



Seed longevity studies in wild type, cultivated and inter-specific recombinant inbred lines (RILs) of soybean [*Glycine max* (L.) Merr.]

Subhash Chandra · Akshay Talukdar · Yashpal Taak · Raju R. Yadav · Manisha Saini · N. S. Sipani

Received: 6 February 2021 / Accepted: 27 June 2021 / Published online: 6 July 2021
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Abstract Loss of seed viability is a serious hurdle in production and ambient seed storage of soybean. Understanding the factors affecting seed viability, and identification of soybean genotype(s) with higher viability is critical for higher soybean production. In this study, seeds of 125 soybean genotypes of three different species [*Glycine tomentella* Hayata, *Glycine max* subsp. *soja* (Siebold & Zucc.) H. Ohashi, and *Glycine max*(L.) Merr.] and 25 RILs (*Glycine soja* × *Glycine max*) were tested for germination immediately after harvest followed by 1, 2 and 3 years of ambient storage. Fresh seeds of all the genotypes recorded 78–99% germination with a mean of 94.02%. However, the mean value of per cent germination of all the genotypes after 1, 2 and 3 years of ambient

storage decreased and found to stand at 79.51%, 52.24% and 29.18%, respectively. Among the genotypes tested, *G. tomentella* accession registered highest seed storability followed by *G. soja*, RILs and *G. max*. After 3 years of storage, 14 wild type genotypes and 3 RILs found to maintain > 70% germination and, were identified as ‘good storers’. Genetic divergence studies via k-mean clustering and principal component analysis grouped all genotypes in three clusters. Seed viability was significantly and negatively correlated with electrical conductivity. Good storers found to be having slow imbibition rate than poor storers in initial hours of imbibition. The good storing genotypes identified in this study will serve as an elite soybean genetic resource in developing the soybean cultivars with better seed longevity.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10722-021-01240-2>.

S. Chandra · A. Talukdar (✉) · Y. Taak · R. R. Yadav · M. Saini
Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India
e-mail: akshay.talukdar1@gmail.com

S. Chandra
ICAR-Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

N. S. Sipani
CORC-Sipani Krishi Anusandhan Farm, Mandasaur, Madhya Pradesh, India

Keywords Genetic resource · Seed longevity · Wild type soybeans · Germination · RILs · Diversity

Introduction

Soybean [*Glycine max* (L.) Merr.] is one of the most important oilseed crop for food, feed and industrial utilities. Owing to its multifarious utilities including medicinal and cosmetic applications, demand for soybean is increasing worldwide. It is found to be the cheapest source of vegetable oil, and protein. Considering the power, properties and limitless

applications, the soybean has been christened as the ‘Golden bean’ (Adsul et al. 2018). However, viability of the soybean seed is a serious concern for the farmers and traders; it rapidly loses viability during ambient storage more severely in the tropic and sub-tropics (Hang et al. 2015), which severely affects seedling vigour, crop stand in the field and eventual yield. The viability is highly influenced by genetic factors, physiological maturity, handling and processing practices and storage conditions (Zhang et al. 2019). High relative humidity and temperature accelerates the deterioration asking for higher seed rate for maintaining the crop stand in the field. Therefore, it is prudent to understand the seed deterioration mechanism so as to develop soybean varieties that can withstand the stresses and maintain viability of the seeds during ambient storage.

Existence of large genotypic variations for traits that affects seed viability has been reported in soybean (*Glycine max* L. Merrill) including factors such as mechanical damages (Zahid 2013), field weathering (Bhatia 1996), physiological characteristics like imbibition kinetics and electrolyte leaching (Kuchlan et al. 2010; Hosamani et al. 2013; Sooganna et al. 2016). The seed traits that enhances seed viability includes hard seed coat (Kumar et al. 2019), small seed size (Hosamani et al. 2013), black seed coat (Liu et al. 2017) and tight attachment of the seed coat to the cotyledons (Kuchlan et al. 2010). Typically, wild type soybean seeds remain viable for longer duration than the cultivated type (Chandra et al. 2017). Attempts have also been made to identify molecular markers linked to the traits considered to influence seed viability (Dargahi et al. 2014; Kumar et al. 2019; Zhang et al. 2019).

Inconsistency in the results of various seed viability related studies is not uncommon. Causes of such discrepancies include variation in the tested genotypes, populations used, approaches followed, criteria adopted, etc. (Chandra et al. 2020). The studies reported earlier have had used seeds stored for one or two years only. Further, no reports could be found where wild type soybeans viz. *Glycine max* subsp. *soja* (Siebold & Zucc.) H. Ohashi and *G tomentella* Hayata were used to understand the viability mechanisms of the seeds in comparison to *G max*. Causes behind better viability of wild type soybeans over cultivated types still are not clear. Therefore, the present study envisaged investigating the longevity of the soybean

using the seeds of wild type, cultivated type and inter-specific RILs stored for one to three years under ambient storage conditions. Genotypic variability for the traits related to seed viability was also investigated for various soybean genotypes.

