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# Influence of seed coat compactness around cotyledons, protein and mineral composition on mechanical strength of soybean [*Glycine max* (L.) Merrill] seed coat

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## ABSTRACT

The critical problem of soybean seed is susceptibility to mechanical processes due to structural limitations of seed and seed coat. The X-radiography of soybean seed revealed the orientation of seed coat around the cotyledons and cultivars like JS 335, JS 9752, JS 71-05, JS 95-60, MACS 124, Pusa 40, Punjab 1 and Type 49 had very compact seed coat around cotyledons. In NRC 12, NRC 37, RKS 18 and SL 525 where the gap between seed coat and cotyledons was significantly very high and non uniform making seed coat very much vulnerable to cracking. Seed coat minerals- calcium, silicon, zinc, iron and lignin content were found to vary significantly among cultivars. Significant genotypic variability for strength of seed coat was observed among the soybean cultivars. A very close relationship was found between strength of seed coat and seed coat compactness. Highly significant correlation was found between mechanical strength and seed coat lignin, iron and zinc content. Quantitative and qualitative variation for seed coat protein among soybean cultivars was revealed and higher protein content was found linked with mechanically strong seed coat. The result revealed that selection for these seed coat parameters in varietal improvement programme may be very fruitful for development of varieties having resistance to field weathering and mechanical damage.

**Key words:** Mineral composition, Seed coat, Seed coat lignin, Seed coat protein, Soybean seed.

## INTRODUCTION

Loss of seed germination due to mechanical damage is critical problem in quality seed production in soybean. Soybean seed coat is very susceptible to mechanical forces during harvesting –threshing and post harvest operations due to its thin seed coat and low lignin content as reported by several researchers. Genetic variability in seed for resistance to mechanical damage among different soybean cultivars had been demonstrated (Carbonell and Kryzanowski, 1995, Kuchlan *et al.,* 2010a and Kuchlan *et al.,* 2010b). Lignin is complex polymer of hydroxylated and methoxylated phenyl propane units linked via oxidative coupling catalyzed by peroxidases. It is a major constituent of cell wall and provides strength to cell and structural support and impermeability to water (Campbell and Sederoff, 1996). Strength of seed coat was a major concern of soybean seed researchers to improve seed quality. Seed coat strength and seed coat permeability were highly influenced by the structural factors and chemical composition of seed coat. Calcium content in the seed coat was found to be positively correlated with water absorption (Saio *et al*,. 1973 and Saio, 1976). Mineral composition plays an important role in cell wall structure and strength of cell wall. Among these, important mineral compositions are calcium, iron, zinc, magnesium and silicon.

Present investigation was undertaken to find out the seed coat lignin, mineral composition and protein content influencing strength of seed coat of soybean and seed coat compactness around the cotyledon for its role in seed coat cracking.

## MATERIALS AND METHODS

The soybean varieties were grown at the experimental farm of Directorate of Soybean Research, Indore. The seeds were manually threshed to avoid any injury to seed and seed coat.

The seed coat strength was measured by Texture Analyzer (Stable Micro System, UK). Mechanical strength of the seed coat was measured as the first break point on the graph of seed cracking using the texture analyzer (Stable Micro System) TA.XT PLUS. The seed moisture of all the cultivars was brought to equilibrium moisture condition (~9%) in desiccators with dry silica gel. The seed was placed on the stationary metallic plate of the instrument using load cell of 500 kg capacity. Test speed 0.5mm/sec and post test speed was 2.0mm/sec. The plane perpendicular to the plane of the hilum was parallel to the surface of the plate. The force was applied with moving the piston downward. The moment the moving piston comes in contact of the seed

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surface, the pressure is created on the surface of the seed. The increase of force is plotted along the Y-axis and the duration of force applied, until the seed is crushed into pieces, is plotted along the X-axis. There is a gradual increase in the graph with the increase of force and after a certain time (fraction of a second), there is a sudden drop on the graph indicating the first break in the seed and that is for the seed coat surface. Again, the force increases along the graph and next drops on the graph are due to compression of seed and subsequently for the pieces of seed.

X-radiography study was undertaken at the Softex X-radiography facility at IARI, Regional Station, Karnal, Haryana. Seeds of soybean varieties were equilibrated at uniform moisture content in desiccators with dry silica and each seed was scanned under X-ray.

