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Influence of Various Seed Amelioration Techniques on Physio-Biochemical Changes during Seed Deterioration in Aged Seeds of Soybean [*Glycine max* (L.) Merill] Mini Core Set

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A B S T R A C T

The laboratory study was conducted to ameliorate the aged seeds of soybean mini core set by using various antioxidants and osmotics *viz*., α-tocopherol (1 %), ascorbic acid (1 %), potassium iodide (2.5 %) and PEG-6000 (1 %). Results revealed that the amelioration with α-tocopherol (1 %) and ascorbic acid (1 %) treatments showed a marked increase in the seed physio-biochemical parameters like seed germination (from 61 % to 74 %), seedling vigour index I (from 783 to 1292), seedling vigour index II (from 330 to 446) and decrease in electrical conductivity (1.80 dS/cm to 1.63 dS/cm) and membrane injury index (53.9 % to 51.8 %) compared to untreated aged seeds. Results of these amelioration treatments indicates the repair and re-synthesizing ability of antioxidants and their role in stabilizing seed deterioration damages in aged seeds. The data's were statistically analyzed by analysis of variance ($P \leq 0.01$).

Introduction

Soybean [*Glycine max* (L.) Merrill.] is an important pulse crop popularly known as miracle crop due to its multiple uses that has around 40 per cent protein and 20 per cent oil in it.

The soybean (*Glycine max L.* Merril) was originated from eastern Asia/China, it is a member of *Leguminacae* family and cultivated soybean *(Glycine max L.* Merril*)* was derived from a wild progenitor *Glycine ussuriensi.* Sub species of soybean are *Glycine gracilis* and *Glycine soja*, cultivated soybean has genome size of 1.1 to 1.15 Gb with chromosome pair of twenty (2n=40).

Soybean seed has been identified as poor storer, because of its delicate (thin) seed coat and vulnerable position of its embryo. Hence it is very much prone for seed deterioration losses. Though, seed deterioration is irreversible and inexorable but its rate and extent can be slowed down to some extent through seed amelioration techniques such as seed priming with antioxidants osmotics and salts *etc*. Beneficial effect of such seed treatment was reflected in better germination and early seedling growth accompanied by greater cellular membrane integrity, counteraction of lipid peroxidation and free radical chain reaction often found to be directly correlated with the maintenance of vigour and viability of seeds (Kapoor *et al.*, 2011).

Therefore an attempt was made to explore such amelioration treatments to enhance performance of low vigour genotypes of soybean germplasm accessions. In the backdrop of this information the present study has been undertaken to study the effect of various amelioration techniques on aged seeds during seed deterioration.

Materials and Methods

The present study was conducted using artificially aged seeds of soybean mini core set (Table 1) which are having different vigour levels. This aged seeds were subjected to various amelioration treatments *viz.*, T_1: Control; T_2: α-tocopherol @ 1 % (organic infusion); T_3: Ascorbic acid @ 1 % soaking; T_4: Potassium iodide @ 2.5 % soaking; T_5: PEG-6000 @ 1 %.

As soybean seeds are prone to soaking injury, seed amelioration were done by placing the seeds between moist papers which were soaked in each treatment, instead of soaking directly in aqueous solution. Then, these seeds were thoroughly washed, surface dried under room temperature and used for further experiments.

Accelerated ageing

Fresh seeds (untreated) were subjected to artificial ageing (Anon., 2010) for a period of 10 days at 45 °C temperature and 95 % RH. Samples were collected at 2 days interval for seed quality studies.

Seed germination (%)

The laboratory germination test was conducted as per the ISTA rules (2010) using between paper method. 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.

Seedling vigour index-I and II

The vigor index I was determined by multiplying the percentage germination and total seedling length and whereas vigour index-II was determined by multiplying percentage germination with total seedling dry weight (Abdul Baki and Anderson, 1973)

Seedling vigour index I = germination (%) x mean seedling length (cm)

Seedling vigour index II = germination (%) x mean seedling dry weight (mg)

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

Electrical conductivity was measured as per the ISTA rules (2010). Fifty seeds of 4 replication 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.