Materials and methodology

Plant material

The experimental material (seeds) used for evaluation of seed longevity and its associated traits was comprised of 150 soybean genotypes (Supplementary Table 1) that included one accession of *Glycine tomentella*, a perennial species of soybean; 19 accessions of *Glycine soja*, immediate progenitor of cultivated soybean (*G. max*); 105 genotypes of *Glycine max* and 25 inter-specific recombinant inbred lines (RILs) produced by crossing *G soja* accession DC2008-1 and *G max* genotype DS9712.

Assay of seed germination and associated traits

Fresh seeds of each soybean species were harvested carefully and maintained with uniform moisture content prior to storage. The seeds of each genotype were packed in water proof brown paper seed envelopes and kept under ambient storage condition (average $25 \pm 5^\circ\text{C}$ and $65 \pm 5\%$ RH). At start of seed storage, associated traits to seed viability viz., seed coat colour, 100 seed weight and seed coat permeability (slow imbibition approach) for the entire soybean lots were recorded using standard methodologies as per Chandra et al. (2017).

During the storage period, seed samples were drawn at one-year interval for 3 years and tested for viability through germination. The germination of the seeds was tested as per ISTA rules (Anon 2013) following between-paper towel method at 25°C in three replications of 100 seeds each. The seeds of the *G soja* and *G tomentella* were scarified manually before putting on the towel while seeds of *G max* and RILs were used directly without scarification. The seeds were evaluated on 8th day and germination percentage was recorded on the basis of normal seedlings. Genotypes having $\geq 70\%$ and $< 70\%$ germination were registered as ‘good’ and ‘poor’ storers, respectively, as per Indian Minimum Seed

Certification Standards (IMSCS) (Tunwar and Singh 1988). Germination percentage was recorded separately of freshly harvested seeds and those of stored for one, two and three years under ambient conditions.

To understand the relationship between seed longevity and seed coat traits, electrolyte conductivity tests were performed on a set of 40 contrasting (good and poor storers) *G. max* genotypes (Supplementary Table 2) as per Anon (2013) with minor modifications. The good and poor storers identified after screening 105 cultivated types, were used in this panel. Three replications of 50 seeds were soaked in 250 ml of double distilled water at 25 °C for 24 h. The seed leachates were collected in 250 ml beaker and the EC was measured at room temperature with a conductivity bridge (Henna-Model 13,502) and expressed as $\mu\text{S}/\text{cm}/\text{g}$ seed. The EC was tested for the freshly harvested as well as 6-months stored seeds separately to identifying promising genotypes for better storability.

To measure the kinetics of water uptake pattern (Hahalis and Smith 1997), seeds of 30 genotypes comprising of 20-soft seeded (10 good-storer *G max* genotypes and 10 poor-storer *G max* genotypes) and 10 hard-seeded (*G soja* and *G tomentella*) genotypes were selected (Supplementary Table 3). Three replications of 2 g seeds were soaked in 25 ml of distilled water at about 25 °C and their weight was recorded at every two hours' interval up to 24 h. The rate of water uptake was calculated as percent increase over the initial weight of seeds over the time.

Data analysis

All the laboratory-based experiments were conducted by adopting Complete Randomized Design (CRD). The data collected from various experiments were analyzed using SAS software package version 9.4 for calculation of Minimum Significant Difference (MSD), ANOVA for storage studies and Pearson correlation coefficient of different parameters. Correlation analysis was performed using the R function (Wei and Simko 2017). The K-means cluster analysis was carried out using SYSTAT software version 13.2. Principal Component Analysis (PCA) was performed with Clustvis (Metsalu and Vilo 2015).

Results

Genetic variation for seed longevity

Significant variation was observed among the soybean genotypes for seed germination, periods of storage in ambient conditions and their interactions (Supplementary Table 4). The germination (%) of the freshly harvested seeds was very high (78–99%) with a mean of 94.05% (Table 1). However, variation in germination was observed in the wild types, cultivated species and the RILs across the storage periods (Table 1). The mean value of germination after one, two and three years of ambient storage was 79.51%, 52.24% and 29.18%, respectively (Table 1 and Supplementary Fig. 1), which indicated a decline in the germination of the seeds under ambient storage.

