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Physical and Biochemical Analysis on Seed Longevity in Soybean (*Glycine max* L. Merrill)

Priyanka M., Parashivamurthy, RamanappaT. M., Siddaraju

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ABSTRACT

The laboratory experiments were comprised of 20 genotypes with five different seed coat colors (Black, Brown, Variegated, Green and Yellow) and seeds were stored for 10 months in polythene bags to evaluate the physical and biochemical changes during seed storage. The results revealed that black colored genotypes showed resistant for seed ageing followed by brown colored genotypes. Yellow colored genotypes are susceptible for seed ageing followed by green colored genotypes. Among the genotypes, for biochemical parameters EC 76756 recorded highest Peroxidase activity (0.703), DPPH (43.32) and decreased Malondialdehyde content (1.907). Whereas for physical traits, EC-76756 have found highest proportion of seed coat to seed (10.01%) and Mechanical strength of seed coat (75.28 N) after 10th months of seed storage and minimum biochemical and physical changes were found in yellow colored genotypes with high lipid peroxidation by-product like Malondialdehyde content.

Keywords Peroxidase activity, DPPH, Malondialdehyde content, Proportion of seed coat to seed, Mechanical strength of seed coat.

Priyanka M.*
Assistant Prof. (Contractual), Department of Seed Science and Technology, College of Horticulture, Mudigere 560007, Karnataka, India

Parashivamurthy
Professor and Head, Department of Seed Science and Technology, College of Agriculture,UAS, Bangalore 560065, Karnataka, India

RamanappaT.M.
Senior Scientist and Scheme Head, AICRP on Breeder Seed Project, NSP, UAS, Bengaluru, India

Siddaraju
Seed Research Officer, STR, National Seed Project, Bengaluru, India
Email: ppriya563@gmail.com
*Corresponding author

INTRODUCTION

Soybean (*Glycine max* L. Merril) is the world's most important seed legume, which contributes about 25% of the global edible oil, which is two-third of the world's protein concentrate for livestock feeding (Agarwal *et al*. 2013). It has earned epithets like ‘‘Cow of the field'' or ‘‘Gold from soil'', "poor man's food" and "wonder crop". Owing to its amino acids composition like glycine, tryptophan and lysine, the protein of soybean is called a complete protein. It has around 40% protein and 20% oil in it. The plant has been classified as an oilseed rather than pulse by an UN Food and Agricultural Organization and known as Golden bean of 20th century. It is originated in Eastern Asia or China (Agarwal *et al.* 2013).

The cultivated soybean (*Glycine max* L. Merril) is a member of Leguminacae and sub family Papilionaceae with twenty chromosome pair (2n = 40). Cultivated soybean is believed to have derived from a wild progenitor *Glycine ussuriensi*. Soybean tops in world production of both oilseeds and edible oil production. It is globally grown over an area of 107.21 m ha with a production of 251mt and productivity of 2447 kg per ha (Anon 2016).

In India soybean is grown over an area of 11.67 m ha with a production of 8.59 mt and productivity of 737 kg per ha (Anon 2016). The major soybean producing states are Madhya Pradesh (831 kg per ha), Maharashtra (557 kg per ha), Rajasthan (829 kg per ha), Karnataka (558 kg per ha) and Andhra Pradesh (1,000 kg per ha). Soybean seed has been identified as poor storer, because of delicate (thin) seed coat and vulnerable position of embryo. Among oilseed crops, soybean is the most extensively studied crop with respect to ageing. Soybean oil with approximately 60% of polyunsaturated fatty acids (PUFA) content is liable to rapid degradation making it a poor storer. The intrinsic factors that are believed to be closely associated with the seed ageing are loss of membrane integrity, alteration of chemical composition, changes in enzyme activities, depletion of food reserves and chromosomal aberrations. Soybean seeds, rich in both fatty acids and proteins are very much prone ageing. With these facts a comprehensive study was conducted to evaluate and assess the physical and biochemical changes occurring during seed ageing in soybean genotypes.

