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RESEARCH ARTICLE

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Effect of Seed Coat Characteristics on Seed Quality in Soybean [*Glycine max* (L) Merrill] Genotypes with Contrasting Seed Longevity Traits

Balachandan Gowda C., S.K. Jain, Monika A. Joshi, Dharmpal Singh

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ABSTRACT

Background: The soybean seed is highly susceptible to field weathering and mechanical damages which adversely affect its longevity. Non-availability of good quality seed and post-harvest maintenance of prescribed level of seed germination and vigour until immediate planting season is the major constraint for soybean seed production. In soybean, the seed coat is one of the main determinants of seed germination, vigour and longevity potentials.

Methods: In view of the same, the present study was conducted during 2015-16 and 2016-17 on 24 soybean genotypes having contrasting seed longevity traits. Freshly harvested seeds were stored for eight months under laboratory ambient environment (25±2°C and 65±5% RH) for evaluation of seed coat properties which are helpful in protecting the seeds from mechanical damage during pre- and post-harvest operations and study its relation with the seed quality.

Result: Black, small-seeded soybean genotypes with white hilum were found to be better storers. Thick seed coat and higher seed coat (%) with more and uniform hours glass cells attributed to better seed quality for these genotypes. Parameters like seed coat (%), lignin content and mechanical strength remained unchanged during eight months laboratory ambient storage. Based on these observations, genotypes AMSS-34 and G-2265 were identified as better genotypes, which may be exploited in soybean crop improvement programme.

Key words: Hour glass cells, Lignin content, Mechanical strength, Seed coat percentage, Seed coat thickness, Seed quality, Soybean.

INTRODUCTION

Soybean [*Glycine max* (L) Merrill] is a native of eastern Asia and belongs to botanical family *Fabaceae*. It contains 20 percent oil and 38-42 percent high quality protein possessing high level of essential amino acids like lysine (5%), minerals (4%), phospholipids (2%) and vitamins A and D (Hymowitz and Harlan, 1983). At physiological maturity, the soybean seed reaches its maximum potential for germination and vigour. This potential is short lived and reduces substantially. A number of seed characters, which are under genetic control such as seed size, per cent hard seededness, permeability and oil content are reported to be associated with seed quality in soybean.

The seed coat is the seed's primary defense against adverse environmental conditions. It is the main modulator of interactions between the internal structures of the seed and the external environment. A hard seed coat protects the seed not only from mechanical stress but also from microorganism invasion and also from temperature and humidity fluctuations during storage. Phenolic compounds in the seed coat contribute to seed hardness and inhibition of microorganism growth. Lignins and phenolic polymers found in the cell wall are believed to contribute to the compressive strength, resistance to degradation by microbial attack and water permeability to the polysaccharide protein matrix of the cell wall (Francisco *et al.*, 2008). The seed coat is one of the main determinants of seed germination, vigour and longevity potentials. However, there are no inter-varietal reports on seed coat characterization. Hence, seed coat characterization of soybean genotypes showing contrasting longevity patterns needs to be addressed to develop soybean varieties with better seed longevity. Therefore, the present study was undertaken to study the Effect of seed coat characteristics on seed quality in soybean [*Glycine max* (L) Merrill] genotypes with contrasting seed longevity traits.

Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi- 110 012, India.

Corresponding Author: Monika A. Joshi, Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi-110 012, India. Email: monikakshat622@gmail.com

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MATERIALS AND METHODS

The present study was carried out both in the experimental farm and laboratory of Division of Seed Science and Technology, IARI, New Delhi during 2015-16 and 2016-2017 using 24 soybean genotypes having contrasting seed longevity traits, comprising 12 good (black-seeded) storers and 12 poor (yellow-orange seeded) as the experimental material, which was procured from Soybean Section, Division of Genetics, ICAR-IARI, New Delhi. About 200 g of untreated freshly harvested soybean seeds from each genotype were packed in muslin cloth bags and stored in the laboratory of Division of Seed Science and Technology under ambient storage environment (average 25±2°C and 65±5% RH). Various parameters were recorded to categorize the soybean genotypes using established methodologies and protocols, as under.