Membrane injury index

Membrane injury index was calculated by the formula given by Blum and Ebercon (1981).

$$MII = (C_1/C_2)*100$$

Where

C_1 = Electric conductivity at 40 ^{0}C for 30 min.
C_2 = Electric conductivity at 100^{0}C for 10 min.

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) in computer. 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 the significant effects of seed amelioration treatments on aged seeds of soybean mini core set. Among the various seed treatments used, the α-tocopherol found to increase the seed germination in PB-5 (89 %), EC57042 (87 %) and TR-5 (86 %) followed by ascorbic acid (88 %) and potassium iodide (86 %), whereas, least or negligible influence was observed in PEG treated seeds (Table 2).

However, highest per cent increase in seed germination was reported for α-tocopherol treatment in AT-156 (13 %) and JS-20-42 (13 %) followed by ascorbic acid (12 %) and potassium iodide (6 %) in JS-20-42. Whereas, PEG showed a least performance of ≤ 5 per cent enhancement in seed germination in almost all the genotypes (DS-72-244, EC-101549 and TAS-92-34) compared to other treatments (Fig. 1).

These differences in response by different genotypes may because of variation in their seed biochemical composition *i.e.* genotypes having higher level of endogenous antioxidants do not respond well for exogenous antioxidants application. The similar results were reported by Taylor *et al.*, (1998) and Kaya *et al.*, (2006).

The present result of enhanced seed germination in low vigour genotypes through antioxidant treatment was supported by Bailly *et al.*, (1998) and Kaya *et al.*, (2006) in sunflower, they reported that priming of aged seeds with antioxidants progressively restores the initial germinative ability and reduces the level of lipid peroxidation.

Significant differences were recorded among the genotypes (aged) and seed treatments for seedling vigour index-I & II. Among various seed treatments α-tocopherol reported the higher SVI-I & II in EC-57042 (1888) (574) and PB-5 (1801) (568) compared to control (1544) (538) (Table 3).

Fig.1 Per cent increase in seed germination through amelioration treatments in aged seeds of soybean genotypes

