Environmental and Experimental Biology (2015) 13: 177–182 *Original Paper*

Antioxidant enzyme activity and germination characteristics of different maize hybrid seeds during ageing

Cyrus Mansouri-Far[1,2], Maryam Goodarzian-Ghahfarokhi[1,3]*, Mohsen Saeidi[1], Majid Abdoli[4]

[1]Department of Agronomy and Plant Breeding, Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran
[2]Faculty of Agricultural Science, Payam-e-Noor University, Karaj, Iran
[3]Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahid Chamran University, Ahvaz, Iran
[4]Young Researchers and Elite Club, Zanjan Branch, Islamic Azad University, Zanjan, Iran

*Corresponding author, E-mail: goodarzian98@gmail.com

Abstract

The aim of this study was evaluation of the effect of ageing on germination traits and activity of antioxidant enzymes in embryo of maize hybrid seeds and the role of these traits in protection of seed vigour. A two-factorial experiment was performed using six maize hybrids, and four accelerated ageing levels (non-aged, aged for 2, 4 and 6 days) at 40 °C temperature and 95% humidity. Mean germination time and electrolyte leakage significantly increased with ageing. Mean germination time and electrolyte leakage of hybrids SC260, SC370 and SC647 increased more than in the other hybrids. Also, antioxidant enzyme activity decreased significantly with ageing. The results indicated severe damage to the cell membrane and antioxidant enzyme acivity in the hybrids under ageing. In general, loss of viability and increase in electrolyte leakage reflected the seed deterioration during storage under accelerated ageing conditions.

Key words: antioxidant enzymes, electrolyte leakage, germination, maize embryo, seed ageing.
Abbreviations: AA, accelerated ageing; CAT, catalase; EC, electrical conductivity; EL, electrolyte leakage; GP, germination percentage; MGT, mean germination time; NBT, nitroblue tetrazolium; $OH^{\bullet}$, hydroxyl radical; O_2^-, superoxide radical; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase.

Introduction

Ageing is known as the process of seed quality loss along with time associated with reduction of seed vigour (Verma et al. 2003). During storage seed vigour and germination slowly decrease. Seed deterioration that occurs during storage causes decrease of seedling establishment and ultimately plant yield in the field (McDonald 1999). Seed deterioration occurs even in optimum storage conditions. Genetic and environmental factors have interacting effects on seed deterioration at harvest and during storage (Bewley and Black 1994). Since seed storage is associated with reduction of seed longevity, conditions must be optimal for conserving genetic and seed commercial storage (Rajjou et al. 2007). Temperature and humidity are two major factors that must be controlled during seed storage (Krishnan et al. 2004).

Germination is the first step in growth and development of crops (Mohsen-Nasab et al. 2010). Germination rate, germination uniformity and seed vigour are important parameters in seed quality and thus affect plant status. During germination, with increasing moisture content of seeds, production of reactive oxygen species (ROS) occurs through two processes: biochemical reactions in mitochondria or glyoxysome, and release of ROS from substances that are produced in different seed tissues during harvest and stored in dry seeds (Zamani et al. 2010). During seed sotrage, ROS such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$), accumulate in ageing seed tissues and have a vital role in seed vigour reduction (Pukacka, Ratajczak 2005). Accumulation of ROS leads to their reaction with unsaturated fatty acids and causes changes in cell membranes, such as lipid peroxidation and ultimately its destruction.

The ability of seeds to produce antioxidative enzymes considerably differs depending on species and genotype. Enzymatic detoxification and repair of cell membranes are the main means to delay ageing (Tavakol Afshari et al. 2007). ROS scavenging enzymatic systems, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) and non-enzymatic antioxidants such as β-carotene, ascorbic acid, α-tocopherol, reduced glutathione can counteract the harmful effects of ROS in plant tissues (Kibinza et al. 2006). Activity of these metabolites and antioxidant enzymes causes high levels of seed resistance to oxidative damage and minimizes damage to cells (Alscher 1989; Tabatabaei 2015).

