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Influence of seed treatment and storage container on physiological and biochemical properties male parental line UMI 1230 seeds in maize hybrid COH (M)6

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Abstract
Investigations were conducted to assess theantioxidant enzyme activity and germination characteristics of male parental line (UMI 1230) in maize hybrid COH (M) 6 seeds obtained from the Agricultural Research Station, Bhavanisagar, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The initial seed qualities of male pare ntal line (UMI 1230) in maize hybrid COH (M) 6 were analyzed and after, the seeds were subjected to different seed treatments *viz.*, Halopolymer @ 4 ml kg^{-1}, Halogenation Mixture @ 3 g kg^{-1}, Carbendazim 2 g $kg^{-1}$and untreated seeds served as control. The seeds were packed in cloth bag and 700 gauge thick polythene containers and stored in ambient condition. Seed samples were drawn at bimonthly interval lsand tested its germination and antioxidant enzyme activity *viz.*, α-amylase content, dehydrogenase activity and superoxide dismutase. The result revealed that male parental line (UMI 1230) seeds in maize hybrid COH (M) 6 treated with halogenation mixture @ 3 g kg^{-1}, recorded maximum seed germination (83%) than control (72%) followed by halogenation mixture @ 3 g kg^{-1} and maintained the antioxidant enzyme activity of seeds up to 10 months of storage. Moisture proof container of 700 gauge thick polythene bag performed as better storer than cloth bag and it was evident that antioxidative enzymes and better cell membrane integrity of aged seeds increased after seed treatments.

Keywords: maize hybrid, parental line UMI 1230, seed treatment, storage container, germination, α-amylase content, dehydrogenase activity and superoxide dismutase

Introduction
Seed deterioration starts even in optimum storage conditions. Genetic and environmental modifications influences seed deterioration at harvest and proceeds in storage (Be w ley and Black 1994) [4]. The ability of seeds to produce antioxidative enzymes considerably differ depending on species and genotype and enzymatic detoxification and repair of cell membranes are the main means to delay ageing (Tavakol Afshari *et al.* 2007) [15].

Demonstrated that early deteriorative changes in seeds under storage was due to various biochemical processes *viz.*, denaturation of biomolecules and accumulation of toxic substances in addition to loss of membrane integrity (Abdul-Baki and Anderson, 1972) [1]. During deterioration, marked changes in the content and activity of certain respiratory enzymes such as catalase, peroxidase, dehydrogenase, cytochrome oxidase and glutamic acid decorboxylase were noticed with decline in viability.

Several authors have emphasized that the main factors responsible for the conservation of seeds during storage are the temperature and the moisture content of the seeds. Harrington (1972) [9], stated that the maintenance of low temperature reduces the activity of enzymes involved in the breathing process and, consequently, the speed of decline in viability of the orthodox seeds during storage. The free radicals are unstable and may react with and damage nearby molecules.

Stored seed subjected to lipid peroxidation showed consistant attack by oxygen, forming hydro peroxides, other oxygenated fatty acids and free radicals, which are unstable and may react with and damage nearby molecules. The total amount of oxygenated fatty acid generated would be proportional to the age of seed (Wilson and McDonald, 1986) [16]. The role of iodine in the stabilization of double bonds of unsaturated fatty acid moieties of lipoprotein

biomembranes and controlling free radicals as a possible reason for viability extension has been suggested by Basu and Rudrapal (1980) [3]. In this study, the dynamics of antioxidant enzyme activity and germination characteristics in deteriorated male parental line (UMI 1230) seeds of maize hybrid COH (M) 6 with different seed treatment during storage were examined. The aims were to investigate antioxidant enzymes activity, ROS damage, lipid peroxidation activity of antioxidative enzymes and membrane integrity in aleurone layer, endosperm and embryo of male parental lines seeds of UMI 1230 during 10 months of storage upon seed treatment and storage containers.