MATERIALS AND METHODS

The material for the present study comprised of 20 soybean (*Glycine max* (L.) Merrill) genotypes by taking seed coat color as main criteria. A laboratory experiment was conducted to evaluate the physical and biochemical changes occurring during seed ageing. This experiment was under taken in Department of Seed Science and Technology, GKVK, UAS, Bangalore, by using freshly harvested seeds which were cleaned and dried to safe moisture level. The genotypes were stored in polythene bags for 10 months and observations were recorded.

Biochemical parameters

Peroxidase activity (A_{436} / *min /g of seed*)

Enzyme extraction

One gram of seeds were extracted in 1 ml of 0.1 M Phosphate buffer with pH 7.0 by grinding with a pre cooled pestle and mortar. The slurry was transferred to eppendorf tubes and kept at 4 ^{0}C for 4 h for enzyme extraction and then tubes are transferred to 20 ^{0}C. The homogenate was centrifuged at 10,000 rpm at 4 ^{0}C for 15 minutes. The supernatant was used as enzyme source. The enzyme extract was stored in ice box till the assay is carried out.

Preparation of reagents

1. Phosphate buffer 0.1 M (pH 7.0)
2. Guaiacol solution (20 mM): 242 µl guaiacol was added to distil water and volume was made to 100 ml. It can be stored in frozen condition for many months.
3. Hydrogen peroxide solution (0.042 %) (12.3 mM): 125 µl of 30 % hydrogen peroxide was added to distilled water and volume was made to 100 ml. It should be prepared at the time of use. Absorbance of 12.3 mM H_2O_2 was adjusted to 0.4 by adding 20 ml of water to it, before using the solution for assay.

Estimation of peroxidase activity

The enzyme assay was carried out according to Sadasivam and Manickam (1996). The reaction mixture was prepared in cuvette by adding 2 ml of 0.1 M phosphate buffer of pH 7.0, Guaiacol-200 µl and 12.3 mM H_2O_2-200 µl. Brought the mixture to 25 ºC and then placed the cuvette in the Spectrophotometer set at 436 nm. Then, add 100 µl of enzyme extract mix it properly with pipette tip, immediately start the stopwatch. Read the initial absorbance at 436 nm and note increase the absorbance for 3 minutes at an interval of 30 seconds by using enzyme kinetics. Water is used as blank during the assay period and enzyme activity was expressed in terms of change in absorbance per minute per gram of seed.

Malondialdehyde content (µM/g of fresh wt)

Enzyme extraction

Malondialdehyde (1, 3-propandial) was measured by a colorimetric method. Two axes were excised from soybeans at 24 h after imbibition, were homogenized in 5 ml of distilled H_2O. An equal volume of 0.5 % TBA in 20 % of trichloroacetic acid solution was added and the sample was incubated at 95^0C for 30 min. The reaction was stopped by putting the reaction tubes in an ice bucket. The samples then were centrifuged at l0,000 rpm for 30 min.

Estimation of malondialdehyde activity

The enzyme assay was carried out according to Sadasivam and Manickam (1996). The supernatant was removed, Absorbance was read at 532 nm and the value for nonspecific absorption at 600 nm was read and subtracted from this. The amount of malondialdehyde present was calculated from the extinction coefficient of 155 Mm/g. It has been pointed out that a number of organic compounds may interfere with the TBA assay for malondialdehyde. Although this problem is reduced by subtracting nonspecific absorption obtained in the assay it was deemed necessary to correlate these data with an analysis of the fatty acid precursor from which malondialdehyde is ultimately produced.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity (%)