Seed coat colour and seed hilum colour

Both the parameters were recorded by using Royal Horticultural Society (RHS) colour chart during the harvest stage under natural day light.

Seed coat percentage

Seed coats from 5 g seeds in two replications were removed carefully with the help of forceps after applying a gentle stroke to seeds placed in-between the muslin cloth. Seed coat and cotyledons were collected and weighed separately. The total weight of seed coat and cotyledon was considered as the seed weight. The proportion of seed coat was calculated as follows:

$$\text{Seed coat percentage} = \frac{\text{Weight of seed coat}}{\text{Weight of seed}} \times 100$$

Mechanical strength of the seed coat

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) following the method of Kuchlan (2006). The seed was placed on the stationary plate of the instrument. The plane perpendicular to the plane of the hilum was parallel to the surface of the plate. The force was applied by gradually increasing the force, which was plotted along the Y-axis. The duration of force applied (until the seed is cracked) was plotted along the X-axis. There was a gradual increase in the graph with the increase of force and at a certain point of time there was a sudden drop on the graph which indicated the first break, on the seed coat surface. Further increase in the force along the graph and next drop on the graph was recorded due to breakage and subsequently for the pieces of seed.

Seed coat thickness and seed coat histology

Various steps as below were undertaken to study these parameters:

Dehydration of samples and block preparation

Dehydration of samples was done using TBA series.

Seed samples

Fixed in FAA → Wash in 30%/50%/70% Ethanol → 55%TBA (2hr) → 75%TBA (overnight) → 85% TBA(1hr) → 95% TBA(1hr) → 85% TBA → 95% TBA(1hr) → 100% TBA (overnight) → Paraffin oil:TBA (1:1) (1hr) Paraffin wax+Paraffin oil+TBA in oven → Paraffin wax (overnight) → Paraffin wax (blocks).

After that paraffin blocks were prepared. These blocks were subjected to microtome sectioning and drying.

Staining techniques

Sections → Xylene (2hr or overnight) → Xylene: Alcohol (1:1) (5 min) 95% Alcohol (5min) → 70% Alcohol (5min) 50% Alcohol (5min) → Safaranin (45 min) → 50% Alcohol (5min) → 70% Alcohol (5 min) → 95% Alcohol (5min) Fast Green (a dip only) → 95% Alcohol (5 min) → Xylene: Alcohol (1:1) (5 min) → Pure Xylene → DPX Mount.

Thickness of the seed coat was measured using phase contrast microscope (Leica software) which is connected to a computer. Finally, using phase contrast microscope, the thickness of parenchyma cells (epidermis), thickness of palisade parenchyma (hypodermis) and length of anticlinal cells were measured (µm). The stained seed coat sections were viewed using phase contrast microscope which is connected to a computer. Photographs were recorded using the above set up.

Lignin content

Lignin content of the seed coat was determined colorimetrically. The sample was extracted in NaOH solution and the aliquot was adjusted to pH 7.0 and 12.3. The amount of lignin was calculated by the difference between *A 245* (pH 7.0) and *A 350* (pH 12.3), *i.e.* E 350/sample. Moisten 100 mg of oven dried experimental material in a mortar with water. Add 2 ml of NaoH to residue and extract 70-80°C for 12-16hr. Cool, add 0.45 ml of 2N HCl and adjust the pH to 7 or 8 with NaoH. Make up the volume to 3 ml with water. Centrifuged at 2000 g for 5 min. Collect the supernatant. To 0.8 ml of extract, add 0.8 ml of 0.1 M sodium phosphate buffer, pH 7.0. To another aliquot of 0.8 ml extract, add 0.8 ml of 0.1N NaoH pH 12.3. Measure the absorbance at 245 and 350. Derive the lignin concentration from the difference between *A245* and *A350* on pH 7.0 and 12.3, samples diluted with buffer and NaoH, respectively. Express the amount of lignin as E 350/sample.

