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ORIGINAL ARTICLE

The Effect of Accelerated Aging on Germination Characteristics, Seed Reserve Utilization and Malondialdehyde Content of Two Wheat Cultivars

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In this study experiment was conducted to evaluated the effect of accelerated aging on germination characteristics, seed reserve utilization and malondialdehyde of two wheat cultivars. The experiment was conducted in factorial with a randomized complete block design with 3 replications. Results of variance analysis showed that, seed aging had significant effects on germination percentage, germination index, normal seedling percentage, mean time to germination, malondialdehyde content, seedling dry weight, weight of utilized (mobilized) seed reserve and electrical conductivity. The highest germination percentage, germination index, normal seedling percentage, seedling dry weight and weight of utilized (mobilized) seed reserve and the minimum mean time to germination, electrical conductivity and malondialdehyde content were attained from Verinak cultivar under control conditions (0 day aging). Results indicates that germination percentage, germination index, normal seedling percentage, seedling dry weight, and weight of utilized (mobilized) seed reserve decreased significantly as seed aging progressed. But, mean time to germination, electrical conductivity and malondialdehyde content increased significantly as seed aging progressed. Also, the decrease in seed reserve mobilization rate was the cause of decreased other traits.

Key words: Malondialdehyde content, Electrical conductivity, Accelerated ageing, Wheat, Germination characteristics

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High quality seed is considered to be one of the most important factors in crop production. One of the components of seed quality is seed vigor (Hampton, 1995). Seed aging is recognized by some parameters like delay in germination and emergence, slow growth and increasing of susceptibility to environmental stresses (Walters, 1998). Seed quality (viability and vigor) decreases under long storage conditions due to aging. It is the reason of declining in germination characteristics (Soltani *et al*., 2008). Aging is manifested as reduction in germination percentage and those seeds that do germinate produce weak seedling (Veselova, Veselovsky, 2003). The aim of seed vigor tests is to distinct low and high vigor seed mass with each other, also these tests provide ways to examine the ability of field performance of different seeds in laboratory conditions (Ghasemi- golezani *et al*, 1996). After genetic structure, seed aging had the most effect on seed vigor (Ghasemi-golezani, 1996). Increasing deterioration severity caused decreasing germination characteristics and seed reserve utilization (Mohammadi *et al*., 2011; Ansari *et al*., 2012). Low vigor seeds emergence lower than high vigor seeds in field conditions and produce less plantlets. Seeds deteriorate and lose their germinability during periods of prolonged aging (Ansari and Sharif Zadeh, 2013; Seiadat *et al*., 2012).The two most important environmental factors influencing the rate of deteriorative processes in seed aging are the relative humidity of the air, which controls seed moisture content, and the temperature (McDonald, 1999).

In seed aging damage to cellular membranes, decrease in mitochondrial dehydrogenases activities, chromosomal aberration and DNA degradation increases (Parrish and Leopold, 1978).

Genetic damage may accumulate until the embryos are unable to germinate and grow. Abdalla and Roberts (1968) suggested that the number of chromosomal aberrations seen in the first cell divisions occurring during germination may be taken as a measure of the damage accumulated during the storage period. Lipid peroxidation is the primary cause of seed damage during storage (Hailstones and Smith, 1988; Gidrol *et al*., 1989). Besides being the main cause of seed damage, lipid peroxidation causes initial biochemical changes in seed that can be observed during storage. Determination of malondialdehyde (MDA) content is the standard method for lipid peroxidation determination. Many authors observed increased content of MDA with prolonged storage period or with accelerated aging (Reuzeau and Cavalie, 1995; Ferguson *et al*., 1990).

Wheat (*Triticum aestivum* L.) is one of the most important crops in Iran, which plays a special role in people’s nutrition. Therefore, the study aimed was to determine the effect of accelerated aging on germination characteristics, seed reserve utilization and malondialdehyde of two wheat cultivars.

MATERIALS AND METHODS

For accelerated aging treatments seeds were then imposed to different accelerated ageing periods of 0, 3 and 6 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 8 days in three replications of 50 seeds. The germinated seeds (2 mm radicle elongation) were counted daily to calculate germination rate. At the end of the germination period, germination percentage, germination index, normal seedling percentage, mean time to germination, malondialdehyde content, seedling dry weight, weight of utilized (mobilized) seed reserve and electrical conductivity were determined.

Also, after 7 days, oven-dried weight of seedlings was determined. The weight of utilized (mobilized) seed (WMSR) reserve was calculated as the dry weight of the original seed minus the dry weight of the seed remnant. Seed reserve utilization efficiency (SRUE) was estimated by dividing seedling dry weight (SLDW) by the utilized seed reserve. The ratio of utilized seed reserve to initial seed dry weight was considered as seed reserve depletion percentage (SRDP) (Soltani *et al*., 2006; Ansari *et al*., 2012).