The percentage of antioxidant activity of seed was assessed by 1, 1 diphenyl-picrylhydrazyl (DPPH) free radical assay. The measurement of the 1, 1 diphenyl-picrylhydrazyl (DPPH) radical scavenging activities was performed according to methodology described by BrandeWilliam *et al*. (1995). The samples were reacted with stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5ml of sample, 3ml of absolute ethanol and 0.3ml of DPPH radical solution 0.5mM in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read (Absorbance (Abs)) at 517 nm after 100 min of reaction using UV spectrophotometer. The mixture of ethanol (3.3ml) and sample (0.5 ml) serve as blank. The control solution was prepared by mixing ethanol (3.5ml) and DPPH radical solution (0.3 ml). The scavenging activity percentage (A %) was determined according to Mensor *et al.* (2001).

$$\text{Activity } \% = 100-((A_{sample} - A_{blank}) \times 100/A_{control})$$

Physical traits of seed

Proportion of seed coat to seed (%)

Seed coats from 20 seeds in two replications were removed carefully with the help of forceps after applying a gentle stroke to seeds placed in-between 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,

$$\frac{\text{Weight of Seed Coat}}{\text{Weight of Seed Coat and Cotyledons}} \times 100$$

Mechanical strength of seed coat (N)

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). The seed was placed on the stationary metallic 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 moving the piston. The increase of force is plotted along the Y-axis and duration of force applied (till the seed is cracked) is 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 is a sudden drop on the graph which indicates the first break on the seed coat surface (Plate 1).

RESULTS AND DISCUSSION

The results revealed that, the seed ageing had signif-

Plate 1. Texture analyzer used for mechanical strength of seed coat (N).

icant effects on seed quality parameters of soybean genotypes. Among the 20 genotypes used for the study, the genotypes with black (EC-76756, EC-57042, IC-501268, TR-5, PB-5) and brown (DS-72-244) colored seed coat showed a highest resistance against the seed deterioration changes compared with variegated, green and yellow colored genotypes. The genotypes having black colored seed coat (Table 1) were found to be highly resistant against deterioration and genotypes with yellow colored were found susceptible for deterioration changes.

Biochemical characteristics

All the genotypes showed significant difference for peroxidase activity over storage period and presented inTable 2.

Peroxidase activity

Among all genotypes, EC-76756 showed highest peroxidase activity in the initial period of storage and after 10th month storage (from 0.998 and 0.703 respectively) followed by IC-501268, EC-57042 (from 0.995 to 0.587, from 0.995 to 0.621 respectively) and PB-5 (from 0.994 to 0.687). 115-B recorded lowest peroxidase activity in the initial period of storage and after 10th month storage (from 0.960 to 0.264) followed by RKS-24 (from 0.963 to 0.278) and JS - 2069 (from 0.966 to 0.295).The maximum percent reduction was found in yellow colored genotype, 11 5-B (72.50) and minimum percent reduction was found in black colored genotype (EC-76756 (29.56).

The rate of reduction in the peroxidase activity

Table 1. Testa color of genotypes

Testa color of genotypes				
Brown	Variegated	Green	Black	Yellow
DS-72-244	RSC-0-71	JS - 9041	EC-57042, TR-5, IC-501268, EC-76756, PB-5	JS-335, JS - 9560, NRC-86, 115-B, DSB-21, RKS-45, NRC-37,RKS-24, JS - 2034, JS - 2069, AGS 29, JS – 2029

Table 2. Performance of soybean genotypes during storage on peroxidase activity (A_{436} /min/ g of seed).

