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Physio-Biochemical Changes During Seed Deterioration in Mini Core Set of Soybean [*Glycine max* (L.) Merill] Germplasm

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ABSTRACT

The laboratory study was carried out in mini core set of 20 soybean genotypes for evaluating the physiological and biochemical changes occurring during seed deterioration by exposing them to artificial seed aging. The results revealed the clear genotypic variability with respect to seed vigour among different soybean genotypes. High vigour genotypes (TR-5, EC-76756, PB-5, EC-101549, IC-501268 and EC-57042) were characterized by smaller seed size (<5.8mm^3) with black and brown testa coloured genotypes showing higher resistance against seed deterioration compared with the poor vigour (AT-156, KDS-869, JS-20-73, NRC-127, CAT-49586, CAT-49, JS-20-42 and AGS-432) large (>6.1mm^3), yellow testa coloured seeds, which were susceptible to deterioration changes. Among the mini coreset higher resistance against seed deterioration was offered by PB-05 with higher seed germination percentage (86%), SVI-I (1562), SVI-II (538), total dehydrogenase activity (1.274 @ A520), peroxidase activity (0.475 A_{430} / min /g of seed). Whereas, least seed germination percentage (60%), SVI-I (783), SVI-II (330) was observed in the genotype JS-20-42.

Keywords : Seed deterioration, seed germination, total dehydrogenase activity, peroxidase activity

SOYBEAN [*Glycine max* (L.) Merril] is the world's most important seed legume, which contributes about 25 per cent 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 per cent protein and 20 per cent 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. 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 mha with a production of 251 MT 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 (1000 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 per cent of poly unsaturated 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 deterioration 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 to these types of deteriorations.

With these facts a comprehensive study was conducted to evaluate and asses the physiological and biochemical changes occurring during seed deterioration in soybean genotypes.

Material and Methods

The material for the present study comprised of 20 soybean [*Glycinemax* (L.) Merrill] genotypes selected from the core population of 98 germplasm accessions by developing a mini core set using power core software (Table I) by taking seed coat colour, hilum colour and malenoldehyde content of the seed as main criteria.

Table I

Particles of soybean mini core set used for the study

Genotypes	Seed coat colour	Hilum colour
EC-76756	Black	Black
EC-57042	Black	Grey
IC-501268	Black	Black
TR-5	Black	Black
PB-5	Black	Black
EC-101549	Brown	Grey
DS-72-244	Brown	Grey
TAS-92-34	Brown	Brown
HIMSOY-1	Green	Grey
CAT-2722	Green	Grey
RSC10-71	Variegated	Black
IC-501185	Variegated	Grey
JS-20-73	Yellow	Brown
AT-156	Yellowish green	Grey
KDS-869	Yellow	Brown
NRC-127	Yellow	Brown
AGS-432	Yellowish green	Brown
CAT-49	Yellow	Brown
CAT-49586	Yellow	Brown
JS-20-42	Yellow	Brown

A laboratory experiment was conducted to evaluate the physiological and biochemical changes occurring during seed deterioration for that the seeds were subjected to accelerated ageing test. 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. Seeds were surface sterilized using 5 per cent sodium hypochlorite solution for 5 minutes and rinsed thoroughly in distilled water and dried at 25 °C for 24 hours in the laboratory.

Accelerated Ageing

Fresh untreated seeds were subjected to artificial ageing as per ISTA procedure for a period of 4 days at 45 °C temperature and 95 per cent RH. Samples were collected after 4 days of aging for evaluation of various seed quality parameters and following observations were recorded.

Seed germination (%)

The laboratory germination test was conducted as per the ISTA (Anon., 2010) using between paper methods of germination. Fifty seeds in eight replications were allowed to germinate at temperature of 25 °C up to 8 days. The germination counts were recorded on 5^{th} and 8^{th} day and per cent germination was expressed on normal seedling basis. Seedlings were evaluated for its vigor by calculating its seedling vigor index (SVI) (Abdul-Baki and Anderson, 1973) by using the following formula.

SVI-I = Germination (%) x Mean seedling length (cm)

SVI-II = Germination (%) x Mean seedling dry weight (mg)

Electrical conductivity ($dScm^{-1}$)

Electrical conductivity was measured as per the ISTA (Anon., 2010). Fifty seeds of 4 replications were weighed on an analytical balance and soaked in 75 ml of distilled water for 24 hours at 25±1 °C. The EC at 25±1 °C was measured using conductivity meter and expressed in desisimens per centimeter.

