Indian J. Genet., 79(1): 48-55 (2019)
DOI: https://doi.org/10.31742/IJGPB.79.1.7

Genetic studies on seed coat permeability and viability in RILs derived from an inter-specific cross of soybean [*Glycine max* (L.) Merrill]

Ashish Kumar, Subhash Chandra[1], Akshay Talukdar*, Raju R. Yadav, Manisha Saini, Shatakshi Poonia and S. K. Lal

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012; [1]Division of Crop Improvement, ICAR-Indian Institute of Soybean Research, Indore 452 001

(Received: November 2018; Revised: February 2019; Accepted: February 2019)

Abstract

Soybean seeds loss viability very rapidly during ambient storage in the tropical and sub-tropical environments. In this study, interrelationship between seed coat permeability and viability over periods of ambient storage was assessed using a set of 217 recombinant inbred lines (RIL) developed from an inter-specific cross between wild type (*Glycine soja*) accession DC2008-1 and cultivated (*G. max*) variety DS9712. *G. soja* seeds were tiny, black, impermeable and highly viable while *G. max* seeds were large, yellow, permeable and poorly viable during ambient storage. Seed coat permeability and viability of the fresh, one-year and two-year-stored seeds (stored in room temperature, av. 25±2°C and 65±5% RH) were tested as per standard protocols in completely randomized design with two replications. Significant variation was found among genotypes for the seed viability, permeability, periods of storage and their interactions. Permeability of the seed coat increased with the period of storage. In the fresh, one-year and two-year-stored seeds, the seed coat permeability was 62.87, 75.17 and 90.52%, respectively. Viability of the seeds was negatively correlated with period of storage and seed size. In the fresh, one-year and two-year-stored seeds, average viability was 90.7, 75.6 and 54.1%, respectively. Scanning electron microscopy (SEM) indicated presence of intact hilum, strong hourglass cells and non-cracked seed coat in the highly viable seeds. A set of 24 RILs were found that maintained higher viability (>80%) with varying degree of permeability after two years of storage. Among the highly viable RILs, more were black seeded. RIL Nos. 7-12-3, 7-24-1, 13-2-2, 13-31-4 found to maintain both viability and permeability in higher order during storage and would pave the way for development of soybean genotypes with high viability and permeability.

Key words: Seed coat ultrastructure, imbibition, hourglass cells, germination, RILs, scanning electron microscopy

Introduction

Soybean [*Glycine max* (L.) Merrill] is the most important oilseed crop in the world. Besides oil (18-22%), it contains protein (38-45%), carbohydrate, ash, nutritional elements and antioxidants largely beneficial for human being. Therefore, it is gaining boundless popularity in the food, health, pharmaceutical and cosmetic industries worldwide. De-oiled cake (DOC) of soybean has been the choice of animal growers as nutritious feed for animal, fowl and fishes. Large scale demand asks for more production. However, insufficient-supply of quality seeds is the biggest hurdle in expansion of soybean cultivation. Loss of seed viability, which starts soon after physiological maturity, compels increasing seed rate resulting in shortage of quality seeds and increase in cultivation cost. Viability and vigor loss is more pronounced in tropical and sub-tropical regions under ambient storage (Singh and Ram 1986; Bhatia 1996). Depending upon genotype, climatic variables and storage condition, seed germination goes below minimum standards in warm and humid climates (Dargahi et al. 2014), leading to poor germination and sub-optimal plant stand in the field (Singh and Ram 1986). Delouche (1974) reported that soybean seed lots cannot be stored for two consecutive planting seasons. In India, seed viability in most of the varieties goes below 70% under ambient storage from harvest to next sowing. Bhattacharya and Raha, (2002) recorded fall of germination to zero in 10 months of storage in ambient condition. Therefore, understanding causes underlying loss of viability in the soybean seed is critical for higher production. Seeds of wild type

---

*Corresponding author's e-mail: akshay.talukdar1@gmail.com

Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

soybean (*Glycine soja* Sieb & Jucc.) lives longer than the cultivated type (*G max* L. Merr.). The seed coat of wild type seeds is impermeable and do not imbibe water immediately, while the seeds of cultivated type imbibe water soon after soaking. Imbibition initiates germination. It was therefore targeted to understand the interrelationship between permeability of seed coat and seed viability in soybean. Seeds of an inter-specific RIL population were stored in ambient conditions for 2 years and thereafter tested for viability. Ultra-structure of the seed coat and its changes over period of storage were studied for possible association with loss of viability. A few RILs with high viability and high seed coat permeability were identified.