Statistical analysis

The analysis of variance was done using single factor analysis using the “F” test. Critical difference (CD) was also used to test the difference between any two means. The mean data of both fresh and stored seeds was jointly analyzed using paired t-test two samples for means to compare whether there was significant difference between the values of fresh and stored seeds.

RESULTS AND DISCUSSION

Seed coat colour and seed hilum colour

Black seeded genotypes performed better as compared to yellow seeded ones. Further, the seed coat colour remained unchanged with advanced seed storage of eight months (Table 1). The black-seeded genotypes with white hilum maintained seed quality higher than IMSCS (70%) than yellow-seeded genotypes with brown to grey hilum after eight months of laboratory ambient storage. These results were in accordance with earlier work of Kumar (2005) and Sooganna (2015) in soybean. Further, black seed coat is also associated with resistance to field weathering (Mugnisjah *et al.,* 1987) and better resistance against pathogen (Starzinger and West, 1982) and hence, is classified as a better storer. Better physiological quality of genotypes with black coats as compared to genotypes with yellow coats was also reported by Bahry *et al.* 2017 in soybean.

Seed coat percentage

With respect to good storers, the average seed coat percentage for fresh seed and after eight months of storage, was recorded to be 9.4146% and 9.4143%, respectively. Genotype G-2614 showed maximum seed coat percentage (10.01%) followed by G-2251 (9.83%), whereas M-11913 recorded least seed coat percentage. However, with respect to poor storers, the average seed coat percentage for fresh seed and after eight months of storage, was recorded to be 7.6059% and 7.6051%, respectively. Genotype 761(137) recorded maximum seed coat percentage (8.244%) followed by genotypes 250 (129) which recorded 8.046% and 241(128) which recorded 7.17%. Further, the black seeded soybean genotypes recorded higher seed coat percentage than yellowish orange genotypes. However, no significant difference was observed between fresh and stored seeds, irrespective of quality of seed. The study recorded black seeded genotypes *viz*. G-2614 and G-2251 with higher seed coat percentage as better performers (Table 1).

Mechanical strength of seed coat

There were significant variations in mechanical strength of seed coat among good and poor storers in the freshly

Table 1: Seed coat colour, hilum colour, mechanical strength and seed coat (%) in soybean genotypes with contrasting longevity pattern.