A clear cut difference was observed in germination of the hard-seeded (*G soja* and *G tomentella*) and soft-seeded (*G max* and RILs) genotypes. The mean germination (%) in the hard-seeded genotypes was 88.5%, 79.50% and 70.65% after one, two and three years of storage, respectively (Table 1). Similarly, the mean germination percentages in the soft-seeded genotypes were 78.12%, 48.05% and 22.80% after one, two and three year of ambient storage, respectively (Table 1). The three species (*G tomentella*, *G soja* and *G max*) also found to vary significantly in germination across storage period. Among the three species, *G tomentella* registered highest germination (94–85%) followed by *G. soja* (88–70%) and *G. max* (77–18%) (Fig. 1). During depiction of distribution pattern of germination percentage of all genotypes, across the storage periods through box plots (Supplementary Fig. 1) it was found that the percent germination window was very narrow for fresh seeds (78–99%) while it was very wide for aged seeds i.e. 10–90% for two years and 0–85% for three years stored seeds.

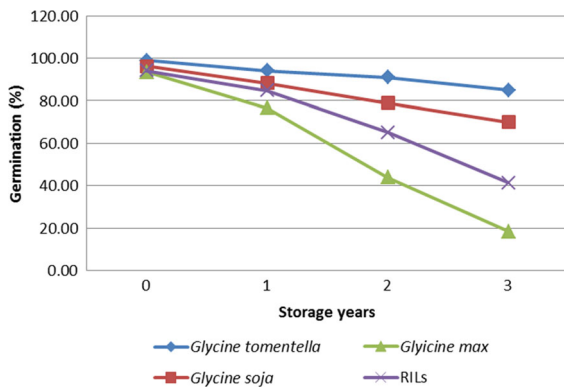
Genetic diversity and principal component analysis

Multivariate analysis was performed using genetic diversity and principal component analyses with three seed related variables viz., seed germination (mean value over the years), 100-seed weight and seed coat permeability. All the 150 soybean accessions were grouped in to 3 clusters using k-means clustering.

Table 1 Descriptive statistics of seed germination of soybean accessions across the years

	100 SW (g)		G0 (%)		G1 (%)		G2 (%)		G3 (%)		Gmean (%)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
<i>Hard-seeded</i>												
<i>G. tomentella</i>	0.53		99.00		94.00		91.00		85.00		92.25	
<i>Glycine soja</i>	1.26	0.56–2.07	96.32	89–99	88.21	77–95	78.95	68–89	69.89	57–83	83.34	72.75–91.5
Total (hard-seeded)	1.22	0.53–2.07	96.45	89–99	88.55	77–95	79.50	68–91	70.65	57–85	83.79	72.75–92.25
<i>Soft-seeded</i>												
<i>Glycine max</i>	8.52	5.06–13.13	93.56	78–99	76.53	48–92	43.96	10–81	18.39	0–69	58.11	36.75–85
RILs	2.30	1.18–3.95	94.17	87–99	84.77	72–95	65.20	35–85	41.33	3–75	71.37	54.33–88.16
Total (soft-seeded)	7.32	1.18–13.13	93.68	78–99	78.12	48–95	48.05	10–81	22.80	0–75	60.66	36.75–88.16
Total accessions	6.51	0.53–13.13	94.05	78–99	79.51	48–95	52.24	10–90	29.18	0–85	63.75	36.75–92.25

100 SW 100 seed weight/seed index, G0 Germination of freshly harvested seeds, G1 Germination of seeds after one year of storage, G2 Germination of seeds after two year of storage, G3 Germination of seeds after three year of storage, Gmean mean germination (%) over the years (0, 1, 2, 3)

**Fig. 1** Aging pattern of seeds of soybean accessions over the years (0, 1, 2, 3 on horizontal axis are years of storage)

Each cluster was found to have varied number of accessions; maximum accessions were found to be in cluster 1 (130nos.), followed by cluster 2 (18nos.) and

cluster 3 (2nos.). The mean value of accessions grouped into each cluster (Table 2) showed that accessions in Cluster-1 had more 100-seed weight and high seed coat permeability whereas Cluster-2 found to have the high seed coat impermeability and high seed germination. The Cluster-3 genotype had highest seed germination and lowest 100-seed weight (Table 2).