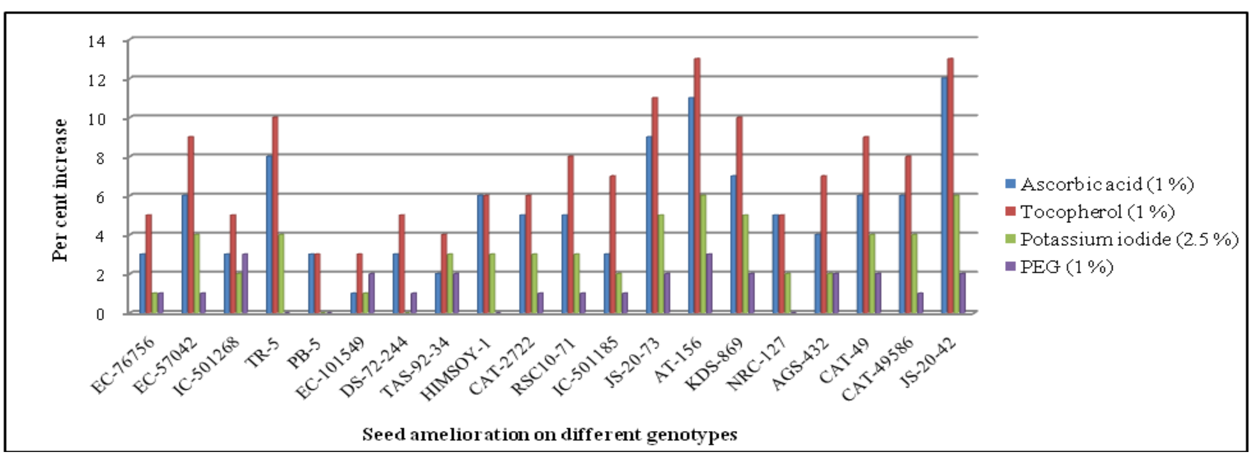

Table.1 List of soybean mini core set used for the study

Sl. No.	Genotypes	Sl. No.	Genotypes
1	EC-76756	11	RSC10-71
2	EC-57042	12	IC-501185
3	IC-501268	13	JS-20-73
4	TR-5	14	AT-156
5	PB-5	15	KDS-869
6	EC-101549	16	NRC-127
7	DS-72-244	17	AGS-432
8	TAS-92-34	18	CAT-49
9	HIMSOY-1	19	CAT-49586
10	CAT-2722	20	JS-20-42

Table.2 Effect of seed amelioration on seed germination (%) of aged seeds of Soybean mini core set

Sl. No.	Genotypes	Control	Ascorbic acid (1%)	Tocopherol (1%)	Potassium iodide (2.5 %)	PEG-6000 (1%)
		4	**4**	**4**	**4**	**4**
1	**EC-76756**	76(8.69)	79(8.86)	81(9.00)	77(8.77)	77(8.60)
2	**EC-57042**	78(8.84)	84(9.16)	87(9.30)	82(9.05)	79(8.87)
3	**IC-501268**	75(8.67)	78(8.83)	80(8.94)	77(8.77)	78(8.60)
4	**TR-5**	76(8.73)	84(9.16)	86(9.25)	80(8.92)	76(8.74)
5	**PB-5**	86(9.28)	89(9.41)	89(9.41)	86(9.22)	86(9.03)
6	**EC-101549**	76(8.69)	77(8.77)	79(8.89)	77(8.57)	78(8.40)
7	**DS-72-244**	75(8.67)	78(8.80)	80(8.94)	75(8.54)	76(8.37)
8	**TAS-92-34**	75(8.44)	77(8.75)	79(8.86)	78(8.57)	77(8.40)
9	**HIMSOY-1**	72(8.50)	78(8.80)	78(8.80)	75(8.67)	72(8.49)
10	**CAT-2722**	71(8.62)	76(8.69)	77(8.77)	74(8.43)	72(8.26)
11	**RSC10-71**	70(8.27)	75(8.63)	78(8.80)	73(8.34)	71(8.17)
12	**IC-501185**	70(8.38)	73(8.51)	77(8.77)	72(8.46)	71(8.29)
13	**JS-20-73**	64(8.33)	73(8.51)	75(8.63)	69(8.27)	66(8.11)
14	**AT-156**	61(8.38)	72(8.46)	74(8.60)	67(8.15)	64(7.99)
15	**KDS-869**	63(8.33)	70(8.37)	73(8.51)	68(8.00)	65(7.84)
16	**NRC-127**	67(8.20)	72(8.49)	72(8.49)	69(8.32)	67(8.15)
17	**AGS-432**	66(8.14)	70(8.34)	73(8.51)	68(8.26)	68(8.09)
18	**CAT-49**	65(8.08)	71(8.28)	74(8.60)	69(8.28)	67(7.94)
19	**CAT-49586**	64(8.02)	70(8.37)	72(8.49)	68(8.22)	65(7.88)
20	**JS-20-42**	60(7.77)	72(8.46)	73(8.51)	66(8.13)	62(7.87)
S. Em±		**0.07**	**0.07**	**0.08**	**0.29**	**0.15**
CD (P=0.01)		**0.26**	**0.29**	**0.34**	**0.73**	**0.34**
CV (%)		**1.08**	**1.19**	**1.39**	**2.90**	**1.01**

Table.3 Effect of seed amelioration on seedling vigour index of aged seeds of soybean mini core set