Total dehydrogenase activity (A_{520})

The total dehydrogenase activity of the seeds was estimated as per the method described by Francaneto *et al*. (1998). Ten seeds of three replications selected randomly were pre-conditioned by soaking in water for 24 hours. Seeds of each treatment were dehusked and immersed in 1 per cent tetrazolium chloride solution in test tubes and incubated for 12 hours in dark. They were then washed thoroughly with distilled water, the red coloured formazan from the stained seeds was extracted by soaking these seeds with 5 ml

of 2-methoxy ethanol for 6-8 hours in an airtight container. The extract was decanted and the colour intensity was measured in spectrophotometer at 520 nm with suitable blank (Methoxy ethanol). The total dehydrogenase activity (TDH) was expressed in OD value.

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

(i) *Enzyme extraction* : One gram of seeds was 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 hours 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.

(ii) *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 test (µM/g of fresh wt.)

(i) *Enzyme extraction* : Malondialdehyde (1, 3-propandial) was measured by a colourimetric method. Two axes were excised from soybeans at 24 hour after imbibition were homogenized in 5 ml of distilled H_2O. An equal volume of 0.5 per cent TBA_2 in 20 per cent of trichloroacetic acid solution was added and the sample was incubated at 95^0 C for 30 min. The reaction was stopped by putting the reaction tubes in an ice bucket. The samples then were centrifuged at l0,000g for 30 min.

(ii) *Estimation of Malondialdehyde activity* : The enzyme assay was carried out according to Heath and Parker (1968). 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.

Statistical analysis

The experimental data was statistically analyzed by adopting the analysis of variance technique appropriate to design as per the methods outlined by Sundararaj *et al*. (1972). Critical differences were calculated at 1 per cent level, where 'F' test was significant. Germination percentages (original values) were transformed into square root transformation. The transformed values were used for statistical analysis.

Results and Discussion

The results revealed that, the seed ageing had significant 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 (EC-101549, DS-72-244, TAS-92-34) coloured seed coat showed a highest resistance against the seed deterioration changes compared with green (HIMSOY-1, CAT-2722), variegated (RSC10-71, IC-501185) and yellow (JS-20-73, AT-156, KDS-869, NRC-127, AGS-432, CAT-49, CAT-49586, JS-20-42) coloured seeds.

The genotypes having grey and black coloured hillum (Table I) were found to be highly resistant against deterioration and genotypes with yellow and brown coloured hillum were found to be susceptible for deterioration changes.

Table II and III represents a summary of the analysis of variance for the evaluation of effect of accelerated ageing in soybean genotypes through the

TABLE II

Effect of accelerated aging on physiological changes occurs during seed deterioration in soybean genotypes

Genotypes	Germination (%)		MSL (cm)		MSDW (mg)		SVI-I		SVI-II	
	Initial	4DAA	Initial	4DAA	Initial	4DAA	Initial	4DAA	Initial	4DAA
EC-76756	91 (9.54)	76 (8.69)	25.56	18.38	7.13	6.10	2328	1346	649	447
EC-57042	95 (9.75)	78 (8.84)	26.86	19.74	7.44	6.33	2553	1544	707	495
IC-501268	92 (9.60)	75 (8.67)	25.85	18.21	7.22	6.22	2380	1352	664	462
TR-5	94 (9.70)	76 (8.73)	26.73	19.12	7.43	6.25	2514	1457	699	477
PB-5	72 (8.50)	86 (9.28)	25.78	18.14	7.27	6.24	1863	1562	525	538
EC-101549	92 (9.60)	76 (8.69)	25.46	17.98	7.18	6.06	2344	1317	661	444
DS-72-244	90 (9.49)	75 (8.67)	25.08	17.54	7.12	6.17	2259	1319	642	464
TAS-92-34	93 (9.65)	75 (8.44)	25.56	18.05	7.21	6.16	2379	1287	671	439
HIMSOY-1	91 (9.54)	72 (8.50)	24.56	15.01	6.95	5.95	2237	1302	633	430
CAT-2722	90 (9.49)	71 (8.62)	23.88	14.75	7.07	5.95	2152	1244	637	442
RSC10-71	92 (9.60)	70 (8.27)	23.87	15.14	7.00	6.02	2198	1171	645	411
IC-501185	90 (9.49)	70 (8.38)	23.76	14.95	7.04	5.88	2141	1192	634	413
JS-20-73	93 (9.65)	64 (8.33)	22.78	13.11	6.85	5.66	2120	978	637	392
AT-156	89 (9.44)	61 (8.38)	22.90	12.01	6.84	5.64	2041	1055	609	396
KDS-869	90 (9.49)	63 (8.33)	22.86	13.04	6.82	5.48	2060	1042	614	380
NRC-127	90 (9.49)	67 (8.20)	21.89	12.50	6.84	5.43	1972	976	616	365
AGS-432	91 (9.54)	66 (8.14)	22.98	12.52	6.84	5.65	2093	1029	623	375
CAT-49	89 (9.44)	65 (8.08)	21.94	13.28	6.80	5.38	1955	867	606	351
CAT-49586	92 (9.60)	64 (8.02)	22.83	12.99	6.87	5.45	2102	836	632	351
JS-20-42	92 (9.60)	60 (7.77)	22.96	12.96	6.76	5.46	2114	783	623	330
S. Em±	0.22	0.07	0.345	0.24	1.01	0.84	62.72	34.35	18.21	12.09
CD (P=0.01)	0.84	0.27	0.983	0.92	3.86	3.21	239.8	131.38	69.65	46.24
CV (%)	3.24	1.08	1.42	1.44	2.09	1.96	2.86	2.90	2.86	2.88

