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THE CHANGES OF GERMINATION CHARACTERISTICS AND ENZYME ACTIVITY OF BARLEY SEEDS UNDER ACCELERATED AGING

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ABSTRACT. Seed aging is the main problem of seed storage. Changes of enzyme activity and reduction of seedling growth are consequence of seed deterioration. An experiment was conducted to evaluate the effects of accelerated aging on germination indexes and enzyme activity of barley seeds. Seeds were incubated in closed plastic boxes for the accelerated aging treatments at 41°C. Three accelerate aging regimes were performed by placing seeds at 41°C and relative humidity (RH) of 90-100 % for 0, 4 and 8 day periods. Our results showed that increasing aging duration resulted higher reduction in germination percentage, germination index, mean time to germination, normal seedling percentage, catalase and ascorbate peroxidase. The highest germination percentage, germination index, normal seedling percentage and enzyme activity were achieved in control conditions (0 day of aging). Under aging conditions, germination percentage, means time to germination, germination index, normal seedling percentage and enzyme activity decrease significantly. Also, our results indicated that seed aging is related to decrease of enzymes and may contribute to low germination efficiency. The general decreases 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, also decrease in antioxidant enzymes is linked to an increased accelerated ageing and decreased germination characteristics. Subsequently, proposed a positive relationship between antioxidant enzyme capacity and the vigour of the seed.

Key words: Germination characteristics; Enzyme activity; Barley seed; Aging.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a common crop grown in the semiarid Mediterranean area, and due to its drought resistance, is one of the most widely grown crops in arid and semiarid regions of the world (Ghazi *et al.* 2007). Seed deterioration can be defined as the loss of quality, viability and vigour either due to aging or effect of adverse environmental factors.

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Seed characteristics decrease under long storage condition due to aging. It is the reason of declining in germination, emergence and seedling growth (Soltani *et al.,* 2008). All organisms undergo aging and it enhances under unfavorable or stress environments. Maximum germination percentage achieves immediately after harvesting and gradually decreases with storage time. Aging is one of the key factors in plant yield loss especially in vegetables. Seed aging is recognized by some parameters like delay in germination and emergence, slow growth and increasing of susceptibility to environmental stresses (Walters, 1998). Using high quality seed improved performance in two ways: first, percent of green seedling derived by high quality seeds is more than weak and exhausted ones and this can be helpful to achieve the desired density in field. Second, vigorous seed had more seedling growth rate and ununiformed emergence in germination time can be minimized (Ghasemi-Golezani *et al.,* 1996). High temperature, ambient relative humidity, and seed moisture content are the main factors influencing seed storage capability (Abdul-Baki, 1980).

Pandey *et al.* (1990) reported that accelerated ageing technique is a widely used tool to test the seed quality. This ageing test of seed vigor can give better indications of probable field emergence for vegetable crop seeds than germination and growth tests. Accelerated ageing initially proposed as a method to evaluate seed storability, this test is rapid, inexpensive, simple and useful for all species (Copeland and McDonald, 2001; Moradi and Younesi, 2009; Siadat *et al.,* 2012). Accelerated ageing techniques have great potential for understanding the mechanism of aging and associated deterioration processes of seeds (McDonald, 1999). Janmohammadi *et al.* (2008) and Ghassemi-Golezani *et al.* (2010) in rape seed and Saha and Sultana (2008) in soybean reported that increasing seed age decreased germination. Most of these studies suggest that decreases occur in the activity of enzymes in aged seeds (Bailly, 2004; Goel *et al.,* 2002; Mc-Donald, 2004).

Therefore, in the present study it has been investigated the changes of germination characteristics and enzyme activity of barley seeds under accelerated aging.

MATERIALS AND METHODS

The study was conducted in the Faculty member, Agricultural and Natural Resources Research Center of Yazd, Iran. For accelerated aging treatments seeds were then imposed to different accelerated ageing periods of 0, 4 and 8 days at 41°C in sealed ageing boxes which had 100% relative humidity. After that, a germination test was conducted.

Standard germination test was carried out at 20°C for 7 days in three replications of 50 seeds. Seeds were germinated on two layers of filter papers (Whatman no. 1) moistened with 5 ml distilled water in Petri dishes. The germinated seeds (2 mm radicle elongation) were counted daily to

calculate germination rate. At the end of the germination period, total and germination percentage, normal seedling percentage, germination index and mean time to germination were recorded.