Maize seeds are often harvested with 22 to 30% moisture content and then dried to 15.5% or less humidity to prevent microbial growth. Kapilan (2015) reported that

the biochemical and enzymatic changes occurring inside the seed and reduction of germination and seedling growth are consequences of deterioration of maize seed.

This study was performed to determine the mechanisms of seed vigour reduction with respect to antioxidant enzyme activity. Germination characteristics, electrolyte leakage as an indicator of membrane damage, enzyme activities were evaluated in seeds of various maize hybrids.

Materials and methods

Plant material, experimental procedure and design

This experiment was carried out at the Plant Physiology Laboratory in the Campus of Agriculture and Natural Resources of Razi University, Kermanshah city, Iran. In order to understand maize seed deterioration mechanisms during storage, a factorial experiment with two factors following completely randomized design with three replications was used. The first factor was maize hybrid (SC704, SC700, SC647, SC500, SC370 and SC260) and the second factor was accelerated ageing (AA) treatment: control (non-aged, A_1), 2 (A_2), 4 (A_3) and 6 (A_4) days of ageing treatment. To promote ageing, seeds were incubated at 40 °C at saturated air humidity (approximately 95 ± 1%) (Komba et al. 2006). A digital humidity meter (TFA, Germany) was used for regulation of air temperature and humidity in germination incubator.

Germination percentage and mean germination time measurements

The ISTA (1999) standard method was used to calculate germination percentage and mean time to germination of seeds. A batch of 25 disinfected seeds was placed in a sterilized Petri dish (9 cm diameter) on filter paper. Then the Petri dishes were placed in a growth chamber at 25 ± 1 °C for 7 days in darkness, and every 24 h distilled water was added as required. Germination and mean germination time in seeds from different ageing treatments and control were calculated (Wiese, Binning 1987). Germinated seeds were recorded daily up to 7 days at the same time of day. Germination percentage (GP) was calculated as the number of germinated seeds at the seventh day, according to the formula:

$$GP = (N_g / N_t) \times 100,$$

where N_g is a total number of germinated seeds, N_t is a total number of seeds evaluated. Mean germination time (MGT) was calculated according to Bailly et al. (2000) using the formula:

$$MGT = \Sigma Dn / \Sigma n,$$

where n is the number of seeds that germinated on day D, and D is the number of days counted from the beginning of emergence.

Enzyme activity measurements

The embryo is the most important, sensitive and vulnerable part of the seed. Seed with damaged cotyledons may survive and form seedlings, but if the seed embryo is damaged, a seedling will not be formed. To evaluate antioxidant enzyme activity in the embryo, seeds were dehydrated for 12 h, then the seed coat was separated and isolated embryos were rolled in foil and immediately frozen in liquid nitrogen and stored at –80 °C. The samples were homogenized in liquid nitrogen and transferred into 15 mL tubes, after which 2.5 mL of the extract buffer (0.1 M Tris, pH 7.8, and 30% glycerol) was added to the samples. They were then centrifuged at 15 000 g for 15 min at 4 °C, and the supernatant retained for enzyme assays.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by the method of Beauchamp and Fridovich (1971). One unit of SOD was defined as the amount of enzyme producing 50% inhibition of nitroblue tetrazolium (NBT). Catalase (CAT, EC 1.11.1.6) activity was estimated by consumption of hydrogen peroxide, which was recorded at 570 nm by a spectrophotometer (Sinha 1972). Peroxidase (POD, EC 1.11.1.7) activity was determined by monitoring the increase of absorbance at 470 nm due to guaiacol oxidation. The reaction mixture consisted of 32 mM potassium phosphate buffer, pH 7.0, 0.1% H_2O_2, 0.25% guaiacol and the extract (Chance, Maehly 1995).