Seed coat lignin was estimated following the protocol of Capeleti *et al.*, 2005, Chen *et al.*, 2000 and Ferrarese *et al.*, 2002. Seeds were initially immersed in water for approximately 12 h in a 50 ml beaker to separate the seed coat from the cotyledons. Seed coats were dried in an oven (16 h, 105 °C) and kept in desiccators to cool down. Dry coats (0.3 g) were homogenized in 50 mM potassium phosphate (7cm$^3$, pH7.0) buffer with mortar and pestle and transferred into a centrifuge tube. The pellet was centrifuged (1,400 *g*, 4min) and washed by successive stirring and centrifugation, as follows: twice with phosphate buffer pH 7.0 (7 cm$^3$); three times with 1% (v/v) Tri ton® X-100 in pH 7.0 buffer (7 cm$^3$); twice with 1 M NaCl in pH 7.0 buffer (7 cm$^3$); twice with distilled water (7 cm$^3$) and twice with acetone (5 cm$^3$). Pellet was left over night in desiccators for drying. Pellet was finally dried in an oven (24 h, 60 °C). Cooled down in vacuum desiccators, the dry matter obtained was defined as the protein free cell wall fraction. To confirm whether protein or other ultraviolet absorbing materials were extracted during the preparation of the cell wall fraction, the supernatant was monitored by absorbance measurement at 280 nm. Further, dry protein free tissue (0.1 g) was placed into a screw-cap centrifuge tube containing the reaction mixture (1.2 cm$^3$ of thioglycolic acid plus 6 cm$^3$ of 2 M HCl) and heated (95 °C, 4 h). After cooling at room temperature, the sample was centrifuged (1,400 *g*, 5 min) and the supernatant decanted. The pellet was washed three times with distilled water (7 cm$^3$) and the product extracted by shaking (30°C, 18 h, 115 oscillations/min) in 0.5 M NaOH (6 cm$^3$). After centrifugation (1,400 *g*, 5 min), the supernatant was stored and mixed with supernatant obtained from a second pellet washed with 0.5 M NaOH (3cm$^3$). The combined alkali extracts were acidified with concentrated HCl (1.8 cm$^3$). The lignothioglycolic acid (LTGA) formed after 4 h at 0°C was recovered by centrifugation (1,400 *g*, 5 min) and washed twice in distilled water (7 cm$^3$). The pellet was dried at 60°C, dissolved in 0.5 M NaOH, and diluted to yield an appropriate absorbance for spectrophotometric determination at 280 nm.

Seed coat protein estimation was done by Kjeldahl method. 0.1 g finely grind seed coat sample was digested with 10 ml concentrated $H_2SO_4$ in presence of 1 spoon $CuSo_4.5H_2O$: $K_2SO_4$ (1:5). The free nitrogen was converted to ammonical form by adding 40-50 ml 40% NaOH solution to the digested sample. Free ammonia was trapped in 20 ml Boric acid solution (4%) in auto-distillation unit. The nitrogen was estimated by titrating against 0.1N HCl.

SDS-PAGE profile of seed coat protein was done at 10% acrylamide gel. 0.1 g seed coat was crushed in 1.0 ml of 25 mM Tris-Glycine buffer and centrifuged at 10000 RPM and 300 µl of supernatant was extracted. 100 µl of supernatant was loaded in SDS-PAGE for seed coat protein profile. The gel was put in 15% trichloro acetic acid (TCA) overnight and after that gel was put in a staining solution containing 100 ml of (15%) TCA and 15 ml of 1% coomasie blue till the bands appear. The staining solution was drained out and distaining solution was poured into the tray containing the gel. The gel was distained till development of clear appearance of the bands.

500 mg seed coat of each variety was digested with tri-acid (sulphuric acid : nitric acid : tricholoroacetic acid at 2:1:1) over hot plate for 30 min. The extracted solution was diluted with distilled water and the volume was made to 100 ml.10 ml of sample solution was taken and to this sample, 10 ml distilled water, 4 ml 8 M KOH and pinch of muroxide (indicator) were added. This sample mixture was titrated against 0.01 M EDTA solution until the red colour is converted to bluish violet colour.The amount of calcium is calculated on the basis of amount of standard EDTA solution required for titration as 1 ml 0.01 M EDTA is equivalent to 0.4008 mg of $Ca^{2+}$. The digested material was used for analysis of iron and zinc by Atomic Absorption Spectrophotometer (GBC-Avanta-AA Series)

Silicon in seed coat was estimated by the protocol of Weaver *et al.* (1968). 5 ml of digested seed coat in tri-acid was taken to a 50 ml volumetric flask and 10 ml of 1(N) $H_2SO_4$ was added to it followed by 10ml of 0.3M $MoO_4$. After 2 min 5 ml of 20% Tartaric acid was added followed by 1 ml 1-amino-2-naphthol-4-sulphonic acid. The volume was made by distilled water and reading was recorded at 820 nm.