MSL: Mean seedling length, MSDW: Mean seedling dry weight, SVI: Seedling vigour index

TABLE III

Effect of accelerated aging on biochemical changes occurs during seed deterioration in soybean genotypes

Genotypes	EC(dS/cm)		TDH(A_{520})		Peroxidase (A_{430} / min /g of seed)	
	Initial	4DAA	Initial	4DAA	Initial	4DAA
EC-76756	0.75	1.58	1.611	1.210	0.722	0.469
EC-57042	0.62	1.30	1.715	1.272	0.738	0.480
IC-501268	0.71	1.41	1.627	1.217	0.717	0.466
TR-5	0.61	1.37	1.711	1.268	0.734	0.478
PB-5	0.58	1.28	1.660	1.274	0.731	0.475
EC-101549	0.70	1.37	1.615	1.227	0.735	0.472
DS-72-244	0.66	1.43	1.636	1.234	0.719	0.467
TAS-92-34	0.74	1.52	1.670	1.248	0.724	0.471
HIMSOY-1	0.71	1.43	1.644	1.197	0.706	0.459
CAT-2722	0.61	1.54	1.608	1.191	0.715	0.465
RSC10-71	0.66	1.48	1.670	1.183	0.727	0.472
IC-501185	0.75	1.58	1.601	1.199	0.719	0.467
JS-20-73	0.81	1.61	1.666	1.090	0.704	0.409
AT-156	0.68	1.68	1.607	1.097	0.706	0.391
KDS-869	0.70	1.78	1.679	1.085	0.708	0.399
NRC-127	0.87	1.80	1.603	0.972	0.711	0.382
AGS-432	0.68	1.66	1.599	1.083	0.694	0.394
CAT-49	0.60	1.82	1.606	1.069	0.710	0.393
CAT-49586	0.77	1.75	1.597	0.992	0.700	0.380
JS-20-42	0.81	1.69	1.593	0.978	0.704	0.370
S. Em±	0.11	0.22	0.13	0.08	0.01	0.13
CD (P=0.01)	0.42	0.84	0.50	0.31	0.04	0.50
CV (%)	1.43	1.44	2.16	1.071	1.45	0.71

EC: Electrical conductivity, TDH: Total dehydrogenase.

assessment of multiple parameters that are indicators of deterioration and antioxidant mechanisms in seed.

Physiological characteristics

The initial germination was above 90 per cent for almost all genotypes (Table II). However, after 4 days of accelerated ageing the seed germination of the black and brown coloured genotypes was reduced by 13 to 15 per cent, apart from germination, seedling vigour index I (from 2450 to 1544) and seedling vigour index II (from 650 to 477) were also significantly reduced, whereas in the green and variegated seeded genotypes seed germination has reduced by 18 per cent along with decline in seedling vigour index I (from 2152 to 1240) and seedling vigour index II (from 637 to 442) and in yellow-seeded genotypes seed germination reduced by 25 per cent along with reduction in seedling vigour index I (from 1972 to 976) and seedling vigour index II (from 616 to 365).

This might be due to the fact that seed deterioration rate is considerably increased by their exposure to highly adverse levels of temperature and relative humidity and also due to the presence of genotypic variability with respect to seed vigour among different soybean genotypes. The same results were confirmed by Vishal singh (2008).