Materials and Methods

The research was conducted at Department of Seed Science and Technology, Tamil Nadu Agricultural University Coimbatore, Tamil Nadu, India. Seeds of male parental line (UMI 1230) in maize hybrid COH (M) 6 were obtained from the Agricultural research station, Bhavani sagar, Coimbatore, India. After assessing the initial quality parameters seeds were subjected to different seed treatments *viz.*, Halopolymer @ 4ml kg^{-1}, Halogenation Mixture @ 3 g kg^{-1}, and Carbendazim 2g kg^{-1} along with control (dry seeds). After the treatment, the seeds were packed in cloth bags and moisture proof container of 700 gauge thick polythene bags and stored in ambient condition, under room temperature. The seed germination and antioxidant enzymes activity were assessed with the seed samples drawn at bimonthly intervals up to 10 months of storage.

Germination test was conducted by following the procedure outlined in ISTA (2011) [10] with roll towel medium using 4 x 100 seeds in a germination room maintained at 25 ± 2°C temperature and 95 ± 3 % relative humidity. Seedlings were evaluated and based on normal seedlings, the germination was calculated and the mean expressed as percentage.

Dehydrogenase activity seeds was done based on the procedure outlined by Kit tock and Law (1968) [12] and seeds were selected randomly and preconditioned by soaking in water for 6 h. Then the seeds were bisected longitudinally into two halves and were steeped in 1% 2, 3, 5 triphenyltetrazolium solution and kept in dark for 4 h at 40°C for staining. The coloured solution was decanted and the colour intensity was measured in a UV-VIS spectrophotometer (Model Systronics 2205) using blue filter (470 nm) and methyl cellosolve as the blank. The OD value obtained was reported as total dehydrogenase activity (Kittock and Law, 1968) [12].

Theα-amylase content of the stored seeds were analysed by the procedure of Paul *et al.* (1970) [14].Seeds were germinated on petriplates adopting top of paper method upto radicle protrusion. The pregerminated seed samples were weighed for 500 mg in four replicates and were homogenised in 1.8 ml of cold 0.02 M sodium phosphate buffer (pH 6.0) and centrifuged at 20,000 rpm for 20 min for extracting the enzymes. The colour change was measured at 620 nm using a UV-VIS spectrophotometer (Model Systronics 2205). The α-amylase content was computed and expressed as mg maltose min^{-1}.

Enzyme extract for superoxide dismutase was prepared by preconditioning the weighed amount of seed samples (0.2 g) p[laced in between the moist germination paper followed by grinding with 2 ml extraction buffer (0.1 M phosphate buffer, pH 7.8, containing 0.5 mM EDTA). Then, the extract was centrifuged for 20 min. at 15,000 rpm and the supernatant was used as enzyme. Superoxide dismutase the assay is based on the formation of blue coloured formazone by nitro-blue tetrazolium and O_2·radical, which absorbs at 560 nm, and the enzyme (SOD) decreases this absorbance due to reduction in the formation of O_2 radical by the enzyme (Dhindsa and Dhindsa, 1981) [7].

Result

The seed germination was significantly differed due to seed treatment storage container, period of storage and its interaction. However, the interaction between the treatment and container were not significant. Irrespective of period of storage and container, after 10 month of storage seed treatment with halogenation mixture @ 3 g kg^{-1} recorded significantly maximum seed germination of 83 per cent compared to untreated control seeds (72 per cent). Between containers, seed stored in 700 gauge thick polythene bag recorded seed germination of 83 per cent when compared to the seed stored in cloth bag (72 per cent).

The seed germination was decreased with increase in storage periods. The germination decreased from 83 to 79 per cent after 10 month of storage. The interaction between treatment and period of storage revealed that the seed treatment with halogenation mixture @ 3 g kg^{-1} registered the higher germination of 83 per cent at the end of the 10 month of storage while the untreated control seeds recorded the lowest germination of 72 per cent. (Table.1).

Significant difference in α-amylase content was observed due to seed treatments, storage containers, period of storage, interaction between container and period of storage, treatment and period of storage. However, other interactions were not significant

Irrespective of period of storage and container, seeds treated with halogenation mixture @ 3 g kg^{-1}registered the maximum α-amylase content (5.11) while untreated seeds recorded the minimum α-amylase content (3.22).

Interaction between container and period of storage expressed that seed stored in 700 gauge polyethylene bag could able to maintain higher level α-amylase content over the period of storage (5.27at P_0and3.57at P_{10}) than cloth bag (5.05at P_0and3.04at P_{10}) (Fig. 1).