Materials and methods

Wild type (*Glycine soja*) accession DC2008-1 was crossed with cultivated (*G. max*) variety DS9712, and the generations were advanced through single seed descent (SSD) approach to produce recombinant inbred lines (RIL) population. The *G soja* seeds are small, black and impermeable, but remain viable for several years in ambient storage. The seeds of DS9712 are medium, yellow and permeable, however losses viability rapidly under storage in ambient condition. Features of the seeds of both the parental genotypes have been given in Table 1. The seeds of the parental genotypes along with the 217 RILs in F_7 generation were used for permeability and viability tests after ambient storage for various period of time.

Seed germination test

Freshly harvested seeds (about 150g) of 217 RILs and their parents were air dried to about 10% moisture contents and packed in muslin cloth bags and stored under ambient conditions of Delhi with an average relative humidity of 65±5% and temperature of 25±2°C. Samples from the fresh seeds (2015), after one year of storage (2016), and two years of storage (2017) were tested for permeability and viability. For germination tests, 50 randomly selected seeds from each line were rolled in moist germination paper and kept at 25°C in an incubator for seven days (ISTA 2011). On the eighth day, the number of germinated seeds with normal seedlings was counted and percentage of germination was obtained. Level of germination (%) was considered as indicative of viability i.e., higher the germination (%), higher is the seed viability.

Permeability test

For testing seed coat permeability, 50 randomly selected seeds from each genotype were collected from fresh, one-year and two-year stored seed lots. The seeds in two replications were rolled in moist germination papers and placed in germination chamber. The rolls were maintained at about 100% humidity and 25°C for 7 days (Kebede et al. 2014). On 8th day, the seeds were checked for imbibition and classification. The seeds that had >80% un-imbibed seeds were classified as “impermeable or hard seed,” and the seeds that had <20% un-imbibed seeds were classified as “permeable or soft seeds” as per Sun et al. (2015). Seed coat permeability and viability of the seeds were evaluated in the laboratories of the Division of Genetics and Division of Seed Science and Technologies, ICAR-IARI, New Delhi.

Ultra-structure of seed coat

The seed coat surface and its ultra-structure of the parental genotypes and a few selected RILs was analyzed using scanning electron microscopy at All India Institute of Medical Sciences (AIIMS), New Delhi. Seed sample fixation was done as per Yuan et al. (2012) with minor modifications. Seed samples of about 1mm^2 were immersed in to 25% glutaraldehyde and 1% p-formaldehyde fixative and were dehydrated in ethanol gradient series (30 min in each) 25%, 50%, 75% and 100%. Samples were subjected to critical point drying (CPD) and then mounted on carbon stubs followed by gold-palladium coating. Samples were finally observed under scanning electron microscope (Model: JEOL-JSM- 6610LV). The images of seed hilar region, hourglass cells and seed coat surface were taken at 100X, 500X and 1000X, respectively.

Data analysis

The experiments were conducted following Completely Randomized Design (CRD), and the data on seed coat permeability and viability from the fresh, one-year and two-year-ambient stored seeds were analyzed using the online available software package OPSTAT CCS HAU, Hisar India (http://hau.ac.in/about/opstat.php).

Results and discussion

Seed viability

Soybean seed reaches highest potential of germination at its physical maturity (Shelar et al. 2008), which starts declining gradually till harvest. After harvest and thereafter viability declines rapidly, rate of which vary with genotype and condition of storage (Surki et al. 2012). In this study, significant variation was observed among the genotypes for its response towards viability under ambient storage. Viability varied significantly with the genotype and period of storage under ambient condition (ANOVA not shown). The average germination in the fresh seeds was 90.7%, which decreased to 75.6% and 54.1% after one year and two years of ambient storage, respectively (Fig. 1). In general, loss of seed viability was more rapid in the one-year-stored seeds (21.5%) than the fresh seeds (18.1%). However, not all the lines lost viability uniformly; some lost it slowly/rapidly than others (Fig. 2). Viability loss in the seeds of *G soja* accession DC2008-1 and *G max* variety DS9712 after 2 years of storage was 2.02% and 68.04%, respectively (Table

Table 1. Selected RILs with seed coat permeability (%) and germination (%) in fresh, one-year and two-year-stored seeds