Genotype	Seed coat colour		Hilum colour		MS (N)		Seed coat (%)	
	Fresh seed	Stored seed	Fresh seed	Stored seed	Fresh seed	Stored seed	Fresh seed	Stored seed
Good storer								
1. AMSS-34	Black	Black	White	White	148.972	148.191	9.155	9.154
2. G-2265	Black	Black	White	White	138.562	138.171	9.291	9.297
3. G-2601	Black	Black	White	White	160.491	160.370	9.409	9.405
4. G-2603	Black	Black	White	White	137.739	137.597	9.291	9.293
5. G-2605	Black	Black	White	White	140.088	140.346	9.167	9.163
6. G-2251	Black	Black	White	White	160.536	160.476	9.750	9.755
7. G-2253	Black	Black	White	White	146.120	146.483	9.346	9.345
8. G-2614	Black	Black	White	White	147.592	147.211	9.944	9.941
9. M-1090	Black	Black	White	White	149.768	149.071	9.768	9.763
10. M-11913	Black	Black	White	White	142.562	142.600	8.970	8.976
11. MACS-1311	Black	Black	White	White	142.806	142.173	9.337	9.335
12.TGX (444-422)	Black	Black	White	White	147.779	143.388	9.543	9.544
Mean					146.918	146.673	9.4146	9.4143
Poor storer								
13. 218 (123)	Yellow orange	Yellow orange	Black	Black	112.329	112.273	7.642	7.644
14. 222 (127)	Yellow orange	Yellow orange	Brown	Brown	118.556	118.276	7.330	7.329
15. 241 (128)	Yellow orange	Yellow orange	Black	Black	103.198	104.010	7.052	7.055
16. 250 (129)	Yellow orange	Yellow orange	Brown	Brown	103.154	103.182	7.943	7.944
17. 732 (135)	Yellow orange	Yellow orange	Black	Black	104.933	104.450	7.451	7.456
18. 761 (137)	Yellow orange	Yellow orange	Black	Black	109.230	109.271	8.244	8.242
19. 871 (145)	Yellow orange	Yellow orange	Black	Black	116.691	116.800	7.255	7.256
20. 876 (146)	Yellow orange	Yellow orange	Black	Black	106.339	106.128	7.855	7.851
21. 888 (150)	Yellow orange	Yellow orange	Brown	Brown	119.314	119.351	7.553	7.554
22. PK-472	Yellow orange	Yellow orange	Brown	Brown	115.587	115.137	7.728	7.724
23. TAMS-38	Yellow orange	Yellow orange	Black	Black	92.450	92.540	7.648	7.643
24. EC-13969	Yellow orange	Yellow orange	Black	Black	111.96	111.966	7.566	7.562
Mean					109.478	109.448	7.605	7.605
MSD @ 5%					16.610	14.076	0.053	0.049

Where,

Fresh seed: At the start of seed storage.

Stored seed: For 8 months under laboratory ambient storage.

Seed coat and hilum colour determination of colour using RHS chart.

MS: Mechanical strength of seed coat, average of five replicates with five seeds in each replicate; N: Newton.

MSD: Minimum significant difference.

