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Seed invigoration with α-tocopherol as a mid-storage correction treatment alleviates seed ageing process in soybean

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Abstract
The germination potential (viability) of soybean is short lived owing to its high susceptibility to mechanical injury and damage occurring during post-harvest handling. This adversely affects the viability and vigour of soybean seed during storage as compared to other oilseed crops and often viability is reduced prior to planting time. Studies on effect of seed invigoration with different antioxidants (Ascorbic acid, α-tocopherol, Butylated Hydroxytoluene) as a mid-storage correction treatment was carried out on four cultivars of soybean *viz*., JS-335, DS-228 and KDS-726 and Type-49 during 2017-18 and 2018-19 at Seed Technology Research Unit, MPKV, Rahuri. Activities of antioxidative enzyme superoxide dismutase activity and lipid peroxidase activity were studied at initial, 120 and 240 days of storage among four soybean varieties. Seed invigorated with α- tocopherol (200 ppm) and ascorbic acid (100 ppm) significantly enhanced activity of superoxide dismutase (SOD) activity. Wherein, the rate of lipid peroxidase activity was delayed at 240 days of storage as compared to control. Among the varied interactions, the seeds of variety Type 49 invigorated with 200 ppm of α-Tocopherol showed significantly higher superoxide dismutase activity and lower lipid peroxidase activity as compared to other treatments. This summarises that, the 200 ppm of α-Tocopherol invigoration treatment enhances the activity of antioxidative enzyme superoxide dimutase (SOD) and reduces lipid peroxidation during storage.

Keywords: Seed invigoration, α-tocopherol, BHT, SOD & MDA

Introduction
Seed is the critical input for achieving sustainable production and efficacy of all other inputs revolves around the supply of quality seed to farmers at right time. High quality seed does not happen by chance. Each stage in seed production from sowing, rouging, weed control, fertility program, harvest, cleaning, processing, storage and transport is critical for achieving it. Seed quality is the collective term for the condition of seed including genetic homogeneity, physical appearance, viability, vigour and uniformity. Seed can play pivotal role in achieving higher productivity, as use of quality seeds alone could increase productivity by 15-20% which highlights the important role of seed in agriculture [1].

Seed deterioration is an irreversible process. It cannot be prevented or reversed, but it can be slowed under specific conditions. Evidence of seed deterioration includes discoloration of seeds, poor germination, poor seedling growth, production of more abnormal seedlings and lower seedling vigour. Physiological changes noticed in seeds are reduced respiration, loss of enzyme activity and membrane damage. The rapid seed deterioration of soybean is thought to be due to lipid peroxidation, subsequently resulting in loss of seed viability [2].

Seed longevity is an important issue for the agricultural prospects of soybean cultivation and lipid peroxidase activity may be a significant factor in this process. There is a known inverse correlation between lipid peroxidase activity and longevity during natural and artificial aging. Seed deterioration during storage and natural aging occurs mainly due to factors that increase respiratory intensity. Therefore, antioxidants such as α- tocopherol could reduce respiratory intensity by blocking the entry of oxygen into the internal tissues of seeds and therefore reduce seed deterioration [3]. Soybean seeds have a lower tocopherol content following ageing, suggesting that tocopherol is consumed and protects the seed against free radical damage [4].

α-Tocopherol application increased super oxide dismutase activity as a result the lipid peroxidation of seed during storage was significantly reduced. Application of α-tocopherol, K_2HPO_4 and salicylic acid improved the storage or keeping quality of soybean seed [5]. SOD and catalase are antioxidant enzymes which protect seeds from peroxidative deterioration [6].

Seed invigoration prevents damaging oxidative reactions, especially free-radical-induced lipid peroxidase activity reactions and repairs the cellular system involved in invigoration [7]. Mid-storage modified hydration-dehydration such as moist sand conditioning-drying and moist sand conditioning-soaking-drying treatments of 5-month-old medium-vigour seed was found to be effective in maintaining vigour, viability and improving field performance. Natural antioxidants such as ascorbates, tocopherols and carotenoids are well known for their role in free radical scavenging activities. Thus, it is essential to know that, which seed invigoration treatment with antioxidants will be able to maintain seed quality and viability during storage.

Keeping this in view, experiment was carried out with three different antioxidants *viz*., Ascorbic acid, α-tocopherol, Butylated Hydroxytoluene and their effect on seed storability and biochemical parameter i.e. superoxide dimutase (SOD) activity and lipid peroxidase activity at initial, 120 and 240 days of storage were studied.