PCA was used to eliminate the redundancy in dataset which revealed that all the three seed traits studied have been loaded on to three components; however, major portion of the variance (91.24%) was explained by the first two components. Out of the three components, only PC1 found to have eigen value > 1 (Supplementary Table 5). The first component (PC1) accounted for 74.9% of the variations and contributed through seed germination and seed coat permeability, rest variation was contributed by PC3 (Supplementary

Table 2 Descriptive statistics of different variables in 3 clusters and no. of accessions in each cluster

Cluster variables	Cluster 1			Cluster 2			Cluster 3		
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
Germination % (Avg.)	60.66	34.25–89.00	12.55	82.89	72.50–90.75	5.07	91.88	89.75–95.25	1.90
Seed coat permeability (%)	99.75	91–100	0.96	1.00	0–7	2.02	51.00	34–68	16.53
100 seed weight (g)	7.32	0.94–13.43	3.03	1.30	0.50–2.37	0.45	0.55	0.43–0.86	0.24
Accessions (No.)	130 (All <i>G. max</i> and all RILs)			18 (18 <i>G. soja</i> accessions)			2 (<i>G. tomentella</i> and <i>G. soja</i> DC 2008–1)		

Table 5). Biplotting of PC1 and PC2 indicated that seed coat permeability contributed highest to the first two principal components, as soybean accessions dispersed in the groups (Fig. 2). Dispersion of the accessions in biplot indicated the presence of fair amount of genetic diversity in the experimental material.

Association of seed longevity with other seed-related traits

Association of seed longevity with germination was tested in the selected 150 genotypes. The germination per cent was found to have a positive association with seed longevity. Germination and corresponding longevity was found to be higher in wild species than the cultivated ones even after prolonged storage. On the other hand, the seed coat permeability (%) found to be in negative correlation (-0.53) with mean germination (%); with increase in period of storage, the permeability increased over the years, while viability of the seeds decreased correspondingly (Fig. 3 and Supplementary Table 6). The 100-seed weight trait had positive correlation (0.58) with seed coat permeability, while it had negative correlation (-0.73) with mean germination per cent; thus small seeds had better germination and vice-versa. The relationship found to be holding true over the period of storage (Fig. 3). The seed coat color also found to have some correspondence with viability of the seeds. It appeared that the seeds with black seed coat remain viable for longer

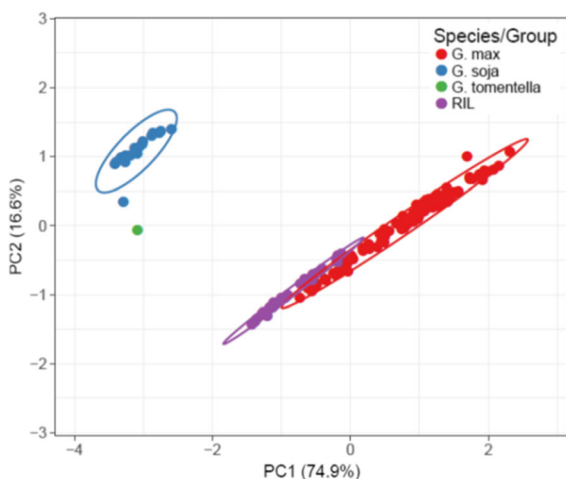


Fig. 2 Bi-plotting of different seed variables loaded on PC 1 and PC 2

period than the seeds with yellow seed coat. Among 22 good storing genotypes identified in cultivated species after two years of storage, 17 appeared to be black and only 5 were yellow seeded. Similarly, out of 10 good storing RILs identified based-on two years of ambient storage, 5 had black seed coat.

Electrical conductance of seed leachates

As an indirect indicator of longevity and vigour of the seeds, electrical conductance (EC) was measured in the seed leachates of identified contrasting genotypes of *G. max*. Initial reading of EC for the controlled seeds ranged from 14.69 to 39.32 $\mu\text{S}/\text{cm}/\text{g}$ with a mean of 25.14 $\mu\text{S}/\text{cm}/\text{g}$, which got doubled to 51.00 $\mu\text{S}/\text{cm}/\text{g}$ (30.35–88.69 $\mu\text{S}/\text{cm}/\text{g}$) after 6 months of storage (Supplementary Table 2 and Fig. 4). However, there was variations in EC readings among the genotypes of good and poor storing categories. Good storing genotypes had lower mean EC values [20.78 $\mu\text{S}/\text{cm}/\text{g}$ (control); 40.37 $\mu\text{S}/\text{cm}/\text{g}$ (after 6 months stored seeds)] than that of the poorly storing genotypes [30.08 $\mu\text{S}/\text{cm}/\text{g}$ (control); 62.45 $\mu\text{S}/\text{cm}/\text{g}$ (six months stored seeds)]. DS74 recorded lowest value (15.20 $\mu\text{S}/\text{cm}/\text{g}$) and EC105790 recorded highest value (30.67 $\mu\text{S}/\text{cm}/\text{g}$) at start of seed storage while G2651 recorded lowest value (30.31 $\mu\text{S}/\text{cm}/\text{g}$) and EC105790 recorded highest value (61.28 $\mu\text{S}/\text{cm}/\text{g}$) among the cultivated good storing genotypes. EC471635 registered lowest EC value (22.23 $\mu\text{S}/\text{cm}/\text{g}$) and (43.25 $\mu\text{S}/\text{cm}/\text{g}$), while VLS61 registered highest EC value 39.32 $\mu\text{S}/\text{cm}/\text{g}$ and 81.22 $\mu\text{S}/\text{cm}/\text{g}$ among the poor storing genotypes at start and after six months' storage, respectively (Supplementary Table 2).