Genotypes	Initial	Peroxidase activity (A436 / min /g of seed) Period of storage (December 2018 to October, 2019) 1	3	5	7	8	9	10	Percent reduction
Black color genotypes									
EC-76756	0.998	0.995	0.982	0.955	0.892	0.851	0.788	0.703	29.56
PB – 5	0.994	0.992	0.971	0.943	0.865	0.820	0.722	0.687	30.89
IC – 501268	0.995	0.993	0.947	0.900	0.833	0.764	0.656	0.587	41.01
TR-5	0.991	0.987	0.933	0.877	0.798	0.740	0.612	0.533	46.22
EC-57042	0.995	0.992	0.965	0.917	0.841	0.770	0.689	0.621	37.59
Yellow color genotypes									
NRC-86	0.975	0.969	0.876	0.787	0.652	0.511	0.432	0.355	63.59
115-B	0.960	0.949	0.830	0.739	0.587	0.424	0.355	0.264	72.50
DSB-21	0.981	0.976	0.897	0.820	0.728	0.600	0.506	0.444	54.74
RKS-45	0.984	0.979	0.905	0.833	0.746	0.632	0.537	0.476	51.63
NRC-37	0.986	0.982	0.911	0.840	0.762	0.673	0.564	0.488	50.51
JS -9560	0.980	0.974	0.889	0.816	0.707	0.588	0.491	0.402	58.98
RKS-24	0.963	0.950	0.844	0.745	0.596	0.453	0.362	0.278	71.13
JS – 2069	0.966	0.957	0.846	0.751	0.603	0.465	0.376	0.295	69.46
JS – 335	0.969	0.962	0.856	0.765	0.627	0.489	0.388	0.321	66.87
JS – 2029	0.973	0.968	0.863	0.783	0.632	0.503	0.421	0.335	65.57
JS - 2034	0.988	0.984	0.917	0.854	0.774	0.692	0.589	0.387	60.83
AGS 29	0.977	0.971	0.881	0.802	0.669	0.521	0.464	0.399	59.16
Brown color genotypes									
DS 72-244	0.993	0.989	0.941	0.897	0.824	0.753	0.623	0.554	44.21
Green color genotypes									
JS - 9041	0.988	0.984	0.917	0.854	0.774	0.692	0.589	0.505	48.89
Variegated color genotypes									
RSC-1071	0.990	0.986	0.924	0.869	0.780	0.721	0.609	0.522	47.27
Mean	0.982	0.976	0.903	0.835	0.730	0.627	0.533	0.458	
S.Em. ±	0.006	0.005	0.015	0.007	0.011	0.007	0.007	0.008	
CD (P= 0.01)	0.021	0.016	0.019	0.029	0.037	0.027	0.027	0.031	
CV (%)	1.21	1.05	2.98	1.59	2.67	2.01	2.31	3.13	

is more in yellow seeded genotypes followed by green and variegated seeded types this might be due to delicate seed coat of yellow colored seeded genotypes which absorbs more water and causes free radicals damage. Peroxidase is the key enzyme which detoxifies the excessive hydrogen peroxide in the seeds. Increased level of peroxidase enzymes protects the cell against the oxidative damage by removal of reactive oxygen species and free radicals. Whereas similar results were revealed by Scialabbal *et al*. (2002) that aged seed lots which maintained high viability showed an increase in peroxidase activity in two distinct parts of the seed, integument and cotyledons upon decline in viability, peroxidase activity also gets declined.

Malondialdehyde content (µM/g of fresh weight)

MDA a major compound responsible for lipid peroxidation differed significantly for ageing. Irrespective of genotype there was an increase in accumulation of MDA from initial seed storage to 10th month of seed storage. Maximum accumulation of MDA observed in a yellow seeded genotype 115-B (0.809 to 5.476 µM/g of fresh weight) from initial seed storage to 10th month of seed storage. Whereas the less accumulation of MDA observed in black colored genotype, EC-76756 (0.547 to 1.907 µM/g of freshweight). Overall black and brown seeded genotypes showed slight increase in MDA content followed by variegated and green seeded genotypes and highest MDA

Table 3. Performance of soybean genotypes during storage on malondialdehyde (µM/g of fresh weight).