Dehydrogenase activity was also significantly influenced by various treatments combinations. Irrespective of period of storage and container, seeds treatment with halogenation mixture @ 3 g kg^{-1}recorded the highest dehydrogenase activity (0.994) while the lowest activity (0.853) was observed in untreated seeds. Between the containers, seeds stored in 700 gauge polyethylene bag maintained higher dehydrogenase activity (0.135at P_0and0.866at P_{10}) than cloth bag (1.131at P_0and0.786at P_{10}) (Fig 2).

Significant difference in superoxide dis mutase was observed due to seed treatments, storage containers, period of storage, interaction between container and period of storage, treatment and period of storage. Irrespective of period of storage and container, seeds treatment with halogenation mixture @ 3 g kg^{-1}registered the maximum superoxide dismutase of enzyme (7.89) while untreated seeds recorded the minimum superoxide dismutase of enzyme (6.32).

Between the containers, seed stored in 700 gauge polyethylene bag maintained higher level of superoxide dismutase of enzyme when compared to cloth bagat all period of storage (8.12at P_0and7.42at P_{10}) (Fig 3).

Discussion

Antioxidant protects and prevents oxidative deterioration of lipids and maintains structural and functional integrity of

cells. Free radicals are atoms, molecules or ions with unpaired electrons, which are highly reactive to chemical reactions with other molecules, in the biology system. The significant lower values of antioxidant enzyme activity indicated better membrane integrity in treated seeds as compared to untreated control during ageing process. Reduction in seed deterioration and improvement in seed vigour by the use of antioxidant like ascorbic acid is primarily due to quenching of free radicals, which prevents the peroxidative damage and enhances the activities of peroxide and radical scavenging enzymes (Hailstone and Smith, 1991) [8]

Lower dehydrogenate activity recorded in seeds stored in cloth bag may be due to higher level of seed deterioration on account of age induced membrane damage of various cell and cell organelles. This results is in agreement with the studies of Chauhan *et al.* (2011) [5, 6] and Pallavi *et al.*, (2003) [13] in wheat and sunflower. Declined TDH activity in seeds could be due to enzymatic changes by losing or gaining certain functional groups, by oxidation of sulf-hydral groups or by conversion of amino acids within the protein structure.

The amylase activity was significantly lower in seeds stored in cloth bags (3.04) and higher in polythene bag (3.57) at the end of 10 months of storage. In cereal seeds, the development of amylase activity constitutes an important event in germination and gradual decline in amylase activity was reported in cloth bags. Similar observation was reported by Agarwal and Kharlukhi (1987) [2].

Specific activity of superoxide dismutaseenzyme decreased significantly after natural ageing on cloth bags where as in polythene bags superoxide dismutaseenzyme activity significantly higher in during storage. This free radical inducing non- enzymatic peroxidation may lead to membrane damages and is likely to cause seed deterioration (Jung and Chiu, 1995) [11]. Protective mechanism that could scavenge the peroxidatively produced free radicals and peroxides prevail within the cellular system to minimize seed deterioration reactions. Superoxide dismutase, peroxidase and catalase enzyme systems provide one such protective mechanism and inhibition of activities of these enzymes is reported to cause faster deterioration of seed (Jung and Chiu, 1995) [11].