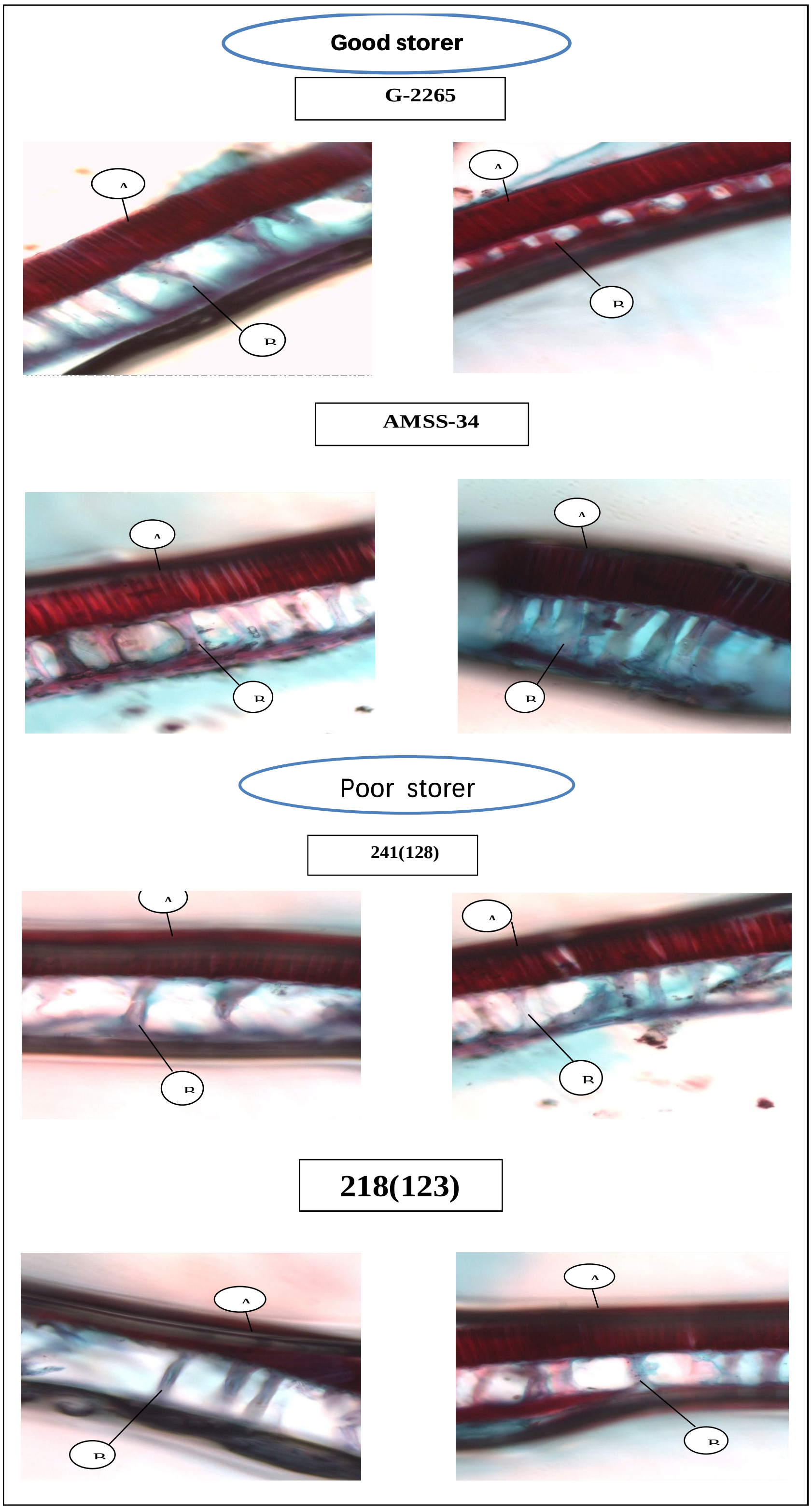

Fig 1: Soybean seed coat cross section view using phase contrast microscope.
A- Epidermis of one cell layer (cutinized palisade cells).
B- A hypodermis of single layer of large cells with thick anticlinal walls having intercellular spaces.

harvested seeds (Table 1). Hard seed coat has been observed to be very beneficial in maintaining the seed viability and vigour. With respect to the mechanical strength of seed coat for freshly harvested seeds, significant variations were recorded among good and poor storers. However, no clear association was reported between the mechanical strength of the seed and their seed quality, after eight months of ambient laboratory stored soybean seeds (Sooganna, 2015). On the contrary, Zahid (2013) reported a significant reduction in soybean seed coat hardness from 36 months stored seeds. This can be attributed to the fact that the black-seeded genotypes are land races which are rich in anthocyanin, an antioxidant and have less oil; which accounts for better storability of black-seeded genotypes with greater mechanical strength.

Seed coat histology

The stained seed coat sections were viewed and photographed using phase contrast microscope. Soybean seed coat consisted of an epidermis of one cell layer (cutinized palisade cells), a hypodermis of single layer of large cells with thick anticlinal walls having intercellular spaces (Fig 1). The pictorial representations revealed that the hour-glass cells, were more in number, uniform in shape and distribution along the cross section of the seed coat in good storer varieties, *viz*. AMSS-34, G-2265 as compared to the yellow-seeded poor storer varieties 218(123), 241(128) (Fig 2).

Seed coat thickness

Thickness of the seed coat was measured using phase contrast microscope (Leica software) which was connected to a computer. The thickness of parenchyma + palisade parenchyma and length of anticlinal cells was measured and is presented in Table 2. Seed coat thickness in soybean genotypes ranged between 193 to 200 ìm. Small seeded soybean seeds have thicker seed coat tissues which helps in better maintenance of viability in contrast to the bold seeds where seed viability is not better maintained.

