

**PHYSIO-BIOCHEMICAL EVALUATION OF SOYBEAN (*Glycine max* L.)
GENOTYPES EXHIBITING VARIABLE SEED SIZE**

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ABSTRACT

Eighteen promising soybean genotypes of different seed size i.e. small, medium and bold were examined for physio-biochemical differences. Among the physiological parameters, the highest mean hundred seed weight, per cent germination, seed vigour index and seed density was recorded in bold seeds. The highest mean seed coat proportion, the lowest mean EC and the lowest mechanical damage by both ferric chloride test and sodium hypochloride test was recorded in small size seeds. Among the biochemical parameters, the highest mean crude protein content was recorded in bold seeds, whereas the highest mean crude fat and crude fibre content was recorded in the medium size seeds. Mean seed coat lignin and seed coat peroxidase activity was the highest in smaller seeds, whereas trypsin inhibitor in defatted flour was the lowest in bold seeds.

KEY WORDS: *Biochemical parameters, Physiological parameters, Seed size, Soybean*

INTRODUCTION

Soybean is often designated as a 'Golden bean' because of its triple use as food, feed and industrial raw material. It becomes a miracle crop of the twentieth century. The seeds of soybean are very nutritious those fully dependent on vegetarian diet on an account of its richness in protein, fat, carbohydrates, minerals and salts. It also provides vitamin A, B and D. Soybean seed also contains some anti-nutritional factors like trypsin inhibitors. Vearasilp *et al.* (2001) reported larger seed size and thinner seed coat had more mechanical damage. Since coating of the soybean seed is very thin and low in lignin content provides little protection to the fragile radicle which lies in a vulnerable

position directly beneath the seed coat. Due to this fact, mechanical damage is one of the causes of great loss in soybean seed quality during harvest and processing (Franca Neto and Henning, 1984). The occurrence of genetic variability in seed resistance to mechanical damage among different soybean cultivars has already been demonstrated (Carbonell and Krzyzanowski, 1995). In addition to lignin, peroxidase played an important role in various metabolic steps during lignin and suberin formation (Quiroga *et al.*, 2000). The predominant trypsin inhibitors in soybeans are located with the main storage protein in cotyledon (Horisberger *et al.*, 1986). Arefrad *et al.* (2013) reported that the genotypes with low levels of trypsin and chymotrypsin

inhibitors especially for Bowman-Birk Protease Inhibitor (BBI) protein could have a significant role from nutritional point of view. Although soybean protein products require heat processing to achieve maximum nutritional value, partially through trypsin inhibitor denaturation, trypsin inhibitor also display anti-carcinogenic properties. The soybean seeds found in variable seed size like small medium and bold might be having different biochemical composition. Thus, in view of above, the present research was undertaken on physio-biochemical evaluation of soybean (*Glycine max* L.) genotypes exhibiting variable seed size.

MATERIALS AND METHODS

The seed materials of 18 soybean genotypes were collected immediately after the harvesting from the Soybean Breeder, Agricultural Research Station, Digraj, Dist. Sangli (Maharashtra). The collected seeds were categorized into three categories viz., small (below 10g), medium (10-12g) and bold (above 12g).

Physiological analysis

Standard procedures were used for physiological analyses of seed viz., germination (%) (Anonymous, 1999), seed vigour index (SVI) (Abdul-Baki and Anderson, 1973), electrical conductivity (EC) (dSm^{-1}) (Loeffler *et al.*, 1988), mechanical damage (%) by ferric chloride test (Agrawal, 1995) and sodium hypochlorite test (Van *et al.*, 2000), seed coat proportion (%) (Hoy and Gamble, 1985), seed density (Deshpande *et al.*, 1993) and 100 seed weight (g).

Biochemical analysis

The biochemical parameter like crude protein, crude fat and crude fibre were determined according to A.O.A.C (2005) method. The seed coat lignin content was determined as per jute titration method given by Hussain *et al.* (2002). The peroxidase activity was assayed as per the procedure given by Cakmak and Horst (1991) with

modifications by Santos *et al.* (2002). Trypsin inhibitor assay was performed as per the method of Erlanger *et al.* (1961).

Physio-biochemical

analyses were carried out with three replications. Statistical analysis carried out using completely randomised design.