Kinetics of water imbibition of seeds

It was found that the hard-seeded genotypes did not uptake water up to 24 h. (no change in seed weight), while the soft seeded genotypes (both good and poor storers) started imbibing water within 2 h of soaking. The imbibition continued at increasing rate up to 4 h. after which it imbibed slowly and almost stopped imbibing after 10 h of soaking (Fig. 5). Genotypic variation in the rate of imbibition was observed. It was found that among the good storing genotypes, rate of imbibition during initial 2 h. ranged from 0.56 to 0.86 g/h as compared to 0.71–0.93 g/h in the poor storing genotypes (Data not presented). Thus, rate of

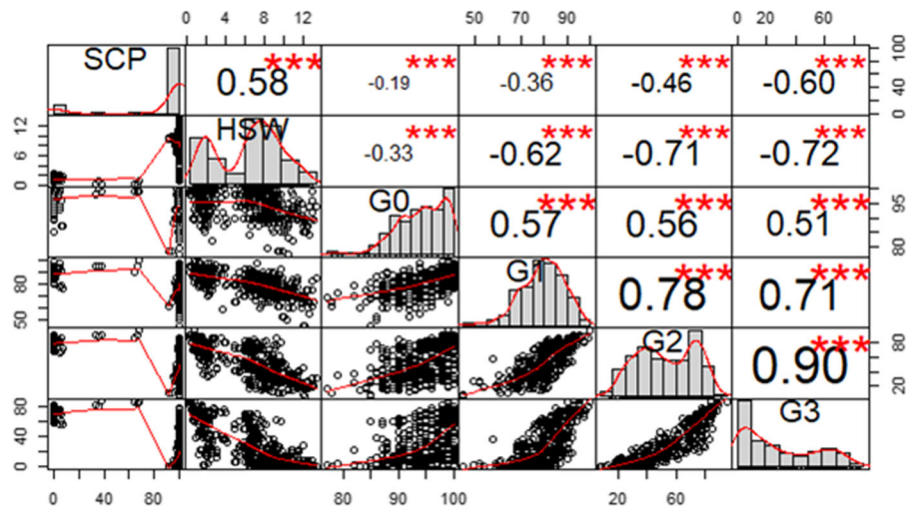


Fig. 3 Graphical depiction of correlation among the seed traits in soybean genotypes (***)Correlation is significant at the 0.01 level; HSW: 100 seed weight; SCP: Seed coat permeability; G0,

G1, G2, G3: Germination (%) after 0, 1, 2, and 3 years of storage respectively)

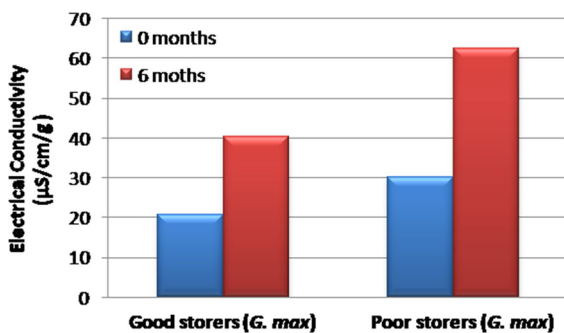


Fig. 4 Electrical conductance from seed leachates on good and poor storers during storage

imbibition in the poor storing genotypes was higher than the good storing genotypes (Supplementary Fig. 2).

Identification of good and poor storers

In this study, out of four groups, *G. tomentella* found to have maximum seed longevity potential followed by *G. soja*. Among the annual wilds, *G. soja* accession DC 2008-1 expressed maximum seed longevity, over the years (Table 3). Based on mean germination per cent over the three years of ambient storage, 10 wild type accessions were identified that maintained more than 70% germination over others (Table 3). Among the cultivated genotypes, 10 genotypes (Table 3.) were identified as good storers that maintained more than

70% germination up to 2 years of ambient storage; however, none of such genotypes had 70% germination after 3 years of ambient storage but they maintained in range of 53–69% germination (Table 3). Similarly, ten genotypes with least viability during storage have also been identified, which was dominated by the cultivated type only (Table 3). Among the RILs, the RIL # 2-6-2, 2-34-4 and 4-11-4 showed 75%, 70% and 73% germination after three year of storage and identified as good storers.