Electrolyte leakage measurement

The intensity of electrolyte leakage from seeds was measured according to Tammela et al. (2005). First, the seed cout was separated and then seeds were placed in batches of 25 in a container to which 50 mL of deionized water was added. The electrical conductivity of containers was measured with an EC meter and reported as μs cm^{-1} (Auld et al. 1988).

Statistical analysis

Data analysis was carried out using MSTAT-C (ver. 1.42) and SAS (ver. 9.1) software. When analysis of variance (ANOVA) showed significant treatment effects, Duncan's multiple range tests was applied to compare the means at $P \leq 0.05$. Correlation analysis using SAS (ver. 9.1) was conducted to determine the relationship between measurement parameters and germination percentage.

Results

Germination percentage (GP) did not significantly differ between maize hybrids (Table 1). Also, the interaction effect of hybrids × ageing treatment was not significant. Germination percentage decreased with increasing the duration of AA (Table 2). Hybrids in treatment A_4 and the control had the lowest and the highest germination percentage, respectively. There was a significant positive correlation between GP and MGT in the A_4 treatment (Table 3).

Mean germination time (MGT) significantly increased with ageing in seeds of maize hybrids. Control and A_4

Table 1. The analysis of variance for the effect of different duration of maize seed aging treatment on peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activity, electrolyte leakage (LE), mean germination time (MGT) and germination percent (GP) in seed embryos of maize hybrids. ns, * and **, non-significant and significant at the 5 and 1% levels of probability, respectively

Source of variation	df	Mean squares					
		POD	CAT	SOD	LE	GP	MGT
Hybrids	5	26520.3 **	13180.8 **	5821.6 **	2. 80 **	48.6 ns	12.9 **
Aging	3	67301.4 **	581.6 **	5645. 2 **	6.12 **	12195.3 **	0.736 **
Hybrids × Aging	15	1440.1 **	32.9 **	173.4 **	0.121 **	5.53 ns	0.219 **
Error	48	353.1	38.9	42.3	0.037	37.5	0.022
CV (%)	–	18.2	11.2	9.11	13.6	14.6	15.4

treatments showed the lowest and the highest MGT, respectively (Table 2). Hybrids SC704 and SC260 had the lowest and highest MGT in control treatment, respectively. Hybrids SC700 and SC370 had the highest MGT in all ageing treatments. In all hybrids, MGT was three times higher in A_4 treatment than in control treatment (Table 2).

A significant positive correlation between MGT and electrolyte leakage and germination percentage was observed in control and A_4 treatments, respectively (Table 3). In addition, there was a significant negative correlation between MGT and SOD activity in the A_2 and A_3 treatments (Table 3).

Measurement of the intensity of membrane electrolyte leakage is the simplest method for assessing the integrity of membranes. The results showed that electrolyte leakage through the seed membrane increased with ageing. Leakage reached a maximum in A_4 treatment (Table 2). Hybrids SC704 and SC370 had the lowest and the highest electrolyte leakage: 1.093 and 1.313 μs cm^{-1} in the control treatment and 2.27 and 2.19 μs cm^{-1} in treatment A_4, respectively. In

Table 2. Effect of maize seed aging duration on peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activity and electrolyte leakage (EL) in maize embryos, as well as mean germination time (MGT) and germination percent (GP) in maize hybrids seeds. Levels of ageing: A_1, control (no aging); A_2, aging for 2 days; A_3, aging for 4 days; A_4, aging for 6 days. Means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range test within each column of each section