RESULTS AND DISCUSSION

Physiological analyses

The data on physiological evaluation of soybean seeds based on seed size reported in Table 1.

The mean 100 seed weight in the various soybean genotypes ranged between 9.09-13.60g. Significant differences were observed among the mean values. The highest mean 100 seed weight was observed in bold size seeds (12.56g) followed by medium and lowest in small seeds. The bold sized genotype, KDS-726 recorded significantly highest hundred seed weight of 13.60 g, whereas the lowest of 9.09 g was recorded in small sized genotype, LVS-2011.117. Kuchlan *et al.* (2010) reported that the 100 seed weight of soybean seed ranges from 8.37 to 14.83. With regards to per cent germination, differences within mean values were not significant. The highest mean per cent germination was observed in bold sized soybean seeds, among which the highest of 90 per cent germination was recorded in KDS-753. The lowest per cent germination was observed in small sized seeds of *Kalitur* and LVS-2011.117. The lower per cent germination may be due to higher lignin and higher mechanical strength. Mean seed vigour index SVI was the highest in bold seeds followed by medium and the lowest by smaller seeds. The bold size genotype KDS-753 recorded significantly the highest values for SVI (1591).

The seed coat proportion in various soybean genotypes ranged between 8.32 to 11.20 per cent. The highest seed coat proportion of 10.03 per cent was recorded in

small seeds followed by medium and bold size seeds. However, significantly the highest and the lowest value of seed coat proportion was found in bold genotypes. Thus, no direct relation was observed between the seed coat proportion and seed size. The results are in agreement with Kuchlan *et al.* (2010). The higher EC represents higher seed damage during storage. The electrical conductivity (EC) of various soybean genotypes ranged from 0.29-0.58 dSm⁻¹. The mean electrical conductivity was found to be the lowest in small size soybean seeds, whereas the highest in bold size seeds. However, the lowest of EC 0.29 dSm⁻¹ was recorded in a bold seeds of Birsa Soya 1, and also in small size genotypes, Kilitur and LVS-2011.117 (0.30 dSm⁻¹). It indicated that EC did not have direct relation with seed size; it may be a genetically governed trait.

The lowest mean per cent mechanical damage was recorded in small sized seeds by both FeCl₃ test and sodium hypochloride test. The bold and medium sized seeds recorded the highest mechanical damage. The differences between mean values of bold and medium size seeds are meagre. The small sized soybean genotype LVS-2011.117 recorded the lowest per cent mechanical damage of 6.0 by sodium hypochloride test and the lowest of 8.0 by FeCl₃ test. The lowest mechanical damage was observed in small sized seeds may be due to the higher seed coat lignin. The highest mean seed density was observed in bold size seeds followed by medium and small seeds. The highest seed density of 1.19g/cm³ was recorded in all bold genotypes. There was not remarkable difference was observed between different seed size. Present results are in agreement with Kuchlan *et al.* (2010).

Biochemical analyses

The data on biochemical evaluation of soybean seeds based on seed size are

reported in Table 2. The crude protein, crude fat and crude fibre content in various soybean genotypes was ranged from 40.50 to 43.63 per cent, 17.57 to 20.11 per cent and 5.11 to 6.40 per cent, respectively. There were not significant differences according to seed sizes. The highest crude protein, crude fat and crude fibre content recorded in genotype, KDS-1035 (43.63%), KDS-726 (20.11 %) and KDS-1041(6.80%), respectively. Results are in agreement with the literature values for crude protein content (Yan-sheng *et al.*, 2012), crude fat content (Raut *et al.*, 1998) and crude fibre content (Redondo-Cuenca *et al.*, 2006).