S. No.	Genotype	Seed color	100-seed weight(g)	Permeability (%)				Germination (%)			
				PP-1	PP-2	PP-3	Av. (%)	GP-1	GP-2	GP-3	Av. (%)
1	DC2008-1	B	0.56	10	28	33	23.66	99	96	92	95.66
2	DS9712	Y	9.12	100	100	100	100	97	70	51	72.66
3	7-12-3	B	2.12	78	85	89	84.00	95	92	80	89.00
4	7-24-1	Y	2.5	88	91	95	91.33	89	82	81	84.00
5	8-10-5	GY	2.1	50	71	74	65.00	92	81	80	84.33
6	9-3-1	G	2.6	18	40	92	50.00	96	90	82	89.33
7	13-1-5	B	2.1	12	45	59	38.67	93	88	83	88.00
8	13-2-2	B	1.9	100	100	100	100.00	96	88	85	89.67
9	13-12-2	B	3.6	14	23	60	32.33	95	94	81	90.00
10	13-16-1	B	2.3	7	28	48	27.67	98	88	84	90.00
11	13-20-2	B	1.7	16	29	67	37.33	97	87	81	88.33
12	13-26-2	GY	2.1	31	43	75	49.67	92	88	81	87.00
13	13-31-4	B	2.6	80	87	87	84.67	92	88	85	88.33
14	13-37-2	Y	1.9	14	24	78	38.67	96	86	85	89.00
15	13-43-2	GY	2.1	63	90	96	83.00	84	82	81	82.33
16	13-48-4	B	2.3	62	70	85	72.33	88	85	81	84.67
17	13-49-2	Y	1.8	14	17	56	29.00	96	90	86	90.67
18	13-63-5	B	2.6	6	18	26	16.67	100	92	88	93.33
19	15-7-2	B	2.3	55	65	78	66.00	92	88	85	88.33
20	15-17-1	Y	2	52	53	57	54.00	88	84	81	84.33
21	15-48-1	B	2.1	15	19	23	19.00	96	94	89	93.00
22	20-3A-1	G	1.8	33	37	71	47.00	99	97	80	92.00
23	20-17-5	B	2.4	27	35	92	51.33	98	88	84	90.00
24	23-13-2	B	2.5	23	65	74	54.00	92	89	85	88.67
25	34-9-4	B	1.3	12	55	75	47.33	95	86	82	87.67
26	34-21-3	Y	2.2	35	42	71	49.33	92	87	81	86.67
	Mean		2.21	37.7	51.3	72.0		93.7	88.0	82.9	
	CD		0.277	7.07	6.70	6.35		5.11	4.57	4.30	

B = Black, G = Green, GY = Green Yellow, Y = Yellow; PP-1 = Permeability in fresh seeds (%); PP-2 = Permeability in one-year-stored seeds (%); PP-3 = Permeability in two-year-stored seeds (%); GP1 = Germination in fresh seeds (%); GP2 = Germination in one-year-stored seeds (%) and GP3 = Germination in two-year-stored seeds (%)

1). Germination of RIL Nos. 15-7-2 and 15-50-1 at harvest was 92 and 90%, respectively. After 1 year of storage, their germination declined to 88 and 41%, which further declined to 85 and 14%, respectively after 2 years of storage (Fig. 2). Keeping Indian minimum seed certification standard (IMSCS) i.e., 70% as base line, the RILs were classified as good-storers (>70% germination) and poor storers (<70% germination). Out of 217 RILs, 158 were categorized as 'good-storers' after one year of storage and 63 were identified as 'good-storers' after two years of ambient storage (Data not shown). A set of 24 RILs that maintained >80% viability even after 2 years of storage is listed in Table 1. Kurdikeri et al. (1996) reported that seed germination and seedling vigor declines with increasing storage period. Loss of viability also depends on the genotype (genetics), species and other varietal characters (Kurdikeri et al. 2000). Contrarily, expression of genes encoding protective chaperones such as heat shock proteins and repression of nuclear and chloroplast genes involved in a range of chloroplast activities contributes towards increase in seed longevity (Lima et al. 2017).

Seed coat permeability

Significant variation for seed coat permeability was observed among the parental genotypes and the RILs tested (ANOVA not shown). Like viability, seed coat permeability also varied with genotypes and periods of storage. Fresh seeds of the *G soja* accession DC2008-1 were impermeable while that of the *G max* genotype DS9712 were permeable. Among the 217 RILs, 85 were permeable and 31 were impermeable, and 101 were intermediate. Permeability in the fresh seeds of the RILs ranged from 3-100% with an average of 62.87%. Similarly, range of permeability in the one-year and two-year-stored seeds were 17-100% and 23-100% with an average of 75.17% and 90.52%, respectively. Permeability status of 24 selected RILs is presented in Table 1. The age of the seed was positively correlated with permeability, however, degree of permeability varied with the genotype and storage period. Mean viability of the selected RILs were higher than the total RILs over the years (Fig. 3). It indicated possibility of genetic improvement of the lines through breeding approach.