Hybrids	Aging	POD (μM min^{-1} mg^{-1} protein)	CAT (μM min^{-1} mg^{-1} protein)	SOD (units mg^{-1} protein)	EL (μs cm^{-1})	GP (%)	MGT (days)
SC704	A_1	279.5 a	218.0 a	123.0 a	1.093 j	97 a	1.20 n
	A_2	194.1 b	99.4 d	107.5 b	1.313 ij	69 b	2.73 hi
	A_3	122.6 ef	87.7 ef	86.6 cd	1.910 gh	52 c	2.87 gh
	A_4	90.4 fgh	54.7 i	71.9 fg	2.270 def	33 d	3.40 cd
SC700	A_1	136.9 de	136.9 de	72.8 efg	1.907 gh	96 a	1.73 k
	A_2	89.6 gh	89.6 gh	58.1 hij	2.203 efg	72 b	3.10 efg
	A_3	49.8 ij	49.8 ij	48.9 jklm	2.877 bc	53 c	3.20 def
	A_4	12.7 jk	12.7 jk	40.9 lmn	3.867 a	34 d	3.83 a
SC647	A_1	198.2 b	111.8 c	94.4 c	1.587 hi	100 a	1.47 lm
	A_2	145.9 cde	76.4 gh	73.3 efg	2.110 fg	76 b	2.37 j
	A_3	93.8 fgh	38.6 jk	66.8 gh	2.393 def	57 c	3.00 fg
	A_4	23.5 jl	14.9 m	37.3 mn	3.137 b	39 d	3.37cde
SC500	A_1	156.7 cd	90.0 def	83.9 cde	1.687 h	94 a	1.70 kl
	A_2	84.5 gh	67.0 h	51.1 jkl	1.860 gh	69 b	3.03 fg
	A_3	41.1 ij	38.3 jk	40.2 lmn	2.433 def	53 c	3.10 efg
	A_4	5.53 k	12.7 m	31.1 n	2.583 cd	35 d	3.23 def
SC370	A_1	211.4 b	124.9 b	116.4 ab	1.313 ij	95 a	1.30 mn
	A_2	178.2 bc	112.6 c	114.2 ab	1.600 hi	70 b	2.50 ij
	A_3	112.8 efg	96.3 de	92.1 c	1.910 gh	54 c	2.73 hi
	A_4	61.6 hi	80.4 fg	79.4 def	2.190 efg	36 d	3.20 def
SC260	A_1	82.5 gh	71.8 gh	64.4 ghi	2.090 fg	97 a	1.80 k
	A_2	59.1 hi	32.6 kl	60.0 hij	2.490 de	69 b	3.57 bc
	A_3	42.4 ij	22.9 lm	53.7 ijk	3.130 b	50 c	3.73 ab
	A_4	21.1 jk	14.4 m	45.4 klm	3.583 a	36 d	3.87 cde

Table 3. Correlation between peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activity, electrolyte leakage (EL), mean germination time (MGT) and germination percent (GP) at the control (non–aged, A_1), 2 (A_2), 4 (A_3) and 6 (A_4) days of aging treatment in the maize. ns, * and **, non–significant and significant at the 5% and 1% levels of probability, respectively

Aging	Traits	POX	CAT	SOD	EL	GP	MGT
A_1	POD	1					
	CAT	0.12 ns	1				
	SOD	0.59 *	0.55 *	1			
	EL	–0.119 ns	0.39 ns	–0.11 ns	1		
	GP	0.39 ns	0.16 ns	0.67 **	–0.43 *	1	
	MGT	0.11 ns	0.22 ns	0.01 ns	0.82 **	–0.12 ns	1
A_2	POD	1					
	CAT	0.06 *	1				
	SOD	0.51 *	0.32 ns	1			
	EL	0.23 ns	0.81 **	0.02 ns	1		
	GP	0.20 ns	0.08 ns	0.33 ns	0.22 ns	1	
	MGT	–0.12 ns	–0.11 ns	–0.76 **	0.32 ns	–0.29 ns	1
A_3	POD	1					
	CAT	–0.05 ns	1				
	SOD	0.41 *	0.20 ns	1			
	EL	0.28 ns	0.70 ns	–0.18 ns	1		
	GP	0.63 **	–0.53 *	0.33 ns	–0.27 ns	1	
	MGT	0.28 ns	0.01 ns	–0.47 *	0.36 ns	–0.25 ns	1
A_4	POD	1					
	CAT	–0.31 ns	1				
	SOD	0.41 *	0.15 ns	1			
	EL	0.51 *	0.24 ns	–0.07 ns	1		
	GP	0.66 **	–0.25 ns	0.29 ns	0.19 ns	1	
	MGT	0.92 **	–0.25 ns	0.22 ns	0.67 **	0.48 *	1