Higher lignin content is important for storage point of view. The seed coat lignin content in the soybean genotypes ranged between 5.06 to 17.38 per cent. The highest mean seed coat lignin was recorded by small size seeds (13.55%) followed by medium (10.64 %) and least in bold seeds (10.54%). The medium size seeds of KDS-1034 recorded significantly the highest seed coat lignin of 17.38 per cent followed by small seed size genotype LVS-2011.117 (17.19%). Most of the higher lignin containing genotypes exhibited higher peroxidase activity may be due to role of peroxidase in lignifications process (Quiroga *et al.*, 2000). The seed coat peroxidase activity in the seed coat of various soybean genotypes was ranged from 398–540 μ moles H₂O₂ decomposed mg⁻¹ protein min⁻¹. In the present investigation, the highest mean seed coat peroxidase activity was observed in small size seeds followed by medium and bold size seeds. The highest seed coat peroxidase activity of 540 μ moles H₂O₂ decomposed mg⁻¹ protein min⁻¹ was recorded in small seeds of *Kalitur*. The values reported by Capeleti *et al.* (2005) for seed peroxidase activity (162-586 μ mol- min⁻¹g⁻¹) are in agreement with present study. The trypsin inhibitor in the grains of various soybean genotypes ranged between 49.70–

64.69 TIU g⁻¹ of defatted flour. The highest mean trypsin inhibitor content was recorded in small sized genotypes followed by medium, whereas the lowest in bold. The lower trypsin inhibitor was recorded in bold seeds of KDS-726. Guillamon *et al.* (2008) studied trypsin inhibitor in the soybean seeds was ranged from 43 to 84 TIU g⁻¹ defatted flour. The higher trypsin inhibitor containing genotypes are better for storage point of view, whereas lower trypsin inhibitor containing genotypes are better for human consumption as it inhibits trypsin secreted in small intestine.

CONCLUSION

In conclusion, within eighteen promising soybean genotypes examined for physio-biochemical parameters, small sized soybean genotypes were better in EC, mechanical damage, seed coat proportion, seed coat lignin and peroxidase activity, whereas bold seeds were better in crude protein, trypsin inhibitor, seed density, 100 seed weight, per cent germination and seed vigour index. The medium sized soybean genotypes were better in crude fat and crude fibre. Thus, present study may useful in developing new soybean varieties with desirable traits. In general, small sized seeds exhibiting most of the characters related to better storage quality, while big sized seeds with nutritional quality.

REFERENCES

A.O.A.C. (2005). Official Methods of Analysis, Association of Official Analytical Chemist International, edn. 18, Gaithersburg, MD, USA.

Abdul-Baki, A. and Anderson, J. D. (1973). Vigour determination in soybean seed by multiple criteria. *Crop Sci.*, **13**: 630-632

Agrawal, R. L. (1995). Seed viability. *Seed Technology*, 2nd Edition, pp.580-582.

Anonymous (1999). International Rules for Seed Testing. *Seed Sci. Tech.*, **13**(2): 299-513.

Arefrad, M.; Nadali, B. J.; Ghorbanali, N. and Seyedkamal, K. (2013). Influence of genotype variation on trypsin and chymotrypsin inhibitors levels of seed storage proteins composition in soybean [*Glycine max* (L.)Merrill]. *Int. J. Agron. Pl. Produ.*, **4**(12): 2877-2884.

Cakmak, I. and Horst, W. J. (1991). Effect of aluminium on lipid peroxidation, superoxidedismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*), *Physiologia Plantarum*, **83**: 463-468.

Capeleti, I.; Bonini, E. A.; Lourdes, M.; Ferrarese, L.; Teixeira, A. C. N., Carlos Krzyzanowski, F. C. and Ferrarese-Filho, O. (2005). Lignin content and peroxidase activity in soybean seed coat susceptible and resistant to mechanical damage. *Acta Physiologiae Plantarum*, **27**: 103-108.

Carbonell, S. A. and Krzyzanowski, F. C. (1995). The pendulum test for screening soybean genotypes for seed resistant to mechanical damage. *Seed Sci. Tech.*, **23**: 331-339.

Deshpande, S. D.; Bal, S. and Oja, T. P. (1993). Physical properties of soybean. *J. Agril. Engg. Res.*, **56**: 89-98.

Erlanger, B. F.; Kokowsky, N. and Cohen, W. (1961). The preparation and properties of two new chromogenic substrates for trypsin. *Arch. Biochem. Biophysics*, **95**: 271-278.

Franca-Neto, J. B. and Henning, A. A. (1984). *Physiological and pathological qualities of soybean seeds*, Embrapa- National Soybean Research Centre, Londrina, Parana Brazil. Pp. 9-39.