Lignin content

The higher the lignin content in the seed coat, the better is the expected resistance to mechanical damage. In the present study, significant difference was recorded for lignin content between good and poor storers; however, no significant change was observed in lignin content after storage for eight months (Table 3). In soybean, seed coat lignin also plays an important role in physical and physiological seed traits (Alvarez *et al* 1997). The soybean seed coat is awfully thin and squat in lignin content and provides little protection to the weak radicle that lies in a susceptible location straight beneath the seed coat.

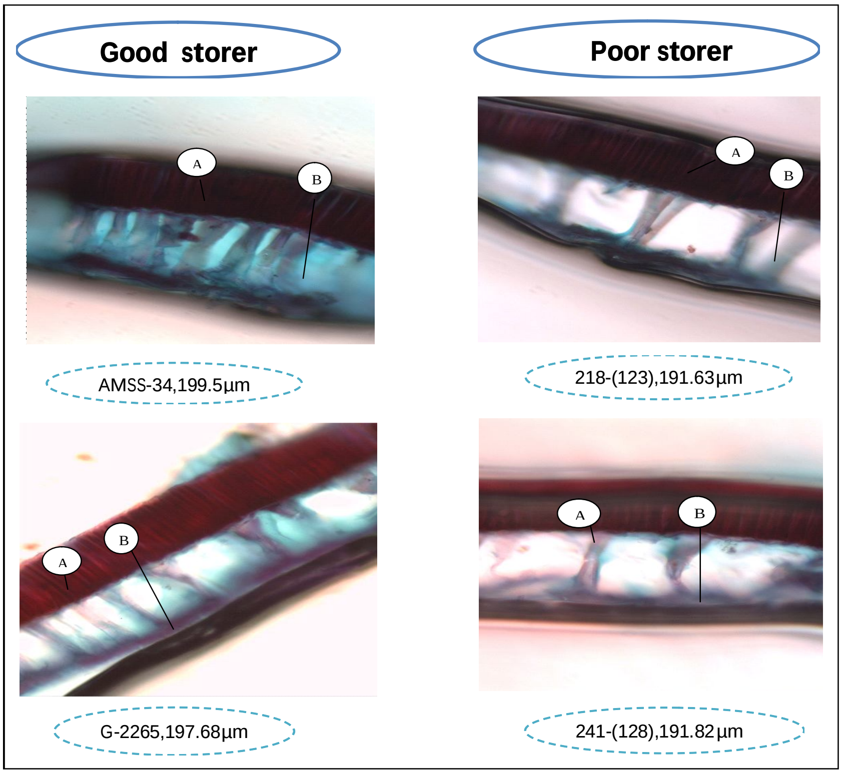

Fig. 2: Soybean seed coat thickness using phase contrast microscope.
A - epidermis of one cell layer (cutinized palisade cells).
B - a hypodermis of single layer of large cells with thick anticlinal walls having intercellular spaces.

Table 2: Seed coat thickness in soybean genotypes with contrasting longevity pattern.

Genotype	Seed coat thickness (µm)		
	Epidermis+palisade	Anticlinal	Total
Good storer			
1. AMSS-34	112.981	87.243	200.224
2. G-2265	111.477	88.924	200.401
Mean	112.229	88.08	200.312
Poor storer			
1. 218 (123)	111.686	83.073	194.759
2. 241 (128)	110.354	82.158	193.012
Mean	111.020	82.865	193.012
MSD @ 5%	15.148	8.168	17.455

Where, Fresh seed: At the start of seed storage, MSD: Minimum significant difference.

Table 3: Seed coat lignin content in soybean genotypes with contrasting longevity pattern.