Viability versus seed coat permeability

Viability or storability of soybean seeds is a complex trait. Several factors *viz*., seed coat permeability, seed coat color, seed size, oil content, reactive oxygen species, etc. have been indicated to have role in enhancing/reducing viability of the seeds. As seed coat is the protective cover of the seed and its embryo, it is critical for viability of the seeds (Mohamed-Yasseen, 1994). In general, seeds with impermeable seed coat, as in wild type genotypes, live longer than others and vice-versa. In this study, it was found that germination was negatively correlated with 100-seed weight (Table 2). Similarly, seed coat permeability appeared to be negatively correlated with seed viability (Table 2). Similar findings were reported by Singh et al. (2008) and Adsul et al. (2018). However, exception does exist. Scanning electron microscopy (SEM) of the hilar region, seed coat surface and the hourglass cells of the seeds indicated remarkable changes in them during storage period. The changes were more prominent in the poor-storing genotypes than others. The permeable seeds of DS9712 developed minute cuticle cracks in the extra hilar regions during storage, which were absent in the seeds of DC2008-1 (Fig. 4). The raphe was cracked and opened slightly in DS9712 (Fig. 4A), while it appeared to be slightly depressed, continuous with the rest of the seed coat, without any outgrowths and nearly closed in DC2008-1 (Fig. 4B). Similarly, slightly depressed areas and pores were observed in dorsal surface of DS9712 seeds, rather smooth surface and absence of pores was observed in DC2008-1 seeds. Seeds of good-storing RILs were almost entirely covered with surface deposits that largely appeared as amorphous (Fig. 5). The seed coat of the good storing RIL No. 15-7-2 was free from pores and depressions (Fig. 5A). The seed coat of poor-

Table 2. Coefficient of correlation among permeability, germination and hundred seed weight in RIL population

	PP1	PP2	PP3	GP1	GP2	GP3	HSW
PP1	1	0.890**	0.619**	-0.264**	-0.517**	-0.441**	-0.033
PP2		1	0.685**	-0.261**	-0.540**	-0.446**	-0.010
PP3			1	-0.259**	-0.398**	-0.478**	-0.017
GP1				1	0.298**	0.151*	-0.053
GP1					1	0.642**	0.085
GP3						1	0.048
HSW							1

PP-1= Permeability in fresh seeds; PP-2= Permeability in one-year-stored seeds; PP-3= Permeability in two-year-stored seeds; GP1=Germination in fresh seeds; GP2= Germination in one-year-stored seeds, GP3= Germination in two-year-stored seeds and HSW= Hundred seed weight