hybrids SC260 and SC700, electrolyte leakage indicated greatest membrane damage with increase of ageing. In all hybrids electrolyte leakage was approximately two times higher in the A_4 treatment than in the control (Table 2). A significant positive correlation was observed between electrolyte leakage and CAT and POD activity in treatments A_2 and A_4 (Table 3).

Lowest POD activity in embryo of hybrids of maize was observed in the A_4 treatment. Hybrids SC704 and SC260 had the highest and lowest POD activity in the control treatment, respectively. Hybrids SC370 and SC500 had the lowest and highest decrease of POD activity in all ageing treatments, respectively (Table 2). There was a significant positive correlation between POD and SOD at all ageing levels (Table 3).

The activity of catalase decreased significantly with increase of ageing. In all treatments the hybrid SC704 had the highest catalase activity, and the lowest enzyme activity was seen in treatment A_4 in hybrid SC260 (Table 2).

Mean superoxide dismutase (SOD) activity in seed embryos was higher in hybrid SC704. Hybrids in the control treatment had the highest SOD activity. A_4 had the lowest SOD activity (Table 2). A significant positive correlation was observed between CAT and SOD activity in the control treatment (Table 3).

Discussion

The main mechanisms for the reduced germination as a result of seed deterioration are not known, but some studies showed that lipid peroxidation caused by oxidative damage can lead to inactivation and/or depletion of key enzymes of recipient protein transport or ion channels, as well as impairment of RNA and DNA synthesis (Basra et al. 2003; Murthy et al. 2003; Lehner et al. 2008).

It is likely that there is a delay in initiation of germination in aged seeds, because of the time needed for membrane damage repair and damage to other parts of the cell, and also for initiation of the antioxidant system activity to prevent the buildup of oxidative stress conditions. This repairing reaction is possible only after seed imbibition; therefore MGT usually increases in aged seeds (Priestly 1986; Kapilan 2015).

Ageing causes irreversible damage to cell structures. The membrane integrity lost by damage of phospholipids leads to increased membrane permeability and exit of electrolytes and other substances, such as enzymes, from cells (Zamani et al. 2010). Fatty acid peroxidation in the cell membranes is another process that causes increased electrolyte leakage (Goel et al. 2003). In studies on peanut (Sung, Jeng 1994), watermelon (Chiu et al. 1995), soybean

(Sung 1996), sunflower (Kibinza et al. 2006) and safflower (Zamani et al. 2010) it was reported that an increase in electrolyte leakage is associated with decrease of seed germination. In the present study, lipid peroxidation and membrane damage resulted in decreased seed germination and increased MGT (Table 2).

It seems that increase of MGT is a symptom of reduced vigour. Results of studies on maize (Lin, Pearce 1990), pigeonpea (Kalpana, Madhava Rao 1994) and wheat (Lehner et al. 2008) were in contrast to the results of our experiment as they did not show cell membrane damage after ageing. It is possible that the differing results may be due to differences in length and other conditions of the ageing treatment and difference in seed characteristics.

The reasons for reduction of antioxidant enzyme activity during seed deterioration are not clear. It seems possible that these effects are due to damage to RNA synthesis, which ultimately leads to decreased synthesis of proteins and inactivation of enzymes. The attack of ROS on all enzymes causes damage to the molecules and also reduces sugar concentration in cells, which can play a significant role in reduction of antioxidant activity (Murthy et al. 2003; Tabatabaei 2015).