**Statistical analysis:** Statistical analysis was performed using MSTAT-C software. The data obtained from the experiment was statistically analyzed by using CRD and correlation analysis of means of different parameter. The critical differences (CD value) for each parameter between cultivars were worked out at 5% significance level.

## RESULTS AND DISCUSSION

**Seed coat compactness around cotyledons:** The x-radiography of seeds revealed the orientation of seed coat around the cotyledons. The outer dark circle was the seed coat and the inner dark are was the cotyledons and the light between coat and cotyledons was the gap. The study revealed significant variation in the compactness of seed coat around cotyledons. There were varieties namely JS 71-05, JS 335, JS 9752, JS 95-60, MACS 124, Pusa 40, Punjab 1 and Type 49 which had very compact seed coat around cotyledons with very less gap between seed coat and cotyledons (Fig 1). In some varieties namely JS 79-81, JS 9305, MAUS 47, MAUS 61-2, PK 471, PS 1042, Pusa 22, Pusa 24, the gap was comparatively more (Fig 1). Very high gap was observed in cultivars NRC 12, NRC 37, RKS 18 and SL 525 where the gap was not uniform throughout the periphery of the seed. It was found that the gap is more in that site of hilum which is opposite to radicle axis. The seed coat wrinkling site was revealed as undulating seed coat in cultivars NRC 37, NRC 12, Pusa 22, Pusa 40, PK 1042, PK 471 and MACS 124 (Fig 1). The seed coat distortion or twist was revealed when the gap between seed coat and cotyledons was higher as in case of RKS 18, NRC 12.

**Seed coat lignin content:** Seed coat lignin content was found to be uniformly distributed among the soybean cultivars ranging from 181.9219 to 564.8621 mg/100g seed coat. The cultivars namely Lee, MACS 450, MAUS 47, VLSoya 1, PS 1042 and Type 49 contained more than 400 mg/100g seed coat. Varieties namely SL 525, RKS 18, NRC 12, JS 79-81, JS 95-60, NRC 37, JS 71-05, Pusa 24, MAUS 2 etc. were having lesser lignin in seed coat (less than 300 mg/100g seed coat). Varieties which had lignin in between 300 to 400 mg/100 g seed coat were PK 471, MAUS 61-2, Type49, JS 9752,Pusa 22, Pusa 40 and MACS124 (Table 1).

**Mechanical strength of soybean seed coat:** The most critical problem in soybean is reported to be damage due to mechanical process of harvesting and processing. Mechanical strength among studied soybean cultivars varied significantly with a range of 90.75 Newton in SL 525 to 149.25 Newton in Type49. Promising cultivars with higher amount of strength (more than 130 Newton) of seed coat were Type49, VL Soya1, MACS 450, Lee, JS 9752 and MAUS 47. Cultivars very poor in seed coat strength were SL 525, RKS 18 and NRC 12 which had less than 100 Newton of strength of seed coat (Table 1). Other cultivars had seed coat strength between 100 to 130 Newton.

**Mineral content of seed coat:** The mineral composition of seed coat of soybean cultivars was studied for silicon, calcium, iron, and zinc content. Calcium content of seed coat varied from 320.64 to 721.44 mg/100 g seed coat (Table 1). Punjab 1, Pusa 22, Type 49, JS 335 and NRC 37 had higher amount (more than 500 mg/100 g seed coat) of calcium in seed coat and varieties namely MACS 124, Pusa 22, MAUS 61-2, MAUS 2, PK 471, RKS 18, SL 525 were having lower