Sl. No.	Genotypes	Control		Ascorbic acid (1%)		Tocopherol (1%)		Potassium iodide (2.5 %)		PEG-6000 (1%)	
		SVI-I	SVI-II	SVI-I	SVI-II	SVI-I	SVI-II	SVI-I	SVI-II	SVI-I	SVI-II
1	EC-76756	1346	447	1534	493	1638	522	1491	475	1418	470
2	EC-57042	1544	495	1771	545	1888	574	1632	525	1560	505
3	IC-501268	1352	462	1550	491	1645	512	1458	481	1459	471
4	TR-5	1457	477	1701	541	1792	566	1565	508	1519	478
5	PB-5	1562	538	1740	558	1801	568	1610	540	1591	543
6	EC-101549	1317	444	1500	482	1593	505	1425	474	1430	477
7	DS-72-244	1319	464	1483	487	1585	514	1370	467	1370	470
8	TAS-92-34	1287	439	1551	479	1648	501	1533	484	1483	482
9	HIMSOY-1	1302	430	1537	470	1590	480	1430	451	1354	431
10	CAT-2722	1244	442	1467	462	1549	484	1416	447	1341	446
11	RSC10-71	1171	411	1373	463	1478	494	1267	446	1221	431
12	IC-501185	1192	413	1337	445	1470	489	1256	433	1223	424
13	JS-20-73	978	392	1219	425	1296	449	1147	397	1057	401
14	AT-156	1055	396	1285	425	1377	456	1199	399	1099	399
15	KDS-869	1042	380	1274	411	1366	445	1214	386	1107	391
16	NRC-127	976	365	1231	427	1274	451	1167	392	1099	369
17	AGS-432	1029	375	1258	418	1358	454	1193	396	1150	387
18	CAT-49	867	351	1204	405	1299	439	1153	382	1052	362
19	CAT-49586	836	351	1205	407	1283	436	1171	383	1045	357
20	JS-20-42	783	330	1231	420	1292	446	1140	374	1000	343
S. Em±		**34.3**	**12.09**	**20.1**	**13.55**	**30.04**	**8.65**	**24.6**	**8.71**	**33.01**	**6.12**
CD (P=0.01)		**97.7**	**34.41**	**81.2**	**54.56**	**120.8**	**34.81**	**99.3**	**35.05**	**132.8**	**24.6**
CV (%)		**2.90**	**2.88**	**2.00**	**4.14**	**2.81**	**2.50**	**1.55**	**1.92**	**2.13**	**1.37**

Where, SVI: seedling vigour index

Table.4 Effect of seed amelioration on seed coat membrane integrity on aged seeds of soybean mini core set

