Indian Journal of Agricultural Sciences **81** (11): 1037–40, November 2011

Change in storage enzymes activities in natural and accelerated aged seed of wheat *(Triticum aestivum)*

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Received: 27 November 2010; Revised accepted: 22 July 2011

ABSTRACT

A study was conducted during 2006–10 on seed of six varieties of wheat (*Triticum aestivum*), viz C 306, PBW 502, WH 542, WH 711, WH 283 and RAJ 3765 were subjected to natural vis-à-vis accelerated ageing conditions and evaluated for change in storage enzymes activities in natural and accelerated aged seed of wheat. The present investigation revealed that the level of various antioxidant enzymes have been studied so as to find the exact cause of seed deterioration. The activity of all the antioxidant enzymes, viz catalase, peroxidase, dehydrogenase and amylase decreased after natural and artificial ageing treatment in all the varieties. Among different ageing treatments, the dehydrogenase activity was recorded less in natural as well as accelerated aged seed lot as compared to fresh lot. In natural aged seed lot catalase and peroxidase activities decreased as the ageing progressed in all the six varieties the rate of decreasing of both the enzymes activity was higher after 18 months of storage. A gradual decline in amylase activity was reported in natural aged seed lot in all six varieties. Dehydrogenase activity was maximum up to 14 months of storage and after that it declined in terms of absorbance among all the varieties. It decreased at faster rate after 18 months of storage.

Key words: Accelerated ageing, Amylase, Catalase, Dehydrogenase, Natural ageing, Peroxidase, Seed quality, Wheat

Dehydrogenase activity was recorded significantly higher in natural aged seed lot in all the varieties as compared to accelerated aged lot. All enzyme activity is positively correlated with germination of wheat as ageing progressed germination also decreased and enzyme activity also decreased which showed significant deterioration in both accelerated as well as in natural aged seed lot.

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). Many of the processes implicated in seed aging during storage appear to be free-radical mediated, and lipid peroxidation is suggested to be a primary cause of deterioration in stored seeds (Wilson and McDonald 1986, McDonald 1999). Some protective mechanisms involving free radical and peroxide scavenging enzymes, such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) have been evaluated within the mechanism of seed aging (Hsu *et al.* 2003, Goel *et al.* 2003, Pukacka and Ratajczak 2007), Loycrajjou *et al.* (2008) reported that ageing induced deterioration increase the extent of protein oxidation thus inducing loss of functional properties of proteins and enzymes. 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, 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. Scialabba *et al.* (2002) reported that peroxidase activity decreased in aged seeds as compare to fresh seeds in radish. Pallavi *et al.* (2003) studied that sharp decline in peroxidase emzyme during ageing in sunflower. Peroxidase and catalase activities were found higher in younger seeds of *Chenopodium rubrum* (Mitrovic *et al.* 2005).

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.

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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.

MATERIALS AND METHODS

The present research work was carried out in the laboratories of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar from 2006 to 2010. Seed material comprised of six varieties of wheat, viz C 306, PBW 502, WH 542, WH 711, WH 283 and RAJ 3765 having germination above minimum seed certification standard (MSCS) was collected at the time of sowing of crop and stored in ambient conditions. For defining the variables for artificial ageing, seed of all six varieties were artificially aged (40±1ºC/72 hr) and observation was recorded after ageing. In case of natural ageing, observation was recorded quarterly on the stored wheat seed in cotton bags in ambient conditions up to one year till germination fall below as compared to fresh seed lot. For standard germination test hundred seeds of each variety in four replicates placed in between sufficient moistened rolled towel papers and kept at 25ºC in seed germinator. The first count was taken on 4th day and final count on 10th day and only normal seedlings were considered for percent germination according to the rules of International Seed Testing Association (ISTA 2003).

For the extraction of peroxidase enzyme, seeds of each variety were imbibed in beaker at 30ºC in the germinator for 24 hr. Two hundred milligram of imbibed seed sample was ground in a chilled pestle mortar by adding 10 ml phosphate buffer (*p*H 7.8) and a pinch of corning sand. The 10 ml homogenate was centrifuged at 12000 rpm for 20 min. at 4ºC. The supernatant obtained was then re-centrifuged at 15 000 rpm for 10 min. The clear supernatant, thus obtained, was used for estimating the activity of peroxidase, following the oxidation of O-dianisidine in the presence of hydrogen peroxide (H_2O_2). 2 Two ml of acetate buffer (*p*H 4.5) and 0.1 ml of O-dianisidine solution was added to 0.05 ml of enzyme extract. Then 0.1 ml of 0.2 M hydrogen peroxide also added to start the reaction. The reading was taken at 470 nm wavelength after every 15 seconds for 1 min. and enzyme unit was expressed as the amount of enzyme required to bring about a change in absorbance of 0.01/min.

