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RESEARCH ARTICLE

Change in storage enzymes activities in natural and accelerated aged seed of maize (*Zea mays* L.)

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SUMMARY

A study was conducted on hybrid maize, *viz.*, Hema. Seeds were subjected to natural *vis-a-vis* accelerated ageing conditions and evaluated for change in storage enzymes activities in natural and accelerated aged seed. The accelerated aging test was carried out at two different temperatures: 41 and 42°C, with four relative humidity levels: 85, 90, 95 and 100 per cent and duration periods of 3, 6, 9 and 12 days. Natural ageing was carried for 12 months. The present investigation revealed that the level of various enzymes have been studied so as to find the exact cause of seed deterioration. The activity of all the enzymes, *viz.*, peroxidase, dehydrogenase and amylase decreased after natural and artificial ageing treatment in all the varieties. Among different ageing treatments, the dehydrogenase activity and amylase activity were recorded less in natural as well as accelerated aged seed lot as compared to fresh lot. In natural aged seed lot peroxidase activity decreased as the ageing progressed. The results revealed that, high level of correlation between loss in seed viability and the decreases that occurred in enzyme activity.

Key Words : Storage enzymes activities, Accelerated aging test, Seeds, Maize

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All seeds undergo aging process during long-term storage which leads to deterioration in seed quality, especially in the humid tropical regions. However, the rate of seed deterioration can vary among various plant species (Merritt *et al*., 2003). Aged seeds show decreased vigour and produce weak seedlings that are unable to survive once reintroduced into a habitat (Atici *et al*., 2007). Seed viability is a matter of great concern and measures to maintain germination efficiency of stored seeds have significant economical implications. A range of pre-harvest, harvest and post-harvest conditions affect seed viability (Basu, 1995). On the other hand, seed ageing which is linked to loss of seed viability with time is inevitable and the best that can be done is to lower its rate (Coolbear, 1995). Many factors contribute to seed ageing. These include genetics, mechanical damage, relative humidity and temperature of the storage environment, seed water content, presence of microflora, seed maturity, etc. The rate of loss of seed viability is mainly a function of temperature and seed moisture content (McDonald, 1999 and 2004). During ageing, seed viability and vigour decrease. Furthermore, the losses of viability and vigour in seeds differ with species and cultivars.

Many hypotheses have been proposed regarding causes of seed ageing such as lipid peroxidation mediated by free radicals, inactivation of enzymes or decrease in proteins, disintegration of cell membranes and genetic damage (McDonald, 1999; Murthy *et al*., 2003; Priestley, 1986; Smith and Berjak, 1995; Walters, 1998). Degradation and inactivation of enzymes due to changes in their

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macromolecular structures is one of the most important hypotheses proposed regarding causes of ageing in seeds (Bailly, 2004; Basavarajappa *et al*., 1991; Basra and Malik, 1994; Goel *et al*., 2002; Kalpana and Rao, 1993; Lehner *et al.*, 2008; McDonald, 2004; Salama and Pearce, 1993). Most of these studies suggest that decreases occur in the activity of enzymes such as superoxide dismutase, catalase, peroxidase and glutathione reductase in aged seeds. The general decrease in enzyme activity in the seed lowers the respiratory capacity, which in turn lowers both the energy (ATP) and assimilates supply of the germinating seed. Biochemical and physiological deterioration during seed aging has been studied mostly under accelerated aging conditions using high temperature and high seed water content (McDonald, 1999 and Hsu *et al.,* 2003). Although these studies allowed important progress towards the understanding of seed aging mechanisms, a major question has been raised whether mechanisms of seed aging are similar under conditions of accelerated aging and natural aging. Present study indicates that the studies on biochemical mechanisms in long-term stored seeds can supply an important contribution to the understanding of natural aging process in seeds.