Guillamon, E.; Mercedes, M. P.; Burbano, C.; Cuadrado, C.; Sanchez, M. C. and Muzquiz, M. (2008). The trypsin inhibitors present in seed of different

- grain legume species and cultivar. *Food Chem.*, **107**: 68–74.
- Horisberger, M.; Clerc, M. F. and Pahud, J. J. (1986). Ultrastructural Localization of Glycinin and Beta Conglycinin in *Glycin max* (Soybean) cv. Mapple Arrow by the immunogold method. *Histochem.*, **85**: 291-294.
- Hoy, D. J. and Gamble, E. E. (1985). The effect of seed size and seed density on germination and vigour in soybeans, (*Glycine max* L. Merrill.). *Can. J. Pl. Sci.*, **65**: 1-8.
- Hussain, M. A.; Huq, M. E.; Rahman, S. M. and Zakaria, A. (2002). Estimation of lignin in Jute by titration method. *Pak. J. Biol. Sci.*, **5**: 521-522.
- Kuchlan, M. K.; Dadlani, M. and Samuel, D. V. K. (2010). Seed coat properties and longevity of soybean seeds. *J. New Seeds*, **11**: 239-249.
- Loeffler, T. M.; Tekrony, D. M. and Egli, D. B. (1988). The bulk conductivity test as an indicator of soybean seed quality. *J. Seed Tech.*, **12**: 37-53.
- Quiroga, M.; Guerrero, C.; Botella, M. A.; Barcelo, A.; Amaya, I.; Medina, M. I.; Alonso, F. J.; Forchetti, S. M.; Tigier, H. and Valpuesta, V. (2000). A tomato peroxidase involved in the synthesis of lignin and suberin. *Pl. Physio.*, **122**: 1119-1127.
- Raut, V. M.; Taware, S. P. and Halvankar, G. B. (1998). Evaluation of soybean germplasm for seed oil content. *Soybean Genet. Newsl.*, **25**: 71-72.
- Redondo-Cuenca, A.; Villanueva-Suarez, M. J.; Rodriguez-Sevilla, M. D. and Mateos-Aparicio, I. (2006). Chemical composition and dietary fibre of yellow and green commercial soybeans (*Glycine max*). *Food Chem.*, **101**: 1216-1222.
- Santos, W. D.; Ferrarese, M. L. L.; Finger, A.; Teixeira, A. C. N. and Ferrarese-Filho, O. (2002). Lignification and related enzymes in *Glycin max* root growth-inhibition by ferulic acid. *J. Chem. Ecol.*, **30**: 1199-1208.
- Van U. D.; Bern, C. J. and Rukunudin, I. H. (2000). Soybean mechanical damage detection. *Appl. Engg. Agric.*, **16**(2): 137-141.
- Vearasilp; Pa-oblek, S. S.; Krittigamas, N.; Thanapornponpong, S.; Suriyong, S. and Pawalzik, E. (2001). Assessment of post harvest soybean seed quality loss. Conference on International Agricultural Research for Development. Deutscher Tropentag – Bonn, October 9-11, 2001.
- Yan-sheng, L. I.; Ming, D. U.; Qiu-ying, Z.; Guang-hua, W.; Masoud, H. and Xiao-bing, L. (2012). Greater differences exist in seed protein, oil, total soluble sugar and sucrose content of vegetable soybean genotypes [*Glycine max* (L.) Merrill] in Northeast China. *Aust. J. Crop Sci.*, **6**(12): 1681-1686.

