.9), reducing sugars (0.21–0.33) and sucrose (5.6–11.8). The free fatty acid and triglyceride content ranged from 31–71 mg 100 g⁻¹ oil and 90.1–93.9 g 100 g⁻¹ oil respectively. The antinutritional components determined include: mg g⁻¹ TIA (41.5–85.0), phytate (2.3–5.6), total phenols (1.0–1.5), flavonols (0.20–0.34) and ortho-dihydroxy phenols (0.10–0.21). A significant variation for the 11S/7S ratio was observed among the 8 soybean genotypes and the values ranged from 0.70 ('SL 768' and 'SL 869') to 2.4 ('SL 794').

Keywords Soybean seeds · Oil · Protein · Carbohydrate · Trypsin inhibitor · Phytic acid · Phenols

Soybean is a major leguminous crop of global importance with widespread applicability and economic value of its

products in the national as well as global market. The total world production of soybean in 2008–09 was 234.65 million tons whereas in India, it was 10.22 million tons with productivity level of 1.06 ton ha⁻¹ (Saxena et al. 2008; SOPA 2009). Soybean contributes about 10% to the domestic oil production in India and country earns substantial foreign exchange through export of soy meal (Joshi 2003). Soybean seed is one of the major food sources for human and livestock. Soy oil can serve as a good source of oleic and linoleic acid, even the partially hydrogenated soy oil contains 25% linoleic and 3% linolenic acid (Lokuruka 2010). Soybean oil is also a good source of vitamin E (Helzlsouer et al. 2000). In addition to its rich protein (35–45%) and oil content (15–25%), soybean seed also contains about 33% carbohydrates, up to 16.6% of which are soluble sugars (Hou et al. 2009). The soy protein has a very significant amino acid composition as it complements to that of cereal. Although soybeans are deficient in methionine but contain sufficient lysine to overcome the lysine deficiency of cereals (Lajalo and Genevise 2002). Galacto-oligosaccharides (Raffinose, stachyose and verbascose) comprise approximately 5% of soybean dry matter while starch represents less than 1% (Karr-Lilienthal et al. 2005). Although soybean is rich in nutrients but its acceptability as raw food is limited due to the presence of antinutritional factors such as trypsin inhibitor, phytic acid and phenols (Liener 1981). Phytic acid (myo-inositol hexakis phosphate) contains 65–80% of total phosphorus in mature soybean seeds (Raboy et al. 2000). It chelates mineral nutrients such as Cu, Zn, Mn, Fe and Ca thus reducing their availability. However, it may play important role as an antioxidant and anti-carcinogenic agent (Ertl et al. 1998). Trypsin inhibitors cause enlargement of the pancreas in rodents and hyper secretion of digestive enzymes that leads to a loss of sulphur rich endogenous proteins trypsin and chymotrypsin and depress

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growth (Guillamon et al. 2008). However, these inhibitors are effective in preventing or suppressing carcinogen induced transformation *in vitro* (Wang and Wikson 1999) and demonstrates potent anti-inflammatory properties (Roy et al. 2010). A number of new soybean genotypes have been developed by plant breeders in Punjab Agricultural University on the basis of yield and disease resistance during past few years but these have not been evaluated for their nutritional composition. The present communication reports the physical and biochemical composition of the seeds of some new soybean genotypes. Such information will be useful in selecting soybean varieties for manufacture of various soy food preparations.

Materials and methods

Soybean seeds of 8 different genotypes (SL 688, SL 525, SL 783, SL 768, SL 799, SL 794, SL 831 and SL 869) were procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India. The crop was raised in the fields of Department of Plant Breeding and Genetics, PAU, Ludhiana by following the recommended Package of Practices in season 2008. The experiment was conducted in replicated trials in a randomized block design with three replications. All the chemicals used in the present investigation were of analytical grade.