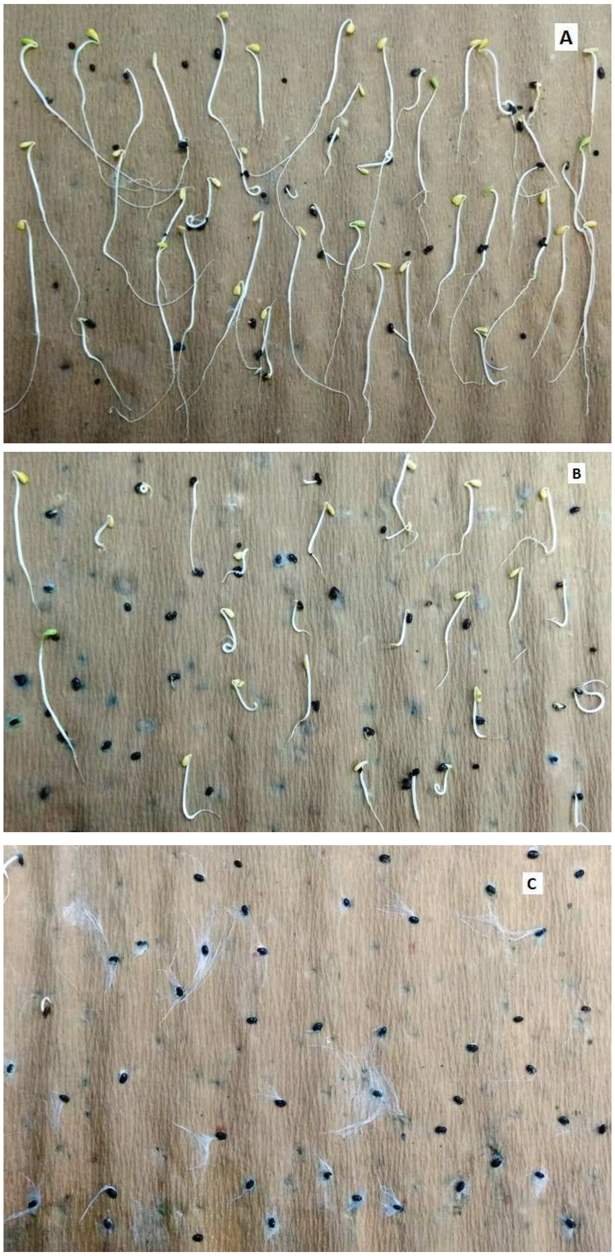

Fig. 1. **Loss of viability in the RILs over period of storage. A= Germination in fresh seeds; B= Germination after one year of ambient storage; and C= Germination after two years of ambient storage**

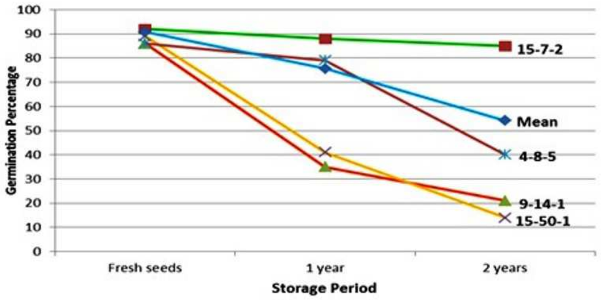

Fig. 2. **Trend of viability loss in seeds during ambient storage over years. RIL Nos. 9-14-1 and 15-50-1 lost viability more rapidly than RIL No.4-8-5. RIL No.15-7-2 lost viability very slowly**

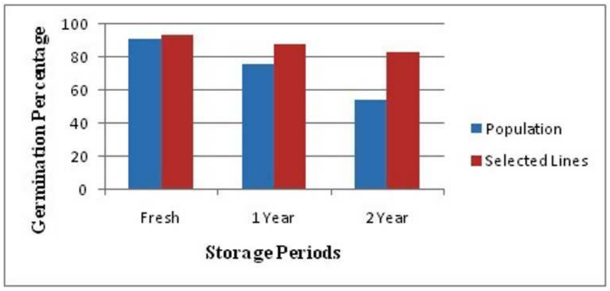

Fig. 3. **Change of mean germination (%) in the selected against total RILs over periods of storage**

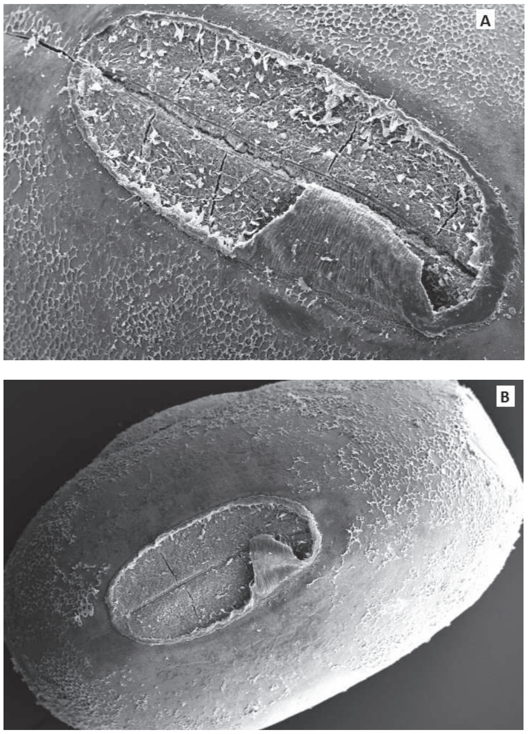

Fig. 4. **SEM of ventral side of seeds. (A) DS 9712: cracks in extra hilar region and raphe; (B) DC 2008-1: closed raphe without any cracks**

storing RIL No.2-34-5 had pores, depression and visible cracks on the surface (Fig. 5B and C). Harris (1987) reported observance of pores in seed coats of the soft-seeded variety Hardee but not in Brachett, a hard-seeded variety. The cuticle of the palisade layer is the key that determines the permeable property of the seed coat of soybean. Usually, the cuticle of a permeable seed coat is mechanically weak and develops small cracks which permit entry of water in