**Table 1:** Mechanical strength, lignin and mineral composition of seed coat of soybean cultivars.

| Varieties | Mechanical strength of seed coat (Newton) | Lignin content of seed coat (mg/ 100 g seed coat) | Calcium content of seed coat (mg/ 100 g seed coat) | Silicon content of seed coat (mg/ 100 g seed coat) | Iron content of seed coat (mg/ 100 g seed coat) | Zinc content of seed coat (mg/ 100 g seed coat) |
|---|---|---|---|---|---|---|
| Bragg | 127.85 | 346.6105 | 400.8 | 49.750 | 38.96 | 9.8 |
| JS 335 | 129.54 | 294.9972 | 521.04 | 58.583 | 38.71 | 9.45 |
| JS 71-05 | 112.14 | 258.9292 | 414.16 | 41.750 | 36.61 | 7.41 |
| JS 79-81 | 114.4 | 242.1124 | 400.8 | 35.250 | 41.31 | 7.37 |
| JS 9305 | 118.43 | 287.0733 | 400.8 | 49.750 | 34.56 | 7.57 |
| JS 95-60 | 104.97 | 250.0056 | 400.8 | 36.583 | 30.38 | 5.59 |
| JS 9752 | 138.25 | 355.6016 | 400.8 | 44.583 | 33.37 | 7.96 |
| Lee | 143.12 | 564.8621 | 400.8 | 53.000 | 37.09 | 9.61 |
| MACS 450 | 145.15 | 513.878 | 407.48 | 48.583 | 34.89 | 10.42 |
| MACS124 | 127.28 | 358.6 | 320.64 | 38.417 | 31.71 | 8.63 |
| MAUS 2 | 119.18 | 278.9124 | 360.72 | 51.250 | 32.53 | 9.42 |
| MAUS 47 | 134.89 | 440.6357 | 400.8 | 43.250 | 41 | 5.92 |
| MAUS 61-2 | 101.21 | 301.159 | 360.72 | 56.583 | 24.15 | 6.79 |
| NRC12 | 95.86 | 216.6522 | 440.88 | 39.873 | 32.2 | 6.57 |
| NRC 37 | 113.49 | 251.5769 | 521.04 | 57.167 | 35.71 | 7.71 |
| PK 471 | 109.87 | 299.3325 | 360.72 | 42.417 | 31.6 | 7.18 |
| PS 1042 | 115.23 | 374.5551 | 400.8 | 33.000 | 37.5 | 8.92 |
| Pusa 22 | 121.01 | 355.2725 | 320.64 | 36.083 | 35.67 | 9.11 |
| Punjab 1 | 106.28 | 281.2983 | 721.44 | 45.917 | 32.46 | 7.47 |
| Pusa 24 | 104.21 | 278.6955 | 400.8 | 42.667 | 31.92 | 8.45 |
| Pusa 40 | 101.32 | 342.0974 | 480.96 | 51.750 | 24.53 | 7.73 |
| RKS 18 | 91.25 | 208.0358 | 360.72 | 45.250 | 31.68 | 7.31 |
| SL 525 | 90.75 | 181.9219 | 400.8 | 47.583 | 28.72 | 7.68 |
| Type 49 | 149.25 | 336.2229 | 521.04 | 45.333 | 38.01 | 9.14 |
| VL Soya1 | 140.14 | 405.042 | 440.88 | 50.500 | 36.61 | 9.72 |
| $LSD_{0.05}$ | 5.683 | 46.31 | 45.10 | 2.349 | 1.274 | 0.4555 |