Mature seeds dried to a moisture content of ~10% (dwb) were stored in paper bags till further analysis. A weighed quantity of seeds was oven dried at 60 °C for 48 h to a constant weight before the final weighing. Physical seed characteristics like 100 seed weight (g), 100 seed volume (ml), water absorption (%), volume expansion (%), swelling capacity (g seed⁻¹) and hydration capacity (ml seed⁻¹) were determined by the methods of Santhan and Shivshankar (1978). Free sugars were extracted from the soybean seed powder with 80% ethanol followed by 70% ethanol. Total soluble sugar levels in the supernatant were determined with the phenol-sulfuric acid reagent (Dubois et al. 1956) using glucose as standard. Reducing sugars content was determined by following the method of Nelson (1944). Starch was extracted from the residue left after sugar extraction by following the method of Clegg (1956) and its content was calculated by multiplying the amount of glucose (determined by Dubois method) by a factor of 0.9. Quantitative estimation of free amino acids was made according to Lee and Takahashi (1966) and nitrogen content (%) was determined by using Kjeldahl method (AOAC 2000). For lipid analysis, a known weight of seeds was crushed and boiled in 1–2 ml of isopropanol to inactivate phospholipases, homogenized in 20 ml of chloroform and methanol (2:1, v/v) and extraction of lipids was done by the method of Folch et al. (1957). A suitable aliquot of lipid extract was evaporated

to dryness to determine the lipid content by weighing. The methods used for the estimation of different lipid classes were as earlier mentioned by Munshi et al. (1990). The fatty acid composition was estimated by converting fatty acids into their ethyl esters (Christie 1972) and analyzed using a AIMIL-Nucon Gas Chromatograph (M/s Nucon Engineers, New Delhi) solid state, equipped with a flame ionization detector fitted with a 2 mm×3 mm stainless steel column, packed with 20% DEGS (diethylene glycol succinate) on 60–80 mesh chromosorb W. The conditions for separation were: Column temperature 180±5°C, nitrogen pressure 37.7 kPa and hydrogen flow 35 ml min⁻¹. The peaks were identified by comparison of their retention times with those of standard fatty acyl esters as well as by using standard fatty acyl esters as internal standards. The peak area was calculated by measuring the height multiplied by the width of peak at half the peak height. The values for each fatty acid are given as g 100 g⁻¹ fatty acids. Duplicate observations were recorded for each sample. Sulphur containing amino acids (methionine and cysteine) were determined by the methods given by Horn et al. (1946) and Gaitonde (1967), respectively. Trypsin inhibitor activity (Kakede et al. 1974), phytate (Vaintraub and Lapteva 1988), total phenols (Swain and Hillis 1959), ortho dihydroxy phenols (Nair and Vaidyanathan 1964) and flavonols (Balabaa et al. 1974) were determined in the seed samples.

The storage proteins of the seeds were extracted and separated into glycinins and β -conglycinins by the method of Pant and Tulsiani (1969) and electrophoretically separated by the method of Laemmli (1970). The protein extract (250–300 μ g) was processed with 6 mol L⁻¹ urea and sodium dodecyl sulfate (SDS)-three volumes, 0.01% bromophenol blue and sucrose and 100 μ g of protein was loaded in each well of the electrophoretic gel. SDS-PAGE was carried out on a 12% running gel (w/v) at 21 mA for 3 h. Protein bands were visualized with Commassie Brilliant Blue G-250 and assessed for their molecular weight by running standard marker proteins. Each gel was compressed and dried in a dryer. The proportions of particular proteins present were determined by densitometry of the dried gels by GS-800 Calibrated Densitometer. The relative amount of each protein or polypeptide was expressed as percentage of total protein in the same gel lane.

All analysis were carried out in triplicate and data were statistically analyzed by one-way ANOVA at the probability of $P<0.05$.

Results and discussion

One hundred seed weight and volume of soybean genotypes ranged from 8.7 to 11.1 g and 8.1 to 12 ml with mean value of 9.73 g and 9.8 ml respectively. Genotypes 'SL 794' and 'SL

768' exhibited the maximum and minimum 100 seed weight respectively (Table 1). Krishna et al. (2003) and Ramteke et al. (2010) reported that mean 100 seed weight of 7 new soybean varieties and 92 released soybean varieties of India was 13 & 11.8 g respectively. The mean 100 seed weight (9.79 g) of genotypes of present study was less as compared to those reported earlier. Genotype 'SL 525' had maximum 100 seed volume whereas 'SL 869' exhibited minimum 100 seed volume. Percent water absorption and percent volume expansion of different soybean genotypes ranged from 94.3 (SL 768) to 119.5 (SL 688) and 70.8 (SL 525) to 159.5% (SL 869) respectively. Genotype 'SL 783' and 'SL 688' exhibited maximum value of swelling capacity (0.13/seed) and hydration capacity (0.12/seed) respectively (Table 1).