For antioxidant enzymes assay, all extraction procedures were carried out at 4°C. About 0.2 g of seed samples were homogenized with 10 ml of phosphate buffer (pH 7), followed by centrifugation at 20,000 g for 15 min. The supernatants were used for determination of enzyme activity. Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometrically, following H_2O_2 consumption at 240 nm (Bailly *et al.,* 1996). Ascorbate peroxidase (APX, EC 1.11.1.7) activity was determined according to the procedures of Al *et al.* (1995). The activities of APX and CAT were expressed per mg protein, and one unit represented 1 µmol of substrate undergoing reaction per mg protein per min.

Data of percentage was subjected to data transformation (arcsine) before the statistical analysis in order to unify the variance of the data (Siadat *et al.,* 2012; Ansari *et al.,* 2012). Data of experiment were subjected to randomized complete design. Statistical analyses on collected data performed with SAS and Microsoft Excel software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ($P < 0.01$) test.

RESULTS AND DISCUSSION

According to our results of variance analysis, effect of priming treatments on germination percentage, germination index, normal seedling percentage and mean time to germination, under aging conditions were significant ($P < 0.01$) (*Tab. 1*). In agreement with the results, earlier reports (Pandey *et al.,* 1990; Bailly, 2004; Goel *et al.,* 2002; McDonald, 2004; Siadat *et al.,* 2012) have shown negative affect cold stress on germination characteristics.

Table 1 - Analysis of variance of studied traits barley seeds under accelerated aging

S.O.V.	df	Germination percenrage	Germination index	Mean time to germination	Normal seedling percentage
Treatment	2	3724**	494.59**	0.68**	4484.77**
Error	6	4	0.3	0.04	0.77
CV%		3.4	2.83	8.69	1.71

** indicate significant difference at 1% probability level.

Our results showed that the highest germination percentage (*Fig. 1*), germination index (*Fig. 2*), mean time to germination (*Fig. 3*), normal seedling percentage (*Fig. 4*) and the minimum were achieved under control conditions (0 day of aging), but with increases of duration of aging this traits reduction. Therefor the minimum this traits were attained under 8 days of aging.

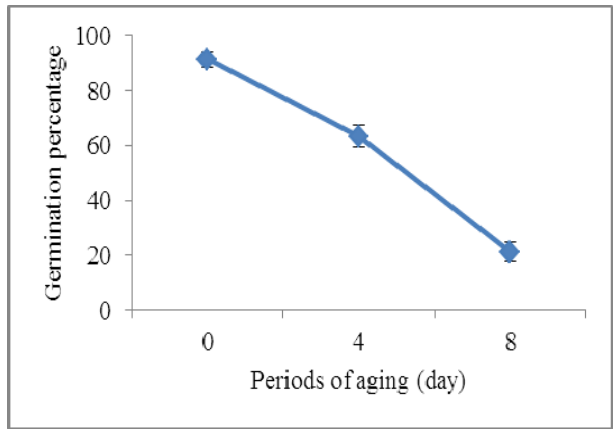

Figure 1 - The effect of accelerated aging on germination percentage of barley seeds

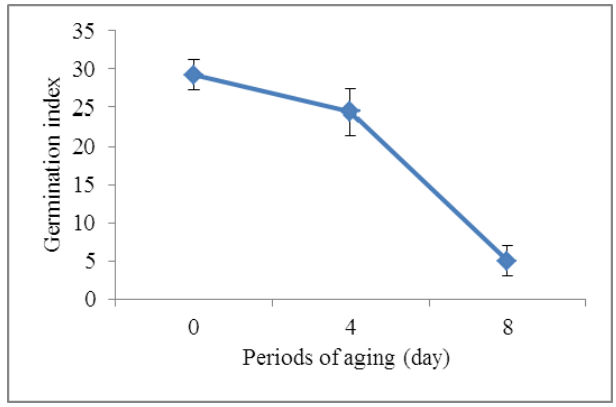

Figure 2 - The effect of accelerated aging on germination index of barley seeds

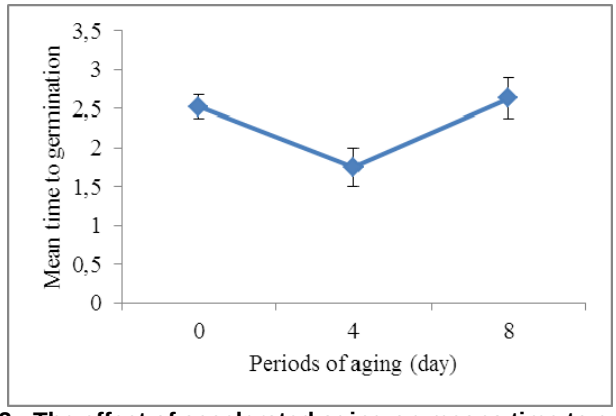

Figure 3 - The effect of accelerated aging on means time to germination of barley seeds