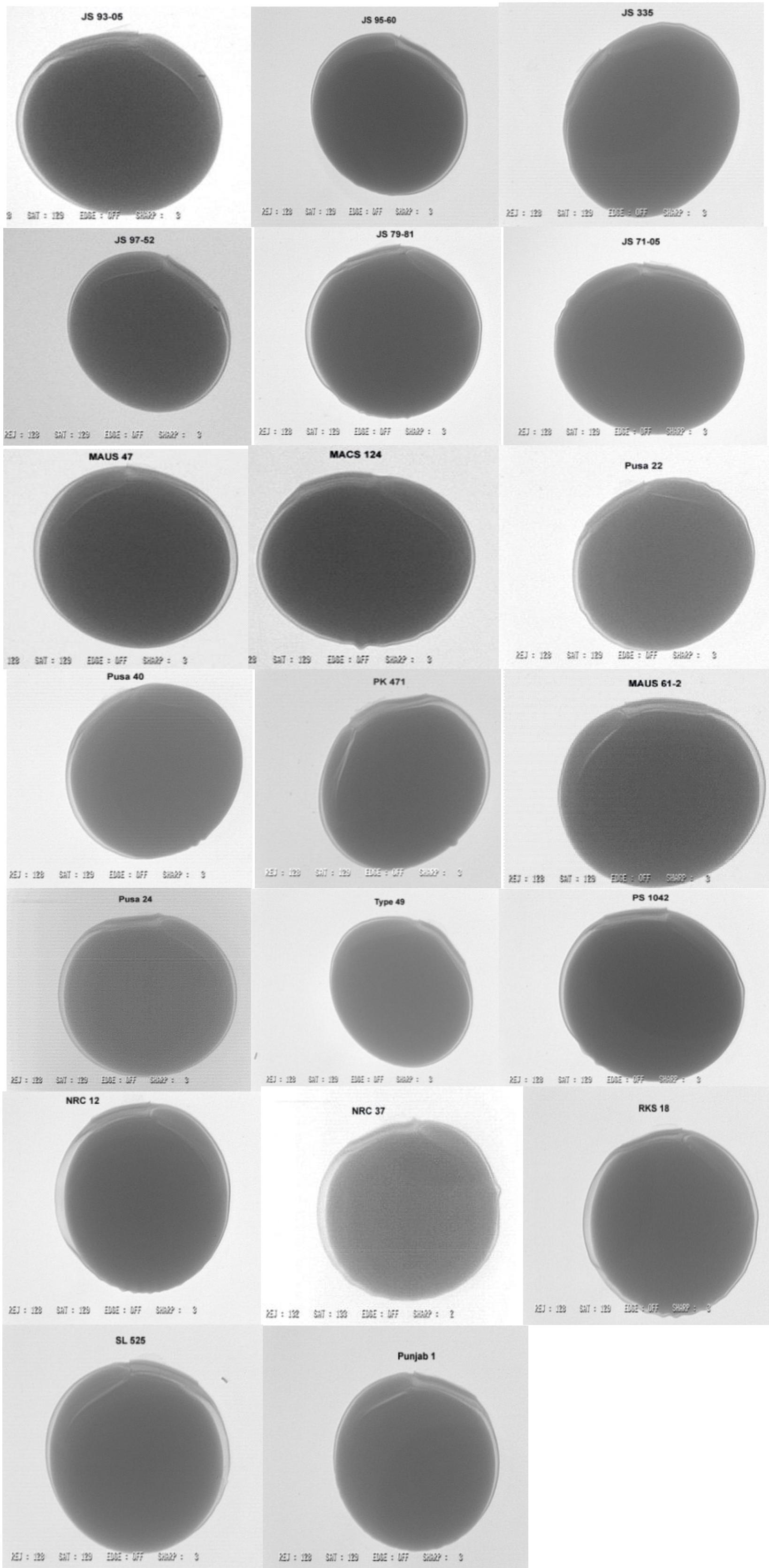


**Fig 1:** X-radiography of soybean seeds showing gap between seed coat and cotyledons

amount (less than 400 mg/100 g seed coat) of calcium in seed coat. Other tested cultivars had calcium content in the range of 400 to 500 mg/100 g seed coat. Similar with calcium content other minerals silicon, iron and zinc content of seed coat significantly varied among the soybean cultivars. The silicon content of seed coat of soybean cultivars varied from 33 to 58.583 mg/100g seed coat (Table 1). Promising cultivars like JS 335, MACS 450, Type 49, Punjab 1, MAUS 61-2, Lee, Pusa 40 and VL Soya1 contained silicon in seed coat more than 45 mg/100g seed coat. Varieties poor in silicon content in seed coat were PK 1042, JS 79-81, Pusa 22, JS 95-60, MACS 124 and NRC 12 which had less than 40 mg/ 100 g seed coat (values ranged from 33 to 39.873). Variation for iron and zinc content among soybean cultivars was in narrow range. Cultivars with higher amount of iron content (above 35 mg/ 100 g seed coat) were JS 79-81, JS 335, Type 49, Lee, VL Soya1. The correlation study among different parameters and mechanical strength revealed very strong correlation with lignin, iron and zinc content (Table 2).