Dehydrogenase activity is also known as tetrazolium reduction ability. The activity of dehydrogenase enzyme is directly correlated with the seed vigour. Pallavi *et al.* (2003) revealed that the absorbance of dehydrogenase enzyme was decreased as the period of storage increased in sunflower. Verma *et al.* (2003) observed that the dehydrogenase activity was reduced as the ageing progressed and was found lowest after four year of storage in *Brassica* spp.The present study, therefore, focused on some biochemical mechanisms in the natural an accelerated aged seed of wheat. Demirkaya *et al.* (2010) a high level of correlation between loss in seed viability and the decreases that occurred in catalase activity in onion, Bhanuprakash *et al.* (2010) also recorded change in enzyme activity due to ageing in bell peper. Hence, the present study was conducted to identify the effect of ageing on physiological activity of seed.

MATERIAL AND METHODS

The present research work was carried out in the laboratories of Department of Seed Science and Technology, University of Agricultural Sciences, Bangalore. For defining the variables for artificial ageing, seeds were artificially aged at two different temperatures (40 and 42°C), four relative humidity levels (85, 90, 95 and 100%) and four periods (3, 6, 9 and 12 days). and observation was recorded after ageing. In case of natural ageing, observation was recorded trimonthly on the stored maize seed in cloth bag in ambient conditions up to one year.

Standard germination:

One hundred (100) seeds per replication were germinated in between paper (BP) in a 25°C germinator. The germination tests were evaluated after 7 days at final count. Numbers of normal seedlings were averaged as the germination percentage (ISTA, 2010).

Total dehydrogenase activity (OD@A_{480}nm):

The total dehydrogenase activity of the seeds was estimated as per the method described by Perl *et al.* (1978). Ten seeds of three replications selected randomly were pre-conditioned by soaking in water for 24 hours. Then ten pre imbibed seeds were randomly selected in each sample, seed was cut longitudinally and soaked in 0.5 per cent tetrazolium chloride solution in a test tube and incubated at 25±1^{0}C under dark for six hours. Then they were washed thoroughly with distilled water, the red colouredformazan from the stained embryos was extracted by soaking these embryos 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 (Model Mini Spec 17) at 480 nm with suitable blank (Methoxy ethanol). The total dehydrogenase activity (TDH) was expressed in absorbance.

~-Amylase activity:

Preparation of seed powder and enzyme extraction:

Seed powder was prepared from different ageing treatment seeds. Immediately after ageing, seeds were washed thoroughly and excess moisture was removed by blotting with tissue paper. Then 0.8 g seeds were frozen in liquid nitrogen and ground with the help of pestle and mortar. While grinding seed itself, 1.0 ml of 0.1 M phosphate buffer of pH 7.0 was added and the ground material was transferred to eppendorf tubes and kept at 4°C overnight and then transferred to -20°C. The slurry was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was used for α-amylase assay.

Enzyme assay:

The α-amylase assay was carried out according to the method of Bernfeld (1955) with slight modification. The enzyme assay of sample was carried out along with blank and control for each sample. For sample analysis 0.1 ml of enzyme extract was taken in a cleaned test tube and 250 µl of 1 per cent soluble starch was added and incubated for 15 min. To this 500 µl of DNS reagent was added to stop the reaction and heated over water bath for 5 min and then cooled under running tap water after this, 250 µl of 40 per cent sodium potassium tartrate was added. Final volume of the reaction mixture was made to 5 ml by adding 3.9 ml of water. Absorbance was read at 560 nm. A control was prepared for each sample similar to that of sample but the reaction was terminated at zero time. Similarly blank was prepared for each sample by omitting starch. Standard curve was prepared by using maltose (0 to 100 µg). One unit of enzymatic activity is defined as one mg of maltose liberated/hour under the standard assay conditions and specific activity as mg

maltose liberated/min/gram seed.

Peroxidase activity:

Enzyme extraction:

One gram of maize seeds subjected to different ageing treatments were extracted in 1ml 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 4ºC for 4 hours for enzyme extraction and then tubes are transferred to 20ºC. The homogenate was centrifuged at 10,000 rpm at 4ºC for 15 minutes. The supernatant was used as enzyme source. The enzyme extract was stored in ice box till the assay is carried out.