Dry matter content of 8 soybean genotypes ranged from 90.9 to 92.9% and it did not vary significantly among different genotypes (Table 2). The seed nitrogen content varied from 6.3 to 7.1% with mean value of 6.6%. Genotype 'SL 783' recorded maximum N content whereas genotype 'SL 794' exhibited minimum N content. Crude protein content of soybean seeds of different genotypes was calculated by multiplying nitrogen content by a factor 6.25 and values varied from 39.4 to 44.4%. Crude protein content of 'SL 783' genotype was significantly higher as compared to other genotypes studied (Table 2). Soybean seed is a rich source of protein and contains optimum amount of all essential amino acids but limiting in methionine and cysteine. The free amino acid content in seeds of different soybean genotypes varied significantly among each other. 'SL 783' seeds exhibited maximum free amino acid content. Total sulfur, methionine and cysteine contents varied significantly among different soybean genotypes. Genotype 'SL 688' exhibited maximum and significantly higher total sulphur and methionine contents

as compared to other genotypes (Table 2). Large variation was seen in Trypsin Inhibitor Activity of soybean genotypes. Genotype 'SL 525' (85 mg g⁻¹) and 'SL 783' (41.5 mg g⁻¹) exhibited maximum and minimum trypsin inhibitor activity respectively with a mean value of 70.6 mg g⁻¹. Guillaumon et al. (2008) reported that trypsin inhibitor among legume seeds can range from negligible as in *Lupinus* spp; to very intense in *Glycine max* cultivars which generally contained highest values of 43–84 TIU mg⁻¹ sample. Whole soybean and defatted soybean have been reported to have 57.2 and 64.8 TIU mg⁻¹ DM respectively (Valdebouze et al. 1980). Kumar et al. (2003) reported that TI activity from defatted soy flour ranged from 42 to 113 mg g⁻¹ and location effects on TI activity were nonsignificant.

The potential health benefit of common bean is attributed to the presence of secondary metabolites such as phenolic compounds that possess antioxidant properties (Azevedo et al. 2003). The content of total phenols, flavonols and ortho-dihydroxy phenols in different genotypes ranged from 1.0–1.5, 0.20–0.34 and 0.10–0.21 mg g⁻¹ respectively. Except for 'SL 525' and 'SL 768', all other genotypes did not show significant variation in their total phenolic content (Table 2). Phytic acid, a major phosphorus storage form in plants, is often regarded as an antinutrient because of strong mineral, protein and starch binding properties thereby decreasing their bioavailability (Weaver and Kannan 2002). The phytic acid content of different soybean genotypes ranged from 2.3 to 5.6 mg g⁻¹. The range of antinutritional factors in soybean have been reported to be 18.3–35 and 1.6–3.1 mg g⁻¹ for phenols and phytic acid respectively (Gehan and Amin 2010).

The starch and total soluble sugar content of seeds didn't differ significantly among different soybean genotypes. Reducing sugar content of seeds showed significant