Genotypes	Initial	1	3	5	7	8	9	10	Percent reduction
	Malondialdehyde (µM/g of fresh weight) Period of storage (December 2018 to October 2019)								
Black color genotypes									
EC 76756	0.547	0.601	0.724	0.891	1.121	1.452	1.645	1.907	71.32
PB – 5	0.570	0.645	0.751	0.946	1.234	1.608	1.983	2.187	73.94
IC – 501268	0.608	0.666	0.795	0.998	1.334	1.846	2.108	2.684	77.35
TR-5	0.657	0.702	0.845	1.107	1.271	1.396	2.256	3.208	79.52
EC-57042	0.584	0.657	0.782	0.957	1.292	1.765	2.098	2.287	74.46
Yellow color genotypes									
NRC-86	0.768	0.812	1.402	1.523	1.808	2.623	3.876	4.987	84.60
115-B	0.809	0.915	1.757	1.892	2.345	3.567	4.306	5.476	85.23
DSB-21	0.720	0.793	0.988	1.375	1.632	2.356	3.087	4.092	82.40
RKS-45	0.703	0.768	0.965	1.246	1.592	2.098	2.982	3.989	82.38
NRC-37	0.691	0.742	0.904	1.187	1.402	1.545	2.767	3.897	82.27
JS -9560	0.728	0.794	0.991	1.403	1.633	2.345	3.287	4.308	83.10
RKS-24	0.815	0.871	1.707	1.865	2.223	3.112	4.109	5.378	84.85
JS – 2069	0.819	0.856	1.676	1.823	2.106	2.987	4.065	5.296	84.54
JS – 335	0.791	0.837	1.608	1.777	1.945	2.823	3.994	5.108	84.51
JS – 2029	0.776	0.823	1.598	1.734	1.878	2.765	3.908	5.074	84.71
JS - 20 34	0.755	0.804	1.309	1.478	1.723	2.505	3.587	4.786	84.22
AGS 29	0.739	0.799	1.070	1.418	1.702	2.487	3.456	4.675	84.19
Brown color genotypes									
DS 72-244	0.629	0.692	0.823	1.007	1.328	1.987	2.199	2.983	78.91
Green color genotypes									
JS - 90 41	0.677	0.723	0.895	1.143	1.397	1.482	2.567	3.765	82.02
Variegated color genotypes									
RSC 10 71	0.663	0.711	0.877	1.121	1.318	1.408	2.345	3.678	81.97
Mean	0.702	0.760	1.123	1.344	1.636	2.185	3.031	3.988	
SEm. ±	0.02	0.01	0.01	0.02	0.02	0.03	0.02	0.04	
CD (P= 0.01)	0.06	0.03	0.04	0.07	0.06	0.10	0.08	0.14	
CV (%)	4.08	1.56	1.45	2.51	1.61	2.10	1.19	1.57	

activity found in yellow seeded genotypes (Table 3) . The maximum percent increase was found in yellow colored genotype, 115-B (85.23) and minimum percent increase was found in black colored genotype, EC-76756 (71.32).

These may be due to variability existing among varieties, it can be noticed that performances of genotypes also influenced the biochemical changes in seeds during ageing. The above obtained results are similar with the results of (Malencic *et al*. 2003). Seed susceptibility to oxidative changes differed, depending on seed fatty acid composition, lipid peroxidation can be considered as indicators of individual soybean genotype susceptibility to oxidative stress (Shruti 2018).

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity (%)

The DPPH radical scavenging activity differed significantly among the genotypes. Among the black colored genotypes, EC-76756 recorded maximum DPPH radical scavenging activity in the initial period and final month of storage (71.09 and 43.32). The minimal was found in 115-B (45.33 and 12.54) in the initial period and final month of storage respectively (Table 4). This might be due to oxidative damage

Table 4. Performance of soybean genotypes during storage on DPPH (1, 1-dipheny l-2- picrylhydrazyl) radical scavenging activity (%).