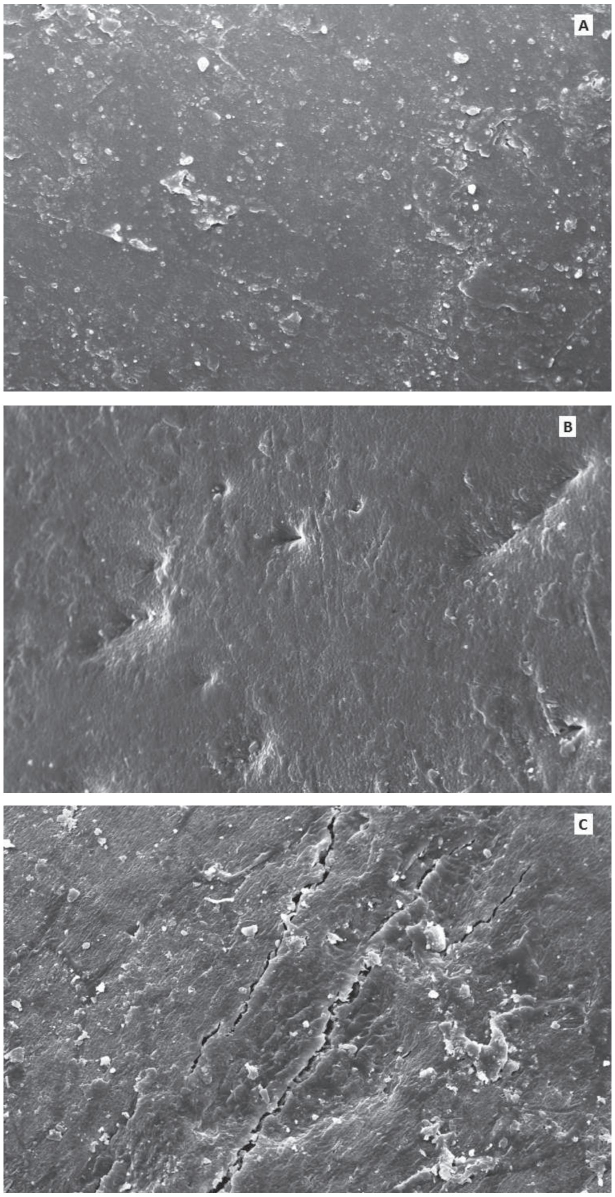

Fig. 5. SEM of seed coat surface. (A) RIL 15-7-2 (good storing): absence of pores and surface deposits; (B) RIL 2-34-5 (poor storing): Pores and depressions on seed coat surface; (C) RIL 2-34-5 (poor storing): cracks in seed coat

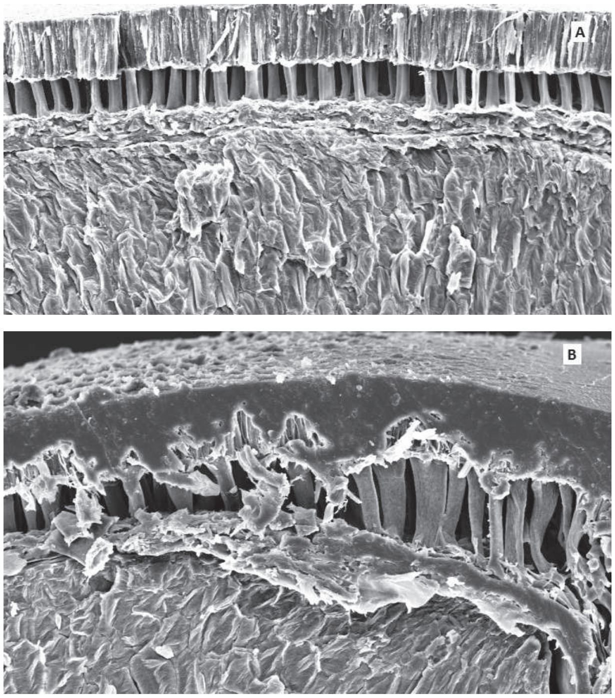

Fig 6. SEM of cross section of hourglass cells. (A) RIL 13-49-2 (good storing): well-shaped and evenly distributed; (B) RIL 19-2A-2 (poor storing): non uniform and uneven distributions of hourglass cells