Further, out of 150 genotypes tested, 20 wild type accessions, 25 RILs and 87 cultivated genotypes showed > 70% germination after one year of ambient storage (Supplementary Fig. 3). Such genotypes can be classified as ‘good storer’ after one year of storage as per methodology. After two years of storage, 17 wild type accessions, 10 RILs and 22 cultivated genotypes met the standard to be called as ‘good storer’. After three years of storage under ambient condition, 14 wild type accessions and 3 RILs qualified to be called as ‘good storer’; however, none of the cultivated type genotype could meet the standard of minimum germination (Supplementary Fig. 3). Thus, the study could identify a set of genotypes (Table 3) that maintained > 70% germination across three years of storage (good storers). Similarly, a set of genotypes were identified that had < 70% germination over three years of storage (poor storer) viz., VLS-61, DSb-19, AMS56,

Table 3 Details of good storing and poor storing genotypes

Genotype	SCC	HSW	G(0)%	G(1)%	G(2)%	G(3)%	G(mean)
<i>Good storers*</i> (wild species)							
<i>G. tomentola</i>	Black	0.5312	99	95	90	85	92.25
DC-2008-1	Black	0.5614	99	95	89	83	91.50
PI-464925-A	Black	1.178	99	95	86	80	90.00
PI-464925-B	Black	0.94	98	94	87	75	88.50
PI-424079	Black	1.301	99	91	84	77	87.75
PI-522183-B	Black	1.007	99	92	84	74	87.25
PI-464889-A	Black	1.044	98	94	83	72	86.75
PI-326582-B	Black	1.178	99	90	81	75	86.25
PI-407292	Black	0.695	99	89	82	73	85.75
PI-366120	Black	1.796	97	92	80	73	85.50
<i>Good storers[#]</i> (cultivated species)							
AMSS 34	Black	6.135	98	92	81	69	85.00
DS 74	Black	6.96	99	91	79	68	84.25
USSL-291	Black	6.405	99	86	78	64	81.75
EC 1023	Yellow	9.41	98	88	80	61	81.75
M253	Black	6.575	99	86	77	60	80.50
UPSL 163	Black	6.18	95	84	75	64	79.50
DS MM 64	Black	7.945	96	85	76	58	78.75
UPSL-736	Yellow	7.595	94	86	76	58	78.50
EC105790	Yellow	6.625	99	86	74	53	78.00
UPSL-34	Black	5.06	98	85	73	55	77.75
<i>Good storers*</i> (RILs)							
2-6-2	Black	2.85	100	93	85	75	88.17
4-11-4	Black	3.81875	99	94	81	73	86.83
2-34-5	Grseen yellow	2.6575	99	95	84	67	86.25
2-34-4	Green yellow	2.374	99	93	76	70	84.41
14-3-5	Black	1.93475	96	86	77	65	80.83
<i>Poor storers[#]</i>							
DSB-19	Yellow	13.13	88	48	11	0	36.75
EC-471635	Brown	9.6	78	62	10	0	37.50
PK 472	Yellow	10.615	88	60	15	1	41.00
PS-1480	Yellow	10.075	88	51	26	3	42.00
MAUS-164	Yellow	12.81	89	65	19	0	43.25
MACS-450	Yellow	10.615	82	70	19	2	43.25
PS-1503	Yellow	12.027	90	63	22	0	43.75
G-2144	Green yellow	6.725	88	70	19	0	44.25
VLS-61	Yellow	8.495	90	68	16	3	44.25
DSB-21	Yellow	12.655	90	60	21	7	44.50

SCC Seed coat colour, HSW 100 seed weight, G0 germination of fresh seeds, G1 germination after 1-year storage, G2 germination after 2-year storage, G3 germination after 3-year storage

*Identified based on mean germination and EC leaching

#Identified based on mean germination

MAUS164 etc. (Table 3). Such genotypes would be useful to study inheritance of the trait through hybridization (Fig. 5).

Discussions

Poor longevity of the soybean seeds poses serious problem in seed market and cause considerable production losses as it affects seed and seedling

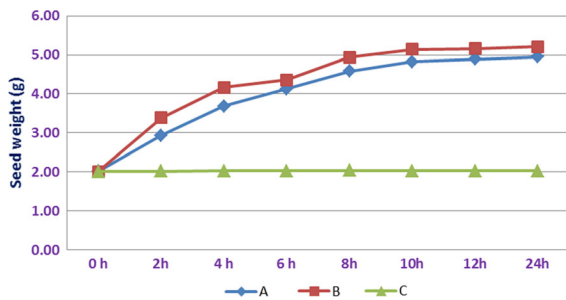


Fig. 5 Dynamics of imbibition in the seeds of RILs (A), G max (B), and G soja (C)

vigour, crop stand in the field and ultimately the seed yield (Zhang et al. 2019). Therefore, improving seed storability in soybean is important to increase overall crop production (Dargahi et al. 2014). Wild species of soybean are excellent reservoir of longevity-related genes and hence needs to be used in breeding program to introgress this trait in cultivated soybean (Zhou et al. 2010; Talukdar et al. 2016; Kumar et al. 2019a).