Enzyme assay:

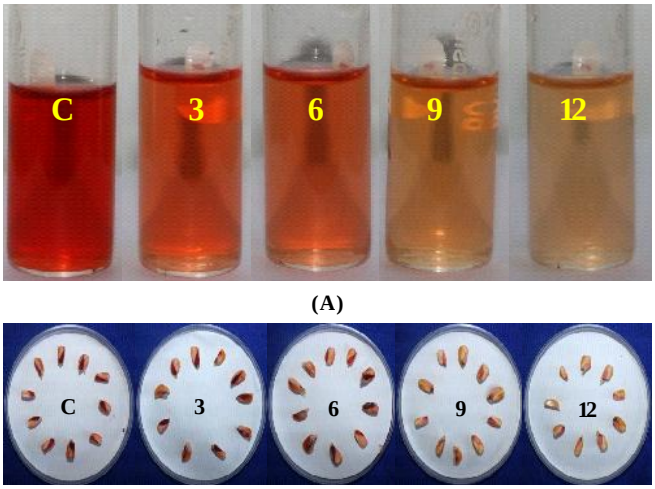
The enzyme assay was carried out according to Sadasivam and Manickam (2008). The reaction mixture was prepared in cuvette by adding 2 ml of 0.1 M phosphate buffer of pH 7.0, Guaiacol-200 µl and 12.3 mM H_2O_2-200 µl. Brought the mixture to 25ºC and then placed the cuvette in the spectrophotometer set 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.

RESULTS AND DISCUSSION

In order to relate antioxidant capacity to germination efficiency, catalase activity was determined in seed extracts from different viability levels.

Dehydrogenase activity:

Table 1 and 2 show the effect of natural and accelerated ageing on dehydrogenase activity test. Among different ageing treatments, the dehydrogenase activity was recorded less in natural as well as accelerated aged seed lot as compared to control (fresh lot). It was significantly higher in natural aged seed lot as compared to accelerated aged lot (Fig. 1a and b). However, in accelerated aged lot maximum activity was recorded in 40ºC (0.419) and minimum was in 42ºC (0.230) irrespective of relative humidity. The trend of the results is in agreement with the studies of Chauhan *et al*. (2011) and Pallavi *et al*. (2003) who also noticed declined TDH activity it could be due to enzymes undergo compositional 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 enzymes may undergo configurational changes such as partial folding or unfolding of ultrastructrure, condensation to form polymers and degradation to sub units *i.e*., absorbance of dehydrogenase enzyme was decreased as the period of storage increased in sunflower. Copeland and McDonald (1995) also reported that continual accumulation of free fatty acids culminated in a reduction of cellular pH and was detrimental to normal cellular metabolism. Furthermore, it denatures enzymes resulting in their loss of activity. Verma *et al*. (2003) observed that the dehydrogenase activity was reduced as the ageing progressed and was found lowest after four year of storage in *Brassica* spp. Mustafa *et al*. (2010) a high level of correlation was found between the loss of seed viability and the decreases that occurred indehydrogenase activity in onion.



(A)

(B)

C: Control; 3: Three days after ageing; 6: six days after ageing; 9: Nine days after ageing; 12: Twelve days after ageing

Fig. 1 : Influence of accelerated ageing on enzyme activity of maize (A) Total dehydrogenase activity affected by accelerated ageing (B) Tetrazolium staining pattern of maize seeds

Amylase activity:

In cereal seeds the development of amylase activity constitutes an important event in germination. It was significantly higher in natural aged seed lot as compared to accelerated aged lot. However, in accelerated aged lot maximum activity was recorded in 40ºC (0.204) and minimum was in 42ºC (0.179) irrespective of relative humidity. A gradual decline in amylase activity was reported in natural aged seedlot as time of ageing increased, similar observation was reported by Agarwal and Kharlukhi (1987) in natural aged gram, chickpea and wheat seeds and Petruzzeliand Taranto (1990) in natural aged and accelerated aged wheat endosperm. Similar results of decrease in α-amylase activity was also reported by Norastehnia *et al*. (2007) who also noticed as deterioration advances, the accumulation of aldehyde compounds especially methyl jasmonte (MeJA) which is potential inhibitor of α-amylase. Higher concentrations of MeJA reduced the concentration of