Sl. No.	Genotypes	Control		Ascorbic acid (1%)		Tocopherol (1%)		Potassium iodide (2.5 %)		PEG-6000 (1%)	
		EC (dS/cm)	MII (%)	EC (dS/cm)	MII (%)	EC (dS/cm)	MII (%)	EC (dS/cm)	MII (%)	EC (dS/cm)	MII (%)
1	**EC-76756**	1.58	47.3	1.498	46.6	1.475	46.2	1.440	47.4	1.434	48.3
2	**EC-57042**	1.30	41.8	1.238	41.2	1.219	40.8	1.275	41.9	1.269	42.7
3	**IC-501268**	1.41	49.3	1.343	46.6	1.323	46.2	1.384	47.4	1.377	48.3
4	**TR-5**	1.37	42.9	1.304	42.3	1.284	41.9	1.343	43.0	1.336	43.8
5	**PB-5**	1.28	38.7	1.217	34.1	1.199	33.8	1.254	36.8	1.247	37.6
6	**EC-101549**	1.37	45.7	1.304	45.0	1.284	44.6	1.343	45.8	1.336	46.7
7	**DS-72-244**	1.43	45.1	1.356	44.4	1.336	44.0	1.397	45.2	1.390	46.0
8	**TAS-92-34**	1.52	46.8	1.442	46.1	1.420	45.6	1.486	46.9	1.478	47.7
9	**HIMSOY-1**	1.43	48.4	1.314	47.0	1.294	46.5	1.353	47.8	1.346	48.6
10	**CAT-2722**	1.54	46.8	1.412	45.4	1.390	44.9	1.454	46.2	1.447	47.0
11	**RSC10-71**	1.48	45.0	1.365	48.5	1.344	48.0	1.405	49.3	1.398	50.2
12	**IC-501185**	1.58	47.9	1.455	46.4	1.433	46.0	1.498	47.2	1.491	48.1
13	**JS-20-73**	1.61	53.9	1.480	52.3	1.457	51.8	1.523	54.1	1.516	54.6
14	**AT-156**	1.68	51.7	1.546	50.2	1.522	49.7	1.592	51.1	1.584	52.0
15	**KDS-869**	1.78	52.8	1.635	51.3	1.610	50.7	1.684	52.1	1.675	53.1
16	**NRC-127**	1.80	50.6	1.655	49.1	1.630	48.6	1.704	50.0	1.696	50.9
17	**AGS-432**	1.66	49.5	1.527	48.0	1.503	47.6	1.572	48.9	1.564	49.7
18	**CAT-49**	1.82	53.9	1.678	52.3	1.652	51.8	1.728	53.2	1.719	54.2
19	**CAT-49586**	1.75	50.6	1.607	49.1	1.582	48.6	1.655	50.0	1.646	50.9
20	**JS-20-42**	1.69	52.8	1.554	51.3	1.529	50.7	1.599	52.1	1.591	53.1
S. Em±		**0.22**	**1.08**	**0.32**	**0.13**	**0.22**	**0.18**	**0.62**	**0.21**	**0.22**	**0.11**
CD (P=0.01)		**0.63**	**4.35**	**0.98**	**0.34**	**0.68**	**0.62**	**1.93**	**0.63**	**0.69**	**0.36**
CV (%)		**1.44**	**2.16**	**3.76**	**1.11**	**2.15**	**1.45**	**2.34**	**2.27**	**2.32**	**4.58**

Where, EC: electrical conductivity, MII: membrane injury index

The positive effect of seed amelioration on seedling vigour index was might be due to reserve mobilization of food material, repair and re-synthesis of various enzymes and rapid growth of embryo results in enhanced germination, seedling growth and seedling dry weight in turn increases the seedling vigour index (Khan, 1992).

Besides physiological parameters, significant changes were also observed in biochemical parameters due to genotypes and their seed treatments. Among various treatments α-tocopherol recorded the least amount of electrical leachates (1.199 dS/cm) and membrane injury index (33.8 %) followed by ascorbic acid (1.217 dS/cm) (34.1 %) and PEG (1.247 dS/cm) (36.8 %) compared to control (1.283 dS/cm) (38.7 %) in PB-5. Higher electrical conductivity was reported for potassium iodide treatment in CAT-49 (1.728 dS/cm) and NRC-127 (1.704 dS/cm) compared to other treatments and genotypes (Table 4).

Irrespective of genotype, antioxidant treatment significantly reduced the electrolyte leachate concentration in all treated seeds of aged genotypes compared to untreated aged seeds. Braccini Adle *et al.*, (2000) in soybean and Khan *et al.*, (1977) have reported that amelioration activates antioxidant enzymes which reduces peroxidation of lipids in seed by stabilizing reactive oxygen species generated in aged seeds thereby results in low damage to membrane and low solute leakage from seed cells due to which increase in electrical conductivity comes under control. These reduced membrane injury index through α-tocopherol treatment indicates the repair and re-synthesizing ability of antioxidants and their role in stabilizing seed membrane damage.

Enhanced electrolyte leakage is often indicated as a symptom of stress related membrane injury, caused by the accumulation of reactive oxygen species. Seed amelioration using antioxidants and osmotics found to be significantly alleviated the adverse effect of ROS, which considerably enhanced membrane stability. These results were confirmed by the results of Noreen *et al.*, (2010) in turnip.

The study could be concluded that the antioxidants like α-tocopherol (1 %) and ascorbic acid (1 %) can be recommended as the efficient seed amelioration treatments against the seed deterioration damages in soybean germplasm accessions.

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