Table 1 Physical seed characteristics of different soybean genotypes

Genotypes	100 seed weight (g)		100 seed volume (ml)		% water absorption	% volume expansion	Swelling capacity/seed	Hydration capacity/seed
	Before Soaking	After Soaking	Before Soaking	After Soaking				
SL688	10.0	21.9	8.5	20.0	119.5	136.1	0.11	0.12
SL525	10.3	20.9	12.0	20.5	103.4	70.8	0.08	0.10
SL783	9.8	21.3	10.5	23.5	116.5	124.6	0.13	0.11
SL768	8.7	16.9	8.7	18.0	94.3	105.9	0.09	0.08
SL799	9.8	21.2	10.0	20.5	115.3	105.0	0.10	0.11
SL794	11.1	22.1	9.6	20.5	99.2	112.4	0.10	0.10
SL831	8.9	18.6	8.2	19.5	108.6	137.8	0.11	0.09
SL869	9.3	19.5	8.1	21.0	110.7	159.5	0.12	0.10
Mean ± SD	9.7±0.77	20.3±1.81	9.8±1.65	20.9±2.40	108.4±8.86	119±26.8	0.11±0.02	0.10±0.01
CD (<i>P</i> <0.05)	0.67	0.25	1.27	2.17	3.15	34.04	0.27	NS

Values for individual treatments are the mean of three replications (*n*=3)

Table 2 Biochemical composition of seeds of different soybean genotypes

Parameters	Genotypes								Mean $\pm$ SD	CD ($P<0.05$)
	SL 688	SL 525	SL 783	SL 768	SL 799	SL 794	SL 831	SL 869		
Dry Matter (%)	92.9	92.2	90.9	91.9	92.3	92.6	92.6	91.9	92.1 $\pm$ 0.62	NS
Nitrogen content (%)	6.8	6.9	7.1	6.7	6.4	6.3	6.4	6.4	6.6 $\pm$ 0.29	0.2
Sulfur content (%)	0.25	0.22	0.20	0.18	0.16	0.18	0.13	0.12	0.18 $\pm$ 0.04	0.03
Crude Protein (%)	42.5	43.1	44.4	41.9	40.0	39.4	40.0	40.0	41.4 $\pm$ 1.82	1.22
Free Amino acids (%)	0.69	0.67	0.78	0.67	0.46	0.38	0.36	0.41	0.55 $\pm$ 0.17	0.09
Methionine (%)	0.25	0.24	0.24	0.20	0.19	0.09	0.17	0.18	0.20 $\pm$ 0.05	0.03
Cysteine (mg%)	39.0	38.0	41.0	39.0	36.0	35.0	33.0	36.0	37.1 $\pm$ 2.59	2.20
Starch (%)	6.7	6.0	6.0	5.8	5.9	5.3	4.3	5.2	5.6 $\pm$ 0.72	1.8
Total Soluble sugars (%)	7.9	5.6	6.8	7.0	7.2	6.4	6.2	6.4	6.7 $\pm$ 0.69	2.8
Sucrose (%)	11.8	9.5	10.1	11.7	7.9	9.8	5.6	6.5	9.1 $\pm$ 3.20	1.33
Reducing sugars (%)	0.29	0.23	0.32	0.33	0.21	0.26	0.26	0.23	0.27 $\pm$ 0.04	0.04
Trypsin Inhibitor Activity(mg g ⁻¹)	75.0	85.0	41.5	71.5	73.5	82.5	82.5	53.5	70.6 $\pm$ 15.4	10.76
Phytic acid (mg g ⁻¹)	5.6	2.3	4.2	4.5	3.6	4.6	2.5	2.8	3.8 $\pm$ 1.16	1.5
Total Phenols (mg g ⁻¹)	1.1	1.3	1.0	1.5	1.2	1.2	1.1	1.1	1.2 $\pm$ 0.20	NS
Flavonols (mg g ⁻¹)	0.20	0.31	0.22	0.34	0.21	0.22	0.25	0.22	0.25 $\pm$ 0.06	NS
Ortho-dihydroxy phenols (mg g ⁻¹)	0.10	0.21	0.12	0.20	0.15	0.20	0.11	0.20	0.20 $\pm$ 0.01	NS

Values for individual treatments are the mean of three replications ($n=3$).

difference among various soybean genotypes except for ‘SL 768’ and ‘SL 783’ that contain significantly higher reducing sugars content as compared to other genotypes. Seeds from different soybean genotypes showed large variation in their sucrose content ranging from 5.6 (SL 831) to 11.8 (SL 688) g 100 g⁻¹ DW. Genotype ‘SL 831’ exhibited lowest starch and sucrose content.