Table 1: Total dehydrogenase (TDH) activity, α-amylase activity and peroxidase activity as influenced by accelerated ageing in maize

Treatments		Germination (%)			TDH activity (A_{480}nm)			α-Amylase activity (mg maltose liberated min^{-1} $gram^{-1}$)			Peroxidase activity (a A_{436}nm min^{-1} $gram^{-1}$)		
		T_1	T_1	Mean	T_1	T_1	Mean	T_1	T_2	Mean	T_1	T_2	Mean
	Control	100	100	100	0.818	0.818	0.818	0.343	0.343	0.343	0.733	0.733	0.733
	A_3	92	88	90	0.457	0.317	0.424	0.293	0.263	0.278	0.317	0.187	0.252
R_1	A_6	74	68	71	0.292	0.180	0.254	0.218	0.176	0.197	0.180	0.110	0.145
	A_9	50	34	42	0.143	0.080	0.126	0.123	0.069	0.096	0.080	0.067	0.073
	A_{12}	02	02	02	0.040	0.041	0.035	0.018	0.012	0.015	0.041	0.022	0.031
	A_3	94	90	92	0.539	0.323	0.494	0.301	0.284	0.293	0.323	0.265	0.294
R_2	A_6	78	71	75	0.349	0.184	0.325	0.228	0.191	0.210	0.184	0.128	0.156
	A_9	55	52	54	0.185	0.090	0.168	0.160	0.142	0.151	0.090	0.084	0.087
	A_{12}	03	02	03	0.072	0.052	0.058	0.019	0.013	0.016	0.052	0.023	0.037
	A_3	96	91	94	0.532	0.363	0.509	0.326	0.286	0.306	0.363	0.315	0.339
R_3	A_6	81	78	80	0.421	0.184	0.384	0.252	0.235	0.244	0.184	0.184	0.184
	A_9	60	51	56	0.224	0.100	0.202	0.161	0.131	0.146	0.100	0.083	0.091
	A_{12}	34	18	26	0.116	0.077	0.099	0.096	0.055	0.076	0.077	0.058	0.067
	A_3	97	94	96	0.670	0.637	0.597	0.340	0.309	0.325	0.637	0.329	0.483
R_4	A_6	89	89	89	0.521	0.255	0.500	0.268	0.265	0.267	0.255	0.228	0.241
	A_9	73	69	71	0.347	0.176	0.316	0.205	0.190	0.198	0.176	0.110	0.143
	A_{12}	38	31	34	0.186	0.079	0.161	0.106	0.068	0.087	0.079	0.065	0.072
					T×R								
	R_1	64	58	61	0.350	0.270	0.331	0.199	0.173	0.186	0.270	0.223	0.247
	R_2	66	63	65	0.393	0.276	0.373	0.210	0.195	0.202	0.276	0.246	0.261
	R_3	74	68	71	0.422	0.291	0.402	0.236	0.210	0.223	0.291	0.274	0.283
	R_4	79	77	78	0.508	0.376	0.478	0.252	0.235	0.244	0.376	0.293	0.334
					T×A								
	A_3	95	91	93	0.550	0.410	0.506	0.315	0.286	0.300	0.410	0.274	0.342
	A_6	81	77	79	0.396	0.200	0.366	0.242	0.217	0.229	0.200	0.162	0.181
	A_9	60	52	56	0.225	0.111	0.203	0.162	0.133	0.148	0.111	0.086	0.098
	A_{12}	19	13	16	0.104	0.062	0.088	0.060	0.037	0.048	0.062	0.042	0.052
	Mean	66	61		0.419	0.230		0.204	0.179		0.230	0.177	
		S.E.±	C.D. (P=0.01)	C.V. (%)	S.E.±	C.D. (P=0.01)	C.V. (%)	S.E.±	C.D. (P=0.01)	C.V. (%)	S.E.±	C.D. (P=0.01)	C.V. (%)
	T	0.150	0.561		0.0015	0.0056		0.001	0.003		0.0014	0.0053	
	R	0.213	0.794		0.0021	0.0079		0.001	0.005		0.0020	0.0075	
	A	0.238	0.888		0.0024	0.0088		0.001	0.005		0.0023	0.0084	
	TR	0.301	1.123	1.70	0.0030	0.0111	2.91	0.002	0.007	3.34	0.0028	0.0106	3.92
	TA	0.336	1.255		0.0033	0.0124		0.002	0.008		0.0032	0.0119	
	RA	0.476	1.776		0.0047	0.0176		0.003	0.011		0.0045	0.0168	
	TRA	0.673	2.511		0.0067	0.0248		0.004	0.015		0.0064	0.0238	

