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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(12): 1118-1121

www.thepharmajournal.com
Received: 07-10-2021
Accepted: 20-11-2021

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Seed coat and biochemical screening of soybean genotypes useful for field weathering and mechanical damage tolerance

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Abstract
The soybean genotypes recorded significant variation for seed coat and biochemical parameters. In seed coat characters like seed coat proportion the genotype Birsa Soya 1 recorded highest proportion of seed coat (10.99%) followed by KDS 1274 (10.76%) and was found lowest 8.02% in KDS 1144 followed by KDS 1097 (8.23%). The highest seed coat hardness was recorded in Birsa Soya 1 (137.16 N) followed by KDS 1274 (128 N) and the highest seed coat thickness was recorded in genotype Birsa Soya 1(12.80 µm) and KDS 1274 (12.80 µm). In seed coat biochemical study the highest lignin content was recorded in black seeded genotype Birsa Soya 1(13.88%) followed by KDS 1274 (11.52%). Whereas the significantly lower seed coat lignin content of 4.26% was recorded in genotype KDS 1144 followed by KDS 1096 (4.41%). Highest peroxidase activity was also showed by black seeded genotypes Birsa Soya 1 (440.20ΔA min^{-1} g^{-1}) followed by KDS 1274 (415 ΔA min^{-1} g^{-1}). The lowest seed coat peroxidase activity of 223ΔA min^{-1} g^{-1} of fresh seed weight was recorded in genotype KDS 980 followed by RCS-11-07 (232.86ΔA min^{-1} g^{-1}). It was observed that black seeded genotypes of soybean had higher seed coat proportion, lignin content, Peroxidase activity than that of yellow seeded soybean genotypes.

Keywords: Seed coat proportion, Seed coat hardness, Seed coat thickness, Lignin content, Peroxidase activity

Introduction
Soybean (*Glycine max* (L.) Merrill) soybean is an important commercial crop. It has a high nutrient content, including protein (40%), oil (18-20%), carbohydrates (30%), saponins (0.4%), fibre (0.5%), lecithins (0.5%) and isoflavonoids such as genistein and daidzein. Isoflavones are non-nutritive substances with medicinal benefits (Radhakrishnan, 2009) [18]. It lowers the risk of cardiovascular disease, osteoporosis, diabetes as well as the severity of menopausal symptoms (Chandrawat *et al*., 2014) [7]. Because of its high protein content, it is known as poor man's meat. Soybean oil is also used for edible purpose. Aside from oil and protein, it has the ability to fix atmospheric nitrogen with the support of root nodule bacteria and add organic matter to the soil, thereby increasing the fertility of soil. Environmental and physiological factors are affecting the seed quality. High quality seed provides better plant stand which is beneficial for production and expansion of this crop. Lignin is of great interest because its deposition in seed coat tissue not only provides mechanical resistance but also protects the cell from attack of microorganisms. Peroxidase is a major component of cell walls providing rigidity for structural support as well as water impermeability (Campbell and Sederoff, 1996) [4]. Lignin content of seed coat determined by gravimetric method was found to be high in soybean cultivars, with high index of resistance to mechanical damage (Alvarez *et al*., 1997) [2].

The seed coat of the soybean seed is very thin and low in lignin content, it provides little protection to the vulnerable radicle that lies directly beneath the seed coat of dicot soybean. As a result, mechanical damage is one of the leading causes of significant loss in soybean seed quality during harvest and processing (Franca Neto and Henning, 1984) [8]. Genetic variability in seed resistant to mechanical damage has already been demonstrated among different soybean cultivars (Carbonell and Krzyzanowski, 1995) [6]. Seed coat tissues may accumulate a significant amount of peroxidase in hourglass cells of the sub-epidermis accounting for at least 5% of the protein in dry seed coat (Schmitz *et al*., 1997) [20]. It has been suggested that peroxidase role is to stiffen the cell wall by forming biphenyl bridges between wall polymers (Fry, 1986) [9]. However study of seed coat lignin and peroxidase with respect to other seed

coat characteristics for its resistance to field weathering and mechanical damage deserves the attention with its practical significance in crop improvement. In view of this an experiment was carried out for screening of 30 soybean genotypes for the biochemical aspects of soybean in relation to susceptibility and resistance to mechanical damage.