Genotype	Lignin content (OD_{245}-OD_{350})	
	Fresh seed	Stored seed
Good storer		
1. AMSS-34	0.498	0.494
2. G-2265	0.499	0.492
3. G-2601	0.430	0.431
4. G-2603	0.425	0.421
5. G-2605	0.477	0.471
6. G-2251	0.470	0.479
7. G-2253	0.552	0.557
8. G-2614	0.420	0.421
9. M-1090	0.437	0.438
10. M-11913	0.440	0.436
11. MACS-1311	0.492	0.492
12.TGX (444-422)	0.476	0.474
Mean	0.468	0.467
Poor storer		
13. 218 (123)	0.360	0.354
14. 222 (127)	0.359	0.355
15. 241 (128)	0.362	0.366
16. 250 (129)	0.357	0.351
17. 732 (135)	0.360	0.357
18. 761 (137)	0.356	0.354
19. 871 (145)	0.353	0.355
20. 876 (146)	0.369	0.361
21. 888 (150)	0.354	0.352
22. PK-472	0.362	0.362
23. TAMS-38	0.358	0.355
24. EC-13969	0.369	0.367
Mean	0.360	0.357
MSD @ 5%	0.050	0.024

Where,
Fresh seed: At the start of seed storage.
Stored seed: For 8 months under laboratory ambient storage.
MSD: Minimum significant difference.

The steadiness of seed coat lignin yet in storage, can be explained by the insolubility and intricacy of the lignin polymer.

CONCLUSION

Genotypic variations were recorded in seed coat characteristics and soybean seed quality. Black-seeded genotypes with white hilum were found to be better performers. Seed coat and hilum colour remained unchanged with seed storage. In the present study, no clear association could be established between the mechanical strength of the seed and their seed quality after eight months of laboratory ambient stored seeds. Transverse sections of the seed coat of soybean seeds depicted three observable cell layers: an outer epidermis, palisade cell layer and an hourglass cell. The hour-glass cells were more in number, uniform in shape and distribution along the cross section of the seed coat in good storers (black-seeded) than those to poor storers (yellow-seeded) genotypes. Black-seeded genotypes with white hilum, thick seed coat and higher seed coat (%) with more and uniform hours glass cells attributed to better seed quality in soybean.

Conflict of interest: None.

REFERENCES

Alvarez, P.J.C., Krzyzanowski, F.C., Mandarino, J.M.G. and Franca-Neto, J.B. (1997). Relationship between soybean seed coat lignin content and resistance to mechanical damage. Seed Science and Technology. 25: 209-24.

Bahry, C.A., Perboni, A.T., Nardino, M. and Zimmer, P.D. (2017). Physiological quality and imbibitions of soybean seeds with contrasting coats. Revista Ciência Agronômica. 48: 125-33.

Francisco, C.K., Jose, D.E., Jose-Marcos, G.M. and Milton, K. (2008). Evaluation of lignin content of soybean seed coat stored in a controlled environment. Revista Brasileira de Sementes. 30: 220-23

Hymowitz, T. and Harlan, J.R. (1983). Introduction of soybeans to North America by Samuel Bowen in 1765. Economic Botany. 37: 371-79

Kuchlan, M.K. (2006). Identification of physical, physiological and biochemical factors and molecular analysis for longevity of soybean seeds. PhD Thesis, ICAR- IARI, New Delhi.

Kumar (2005). Studies on storability and vigour parameters in soybean. MSc Thesis. ICAR- IARI New Delhi.

Mugnisjah, W.Q., Shimano, I. and Matsumoto, S. (1987). Studies on the vigour of soybean seeds.II Varietal differences in seed coat quality and swelling components on seed during moisture imbibition. Journal of the Faculty of Agriculture of Kyushu University.13: 227-34

Sooganna (2015). Phenotyping of soybean genotypes for seed quality and seed vigour. PhD thesis, ICAR- IARI, New Delhi.

Starzinger, E.K. and West, S.H. (1982). An observation in the relationship of soybean seed colour to viability maintenance. Seed Science and Technology. 10: 301-05.

Zahid (2013). Physiological phenotyping of soybean [*Glycine max* (L) Merrill] genotypes for seed longevity. M Sc Thesis, ICAR- IARI, New Delhi.