In the present investigation, genotypes varied for storage potential; the wild species (*G. soja* and *G. tomentola*) maintained higher mean germination per cent (88.5%) after one year of storage than the cultivated soybean (*G. max*) (76.53%). Hosamani et al. (2013) also reported around 79% mean germination after one year of storage in an investigation of 33 diverse *G. max* genotypes. Sooganna et al. (2016) reported observance of 79% germination after eight month of storage of 20 soybean genotypes. Thus, genetic makeup of soybean genotypes essentially determines the viability of the seeds during storage. After two year of storage, barring a few, most of the cultivated genotypes lost viability to the tune of about 56%. Nevertheless, 22 genotypes were identified that maintained more than 70% germination even after 2 years of ambient storage. Kumar (2005) also identified 104 good storing genotypes after two year of storage in ambient storage conditions through screening of 693 *G. max* accessions. Loss of germination was more conspicuous after three years of storage. As a result, none of the *G. max* genotypes maintaining > 70% germination could be identified; however, a few genotypes viz., DS74, AMS34, EC1023, UPSL163, UPSL291 etc. recorded more than 60% germination. Saman et al. (2014) also observed similar

kind of result (65% germination) in two genotypes i.e. Williams and L17, after 30 months of storage.

It was observed that the wild type genotypes lived long; however, the genotypes also had genetic variations for the seed viability traits. Among the twenty accessions, germination after three years of storage ranged from 57 to 85%. This variation was contributed primarily by genetic makeup of wild genotypes, as all the *G. soja* accessions had similar hard seed coat (Chandra et al. 2017). Genetic variation for seed longevity within *G. soja* was reported previously by Zhou et al. (2010) and Talukdar et al. (2016). Zhou et al. (2010) observed germination about 28% and 9% in *G. soja* accessions namely DS and QN, respectively, after 10 years of seed storage. Talukdar et al. (2016) recorded seed viability in range of 0–50% after 7 years of ambient storage during investigation of 29 *G. soja* accessions. The RILs with permeable seed coat derived from *G. soja* × *G. max* demonstrated the genetic potential of the wild type in enhancing the seed viability of the soybean seeds. The wild-type parent of RILs i.e. *G. soja* accession DC 2008-1 showed 95%, 89% and 83% germination after one, two and three years of ambient storage, respectively, while cultivated parent i.e. *G. max* cv. DS9712 recorded 85%, 52% and 11% germination after 1, 2 and 3 years of storage, respectively. RILs derived from this cross showed average seed viability of 84.77% (77–95%), 65.20% (35–85%) and 41.75% (3–75%) after 1, 2 and 3 years of storage, respectively. This observation revealed that soft-seeded RILs showed germination up to 75% even after 3 years of storage due to genes introgressed from the wild-type parent rather than hard-seededness per se.

In this study, the 100-seed weight of the tested genotypes ranged from 0.53 to 13.13 g with a mean of 6.51 g (Table 1). Among the genotypes, lowest 100-seed weight was recorded for the wild type accession of *G. tomentella* (0.53 g) and the highest was recorded for the cultivated genotype Dsb 19 (13.13 g). Seeds of RILs were also small but larger than the wild types and its 100-seed weight ranged from 1.18 (RIL#7-28-2) to 3.95 g (RIL#34-30-1) with mean of 2.30 g (Table 1). As per DUS guidelines (2009), seeds of the wild type accessions and RILs were grouped as small; however 105 *G. max* genotypes were grouped in all three categories i.e. small (84 nos.), medium (19nos.) and large (2 nos.). The small seed size and black seed coat colour may contribute towards better

seed longevity (Hosamani et al. 2013; Sooganna et al. 2016). However, RILs with better seed viability and acceptable seed size (2.8–3.8 g) and non-black (green–yellow) seed coat was also obtained in this study. Thus, the *G. soja* can contribute towards enhancing greater seed longevity in cultivated soybean. In Rice, Xue et al. (2008) and Jiang et al. (2011) identified QTLs for storability using wild genotypes and developed improved breeding line by introgressing these QTLs. In India, wild soybean germplasm has been least used in genetic studies and breeding programs (Yashpal et al. 2015). Successful utilization of wild types to improve the traits of cultivated type has been reported in tomato (Tanksley et al. 1996), soybean (Concibido et al. 2003) and rice (Gaikwad et al. 2014). This study demonstrates the potential of wild-type germplasm as source of viability-related gene(s) to improve the storability of elite cultivars.