Material and Methods

The thirty soybean genotypes used for the present investigation were obtained from the Soybean Breeder, Agricultural Research Station Kasbe Digraj, Dist. Satara (MS). Grown on the gross plot size 0.60 x 3.30 m^2 with recommended package of practice and fertilizer dose. The freshly harvested seeds were used for seed coat parameters as well as biochemical analysis. The seed hardness i.e. The mechanical strength of the seed coat was measured as the first break point on the seed cracking using a texture analyzer according to the method of Kuchlan *et al.* (2006) [13]. The seed coat proportion was recorded according to the formula given by (Hoy and Gamble, 1985) [10] i.e. division of weight of seed coat to weight of seed coat and cotyledons X 100. Seed coat thickness was measured by of screw gauge micro meter and expressed in micrometer (µm). The biochemical parameters *viz*., lignin content was determined as per titration method given by (Hussain *et al*., 2002) [11]. The seed coat peroxidase activity was assayed as per the procedure given by Cakmak and Horst (1991) [3] with modifications by Santos *et al.* (2002) [19]. The data was statistically analyzed through analysis of variance (ANOVA) technique for factorial controlled randomized design and presented at 5% level of significance (P = 0.05) by the procedure prescribed by Panse and Sukhatme (1995) [17] and the percentage data was transformed into arcsine values and utilized for statistical analysis (Snedecor and Cochron, 1968) [21].

Result and Discussion

A. Performance of soybean genotypes for seed coat parameters: The performance of different soybean genotypes for different seed coat parameters are presented in Table 1.

Table 1: Performance of soybean genotypes for seed coating parameters

Sr. No.	Genotype	Seed hardness (N)	Seed coat thickness (µm)	Seed coat proportion (%)
1	KDS 980	81.00	12.43	8.73 (17.18)
2	KDS 992	105.00	12.26	9.20 (17.65)
3	KDS 1096	87.00	12.33	9.79 (18.23)
4	KDS 1097	82.00	12.10	8.23 (16.67)
5	KDS 1114	96.66	11.60	8.80 (17.25)
6	KDS 1142	104.66	12.34	9.16 (17.62)
7	KDS 1144	69.00	11.93	8.02 (16.45)
8	KDS 1149	85.00	12.66	9.40 (17.85)
9	KDS 1150	79.33	12.33	9.21 (17.66)
10	KDS 1272	109.00	12.50	9.60 (18.04)
11	KDS 1273	108.00	12.66	9.50 (17.95)
12	KDS 1274	128.00	12.80	10.76 (19.15)
13	MAUS 71	111.33	12.46	9.76 (18.21)
14	MAUS 612	95.00	12.40	9.53 (17.98)
15	NRC 147	108.00	12.32	10.12 (18.54)
16	MACS-NRC-1667	118.33	12.46	9.86 (18.30)
17	MACS 450	120.66	12.53	8.74 (17.19)
18	MACS 1460	107.16	12.35	9.40 (17.85)
19	MACS 1520	99.20	12.06	8.70 (17.15)
20	RCS-11-07	104.13	12.16	9.06 (17.51)
21	EC 390976	98.36	12.60	9.52 (17.97)
22	EC 390977	104.43	12.73	9.26 (17.72)
23	Birsa Soya 1	137.16	12.80	10.99 (19.36)
24	Type 49	114.16	12.40	9.10 (17.55)
25	DSb 21	106.90	12.23	9.49 (17.94)
26	KDS 726	115.10	12.48	9.84 (18.28)
27	KDS 753	111.20	12.40	9.26 (17.72)
28	DS 228	104.10	12.20	9.18 (17.64)
29	JS 9305	107.43	12.63	9.58 (18.03)
30	JS 335	98.50	12.50	8.90 (17.35)
	Mean	104.83	12.39	9.35 (17.80)
	SE (±)	1.231	0.063	0.091
	CD at 5%	3.483	0.179	0.258

*Figures in parenthesis indicate arcsine transformed value

Seed Hardness (N)

The data on seed hardness that is mechanical strength varied significantly among the 30 soybean genotypes. The significantly higher seed hardness of 137.16 N was recorded by genotype Birsa Soya1 followed by KDS 1274 (128 N). Whereas the lowest seed hardness of 69.00 N was recorded by genotype KDS 1144 followed by KDS 1150 (79.33 N). The black seeded genotypes Birsa Soya 1 and KDS 1274 recorded highest seed hardness implying there resistance to field weathering and mechanical damage. Similar results were observed by Kuchlan *et al.* (2010) [14] in different soybean genotypes.

Seed coat thickness (µm)

The data on seed coat thickness was ranged between 11.60 to 12.80 µm. The significantly higher seed coat thickness of

12.80 µm was recorded by genotypesBirsa Soya 1 and KDS 1274 whereas the lowest seed coat thickness of 11.60 µm was recorded in KDS 1114 followed by KDS 1144 (11.93 µm). Higher lignin content coupled with thick seed coat may be responsible for high mechanical strength of seed. Similar results were observed by Agarwal and Menon (1974) [1] in different soybean genotypes.

Seed coat proportion

The significantly highest seed coat proportion of 10.99% was recorded in genotype Birsa Soya 1 followed by KDS 1274 (10.76%). While it was found lowest 8.02% in KDS 1144 followed by KDS 1097 (8.23%). Kuchlan *et al*. (2010) [14] studied that the seed coat proportion of soybean seeds ranged from 7.07 to 11.25%. It was further observed that black seeded genotypes of soybean had higher seed coat proportion than that of yellow seeded soybean genotypes.