	DPPH (1, 1-dipheny l-2- picrylhydrazyl) radical scavenging activity (%) Period of storage (December 2018 to October 2019)								
Genotypes	Initial	1	3	5	7	8	9	10	Percent reduction
Black color genotypes									
EC-76756	71.09	69.88	64.69	60.15	57.88	54.00	49.23	43.32	39.06
PB -5	70.23	68.62	63.33	59.81	55.03	52.04	48.65	42.32	39.74
IC - 501268	68.56	65.44	61.67	57.56	53.00	49.72	45.18	38.56	43.76
TR-5	64.31	63.22	59.45	55.48	51.08	47.23	41.18	35.45	44.88
EC-57042	69.45	67.00	62.85	58.85	54.01	51.67	47.66	41.34	40.48
Yellow color genotypes									
NRC-86	51.00	49.37	45.15	41.58	36.58	32.17	27.35	21.13	58.57
115-B	45.33	43.02	38.67	34.23	27.03	23.25	18.94	12.54	72.34
DSB-21	58.53	56.29	51.33	47.02	41.11	38.54	33.21	27.64	58.57
RKS-45	60.00	57.75	53.55	48.68	43.11	40.59	35.23	28.76	72.34
NRC-37	61.56	58.00	53.69	49.21	45.00	41.25	36.00	31.25	58.57
JS -9560	56.63	54.22	50.38	46.32	41.11	37.54	32.18	25.43	55.09
RKS-24	46.00	43.16	39.39	35.43	29.34	25.12	20.76	13.01	71.72
JS – 2069	47.71	45.31	41.72	36.45	31.39	27.34	21.89	16.45	65.52
JS – 335	49.87	47.09	42.83	37.00	31.71	28.65	22.11	18.69	62.52
JS – 2029	50.91	48.66	43.58	39.87	34.89	30.41	25.54	19.47	61.76
JS - 2034	53.22	51.09	47.44	43.22	39.51	35.31	28.76	28.76	45.96
AGS 29	55.00	53.29	49.62	45.03	40.88	36.44	30.87	24.32	55.78
Brown color genotypes									
DS 72-244	67.32	64.11	61.33	57.02	52.09	48.76	43.22	37.76	43.91
Green color genotypes									
JS - 9041	62.14	58.67	54.22	50.73	47.25	43.76	38.55	32.11	48.33
Variegated color genotypes									
RSC 1071	63.00	61.04	56.84	52.60	48.65	45.27	40.00	34.12	45.84
Mean	58.59	56.26	52.09	47.81	43.03	39.45	34.33	28.62	
S.Em. ±	0.77	0.52	0.92	0.90	0.94	0.54	0.26	0.30	
CD (p= 0.01)	2.96	1.98	3.51	3.44	3.59	2.06	0.99	1.04	
CV (%)	2.29	1.59	3.05	3.26	3.78	2.36	1.30	1.80	

causes reduction in the antioxidant synthesis over the storage period as there will be gradual increase in MDA and free radical production. The results are in agreement with Kartika (2016) that yellow seeded genotype having least percent antioxidant activity and this was found associated with total phenolic compounds.

Proportion of seed coat to seed

The highest proportion of seed coat to seed was found in black colored genotypes followed by brown and variegated genotypes. The least proportion of seed coat to seed was recorded in yellow colored genotype followed by green genotype. In the initial period of storage, maximum proportion of seed coat to seed was observed in EC-76756 (12.31) followed by PB-5 (12.12). The minimum proportion of seed coat to seed was observed in 115-B (8.81) followed by RKS-24 (8.93). After 10th month of seed storage, EC-76756 recorded maximum (10.01) proportion of seed coat to seed and lowest was seen in 115-B (6.30). The maximum percent reduction in proportion of seed coat to seed was found in yellow colored genotype, 115-B (29.45) and minimum percent reduction was found in black colored genotype, PB-5 (18.56).