The enzyme extract was prepared as described earlier for peroxidase. The catalase activity was assayed by the method as described by Aebi (1983) based on the reduction of potassium dichromate to chromic acetate by hydrogen peroxide.0.5 ml of H_2O_2 and 1.0 ml of phosphate buffer (*p*H 7.0) was added in 0.5 ml of enzyme extract in a side mouthed test tube. This was mixed rapidly and then incubated at 37ºC for 5 min. The test tubes were then taken out and 4 ml of dichromate acetic acid reagent was added. These were then heated for 10 min. in a boiling water bath. The colour which changed to green due to the formation of chromic acetate after cooling was measured by systronic spectrophotometer 169 at 570 nm.

The activity of catalase has been expressed as the amount of enzyme required to bring about a change in absorbance by 0.01/min. Amylase enzyme was assayed by method of Shuster and Gifford (1962). For extraction of enzyme four gm of seed was homogenized with 8 ml of chilled 0.2 M tris-HCL buffer (*p*H7.5) containing 0.1M each of cystein and EDTA. The homogenate was centrifuged at 15 000 rpm in a centrifuge at a temperature of 4°c for 15 min. The supernatant was used for enzyme assay. One ml of enzyme extract was incubated with 1 ml of starch at 30°c for 20 min. The reaction was terminated by using 0.5 ml of 2 N-HCl. One ml of iodine reagent was added in it and the volume was made 5 ml with distilled water. Optical density was recorded at 620 nm against reagent blank in systronic spectrophotometer 169.

Tetrazolium reduction ability by the enzyme dehydrogenase was determined by the method of Kittock and Law (1968).One gram seed of each seed lot replicated thrice were ground to pass through a 20 mesh screen. 200 mg flour was soaked in 5 ml of 0.5% tetrazolium solution at 38ºC for 3–4 hr. Then it was centrifuged at 10000 rpm for 3 min. and the supernatant was poured off. The formazan was extracted with 10 ml acetone for 16 hr, followed by centrifugation and absorbance of the solution was determined by systronic spectrophotometer 169 at 480 nm. These observations were expressed as change in O D/g/ml.

RESULTS AND DISCUSSION

Specific activity of peroxidase enzyme decreased significantly after natural and accelerated ageing treatments in all the six varieties of wheat. In natural aged seed lot peroxidase activity was significantly higher in PBW 502 (1.71) an minimum was in RAJ 3765 (1.122) and in accelerated aged lot maximum peroxidase activity was also recorded in PBW 502 (1.043) and minimum was recorded in RAJ 3765 (0.992) .Catalase activity of the natural as well as accelerated aged seed was recorded significantly lower than that of fresh seeds. In natural aged seed lot maximum catalase activity was recorded in PBW 502 (0.265) and minimum in RAJ 3765 (0.200) and after accelerated ageing the maximum catalase activity was also recorded in PBW 502 (0.226) and minimum in RAJ 3765 (0.194).Specific activity of amylase enzyme decreased significantly after natural and accelerated ageing treatment in all the six varieties of wheat. In natural aged lot amylase activity was significantly higher in PBW 502 (0.482) and minimum was recorded in RAJ 3765 (0.379)

and in accelerated aged seed lot maximum amylase activity was also observed in PBW-502 (0.322) and minimum was in RAJ 3765 (0.291).

Table 1 shows 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 fresh lot. It was significantly higher in natural aged seed lot in all the varieties as compared to accelerated aged lot. However, in natural aged seed lot, among different varieties of wheat maximum dehydrogenase activity was observed in PBW 502 (0.462) and minimum in RAJ 3765 (0.348) and in accelerated aged lot maximum activity was recorded in PBW 502 (0.445) and minimum was in RAJ 3765 (0.214). The present investigation revealed that the level of various antioxidant enzymes have been studied so as to find the exact cause of seed deterioration. The activity of all the antioxidant enzymes, viz catalase, peroxidase, dehydrogenase and amylase decreased after artificial ageing treatment in all the varieties. Similar decrease in the activity of catalase and peroxidase were reported by Scialabba *et al.* (2002) that peroxidase activity decreased in aged seeds as compare to fresh seeds in radish. Pallavi *et al.* (2003) studied that sharp decline in peroxidase emzyme during ageing in sunflower. Peroxidase and catalase activities were found higher in younger seeds of *Chenopodium rubrum* (Mitrovic *et al.* 2005).