Table 2: Total dehydrogenase (TDH) activity, α-amylase activity and peroxidase activity as influenced by natural ageing in maize

Duration of storage (months)	Germination per cent (%)	Total dehydrogenase activity (A_{480}nm)	~ -Amylase activity (mg maltose liberated min-1 gram-1)	Peroxidase activity (a A_{436}nm min^{-1} $gram^{-1}$)
Control	99.8	0.818	0.343	0.733
3	97.5	0.681	0.319	0.554
6	95.3	0.549	0.311	0.421
9	93.3	0.504	0.269	0.303
12	90.5	0.445	0.232	0.214
S.E.±	1.449	0.01	6.859	0.005
C.D. (P=0.01)	6.039	0.041	28.582	0.02
C.V. (%)	3.667	3.32	4.699	2.303

the enzyme protein and also the consequence of the inhibition of gibberellin biosynthesis, since GA stimulates α-amylase synthesis and its secretion from aleurone layers in cereals.

Peroxidase activity:

Specific activity of peroxidase enzyme decreased significantly after natural and accelerated ageing treatments. In natural aged seed lot peroxidase activity was significantly higher in control (0.733) an minimum was in 12 month of ageing (0.214) and in accelerated aged lot maximum peroxidase activity was also recorded in control (1.733) and minimum was recorded in 42ºC (0.177) irrespective of relative humidity. The ageing period had an significant effect on peroxidase activity. The decline in scavenging enzymes particularly peroxidase could be due to lipid peroxidation and sugar hydrolysis (formation of reducing sugars) was coupled to the Maillard reactions during seed ageing. Carbonyl intermediates as a result of Millard reaction may attack the enzyme complex and modify the native structure of enzyme either by adding or drawing the certain functional groups from enzyme structure. Thus, alter the macromolecular structure and reduces the efficiency of peroxidases Narayanamurthy and Sun (2000). Cyanide production is often associated with deleterious mechanisms. It will inhibit the activity of heme proteins from peroxidases (Ellis and Dunford, 1968; Job and Ricard, 1975) and make it inefficient. Demirkaya *et al*. (2010) studied that a high level of correlation between loss in seed viability and the decreases that occurred in antioxidant enzyme activity in onion, Bhanuprakash *et al*. (2010) also recorded change in enzyme activity due to ageing in bell peper, Cakmek *et al*. (2010) also studied the decrease germination ability of aged legume seed were correlated with decrease in activity of enzymatic antioxidant studies. These results support the hypothesis of Bailly *et al*. (1996) that a decrease in antioxidant enzymes is linked to an increased lipid peroxidation and accelerated ageing. Subsequently, Bailly *et al*. (2000 and 2002) proposed a positive relationship between antioxidant enzyme capacity and the vigour of the seed.

Consequently, our results support the hypothesis of inactivation of free radical scavenging enzymes (*i.e.*, SOD) during ageing and showed a direct relationship with the germination efficiency of ageing maize seeds.

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