The results of the present experiment showed that seed vigour reduction is associated with reduction of the SOD, CAT and POD activity (Table 2). These three enzymes are part of the antioxidant defense system, there are likely other defense mechanisms that are damaged in the ageing process, leading to reduction of SOD, CAT and POD activity, and decrease of antioxidant defense system activity. Antioxidant enzymes activity decreased noticeably during seed deterioration but never reached zero. Previous studies on beech (Pukacka, Ratajczak 2005), onion (Rao et al. 2006), safflower (Zamani et al. 2010) and maize (Kapilan 2015) seeds showed that ageing is associated with a reduction of antioxidant enzyme activity. It is evident that seed ageing is associated with lower antioxidant defense system activity, but not with a complete loss of this capacity.

In conclusion, the present results demonstrate that ageing of maize seed is associated with changes in antioxidant enzyme activity and electrolyte leakage. The hybrid SC704 showed least changes in enzyme activity and had the lowest electrolyte leakage and mean germination time during the experiment. Hybrid SC370 was most resistant to ageing and hybrids SC700 and SC260 were the most susceptible hybrids tested. In general, lipid peroxidation and electrolyte leakage play an important role in maize seed deterioration in accelerated ageing.

Acknowledgements

The authors would like to thank their colleagues in Agricultural Faculty, Razi University, Iran. Also, we thank the editor and the anonymous reviewers of the Environmental and Experimental Biology, for their helpful comments, suggestions and corrections on this paper.

References

Alscher R.G. 1989. Biosynthesis and antioxidant function of glutathione in plants. *Plant Physiol.* 77: 457–464.

Auld D.L., Bettis B.L., Crock J.E., Kephart D. 1988. Planting date and temperature effects on germination, and seed yield of chickpea. *Agron. J.* 80: 909–914.

Bailly C., Benamar A., Corbineau F., Come D. 2000. Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. *Seed Sci. Res.* 10: 35–42.

Basra S.M.A., Ahmad N., Khan M.M., Iqbal N., Cheema M.A. 2003. Assessment of cotton seed deterioration during accelerated ageing. *Seed Sci. Technol.* 31: 531–540.

Beauchamp C., Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* 44: 276–287.

Bewley J.D., Black M. 1994. *Seeds: Physiology of Development and Germination.* Plenum Press, New York.

Chance B., Maehly A.C. 1955. Assay of catalases and peroxidase. *Methods Enzymol.* 2: 764–775.

Chiu K.Y., Wang C.S., Sung J.M. 1995. Lipid peroxidation and peroxide-scavenging enzymes associated with accelerated ageing and hydration of watermelon seeds differing in ploidy. *Physiol. Plant.* 94: 441–446.

Goel A., Goel A.K., Sheoran I.S. 2003. Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. *J. Plant Physiol.* 160: 1093-1100.

ISTA. 1999. International rules for seed testing. International Seed Testing Association. *Seed Sci. Technol.* 27S: 1–333.

Kalpana R., Madhava Rao K.V. 1994. Absence of the role of lipid peroxidation during accelerated ageing of seeds of pigeonpea (*Cajanus cajan* (L.) Millsp.) cultivars. *Seed Sci Technol.* 22: 253-–260.

Kapilan R. 2015. Accelerated ageing declines the germination characteristics of the maize seeds. *Scholars Acad. J. Biosci.* 3: 708–711.

Kibinza S., Vinel D., Come D., Bailly C., Corbineau F. 2006. Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. *Physiol. Plant.* 128: 496–506.

Komba C.G., Brunton B.J., Hampton J.G. 2006. Accelerated ageing vigour testing of kale (*Brassica oleracea* L. var. *acephala* DC) seed. *Seed Sci. Technol.* 34: 205–208.

Krishnan P., Nagarajan S., Moharir A.V. 2004. Thermodynamic characterization of seed deterioration during storage under accelerated ageing conditions. *Biosyst Eng.* 89: 425-433.

Lehner A., Mamadou N., Poels P., Come D., Bailly C., Corbineau F. 2008. Change in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains. *J. Cereal Sci.* 47: 555–565.