There is a slight resistance in the reduction of proportion of seed coat to seed in black colored genotypes and brown coloured genotype compare

Table 5. Performance of soybean genotypes during storage on proportion of seed coattoseed (%)

Genotypes	Initial	Proportion of seed coattoseed (%) Period of storage (December, 2018 to October 2019) 1	3	5	7	8	9	10	Percent reduction
Black color genotypes									
EC-76756	12.31	12.21	11.90	11.44	10.90	10.67	10.22	10.01	18.68
PB-5	12.12	12.01	11.78	11.32	10.72	10.54	10.13	9.87	18.56
IC – 501268	11.51	11.38	11.00	10.63	10.17	9.87	9.44	9.17	20.33
TR-5	11.08	10.97	10.62	10.23	9.83	9.61	9.24	9.00	18.77
EC-57042	11.76	11.62	11.20	10.82	10.30	10.15	9.77	9.53	18.96
Yellow color genotypes									
NRC-86	9.54	9.35	9.00	8.53	8.02	7.85	7.34	7.02	26.42
115-B	8.81	8.70	8.27	7.84	7.34	7.01	6.71	6.30	29.45
DSB-21	10.11	10.00	9.66	9.22	8.75	8.53	8.10	7.83	22.55
RKS-45	10.43	10.27	9.87	9.41	8.93	8.61	8.24	8.00	23.30
NRC-37	10.77	10.42	10.00	9.67	9.11	8.85	8.47	8.18	24.05
JS -9560	10.00	9.82	9.46	9.00	8.64	8.33	7.98	7.65	23.50
RKS-24	8.93	8.77	8.30	7.90	7.38	7.07	6.74	6.47	26.56
JS – 2069	9.02	8.93	8.43	8.01	7.45	7.17	6.93	6.56	27.27
JS – 335	9.21	9.09	8.54	8.12	7.73	7.42	7.03	6.76	26.60
JS – 2029	9.32	9.16	8.82	8.38	7.96	7.64	7.20	6.96	25.32
JS - 2024	9.73	9.55	9.01	8.73	8.24	8.03	7.76	7.36	24.36
AGS 29	9.87	9.63	9.33	8.88	8.40	8.17	7.84	7.51	23.91
Brown color genotypes									
DS 72-244	11.23	11.11	10.89	10.45	10.03	9.82	9.30	9.01	19.77
Green color genotypes									
JS - 9041	10.65	10.51	10.15	9.87	9.32	9.07	8.80	8.51	20.09
Variegated color genotypes									
RSC-1071	10.98	10.72	10.43	10.06	9.50	9.28	9.05	8.78	20.04
Mean	10.36	10.21	9.83	9.42	8.93	8.68	8.31	8.02	
SEm. ±	0.11	0.12	0.17	0.12	0.08	0.08	0.10	0.11	
CD (p= 0.01)	0.42	0.39	0.62	0.37	0.30	0.30	0.38	0.43	
CV (%)	1.83	2.05	2.99	2.24	1.52	1.54	2.05	2.43	

to yellow colored genotypes.The rate of reduction is slower in black colored genotypes and more in yellow colored genotypes this could be due to thicker seed coat of black colored genotypes than that of yellow-seeded lines. Similar findings were reported by Kuchlan *et al.* (2010) the black-seeded varieties had highest seed coat to seed proportion and they were resistant to field weathering because of thicker seed coat.

Mechanical strength of seed coat (N)

Mechanical strength of seed coat differed significantly among the 20 genotypes and presented in Tables 5 and 6. There is a gradual decrease in mechanical strength of seed coat from initial period of storage to 10th month of seed storage. The maximum mechanical strength of seed coat was observed in black colored genotypes (92.98) followed by brown (88.52) and

Table 6. Performance of soybean genotypes during storage on mechanical strength of seed coat (N)