A. Performance of soybean genotypes for biochemical parameters useful for tolerance to field weathering and mechanical damage: The performance of different soybean genotypes for different seed coat parameters are presented in Table 2.

Table 2: Performance of soybean genotypes for biochemical parameters

Sr. No.	Genotype	Seed coat lignin (%)	Peroxidase activity (ΔA min^{-1} g^{-1})
1	KDS 980	4.80 (12.65)	223.00
2	KDS 992	6.69 (14.99)	358.53
3	KDS 1096	4.41 (12.11)	311.93
4	KDS 1097	4.58 (12.36)	268.80
5	KDS 1114	4.90 (12.78)	293.40
6	KDS 1142	5.50 (13.56)	282.33
7	KDS 1144	4.26 (11.91)	290.06
8	KDS 1149	5.44 (13.49)	288.06
9	KDS 1150	4.54 (12.30)	356.20
10	KDS 1272	8.91 (17.37)	254.73
11	KDS 1273	8.82 (17.27)	284.00
12	KDS 1274	11.52 (19.84)	415.46
13	MAUS 71	5.96 (14.13)	280.53
14	MAUS 612	5.73 (13.84)	293.00
15	NRC 147	6.52 (14.79)	333.40
16	MACS-NRC-1667	7.00 (15.34)	295.86
17	MACS 450	7.11 (15.46)	344.60
18	MACS 1460	5.74 (13.86)	244.73
19	MACS 1520	4.85 (12.72)	273.06
20	RCS-11-07	5.00 (12.92)	232.86
21	EC 390976	5.85 (13.99)	275.73
22	EC 390977	5.60 (13.68)	237.00
23	Birsa Soya 1	13.88 (21.86)	440.20
24	Type 49	7.41 (15.79)	337.00
25	DSb 21	5.83 (13.97)	286.46
26	KDS 726	7.75 (16.17)	365.73
27	KDS 753	7.49 (15.89)	343.53
28	DS 228	5.46 (13.51)	256.86
29	JS 9305	5.88 (14.04)	277.60
30	JS 335	5.98 (14.15)	269.80
	Mean	6.45 (14.56)	300.48
	SE (±)	0.167	5.687
	CD at 5%	0.473	16.087

*Figures in parenthesis indicate arcsine transformed value

Seed coat lignin (%)

The data pertaining to seed coat lignin content varied significantly among the 30 soybean genotypes. The data on seed coat lignin content was ranged between 4.26 to 13.88%. The significantly higher seed coat lignin content of 13.88% was recorded by genotypeBirsa Soya 1 followed by KDS 1274 (11.52%). Whereas the significantly lower seed coat lignin content of 4.26% was recorded in genotype KDS 1144 followed by KDS 1096 (4.41%). The results observed by Marwanto and Marlinda (2003) showed that black coloured genotypes had higher lignin content. The genotypes with high lignin content positively correlated with seed hardness implying involvement of lignin content with tolerance to field weathering and mechanical damage in soybean genotypes. Similar results were reported by Huth *et al.* (2016) [12] and Kuchlan *et al*. (2018) [15] in different soybean genotypes.

Seed coat peroxidase activity (ΔA min^{-1} g^{-1})

The seed coat peroxidase activity varied significantly among the soybean genotypes. The peroxidase activity was ranged between 223 to 442.20 ΔA min^{-1} g^{-1} of fresh seed weight. The highest seed coat peroxidase activity of 442.20ΔA min^{-1} g^{-1} of fresh seed weight was recorded in Birsa Soya 1 followed by KDS 1274 (415.46). Whereas the lowest seed coat peroxidase activity of 223ΔA min^{-1} g^{-1} of fresh seed weight was recorded in genotype KDS 980 followed by RCS-11-07 (232.86ΔA min^{-1} g^{-1}). Capeleti *et al*. (2005) [5] reported that the soybean seed coat peroxidase activity ranged from 162-586 µmol$min^{-1}$$g^{-1}$. In present research highest lignin and peroxidase activity was recorded in black coloured genotypes. Similar results were reported by Thmobre *et al.* (2017) in different soybean genotypes.

Conclusion

Significant variation was observed in all the 30 soybean genotypes for seed coat and biochemical parameters useful for varietal screening for field weathering and mechanical damage during threshing and processing practices. The genotype Birsa Soya 1 and KDS 1274 recorded the highest seed coat proportion (10.99% and 10.76%), seed hardness(137.16 N and 128 N), seed coat lignin (13.88% and 11.52%) and seed coat peroxidase activity (440.20 and 415.46 ΔA min^{-1} g^{-1}) respectively.

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