In case of black seeded genotypes, the variety PB-5 (more hard seeds) showed an increased germination of 13 per cent after 4 days of ageing, this may be due to its hard testa which imparts low permeability and decreased the rate of imbibitions. The similar results obtained by Tian *et al*. (2008) who reported that soybean seed with low seed coat permeability tended to imbibe water at slower rate and this delayed imbiber might resist absorption of moisture and protect seed from deterioration.

Biochemical characteristics

Besides physiological changes, significant changes in biochemical parameters were also observed due to accelerated aging (Table III). Fresh seeds which are aged for four days showed gradual increase in electrical conductivity from 0.62 to 1.31 $dScm^{-1}$ in black seeded genotypes, 0.64 to 1.54 $dScm^{-1}$ in green and variegated seeds and a higher electrical leakage of 0.68 to 1.75 $dScm^{-1}$ in yellow seeded genotypes. An increase in electrolyte leachates from seeds was associated with a decrease in seed membrane integrity and it is more pronounced in large seeds (Jyoti and Malik, 2013).

Physical injury to seed coat and seed size are reported to adversely affect the electrical conductivity test results (Moradshaban *et al.*, 2013). In current study also, high vigour genotypes with lower electrolyte leakage were characterized by smaller seed size and black testa colour as compared to low vigour genotypes, which recorded higher electrolyte leakage, larger seed size and yellow testa colour.

The results confirmed the possibility of determining the levels of deterioration in seed via determination of MDA derivatives content, TDH and peroxidase activity in seeds. The activity of the enzymes indicates deterioration in seeds and their results revealed the significance difference in reduction of level of antioxidants (Table III). Among 20 genotypes high and medium vigour genotypes showed a slight reduction in the activity of enzymes like dehydrogenase (1.71 to 1.26 @ 520nm) and peroxidase (0.73 to 0.47 ΔA430nm / min /g of seed) whereas, in low vigour yellow coloured genotypes showed a drastically decrease in activity of dehydrogenase (1.59 to 1.08 OD @ 520nm) and peroxidase (0.71 to 0.38 ΔA430nm / min /g of seed) enzymes after four days of accelerated aging.

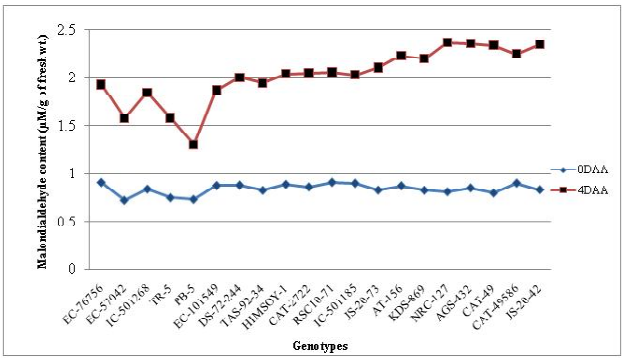

Fig. 1 :Effect of accelerated aging on malondialdehyde content in soybean genotypes

The continuous decline of these antioxidants might be due to seed senescence which increases the activity of free radicals and scavenger enzymes like catalase and peroxides that suppresses the activity of

these antioxidants. The similar results were obtained by the Tian *et al*. (2008). Along with the constitution of seed testa (higher lignin), the higher activity of these antioxidants (dehydrogenase, SOD, peroxidase) in black coloured genotypes is one of the reason for their higher vigour and storability (Francisco *et al*., 2008).

Apart from these enzymes, study also revealed the accumulation of major compound responsible for lipid peroxidation *i.e.*, malondialdehyde content (MDA). There is a significant increase in the MDA content in embryos and cotyledons after four days of artificial aging. The higher activity of MDA content (0.85 to 2.35 µM/g of fresh wt.) in low vigour genotypes after aging and slight increase of MDA content (0.72 to 1.57 µM/g of fresh wt. and 0.86 to 2.02 µM/g of fresh wt.) in high and medium vigour genotypes, respectively (Table III).

This might be due to the differences existing among varieties. It can be noticed that performances of genotypes also influenced the biochemical changes in seeds during deterioration. The above obtained results are in confirmity with the results of Sital *et al*., 2008. Seed susceptibility to oxidative changes differed, depending on seed fatty acid composition and lipid peroxidation can be considered as one of the indicators of individual soybean genotype susceptibility to oxidative stress (Sital *et al*., 2008).

Based on the above discussion it is concluded that, among the 20 soybean genotypes used for the study, they can be classified in to three vigour levels based on the assessment of their physiological and biochemical changes during seed deterioration. The genotypes with Black and brown coloured seed coat are grouped as high viogour seeds, green and variegated coloured genotypes as medium vigour and yellow coloured seeds as low vigour.

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