Legume seeds contain 2–21% fat with beneficial composition of exogenic unsaturated fatty acids: linoleic (21–53%) and linolenic (4–22%) acids (Campos-Vega et al. 2010). The oil content (%) in soybean genotypes ranged from 14.0 to 18.7%. Genotypes ‘SL 688’ and ‘SL 869’ recorded the maximum and minimum amount of oil respectively. ‘SL 869’ and ‘SL 831’ contained significantly lower oil content as compared to other genotypes. Earlier studies by different authors reported percent mean protein and oil content of 39.8 and 20.5 (Krishna et al. 2003) and 40.2 and 18.3 (Ramteke et al. 2010) respectively in different soybean varieties released in India. Although the protein content of genotypes evaluated in the present study was found consistent with earlier studies but some of the genotypes viz. ‘SL 799’, ‘SL 794’, ‘SL 831’, and ‘SL 869’ showed lower oil content as compared to mean oil content reported by these authors. Guleria et al. (2007) have reported that both oil and protein content showed positional effect and seeds located at apical positions on soybean stem axis had higher percentage of protein and lower oil content than those located at basal portion of the plant.

Soybean genotypes exhibited wide variation in their lipidic composition (Table 3). Genotype ‘SL 783’ contained significantly higher amount of phospholipids and sterols as compared to other genotypes studied. Genotype ‘SL 688’ exhibited maximum glycolipid content of 2.4 g 100 g⁻¹ oil whereas genotype ‘SL 794’ exhibited minimum glycolipid content of 1.1 g 100 g⁻¹ oil. The free fatty acid content in different genotypes ranged from 31 to 71 mg 100 g⁻¹ oil and genotypes ‘SL 688’ and ‘SL 869’ exhibited maximum and minimum free fatty acid content respectively. The triglyceride content was calculated after subtracting the sum total of phospholipids, glycolipid, sterol and free fatty acids from 100. Triglyceride content ranged from 90.1 to 93.9 g 100 g⁻¹ oil. Genotypes ‘SL 794’ and ‘SL 783’ exhibited maximum and minimum values for triglyceride content respectively. Triglyceride content of ‘SL 794’ was significantly higher as compared to other genotypes except for ‘SL 799’ and ‘SL 869’.

Fatty acid composition studies are important for accessing the quality and stability of soybean oil. Soybean genotypes also showed variation in their fatty acid composition. Maximum linoleic acid was found in genotype ‘SL 831’ and minimum content was found in genotype ‘SL 799’. Genotype ‘SL 688’ exhibited a higher content of saturated fatty acids such as palmitic and stearic acid as compared to other genotypes. Maximum oleic acid content (30.4 mg 100 g⁻¹ oil) was recorded in genotype ‘SL 525’.

Table 3 Oil content (g 100 g⁻¹ DW), lipidic composition and fatty acid composition of different soybean genotypes

Parameters	Genotypes									CD $P<0.05$
	SL 688	SL 525	SL 783	SL 768	SL 799	SL 794	SL 831	SL 869	Mean $\pm$ SD	
Oil content	18.7	18.5	17.5	18.0	15.0	14.9	14.1	14.0	16.3 $\pm$ 2.03	0.79
Lipidic Composition (g 100 g ⁻¹ oil)										
Phospholipids	0.25	0.25	0.31	0.21	0.20	0.20	0.19	0.20	0.23 $\pm$ 0.04	0.04
Glycolipids	2.4	2.1	2.0	1.7	1.4	1.1	1.8	1.4	1.7 $\pm$ 0.43	0.29
Sterols	5.4	6.5	7.5	6.3	5.4	4.7	6.0	4.8	5.8 $\pm$ 0.94	1.04
Free Fatty Acids*	71	65	62	60	40	59	68	31	57 $\pm$ 14.1	13.1
Triglyceride	91.9	91.1	90.1	91.7	92.9	93.9	91.9	93.6	92.1 $\pm$ 1.27	1.24
Fatty acid Composition (g 100 g ⁻¹ fatty acids)										
Palmitic Acid(16:0)	12.9	11.5	12.5	12.9	12.3	12.7	11.2	10.6	12.0 $\pm$ 0.82	
Stearic Acid (18:0)	4.0	3.9	3.6	4.0	3.5	3.0	ND	3.4	3.6 $\pm$ 0.38	
Oleic Acid(18:1)	23.8	30.4	24.5	23.8	29.6	26.5	26.1	20.9	5.4 $\pm$ 3.40	
Linoleic Acid(18:2)	53.6	48.6	52.7	53.0	46.3	50.1	58.8	58.8	54.2 $\pm$ 6.10	
Linolenic Acid(18:3)	5.5	5.3	6.0	5.7	5.6	5.4	3.6	6.3	4.9 $\pm$ 1.38	