Material and Methods

Seeds of four soybean varieties *viz*., *viz*., JS-335, DS-228, KDS-726 and Type-49 were multiplied at Post Graduate Institute Research Farm, Department of Agricultural Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri during *kharif* 2016 and 2017. The harvested seeds from each treatment and replication were sun dried separately and threshed manually by beating with wooden stick. The seeds were cleaned and sun dried before storage. Freshly harvested soybean seeds of four varieties were stored in HDPE (high density polyethylene) bags for four months. At 120 Days of storage, seeds were treated with antioxidant treatments (Ascorbic acid, α-tocopherol, Butylated Hydroxytoluene) as mid- storage corrections and further stored until germination percentage reduced below 70% as prescribed in Indian Minimum Seed Certification Standards, 2013 [8].

For mid-storage correction treatments, soybean seeds were sand conditioned with white sand, which was pre-sterilized in autoclave for 8 hours to avoid any contamination to treated seeds. Pre-moist sand was mixed with seeds in 3:1 ratio and kept for 12 hours at room temperature. Further conditioned seeds were soaked in different antioxidant solution for 1 hour. After that, invigorated seeds were shade dried for 72 hours to bring back to original moisture content.

Superoxide dismutase activity was measured (3 days old seedlings) using the method described by Dhindsa *et al*., (1981) [9]. Lipid peroxidase activity was measured as described by Heath and Packer, (1968) [10].

Result and Discussion

Among the four varieties, seeds of Type-49 recorded higher superoxide dismutase activity at initial, 120 and 240 days of storage as compared to other varieties *viz*., JS-335, DS-228, KDS-726. Wherein, among the various treatments, at 120 days of storage, the seeds invigorated with α-tocopherol (200 ppm) (T_5) recorded significantly higher superoxide dismutase activity as 61.19 (µm SOD units/mg of protein) followed by ascorbic acid (100 ppm) (T_2) as 60.08 (µm SOD units/mg of protein) and α-tocopherol (100 ppm) (T_4) 58.53 (SOD units/mg of protein) on pooled basis, respectively.

At 240 days of storage, seeds invigorated with α-tocopherol (200 ppm) (T_5) recorded significantly higher superoxide dismutase as 59.37 (SOD units/mg of protein) followed by ascorbic acid (100 ppm) (T_2) as 57.65 (SOD units/mg of protein) followed by α-tocopherol (100 ppm) (T_4) 56.40 (SOD units/mg of protein) (Table 1).

Table 1: Effect of varieties (V), seed invigoration treatments (T) and their interactions (V x T) on superoxide dismutase activity (SOD units/mg of protein)

Variety	Initial			120 DAS			240 DAS		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
JS 335	56.18	56.75	56.47	52.76	50.98	51.87	48.96	50.98	49.97
DS 228	67.19	67.85	67.52	55.27	53.43	54.35	52.17	53.43	52.80
KDS 726	59.39	60.00	59.70	52.99	51.77	52.38	50.70	51.77	51.23
Type 49	74.62	69.31	71.96	68.04	66.34	67.19	58.44	66.34	62.39
S.Em±	0.203	0.361	0.359	0.356	0.208	0.357	0.162	0.208	0.228
CD @1%	0.761	1.355	1.326	1.338	0.780	1.321	0.607	0.780	0.843
Treatment									
T1-Hydropriming	58.61	57.95	58.28	53.19	52.95	53.07	48.08	52.95	50.51
T2- Ascorbic Acid (100 ppm)	68.32	67.39	67.86	61.83	58.32	60.08	56.98	58.32	57.65
T3- Ascorbic Acid (200 ppm)	64.91	64.67	64.79	58.55	56.27	57.41	53.39	56.27	54.83
T4- α- Tocopherol (100 ppm)	66.42	66.08	66.25	59.41	57.65	58.53	55.15	57.65	56.40
T5- α-Tocopherol (200 ppm)	72.77	71.12	71.94	61.82	60.56	61.19	58.19	60.56	59.37
T6- Butylated Hydroxytoluene (100 ppm)	64.27	62.51	63.39	56.44	54.52	55.48	51.60	54.52	53.06
T7- Butylated Hydroxytoluene	63.48	61.83	62.66	54.99	54.24	54.61	50.08	54.24	52.16
T8- Control	55.97	56.27	56.12	51.89	50.53	51.21	47.08	50.53	48.80
S.Em±	0.287	0.510	0.507	0.504	0.294	0.505	0.229	0.294	0.322
CD @1%	1.148	2.044	2.030	2.019	1.176	2.023	0.916	1.176	1.291
Variety x Treatment interaction									
S.Em±	0.57	1.02	1.01	1.01	0.59	**1.01**	0.46	0.59	0.64
CD @1%	2.15	3.83	3.75	3.79	2.21	**3.74**	1.72	2.21	2.38