to the seed. On the other hand, the cuticle of an impermeable seed coat is mechanically strong and does not crack under normal circumstances (Ma et al. 2004). SEM further revealed that the hour-glass cells, which provide cushioning effect to the seeds and impart protection against hydration damage and field weathering, were rather uniform in shape and distribution along the cross section of the seed coat in good-storing genotypes (Fig. 6). These cells were found more in number and well-shaped without any distortion (Fig. 6A). Contrarily, the hourglass cells were broken, less in number and not uniform in shape in the poor-storing genotypes (Fig. 6B). Pereira and Andrew (1985) observed that the hourglass cells lying between the internal palisade and parenchyma cell layers get distorted at the seed coat wrinkle site damaging that seed coat. Complete, unbroken and ordered hourglass cells, perhaps, prevents damage caused by hydration and dehydration. There were amorphous deposits on the seed coat of the good-storing RILs, which were absent in the poor-storing RILs. Calero et al. (1981) reported that the presence of certain waxy deposits can contribute towards seed coat impermeability too. The cracks in the seed coat also permit exchange of air and leachate out of the seeds. Conduction of electrical conductivity (EC) test has shown that it is more in the permeable seeds than others (Sooganna et al. 2016). Thus permeable seed coat appeared to be contributing towards loss of viability in the seeds. In this study, mean germination of the fresh seeds was 90.76% which reduced to 54.18% in two years of storage. Similarly, mean permeability of the fresh seeds was 62.87% which increased to 90.53% in two year of storage. Thus, more porous was the seed coat, less viable were the seed, and vice-versa (Fig. 5). During storage, the seed coat losses its strength and become permeable due to biochemical changes. Microbes also attack old seeds more than fresh seeds as week seed coat fail to defend the fungal attack (Kulik et al. 1991). The impermeable seed coat protects the seeds against microbial attack and eventual decay (Tyler 1997). However, there is substantial disagreement concerning the mechanisms and related structures that control the permeability properties of soybean seed coats (Ma et al. 2004). At molecular level, a single nucleotide polymorphism (SNP) has been reported to convert the impermeable wild type seeds to permeable one. A base substitution (T→G; Isoleucine→Serine) in Endo-1,4-β-Glucanase gene led to accumulation of β-1,4-glucan derivatives such as xyloglucan which resulted in impermeability of seed coat in soybean (Jang et al. 2015). Sun et al. (2015) also indicated involvement of similar mechanism in seed coat permeability in soybean. However, involvement of some other mechanisms also can't be ruled out (Jang et al. 2015; Sun et al. 2015).

Identification of good storing RILs with partial and complete permeability

Like many other legumes, soybean too produces impermeable or hard seeds. However, 'hard seeds' are not ideal for the food processing industry as it imbibe water very slowly. It is more critical when the whole seeds are processed to produce the food products such as soya milk, soya sauce, tofu, miso, etc. Permeable or soft-seeds, on the other hand, are susceptible to mechanical damage during pre-processing of the seeds leading to economic losses. In this study, a few interspecific RILs *viz*., 7-12-3, 7-24-1, 13-2-2, 13-31-4, etc. was identified that maintained >80% viability even after two years of ambient storage and were fairly permeable (Table 1). Genetic improvement of such lines will lead to development of soybean varieties with higher seed viability during storage.

Acknowledgement

The first author is thankful to the Indian Council of Agricultural Research (ICAR) and ICAR-IARI, New Delhi, India for the grant of Senior Research Fellowship and facilities during Ph. D. programme. National Agricultural Science Fund (NASF), ICAR, New Delhi is duly acknowledged for the financial grant for this study.

Authors' contribution

Conceptualization of research (AT); Designing of the experiments (AT, AK); Contribution of experimental materials (AT); Execution of field/lab experiments and data collection (AK, RRY, SP, SC, MS); Analysis of data and interpretation (AK, SKL, AT); Preparation of the manuscript (AK, AT).

Declaration

The authors declare no conflict of interest.

References

Adsul A. T., Chimote V. P. and Deshmukh M. P. 2018. Inheritance of seed longevity and its association with other seed-related traits in soybean (*Glycine max*). Agric Res., **7**: 105-111.

Bhatia V. S., Tiwari S. P. and Joshi O. P. 1996. Inter-relationship of leaf photosynthesis, specific leaf weight and leaf anatomical characters in soybean. Indian J. Pl. Phy., **1**: 6-9.

Bhattacharya K and Raha S. 2002. Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. Mycopathologia, **155**: 135-141.

Calero E., West S. H. and Hinson K. 1981. Water Absorption of Soybean Seeds and Associated Causal Factors 1. Crop Sci., **21**(6): 926-933.