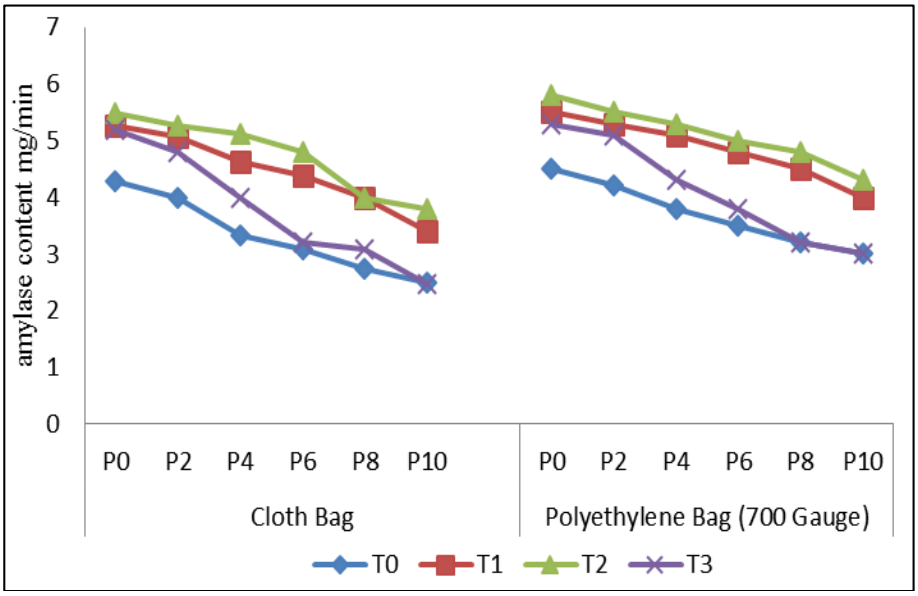

Fig 1: Effect of seed treatment, storage container and period of storage on α – amylase content (mg maltose min^{-1}) in maize parental line (UMI 1230) of maize COH (M) 6

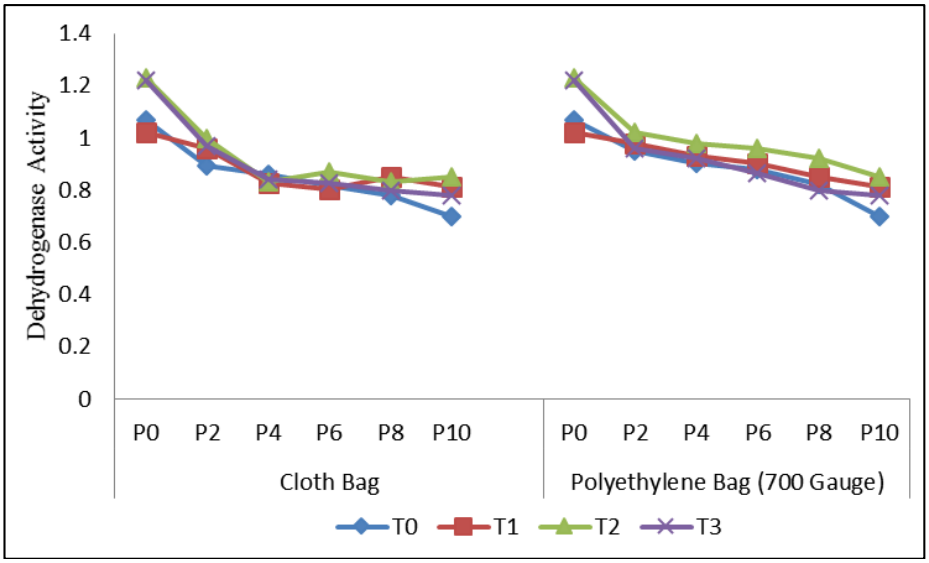

Fig 2: Effect of seed treatment, storage container and period of storage dehydrogenase activity in (OD value) of maleparental line (UMI 1230) in maize COH (M)6.

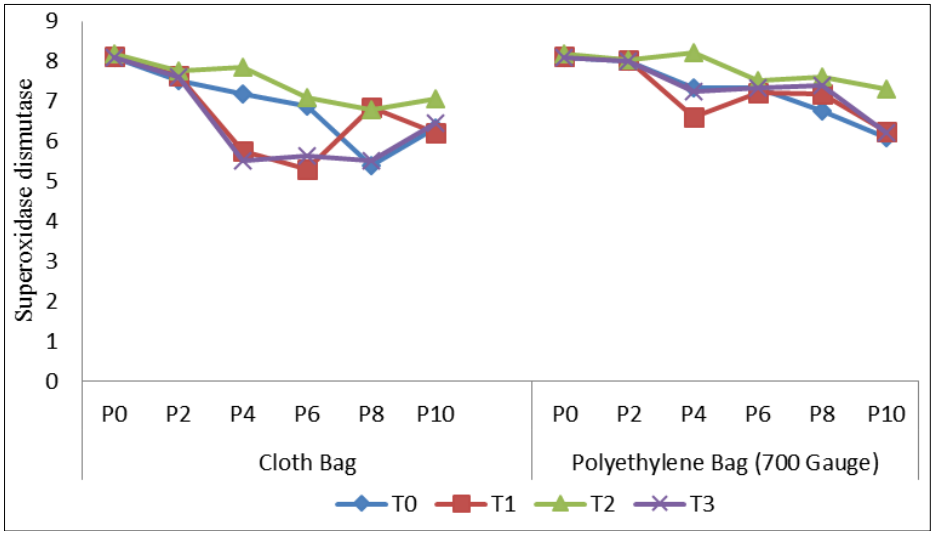

Fig 3: Effect of seed treatment, storage container and period of storage on superoxide dismutase (unit of enzyme) in male parental line (UMI 1230) of maize COH (M) 6