In this investigation, color of the seed coat seemed to influence storability of soybean seeds in some genotypes, if not all. Usually, wild type genotypes are black and stores better whereas, cultivated genotypes are primarily yellow and stores poorly. Among the 22 good storers identified in this study, 72% were black and rest 28% were yellow (Supplementary Fig. 4). Similar findings have also been reported by Hosamani et al. (2013) and Sooganna et al. (2016) in soybean. The black seeds show more storability due to activity of some free fatty acids and phenolic compounds (Liu et al. 2017). In present investigation, the seed coat permeability found to have negative correlation with seed viability, which is in agreement with Kumar et al. (2019). The seed size has positive correlation with seed coat permeability and negative correlation with seed viability; similar kind of associations also has been reported by Mugnisjah et al. (1987), Tiwari and Joshi (1989), Chandra et al. (2017), Kumar et al. (2019).

Based on seed coat permeability (%), the group of wild genotypes and the combined group of cultivated types and RILs appeared to be different from one another. In this study, all *G. soja* accessions (except DC2008-1) were impermeable and *G. tomentella* and DC 2008-1 (*G. soja*) appeared to be intermediate-permeable; however all *G. amx* and RIL were found to be permeable on the basis of 7 days ‘slow imbibition’ as per our earlier report (Chandra et al. 2017). The water uptake in the soft-seeds was very rapid in the first few hours of imbibition followed by a lag phase.

The rate of imbibition, however, varied considerably among the soft seeded genotypes. Rapid imbibition may lead to hydration damage causing dip in germination percentage among the poor storing genotypes. The good storing genotypes, on the other hand, maintained slow imbibition at the initial phase thus preventing reduction in viability of the seeds. Therefore, seeds that absorb moisture slowly might also be expected to have better storability.

In this study, it was found that electrolyte leakage as measured through EC had higher values in the poor storing genotypes than the good ones. A significant linear relationship between electrolyte leakage from imbibed seed and germinability has been reported in soybean (Dadlani and Agrawal 1983; Sooganna et al. 2016). Sano et al. (2015) reported that loss of seed viability is often associated with oxidation of various bio-molecules viz., glucose, oils and fatty acids etc. Thus, electrical conductance (EC) of the seed leachates can be used as a marker for screening soybean genotypes for seed storability.

Knowledge of genetic divergence in the available soybean species is of immense importance for selecting the parents to be used in breeding programme including wide hybridization for obtaining desirable genetic recombination for seed longevity and associated traits. The K-means cluster analysis grouped the genotypes in to three clusters. The genotypes from the cluster 1 (soft-seeded) and cluster 2 (hard-seeded) were contrasting regarding seed coat permeability, 100-seed weight and germination (%). These genotypes can be used to develop mapping population for understanding genetics of these traits. Further, wild annual i.e. DC 2008-1 from cluster 3 can be used to introgress intermediate seed coat impermeability in the elite cultivars to improve their viability. In the current study PCA analysis revealed that first two components explains > 90% of the total variation. In the first two PCs, maximum percentage of variation was contributed by seed coat permeability. Genotypes far from the origin exhibited more variability for seed related traits and could be utilized as diverse parents in broadening the genetic base of soybean through hybridization. Similar type of divergence studies was also conducted by Naik et al. (2016) in *G. max* lines to identify the diverse lines.

Conclusion

One of the major obstacles for soybean cultivation in India is the non-availability of good quality seed and maintenance of prescribed level of seed germination and vigor after harvest until the planting of seeds in the succeeding season. The information generated in this investigation may be utilized during evaluation of seed storability traits in soybean species. Identified good storer genotypes could be used to introgress seed storability trait in to the elite cultivars as well to develop mapping population by hybridizing with the poor storers to understand the genetics of the storability traits.

Acknowledgements First author is grateful to the UGC, New Delhi for Junior Research Fellowship (JRF) during his doctoral study and authors are grateful to the Collaborative Outstation Research Centre-Sipani Krishi Anusandhan Farm (SKAF), Mandasour, MP for providing the seed materials of the wild soybean accessions. The funding from the National Agricultural Science Fund (NASF), Indian Council of Agricultural Research, New Delhi is duly acknowledged.

Author contributions Conceptualization of research: AT, SC; Designing of the experiments: AT, SC; Contribution of experimental materials: AT, NSS; Execution of lab experiments and data collection: SC, RRY, YT, MS; Analysis of data and interpretation: SC, YT; Preparation of the manuscript: SC, AT.

Funding Authors are grateful to the National Agricultural Science Fund, ICAR, New Delhi for funding the program.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval We declare that we have maintained the ethical standard while preparing and submitting the manuscript to the GRES.

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