Table 1: Physiological evaluation of soybean seeds based on seed size

Sr. No.	Genotypes	Hundred Seed Weight (g)	Germination (%)	SeedCoat Proportion (%)	Seed Vigour Index-I	Mechanical Damage		Electrical Conductivity (dSm ⁻¹)	Seed Density (g/cm ³)
						By Ferric Chloride Test (%)	By Sodium Hypochloride Test (%)		
Small size genotype									
1.	Kalitur	9.56	83	10.13	1461	8.0	7.0	0.30	1.15
2.	LVS-2011.117	9.09	83	10.17	1401	8.0	6.0	0.30	1.15
3.	KDS-730	9.98	89	9.63	1561	10.0	10.0	0.56	1.17
4.	KDS-1025	9.48	85	10.17	1481	10.0	8.0	0.32	1.13
	Mean	9.52	85	10.03	1476	9.0	7.7	0.37	1.15
Medium size genotype									
5	VLS-65	11.23	83	9.93	1421	10.0	9.0	0.31	1.18
6.	EC-456600	11.26	83	9.34	1561	16.0	14.0	0.53	1.18
7.	EC-528628	10.60	89	9.75	1509	10.0	11.9	0.53	1.17
8.	KDS-1034	11.00	85	10.45	1471	8.0	10.0	0.31	1.17
9.	JS-9305	10.14	85	9.20	1571	16.0	14.0	0.56	1.17
10.	KDS-344	10.50	83	8.72	1501	17.0	14.0	0.55	1.18
11.	KDS-1041	10.60	83	10.06	1502	8.0	10.0	0.31	1.18
12.	KDS-1042	10.60	89	9.70	1501	9.8	8.0	0.30	1.18
	Mean	10.74	85	9.64	1504	11.9	11.4	0.43	1.18
Bold size genotype									
13.	Birsa Soya 1	12.00	84	11.20	1491	8.0	10.0	0.29	1.19
14.	KDS-753	12.75	90	8.53	1591	16.0	14.0	0.58	1.19
15.	KDS-726	13.60	89	8.87	1521	16.0	14.0	0.58	1.19
16.	KDS-1035	12.70	84	10.19	1470	8.0	6.0	0.33	1.19
17.	KDS-1031	12.17	84	9.96	1491	7.9	8.0	0.31	1.19
18.	EC-34147	12.14	89	8.32	1531	14.0	13.0	0.54	1.19
	Mean	12.56	86.66	9.51	1515	11.6	10.8	0.44	1.19
	Range	9.09-13.60	83-90	8.32-11.20	1401-1591	7.9-17.0	6.0-14.0	0.29-0.58	1.13-1.19
	S.E±	0.11	0.71	0.10	0.72	0.26	0.23	0.01	0.010
	CD at 1%	0.33	2.10	0.30	2.12	0.78	0.67	0.03	0.03

Table 2: Biochemical evaluation of soybean seeds based on seed size

Sr. No.	Genotypes	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Seed Coat Lignin (%)	Peroxidase ($\mu\text{moles H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1})	Trypsin Inhibitor (TIU g^{-1} defatted Flour)
Small size genotype							
1.	Kalitur	43.10	18.77	5.50	16.64	540	62.87
2.	LVS-2011.117	42.20	18.68	6.30	17.19	533	61.87
3.	KDS-730	41.03	19.67	5.60	7.53	420	50.24
4.	KDS-1025	43.00	17.57	6.30	13.05	504	61.67
	Mean	42.33	18.67	5.93	13.55	499	59.16
Medium size genotype							
5	VLS-65	43.30	18.82	6.50	16.11	530	61.87
6.	EC-456600	40.50	18.30	5.11	6.72	422	53.85
7.	EC-528628	41.00	18.80	5.50	6.58	432	50.60
8.	KDS-1034	41.10	19.07	6.50	17.38	514	62.97
9.	JS-9305	43.07	20.03	5.90	5.06	433	51.70
10.	KDS-344	43.30	19.80	5.20	5.06	402	51.80
11.	KDS-1041	42.37	19.13	6.80	13.52	521	60.17
12.	KDS-1042	42.60	19.12	6.40	14.71	511	59.30
	Mean	42.16	19.13	5.99	10.64	471	56.53
Bold size genotype							
13.	Birsa Soya 1	18.77	18.21	6.40	15.67	533	64.69
14.	KDS-753	41.87	19.67	5.50	5.22	421	50.53
15.	KDS-726	43.38	20.11	5.20	9.23	398	49.70
16.	KDS-1035	43.63	18.40	6.50	11.67	516	57.67
17.	KDS-1031	42.70	18.61	5.80	14.56	503	57.46
18.	EC-34147	42.32	18.13	5.60	6.89	420	51.85
	Mean	42.65	18.85	5.83	10.54	465	55.32
	Range	41.03-43.63	17.57-20.11	5.11-6.80	5.06-17.38	398-540	49.70-64.69
	S.E $\pm$	0.42	0.18	0.06	0.10	0.70	0.36
	CD at 1%	1.20	0.54	0.18	0.30	2.10	1.03

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