	Mechanical strength of seed coat (N) Period of storage (December 2018 to October 2019)								
Genotypes	Initial	1	3	5	7	8	9	10	Percent reduction
Black color genotypes									
EC-76756	92.74	91.19	89.64	85.26	82.21	81.17	78.74	75.28	18.83
PB-5	92.98	90.41	88.36	83.61	81.58	80.20	77.52	75.25	19.08
IC – 501268	89.04	87.79	85.85	80.15	78.25	77.98	75.74	72.05	19.08
TR-5	87.30	86.47	84.91	78.11	74.16	72.63	71.20	70.38	19.38
EC-57042	90.94	89.86	87.44	82.48	79.71	78.54	76.63	73.17	19.54
Yellow color genotypes									
NRC-86	75.85	75.34	72.99	68.03	64.16	63.37	61.07	59.38	21.71
115-B	70.09	68.65	65.83	63.03	59.58	58.33	56.68	53.40	23.81
DSB-21	80.52	79.66	77.51	73.68	69.29	68.41	64.52	62.58	22.28
RKS-45	82.70	81.70	78.45	74.03	70.47	69.52	65.99	63.71	22.96
NRC-37	83.74	82.44	80.92	75.15	71.06	70.54	67.73	65.27	22.06
JS – 9560	78.80	77.62	75.78	72.11	68.69	67.42	64.08	62.14	21.14
RKS24	70.95	69.68	67.77	65.06	60.93	59.63	57.63	54.27	23.51
JS – 2069	72.89	71.81	70.96	67.05	61.38	59.55	58.61	55.93	23.27
JS – 335	74.87	73.76	71.03	68.03	62.93	61.31	59.65	56.27	24.84
JS – 2029	76.68	75.18	72.18	69.92	63.24	62.54	60.42	58.69	23.46
JS - 2034	76.91	75.80	73.79	70.11	66.25	65.31	62.41	60.25	21.66
AGS 29	77.67	76.75	74.77	71.43	67.38	66.20	63.20	61.17	21.24
Brown colour genotypes									
DS 72-244	88.52	86.81	85.47	79.36	76.48	75.65	73.52	71.02	19.77
Green color genotypes									
JS - 9041	84.92	84.00	82.73	76.37	72.49	70.67	68.63	66.46	21.74
Variegated color genotypes									
RSC-1071	85.97	85.30	83.59	77.48	73.60	71.51	70.18	67.25	21.78
Mean	81.70	80.51	78.49	74.02	70.19	69.024	66.70	64.09	
SEm. ±	1.59	0.93	0.84	0.52	0.54	1.38	1.38	0.91	
CD (p= 0.01)	4.77	2.79	2.52	1.07	1.62	4.19	4.21	2.84	
CV (%)	3.37	2.00	1.85	1.23	2.31	3.45	3.60	2.47	

variegated (85.97) in the initial period of storage. The minimal was observed in yellow colored genotypes (70.09) followed by green colored genotypes (84.92) in the initial period of storage. After 10th month of seed storage, maximum mechanical strength of seed coat was observed in EC-76756 (75.28) followed by PB-5 (75.25). The minimal mechanical strength of seed coat was observed in 115-B (53.40) followed by RKS-24(54.27) and JS - 2069 (55.93). The maximum percent reduction was found in yellow coloredgenotype, 115-B (23.81%) and minimum was found in black coloredgenotype, EC-76756 (18.83 %).

The decrease in mechanical strength of seed coat might be due to decrease in calcium content which imparts less resistant for field weathering and leads to ageing of seed. Similar results were found with Assou and Kueneman (1984) that black-seeded lines of soybean were resistant to field weathering because of thicker seed coat than that of yellow-seeded lines.

CONCLUSION

Among black color genotypes, EC-76756 followed by PB-5are resistant for field weathering because they have maximum mechanical strength of seed coat and minimum MDA. Among yellow color genotypes, 11 5-B followed by RKS-24 are susceptible for seed longevity (poor storers) because of its delicate seed coat and minimum mechanical strength of seed coat and maximum MDA.Best accessions identified (EC-76756, EC-57042, IC-501268, TR-5, PB-5 and DS-72-244) for resistant against seed deterioration can be used as parental lines to develop mapping population for seed longevity.

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