Dargahi H., Tanya P. and Srinives P. 2014. Mapping of the genomic regions controlling seed storability in soybean (*Glycine max* L.). J. Genet., **93**(2): 365-370.

Delouche J. C. 1974. Maintaining soybean seed quality. In Soybean: production, marketing and use, Y-69. TVA Bull. V-19. Alabama: Muscle Sholas, pp: 46-62.

Harris W. M. 1987. Comparative Ultrastructure of Developing Seed Coats of "Hard-Seeded" and "Soft-Seeded" Varieties of Soybean (*Glycine max* L.). Botanical Gazette, **148**(3): 324-331.

ISTA. 2011. International Seed Testing Rules, published by International Seed Testing Association, Zurich, Switzerland.

Jang S. J., Sato M., Sato K., Jitsuyama Y., Fujino K., Mori H. and Abe J. 2015. A single-nucleotide polymorphism in an endo-1, 4-β-glucanase gene controls seed coat permeability in soybean. PLoS One., **10**(6): e0128527.

Kebede H., Smith J. R. and Ray J. D. 2014. Identification of a single gene for seed coat impermeability in soybean PI 594619. Theor. Appl. Genet., **127**(9): 1991-2003.

Kulik M. M. and Yaklich R. W. 1991. Soybean seed coat structures: relationship to weathering resistance and infection by the fungus Phomopsis phaseoli. Crop Sci., **31**: 108-113.

Kurdikeri M. B., Merwade M. N. and Channaveeraswamy A. S. 2000. Maintenance of viability in different crop species under ambient storage. Seed Res., **28**(1): 109-110.

Kurdikeri M. B., Basavaraj G. T., Hiremath M. V. and Aswathanarayan S. C. 1996. Storability of soyabean (Glycine max (L.) Merill) seed under ambient condition. Karnataka J.Agric. Sci., **9**: 552-554.

Lima J. J. P., Buitink J., Lalanne D., Rossi R. F., Pelletier S., Da Silva E. A. A. and Leprince O. 2017. Molecular characterization of the acquisition of longevity during seed maturation in soybean. PloS One., **12**(7): e0180282.

Ma F., Cholewa E. W. A., Mohamed T., Peterson C. A. and Gijzen M. 2004. Cracks in the palisade cuticle of soybean seed coats correlate with their permeability to water. Annals Bot., **94**(2): 213-228.

Mohamed-Yasseen Y., Barringer S. A., Splittstoesser W. E., Costanza S. 1994. The role of seed coats in seed viability. Botanical Review, **60**: 426-439.

Pereira L. and Andrew. G. 1985. Comparison of non-wrinkled and wrinkled soybean seed coats by scanning electron microscopy. Seed Sci. Tech., **13**: 853-860.

Shelar V. R., Shaikh R. S. and Nikam A. S. 2008. Soybean seed quality during storage: a review. Agric. Rev., **29**(2): 125-131.

Singh R. K., Raipuria R. K., Bhatia V. S., Rani A., Pushpendra, Husain S. M., Chauhan D., Chauhan G. S. and Mohopatra T. 2008. SSR markers associated with seed longevity in soybean. Seed Sci. Tech., **36**: 162-167.

Singh R. K. and Ram H. H. 1986. Inheritance study of soybean seed storability using an accelerated aging test. Field Crops Res., **13**: 89-98.

Sooganna S., Jain S., Bhat K., Lamichaney A. and Lal S. K. 2016. Characterization of soybean (*Glycine max* L.) genotypes for seed longevity using SSR markers. Indian J. agric. Sci., **86**(5): 605-10.

Sun L., Miao Z., Cai C., Zhang D., Zhao M., Wu Y., Zhang X., Swarm S. A., Zhou L., Zhang Z. J. and Nelson R. L. 2015. *GmHs1-1*, encoding a calcineurin-like protein, controls hard-seededness in soybean. Nature Genet., **47**(8): 939-943.

Surki A. A., Sharifzadeh F. and Afshari R. T. 2012. Effect of drying conditions and harvest time on soybean seed viability and deterioration under different storage temperature. African J. Agrl. Res., **7**(36): 5118-5127.

Tyler J. M. 1997. Effect of impermeable seed coat on germination of seed from early maturing soybean. Seed Tech., 45-50.

Yuan T. C., Huang R. N., Kuo Huang L. L., Kuo T. C., Yang Y. Y., Lin C. Y. and Chen S. J. 2012. A simple cryo holder facilitates specimen observation under a conventional scanning electron microscope. Microscopy Res. Tech., **75**(2): 103-111.