**Seed coat protein content and protein profile:** In the present study it was found that seed coat contained substantial amount of protein in soybean varieties ranging from 7.88 to 11.38%. Varieties which contained higher amount of protein (>10%) in seed coat were MACS 450, JS 9752, TAMS 38, MAUS 32, PS 1347, PS 1042 *etc*. The varieties with lower range of protein content (less than 9%) were PK 471 and Pusa 24 (Table 3). The SDS-PAGE revealed protein from 14 kDa to about 80 kDa (Fig 2). The twin bands at 70 to 80 kDa were conserved among most of the varieties. The seed coat guaiacol-peroxidase isoforms was expressed at 42 kDa in the cultivars positive for peroxidase. Variations for protein bands between 29 to 40 kDa and for ~51 kDa were found to vary among the cultivars (Fig. 2).

Soybean seed is most sensitive to mechanical injury. There are several report regarding mechanical damage to soybean seed influenced by seed moisture content (Paulsen *et al*., 1981, Singh and Singh, 1981 and Prakobboon, 1982), presence and distribution of hourglass cells in seed coat (Pereira and Andrew, 1985, Kuchlan *et al*., 2010b), thickness of seed coat (Agrawal and Menon, 1974) but rarely any one has reported the compactness of seed coat in relation to mechanical damage. Compactness of seed coat determines amount of depression of seed coat on application of

**Table 2:** Correlation between mechanical strength, lignin content and mineral composition of seed coat in soybean cultivars.

| | Strength | Lignin | Ca | Si | Fe | Zn |
|---|---|---|---|---|---|---|
| Strength | 1.000 | 0.776 | 0.016 | 0.149 | 0.610 | 0.605 |
| Lignin | | 1.000 | -0.105 | 0.127 | 0.315 | 0.519 |
| Ca | | | 1.000 | 0.299 | 0.083 | -0.018 |
| Si | | | | 1.000 | -0.179 | 0.274 |
| Fe | | | | | 1.000 | 0.319 |
| Zn | | | | | | 1.000 |

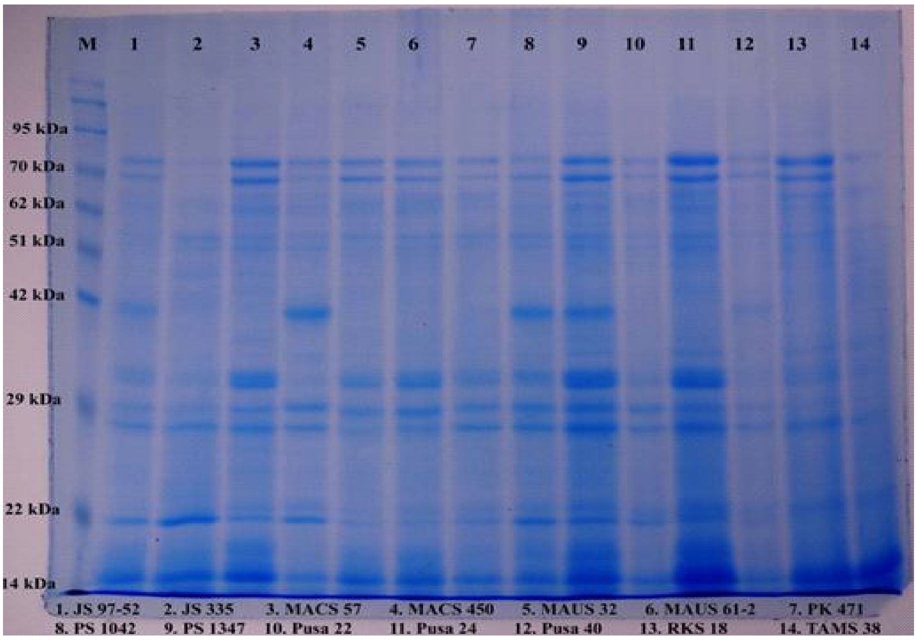


**Fig 2:** Seed coat protein profile

**Table 3:** Nitrogen and protein content of seed coat of soybean cultivars

| Variety | $N_2$ Content (%) | Protein Content (%) |
|---|---|---|
| JS 9752 | 1.7504 | 10.94 |
| JS 335 | 1.5408 | 9.63 |
| MACS 57 | 1.2608 | 7.88 |
| MACS 450 | 1.8208 | 11.38 |
| MAUS 32 | 1.6816 | 10.51 |
| MAUS 61-2 | 1.4704 | 9.19 |
| PK 471 | 1.4016 | 8.76 |
| PS 1042 | 1.6112 | 10.07 |
| PS 1347 | 1.6464 | 10.29 |
| PUSA 22 | 1.5408 | 9.63 |
| PUSA 24 | 1.4016 | 8.76 |
| PUSA 40 | 1.5731 | 9.83 |
| RKS 18 | 1.6112 | 10.07 |
| TAMS 38 | 1.6816 | 10.51 |
| $LSD_{0.05}$ | | 0.6349 |