At 120 days of storage period, among the interaction of varieties and seed invigoration treatments (V x T), V_4T_5 recorded significantly higher superoxide dismutase activity (73.87 SOD units/mg of protein) followed by interaction V_4T_2 recorded as (71.45 SOD units/mg of protein) and V_4T_4 as (69.79 SOD units/mg of protein). Whereas, at 240 days, interaction of V_4T_5 recorded significantly highest superoxide dismutase activity (69.91 SOD units/mg of protein) followed

by V_4T_2 recorded as (67.25 SOD units/mg of protein) and V_4T_4 recorded as (65.61 SOD units/mg of protein) on pooled basis, respectively.

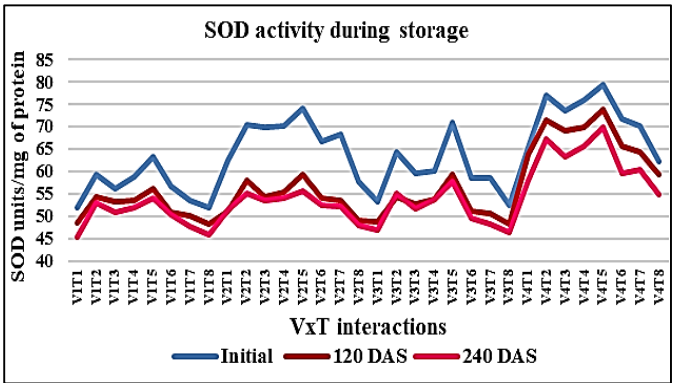

Fig 1: Effect of varieties (V), seed invigoration treatments (T) and their interactions (V x T) on superoxide dismutase activity in seedlings of soybean at initial, 120 and 240 days of storage

Lipid peroxidase activity increases with advancement of storage period. From the Table 2, it was found that the interaction effects of varieties and seed invigoration treatments on lipid peroxidise activity (nmoles of MDA) were found significant in both the years and on pooled basis. Among the varieties, seeds of Type-49 recorded lower lipid peroxidase activity followed by seeds of DS-228 at initial, 120 and 240 days of storage (Table 2).

At 120 days of storage period, the seeds invigorated with α-tocopherol (200 ppm) (T_5) recorded significantly lower lipid peroxidase activity as (17.27 nmoles of MDA/g) followed by ascorbic acid (100 ppm) (T_2) as (18.40 nmoles of MDA) and α-tocopherol (100 ppm) (T_4) (19.11nmoles of MDA) on pooled basis, respectively. Seeds treated with different antioxidants maintained lower lipid peroxidase activity at the 240 days of storage period. Seeds invigorated with α-tocopherol (200 ppm) (T_5) recorded minimum lipid peroxidase activity as 21.45 (nmoles of MDA) followed by ascorbic acid (100 ppm) (T_2) as 22.82 (nmoles of MDA) and α-tocopherol (100 ppm) (T_4) 23.54 (nmoles of MDA). However, higher lipid peroxidase activity was recorded in control (T_8) as 26.59 (nmoles of MDA) at the 240 days of storage period.

Table 2: Effect of varieties (V), seed invigoration treatments (T) and their interactions (V x T) on lipid peroxidase activity (nmoles of MDA)