Values for individual treatments are the mean of three replications ($n=3$); *mg 100 g⁻¹ oil

The storage proteins in soybean mainly consist of conglycinin (7S) and glycinin (11S) which contribute to approximately 75% of total storage protein (Murphy and Resurreccion 1984). The SDS-PAGE pattern of storage proteins showed that different genotypes accumulated storage proteins due to differential response of protein production and quality among various genotypes. The difference in storage protein content between genotypes was due to changes in both β -conglycinin (7S) and glycinin (11S) proteins. Glycinin (11S) is a hexameric protein made of 5 subunits and each subunit is composed of acidic and basic polypeptides linked together by disulfide bonds (Kitamura and Kaizuma 1981). The β -conglycinin is a trimeric protein composed of three subunits α , α' and β (Thanh and Shibasaki 1977). The β -subunit of β -conglycinin storage protein is of extremely poor quality, being devoid of sulfur containing amino acids (methionine and cysteine). A significant variation for the 11S/7S ratio

was observed among the 8 soybean genotypes and the values ranged from 0.70 ('SL 768' and 'SL 869') to 2.4 ('SL 794') (Table 4). 11S/7S ratio can be used as a criterion of indirect selection for high quality protein. On the basis of this, high or low 11S or 7S genotypes/lines can be selected. 'SL 794' has 11S/7S ratio of 2.4 which is comparable to the earlier observations of Kitamura and Kaizuma (1981) and Carrao-Panizzi et al. (2008). Content of β -conglycinin fractions, α' , α and β in 'SL 794' were respectively 7.6, 0.97 and 2.9% and glycinin acidic and basic subfractions were respectively 12 and 15.6% (Table 4). Fehr et al. (2003) reported that cultivar Vinton 81 has 11S/7S ratio of 2.04 and good for tofu processing due to its high content of glycinin subfraction. So genotypes 'SL 783' and 'SL 794' can be compared to these cultivars for having similar properties. These differences for the protein fractions among genotypes can be genetically manipulated to improve the protein quality of soybean genotypes. Since

Table 4 Mean values of protein components (%) in seeds of soybean genotypes

Genotype	β -conglycinin (7S)			Total 7S	Glycinin (11S)		Total 11S	11S/7S Ratio
	α'	α	β		Acidic	Basic		
SL 688	5.9	7.0	5.9	18.8	7.1	16.6	23.7	1.3
SL 525	5.5	7.8	7.1	20.4	15.4	6.3	21.7	1.1
SL 783	9.0	1.0	1.3	11.3	12.2	12.4	24.6	2.2
SL 768	15.3	11.6	7.7	34.6	8.3	15.8	24.1	0.70
SL 799	11.5	6.3	7.5	25.3	13.1	13.8	26.9	1.1
SL 794	7.6	0.97	3.0	11.6	12.0	15.6	27.6	2.4
SL 831	13.3	9.3	6.5	29.1	9.3	12.8	22.1	0.76
SL 869	3.6	17.3	17.3	38.2	14.6	11.8	26.4	0.70

Values given are the mean of two replications ($n=2$)

glycinin (11S) fraction contains higher content of sulfur containing amino acids than β -conglycinin (7S), it is possible to improve the amino acid balance of soybean protein.

Among different soybean genotypes, 'SL 783' showed maximum protein content and 11S/7S ratio of 2.2 with minimum amount of sulfur deficient β subunit of β -conglycinin. Values of total sulphur, methionine and cysteine contents in 'SL783' were found to be higher than the mean values with a low amount of total phenolic content and Trypsin inhibitor activity.

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