mechanical forces on the surface of seed. If the gap is more, the depression of seed coat will be more and chance of cracks is more. The seed coat strength was found high with cultivars having compact seed coat and lower mechanical strength of seed coat with higher gap between seed coat and cotyledons. Most biological materials behave viscoelastically which helps avoid mechanical damages (Mohsenin, 1986). In dry seeds the coefficient of elasticity of seed coat is high and seed coat becomes fragile as the seed moisture content decreases (Newberg *et al*., 1980 and Prakobboon, 1982). Therefore, higher gap between seed coat and cotyledons make seed coat more vulnerable to mechanical injury. The gap was high at the site opposite to the area of radicle axis. Famili *et al*. (1980) reported that damage was greatest when the compressive load was applied to the end along the long axis, and was least when applied to the sides of the cotyledons. X-radiographs revealed the gap between the seed coat and cotyledons was more along the long axis and forces applied along this long axis could cause more seed coat cracking. X-radiography revealed distorted or twisted seed coat in RKS 18, NRC 12 which might had occurred due to wetting and drying phenomenon during seed maturity in field and the risk of seed coat distortion or twist was more with higher seed coat gap.

Lignin is found in the seed coat tissue since it is a constituent of cell walls and its occurrence affects the impermeability of soybean seeds (Tavares *et al*., 1987). Its content in the soybean seed coat varies among genotypes (Alvarez *et al*., 1997 and Panobianco *et al*., 1999). Similar result was also observed in this study and significant variation was recorded for seed coat lignin content among the cultivars. High amount of lignin (more than 400 mg/100g seed coat) content in Lee, MACS 450, MAUS 47, VL Soya 1 was associated with higher strength of seed coat which is in concurrence with the findings of Capeleti *et al*. (2005). Significant variability for mechanical strength of seed coat was observed among the cultivars and strength above 130 Newton should be selection criteria for developing cultivars resistant to mechanical damage. The occurrence of genetic variability in seed resistance to mechanical damage among soybean cultivars had been demonstrated (Carbonell and Krzyzanowski, 1995 and Kuchlan *et al.,* 2010a).

VL Soya 1, a black seeded soybean cultivar which is having bold seed size is also having higher seed coat strength due to higher lignin content in seed coat. Thus, this line may have very good potential in soybean breeding programme for developing bold seeded varieties with better seed coat strength.

Genetic variability was observed for seed coat minerals – calcium, silicon, iron and zinc content. High significant correlation was observed between mechanical strength and lignin content of seed coat as well as iron and zinc content. Plant nutrition management should be taken care of otherwise lack of minerals in seed coat may impact on strength of seed coat. Zhang *et al.,* (2009) reported genetic variation for calcium content (0.21 to 0.38%) in soybean seed and QTL markers for proper calcium content, as high calcium content in seed was associated with hardness. Therefore, calcium content ~ 500 mg/100 g seed coat and silicon content above 50 mg/100g seed coat may have significant impact on seed coat strength.

The seed coat serves as a multifunctional organ with a role in protection and for the supply of nutrients to the embryo sac during development. The composition of the legume seed coat differs from other seed tissues in many ways including its protein composition. An abundant 24 kDa protein (SC24) has been purified and identified from soybean seed hulls (Sangeeta-Dhaubhadel *et al*., 2005). Several other workers also reported other functional protein in seed coat. The presence of protein and variation have critical role in seed coat properties. Sessa and Wolf (2001) reported that soybean seed coat contain 1.56 % kjeldahl nitrogen and seed coat contains 8 kDa Bowman-Birk inhibitor (BBI) and peroxidase along with three unknown proteins, each of which possessed an estimated MW of less than 10 kDa. The nitrogen content of seed coat in the range of 1.26 to 1.75% was in concurrence to the result of Sessa and Wolf, (2001). But present study revealed several high molecular weight proteins of about 80 kDa in seed coat. Therefore, seed coat protein had significant genotypic variation to play key role in seed coat properties and there is scope for further investigation of role of such high molecular weight proteins in soybean seed coat.