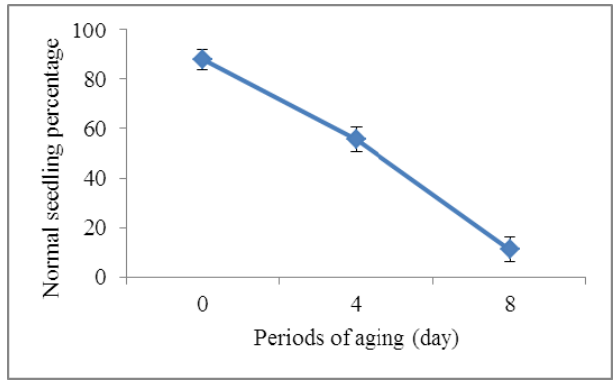

Figure 4 - The effect of accelerated aging on normal seedling percentage of barley seeds

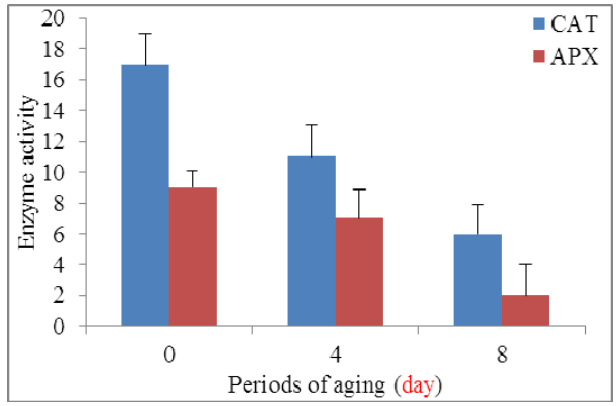

Figure 5 - The effect of accelerated aging on enzyme activity of barley seeds

Increasing seed age decreased germination and this result is in accordance with Janmohammadi *et al.* (2008) and Ghassemi-Golezani *et al.* (1996) in rapeseed and Saha and Sultana (2008) in soybean. Also, earlier reports (Pandey *et al.*, 1990; Bailly, 2004; Goel *et al.*, 2002; McDonald, 2004; Moradi and Younesi, 2009; Siadat *et al.*, 2012) have shown negative effect of aging in relation to seed performance, germination percentage and seedling indices. Akhter *et al.* (1992) suggested that decreasing in GP was related to chromosomal aberrations that occur under long storage conditions. Decreasing of GP in aged seeds can be due to reduction of α-amylase activity and carbohydrate contents (Bailly, 2004) or denaturation of proteins (Nautiyal *et al.*, 1985). According to Abdalla and Roberts (1968) barley and pea seeds treated with different combinations of accelerated ageing treatment showed that the amount of genetic damage was solely a function of loss of viability. Also, our results showed that enzyme activity decreased in

seeds after aging (*Fig. 5*). Most of these studies suggest that decreases occur in the activity of enzymes in aged seeds (Bailly, 2004; Goel *et al.,* 2002; McDonald, 2004). Kibinza *et al.* (2011) reported that the CAT is a key enzyme in seed recovery from ageing during priming.

CONCLUSIONS

In general, our results clearly indicate that decline in germination characteristics in response to aging is a consequence of decline in enzyme activity in barley seeds. The highest germination characteristics and enzyme activity were attained under control conditions (0 day of aging). Decreasing of germination percentage in aged seeds can be due to reduction of enzyme activity. 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, also decrease in antioxidant enzymes is linked to an increased accelerated ageing and decreased germination characteristics. Subsequently, proposed a positive relationship between antioxidant enzyme capacity and the vigour of the seed. Therefore, several changes in the enzyme macromolecular structure may contribute to their lowered germination efficiency.

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