Lin S.S., Pearce R.S. 1990. Changes in lipids in bean seeds (*Phaseolus vulgaris*) and corn caryopses (*Zea mays*) aged in contrasting environments. *Ann. Bot.* 65: 451–456.

McDonald M.B. 1999. Seed deterioration: physiology, repair, and assessment. *Seed Sci. Technol.* 27: 177–237.

Mohsen-Nasab F., Sharafi-Zadeh M., Siadat A. 2010. Study the effect of ageing acceleration test on germination and seedling growth of wheat cultivars in controlled conditions (*in vitro*). *J. Crop Physiol.* 2: 59–71. (in Persian)

Murthy U.M.N., Kumar P.P., Sun W.Q. 2003. Mechanisms of seed ageing under different storage conditions for *Vigna radiata* (L.) Wilczek: lipid peroxidation, sugar hydrolysis, Maillard

reactions and their relationship to glass state transition. *J. Exp. Bot.* 54: 1057–1067.

Priestly D.A. 1986. *Seed Ageing: Implication for Seed Storage and Persistence in the Soil.* Cornell University Press. Ithaca, NY.

Pukacka S., Ratajczak E. 2005. Production and scavenging of reactive oxygen species in *Fagus sylvatica* seeds during storage at varied temperature and humidity. *J. Plant Physiol.* 162: 873–885.

Rajjou L., Lovigny Y., Job C., Belghazi M., Groot S., Job D. 2007. Seed quality and germination. In *Seeds: Biology, Development and Ecology*. Adkins S., Ashmore S., Navie S.C. (eds) CAB International, 324–332.

Rao R.G., Singh P.M., Mathura Rai S. 2006. Storability of onion seeds and effects of packageing and storage conditions on viability and vigor. *Sci. Hortic.* 110: 1–6.

Sinha A.K. 1972. Colorimetric assay of catalase. *Anal. Biochem.* 47: 389–394.

Sung J.M. 1996. Lipid peroxidation and peroxide-scavenging in soybean seeds during ageing. *Physiol. Plant.* 97: 85–89.

Sung J.M., Jeng T.L. 1994. Lipid peroxidation and peroxide-scavenging enzymes associated with accelerated ageing of peanut seed. *Physiol. Plant.* 91: 51–55.

Tabatabaei S.A. 2015. The changes of germination characteristics and enzyme activity of barley seeds under accelerated ageing. *Cerc. Agron Mold.* 48: 61–67.

Tammela P., Vaananen P.S., Lakso I., Hopia A., Vourela H., Nygrem M. 2005. Tocopherols, tocoterienols and fatty acids as indicators of natural ageing in *Pinus sylvestris* seeds. *Scand. J. Forest Res.* 20: 378–384.

Tavakol Afshari R., Ghasem F., Majnoun Hosseini N., Alizadeh H., Bihamta M.R. 2007. Some effects of seed ageing on germination characteristics and activities of catalase and peroxidase antioxidant enzymes in barley genotypes (*Hordeum vulgare*). *Iranian J. Agric. Sci.* 38: 337–346. (in Persian/

Verma S.S., Verma U., Tomer R.P.S. 2003. Studies on seed quality parameters in deteriorating seeds in brassica (*Brassica campestris*). *Seed Sci. Technol.* 31: 389–398.

Wiese A.M., Binning L.K. 1987. Calculating the threshold temperature of development for weeds. *Weed Sci.* 35: 177–179.

Zamani A., Sadat Nouri S.A., Tavakol Afshari R., Iran Nezhad H., Ali Akbari G., Tavakoli A. 2010. Lipid peroxidation and antioxidant enzymes activity under natural and accelerated ageing in safflower (*Carthamus tinctorius* L.) seed. *Iranian J. Agric. Sci.* 41: 545–554. (in Persian)

Received 14 October 2015; received in revised form 22 November 2015; accepted 15 December 2015