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**REFERENCES**


Agrawal, P.K. and Menon, K. (1974). Lignin content and seed coat thickness in relation to seed coat cracking in soybean. *Seed Res.*, **2:** 64-65.

Alvarez, P.J.C., Krzyzanowski, F.C., Mandarino, J.M.G. and Franca, N.J.B. (1997). Relationship between soybean seed coat lignin content and resistance to mechanical damage. *Seed Sci. Tech.,* **25:** 209-214.

Campbell, M.M. and Sederoff, R. (1996). Variation in lignin content and composition- mechanism of control and implications for genetic improvement of plants. *Plant Physio.*, **110:** 3-13

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

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

Chen, M., Sommer, A.J. and McClure, J.W. (2000). Fourier transform-IR determination of protein contamination in thioglycolic acid lignin from radish seedlings, and improved methods for extractive-free cell wall preparation. *Phytochemical Analysis*, **11:** 153-159.

Famili, A., Huber, S.G., Holmes, R.G. and Herum, F.L. (1980). Effect of compression strain on soybean seed quality. *Transaction of ASAE*, **80:** 3540.

Ferrarese, M.L.L., Zottis, A. and Ferrarese-Filho, O. (2002). Protein-free lignin quantification in soy bean (*Glycine max* L.) roots. *Biologia*, **57:** 541-543.

Kuchlan, P., Husain, S.M. and Chauhan, G.S. (2010a). Evaluation of soybean (Glycine max) genotypes for seed longevity. *Indian J. of Agric. Sci*., **80:** 141-145.

Kuchlan, M.K., Dadlani, M. and Samuel, D.V.K. (2010b). Seed Coat Properties and Longevity of Soybean Seeds. *J. New Seeds*, **11:** 239-249.

Mohsenin, N.N. (1986). Physical Properties of Plant and Animal Materials. 2nd ed.; Gordon and Breach Science Publishers, New York.

Newberg, R.S., Paulsen, M.R. and Nave, N.R. (1980). Soybean quality with rotary and conventional threshing. *Transaction ASAE*, **23:**303-308.

Panobianco, M., Vieira, R.D., Krzyzanowski, F.C. and Franca-Neto, J.B. (1999. Electrical conductivity of soybean seed and correlation with seed coat lignin content. *Seed Sci. Tech.,* **27:** 945-949.

Paulsen, M.R., Nave, Q.R. and Gray, L.E. (1981). Soybean seed quality as affected by impact damage. *Transaction of ASAE*, **22:** 1475-1479.

Pereira, L.A.G. and Andrew, C.H. (1985) Comparison of non-wrinkled and wrinkled soybean seed coats by scanning electron microscopy. *Seed Sci. Tech.,* **13:** 853-859.

Prakobboon, N. (1982). A study of abnormal seedling development in soybean as affected by threshing injury. *Seed Sci. Tech.,* **10:**495-500.

Saio, K., Arai, K., and Watanabe, T. (1973). Fine structure of soybean seed coat and its changes on cooking. *Cereal Sci. Today*, **18:** 197-201.

Saio, K. (1976). Soybeans resistant to water absorption. *Cereal Foods World*, **21:** 168-173.

Sangeeta-Dhaubhadel, Kuflu, K., Romero, M.C. and Gijzen, M. (2005). Soybean seed protein with carboxylate-binding activity. *J. Experimental Botany*, **56:** 2335-2344.

Sessa, D.J. and Wolf, W.J. (2001). Bowman–Birk inhibitors in soybean seed coats. *Industrial Crops Production*, **14:** 73–83.

Singh, K.N. and Singh, B. (1981). Effect of crop and machine parameters on threshing effectiveness and seed quality of soybean. *J. Agric. Eng. Res.*, **26:** 349-355.

Tavares, D.Q., Miranda, M.A.C., Umino, C.Y. and Dias, G.M. (1987). Structural characteristics of seed coat of permeable and impermeable of seeds of soybean breeding line. *Revista Braseleira de Botanica*, **10:** 147-153.

Weaver, R.M., Syers, J.K. and Jackson, M.L. (1968). Determination of silica in citrate-Bicarbonate-dithionate extract of soils. *Soil Sci. Soc. American Proc.*, **32:** 497-501.

Zhang, B., Chen, P., Shi, A., Hou, A., Ishibashi, T. and Wang, D. (2009). Putative quantitative trait loci associated with calcium content in soybean seed. *J. Heredity*, **100:** 263–269