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.

In case of natural aged seed catalase and peroxidase activities decreased as the ageing progressed in all the six varieties. The rate of decreasing of both the enzymes activity was higher after 18 months of storage. In the present study, the level of various enzymes have been studied so as to find the exact cause of seed deterioration under natural ageing. In general, decrease in enzyme activity in seed lowers its respiratory potential, which in turn lowers both the energy (ATP) and food supply to the germinating seed. Several changes in the enzyme macromolecular structure may contribute to their lower effectiveness. They may 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. However, the basic reason for reduced enzymatic activities in deteriorating wheat seed is yet to be established.

In cereal seeds the development of amylase activity constitutes an important event in germination. A gradual

Table 1 Effect of natural and accelerated ageing on enzyme activity of wheat

Variety	Catalase				Amylase				Peroxidase				Dehydrogenase			
	Ageing															
	Fresh	Natural	Accelerated	Mean	Fresh	Natural	Accelerated	Mean	Fresh	Natural	Accelerated	Mean	Fresh	Natural	Accelerated	Mean
C 306	0.288	0.242	0.251	0.26	0.618	0.434	0.288	0.441	1.214	1.153	1.026	1.131	0.392	0.359	0.318	0.423
PBW 502	0.422	0.265	0.226	0.304	0.627	0.482	0.322	0.483	1.243	1.171	1.043	1.153	0.605	0.462	0.445	0.480
WH 542	0.234	0.219	0.211	0.221	0.586	0.395	0.292	0.424	1.204	1.132	1.002	1.113	0.604	0.349	0.401	0.475
WH 711	0.237	0.217	0.211	0.221	0.573	0.398	0.298	0.423	1.218	1.144	1.022	1.128	0.565	0.419	0.308	0.431
WH 283	0.223	0.216	0.205	0.215	0.597	0.383	0.294	0.424	1.234	1.157	1.045	1.145	0.364	0.349	0.218	0.313
RAJ 3765	0.208	0.2	0.194	0.201	0.609	0.379	0.291	0.426	1.186	1.122	0.992	1.1	0.356	0.348	0.214	0.236
Mean	0.269	0.226	0.216		0.602	0.412	0.297		1.217	1.147	1.022		0.481	0.381	0.317	
Interaction	Catalase				Amylase				Peroxidase				Dehydrogenase			
Factors	CD	SE(d)	SE(m)		CD	SE(d)	SE(m)		CD	SE(d)	SE(m)		CD	SE(d)	SE(m)	
Varieties (A)	0.002	0.001	0.001		0.004	0.002	0.001		0.006	0.003	0.002		0.008	0.004	0.003	
Treatment (B)	0.003	0.002	0.001		0.006	0.003	0.002		0.009	0.004	0.003		0.012	0.006	0.004	
Factor(A × B)	0.006	0.003	0.002		0.010	0.005	0.003		N.S.	0.007	0.005		0.020	0.010	0.007	

decline in amylase activity was reported in natural aged seed lot in all six varieties as time of ageing increased similar observation was reported by Agarwal and Kharlukhi (1987) in natural aged gram, chickpea, and wheat seeds, and Ppetruzzeli and Taranto (1990) in natural aged and accelerated aged wheat endosperm. Dehydrogenase activity was maximum up to 14 months of storage and after that it declined in terms of absorbance among all the varieties. It decreased at faster rate after 18 months of storage. Similar results were recorded by Plallavi *et al.* (2003) in sunflower seeds, Narwal (1995) in okra, Verma *et al.* (2003) in *Brassica* spp. Demirkaya *et al.* (2010) studied that 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, Cakmek *et al*. (2010) also studied the decrease germination ability of aged legume seed were correlated with decrease in activity of enzymatic antioxidant studies.. Hence, the present study was conducted to identify the effect of ageing on physiological activity of seed.

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