Variety	Initial			120 DAS			240 DAS		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
JS 335	16.19	15.22	15.71	21.99	20.80	21.39	25.09	25.86	25.48
DS 228	13.25	14.16	13.71	19.38	20.08	19.73	22.76	23.76	23.26
KDS 726	15.58	14.35	14.96	21.38	20.58	20.98	24.67	25.45	25.06
Type 49	13.22	13.77	13.50	19.03	18.18	18.61	22.40	23.01	22.70
SEm±	0.171	0.003	0.148	0.072	0.038	0.070	0.059	0.037	0.061
CD @1%	0.643	0.013	0.549	0.269	0.142	0.259	0.222	0.141	0.224
Treatment									
T1-Hydropriming	15.61	15.35	15.48	21.70	21.87	21.78	25.08	26.32	25.70
T2- Ascorbic Acid (100 ppm)	13.22	13.03	13.13	19.00	17.80	18.40	22.25	23.38	22.82
T3- Ascorbic Acid (200 ppm)	14.45	14.00	14.22	20.25	19.41	19.83	23.64	24.32	23.98
T4- α-Tocopherol (100 ppm)	13.67	13.80	13.74	19.70	18.51	19.11	23.19	23.90	23.54
T5- α-Tocopherol (200 ppm)	12.32	12.19	12.26	18.09	16.45	17.27	20.38	22.51	21.45
T6- Butylated Hydroxytoluene (100 ppm)	15.03	15.03	15.03	20.58	20.58	20.58	24.06	24.70	24.38
T7- Butylated Hydroxytoluene (200 ppm)	15.61	15.22	15.42	20.89	21.29	21.09	24.22	24.86	24.54
T8- Control	16.58	16.38	16.48	23.34	23.36	23.35	27.01	26.17	26.59
SEm±	0.242	0.005	0.210	0.101	0.054	0.099	0.084	0.053	0.086
CD 1%	0.970	0.020	0.841	0.405	0.215	0.397	0.334	0.212	0.343
Variety x Treatment interaction									
SEm±	0.48	0.01	0.42	0.20	0.11	0.20	0.17	0.11	0.17
CD 1%	1.82	0.04	1.55	0.76	0.40	0.73	0.63	0.40	0.63

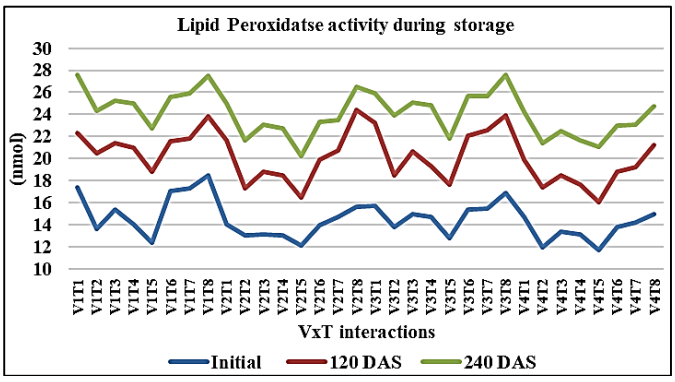

Fig 2: Effect of varieties (V), seed invigoration treatments (T) and their interactions (V x T) on lipid peroxidase activity (nmoles of MDA)

At 120 days of storage period, (Fig 2) among the interaction of varieties and seed invigoration treatments (V x T), interaction of V_4T_5 recorded significantly lowest lipid peroxidase activity (16.06 nmoles of MDA) followed by interaction V_4T_2 recorded as 17.42 nmoles of MDA followed by V_4T_4 as 17.67 nmoles of MDA. Whereas, at 240 days, interaction of V_4T_5 recorded significantly lowest lipid peroxidase activity (21.03nmoles of MDA) followed by interaction V_4T_2 recorded as (21.41 nmoles of MDA) followed by interaction V_4T_4 recorded as (21.67 nmoles of MDA) on pooled basis, respectively. This might be due to tocopherols reduces respiratory intensity by blocking the entry of oxygen into the internal tissues of seeds and therefore reduce seed deterioration [3]. Malondialdehyde (MDA), product of lipid peroxidation is an indicator of biochemical degradation of phospholipids of cell membrane and unit membrane of cell macromolecules. Higher the MDA content higher is the degree of seed deterioration. Antioxidant, controlled the lipid peroxidation of cells thus protected it from biochemical degradation [5]. Similarly, lipid peroxidation was decreased with response to application of α-tocopherol on *Vicia faba* [11].

Conclusion

Activities of different antioxidative enzyme activity i.e. superoxide dismutase activity and lipid peroxidase activity differed significantly due to varieties, invigoration treatment and its interactions. During storage period, superoxide dismutase activity declined with advancement of storage, while lipid peroxidase activity increased with advancement of storage period. Among the four varieties, Type-49 exhibited higher superoxide dismutase and lower lipid peroxidase activity. Among the various treatments, seed invigorated with α-Tocopherol (200 ppm) exhibited higher superoxide dismutase activity and lower lipid peroxidise activity as compared with other treatments. Among the varied interactions, the seeds of variety Type 49 invigorated with 200 ppm of α-Tocopherol at 120 and 240 days of storage showed significantly higher superoxide dismutase activity and lower lipid peroxidise activity, which summarises that the 200 ppm of α-Tocopherol invigoration treatment enhances the activity of different antioxidative enzymes and reduces lipid peroxidase activity during storage.

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