Table 1: Effect of seed treatment, storage container and period of storage on germination (%) of male parental line (UMI 1230) in maize COH (M) 6

Seed Treatment (T)	Storage Container (C)													
	Cloth Bag							Polyethylene Bag (700 Gauge)						
	Period of storage (p)													
	P_0	P_2	P_4	P_6	P_8	P_{10}	Mean	P_0	P_2	P_4	P_6	P_8	P_{10}	Mean
T_0 -Control	90 (71.56)	87 (68.86)	83 (65.64)	67(54.93)	56(48.44)	35 (36.27)	70 (56.78)	91 (72.54)	89 (70.63)	85 (67.21)	70 (56.78)	60 (50.76)	38 (70.63)	72 (58.05)
T_1 -Halopolymer @ 4 ml kg^{-1}	90 (71.56)	88 (69.73)	82 (64.89)	68 (55.55)	64 (54.13)	44 (41.55)	73 (58.69)	90 (71.56)	90 (71.56)	90 (71.56)	71 (57.41)	67 (54.93)	54 (47.29)	77 (61.34)
T_2 - \Halogenation Mixture @ 3 g kg^{-1}	92 (73.57)	90 (71.56)	87 (68.86)	80 (63.43)	76 (60.66)	50 (45.00)	79 (62.75)	92 (73.57)	90 (71.56)	90 (7.56)	83 (65.64)	81 (64.15)	63 (52.53)	83 (65.64)
T_3 - Carbendazim 2 g kg^{-1}	90 (71.56)	90 (71.56)	80 (63.43)	70 (56.78)	65 (53.72)	34 (35.66)	72 (58.05)	90 (71.56)	90 (71.56)	85 (67.21)	74 (59.34)	70 (56.78)	50 (45.00)	76 (60.66)
Mean	91 (72.54)	89 (70.63)	83 (65.64)	71 (57.14)	65 (53.72)	41 (39.81)	74 (59.34)	91 (72.54)	90 (71.56)	88 (69.73)	75 (60.00)	70 (56.78)	51 (45.57)	77 (61.34)

Treatment (T)	T x P Interaction Mean						Treatment (Mean)
	P_0	P_2	P_4	P_6	P_8	P_{10}	
T_0	90(71.56)	88(69.73)	84(66.42)	69(56.16)	58(49.60)	38(38.06)	71(57.42)
T_1	90(71.56)	89(70.63)	46(42.70)	70(56.78)	66(54.33)	54(47.29)	69(56.17)
T_2	92(73.57)	90(71.56)	89(70.63)	82(64.89)	79(62.72)	63(52.54)	83(65.65)
T_3	90(71.56)	90(71.56)	83(65.64)	72(58.05)	68(55.55)	50(45.00)	76(60.67)
Period of Mean	91(72.57)	89(70.63)	76(60.66)	73(58.69)	68(55.55)	51(45.57)	77(61.34)
Level of significance							
	T	C	P	T x C	C x P	T x P	T x C x P
SEd	0.562	0.397	0.688	0.795	0.973	1.377	1.947
CD (P = 0.05)	1.108	0.784	1.358	NS	1.920	2.716	3.841

Figures in parentheses indicate arcsine transformed values

Conclusion

Among the seed treatments, seeds treated with halogenation mixture @ 3 g kg^{-1} recorded higher seed quality parameters followed by different seed treatment throughout the storage period. Seeds treated with the halogenation mixture @ 3 g kg^{-1} maintained higher germination (83%) at the end of 10 month of storage with higher antioxidant enzymes *viz.* superoxide dismutase, dehydrogenase activity and α – amylase content during the seed storage and less ROS damage and minimum lipid peroxidation results the activity of antioxidative enzymes and better cell membrane integrity in male parental line (UMI 1230) in maize hybrid COH (M) 6.

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