The electrical conductivity (EC) test was performed according to the method of Hampton and TeKrony (1995). Four samples of 50 sound looking, unbroken seeds were weighed, and then each sample was placed in 250 ml of distilled water in a 500 ml Beakers. Beakers were then sealed and kept at controlled temperature of 20 ºC for 24 h. The electrical conductivity of the seed leachates was then measured using an EC-meter. The electrical conductivity of seed leachates was expressed per gram of seed weight as µS cm-1 g-1 for each sample.

One hundred milligrams of seeds were hand-homogenized using a mortar and pestle with 4.0 mL of 5% (w/v) Trichloroacetic Acid (TCA) at 4°C to precipitate proteins and then centrifuged at 14,000 rpm for 20 min. The supernatants were separated and used for MDA determination (Yeh *et al*., 2005).

MDA was determined by adding 0.8 mL of 20% (w/v) trichloroacetic acid in 0.5% (w/v) thiobarbituric acid to 0.2 mL of extracted sample (supernatant) and 3.0 mL of distilled water. The reaction was carried out at 95°C for 30 min and then terminated by soaking in ice-cold water and monitored by spectrophotometer at 532 nm (Heath and Packer, 1968). Stock standard solutions of MDA were prepared at a concentration of 100 mg L^{-1}. The MDA was determined from the calibration curve in the ranges 2-10 mg L^{-1}.

The experimental design was laid out in a completely randomized design factorial (seed treatment × drought stress) with three replicates. All data were analyzed statistically by analysis of variance using MSTAT-C software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ($P < 0.05$) test.

RESULTS AND DISCUSSION

According to our results of variance analysis, seed aging had significant effects on germination percentage, germination index, normal seedling percentage, mean time to germination, malondialdehyde content, seedling dry weight, weight of utilized (mobilized) seed reserve and electrical conductivity (Tab. 1). In agreement with the results, earlier reports (Bailly, 2004; Goel *et al*., 2002; McDonald, 2004; Seiadat *et al*., 2012; Ansari and Sharif Zadeh, 2013) have shown negative affects aging on germination characteristics. Figure 1 to 8 shows the effect of seed aging on these traits. The highest germination percentage (Fig. 1), germination index (Fig. 2), normal seedling percentage (Fig. 4), seedling dry weight (Fig. 7), and weight of utilized (mobilized) seed reserve (Fig. 8) and the minimum mean time to germination (Fig. 3), electrical conductivity (Fig. 5) and malondialdehyde content (Fig. 6) were attained from Verinak cultivar under control conditions (0 day aging).

Results indicates that germination percentage (Fig. 1), germination index (Fig. 2), normal seedling percentage (Fig. 4), seedling dry weight (Fig. 7), and weight of utilized (mobilized) seed reserve (Fig. 8) decreased significantly as seed aging progressed. But, mean time to germination (Fig. 3), electrical conductivity (Fig. 5) and malondialdehyde content (Fig. 6) increased significantly as seed aging progressed.

Increasing seed age decreased germination characteristics and our results are in agreement with Jan-Mohammadi *et al*. (2008) and Ghassemi-Golezani *et al*. (2010) in rapeseed, Bhattacharjee *et al*. (2006) in common bean and sunflower, Saha and Sultana, (2008) and Mohammadi *et al*. (2011) in soybean, Ansari and Sharif Zadeh (2013) in secale montane.

Also many studies have shown that negative effects of stress conditions such as aging, drought and salinity stress in relation to seed performance, germination characteristics and seed reserve utilization (Mohammadi *et al*., 2011; Ansari *et al*., 2012; Ansari and Sharif Zadeh, 2013; Soltani *et al*., 2006).

Akhtar *et al*. (1992) showed that decreasing in germination characteristics were related to chromosomal aberrations that occur under long storage conditions. Decreasing of germination percentage 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), the amount of genetic damage was solely a function of loss of viability in barley and pea seeds treated with different combinations of accelerated ageing treatment.

Increasing seed age increased electrical conductivity and malondialdehyde content and our results are in agreement with the results, earlier reports (Mohammadi *et al*., 2011; Reuzeau and Cavalie, 1995; Ferguson *et al*., 1990) have shown increasing seed age increased electrical conductivity and malondialdehyde content.

Table 1 Variance analysis of studied traits in two wheat cultivars under aging conditions

S.O.V	df	GP	GI	MTG	NSP	MDA	SDW	WUS	EC
Cultivar (A)	1	80.22**	8.98**	0.0003ns	32.27**	8896.22**	1400.54**	0.001**	0.00002**
Aging (B)	2	7874**	997.76**	550.5**	2711.4**	643.42**	906.98**	0.01**	0.0005**
A × B	2	20.22*	1.12*	9.2**	6.07*	0.604**	175.74**	0.0003**	0.0000006*
Error	12	37.33	0.26	0.0005	3.33	0.24	1.47	0.000007	0.0000001
C.V %	-	2.86	2.68	1.01	3.38	3.62	3.87	4.37	2.1

** and *, indicates significant difference at 1% and 5% probability level respectively. (GP, GI, MTG, NSP, MDA, SDW, WUS and EC indicates germination percentage, germination index, normal seedling percentage, mean time to germination, malondialdehyde content, seedling dry weight, weight of utilized (mobilized) seed reserve and electrical conductivity respectively)

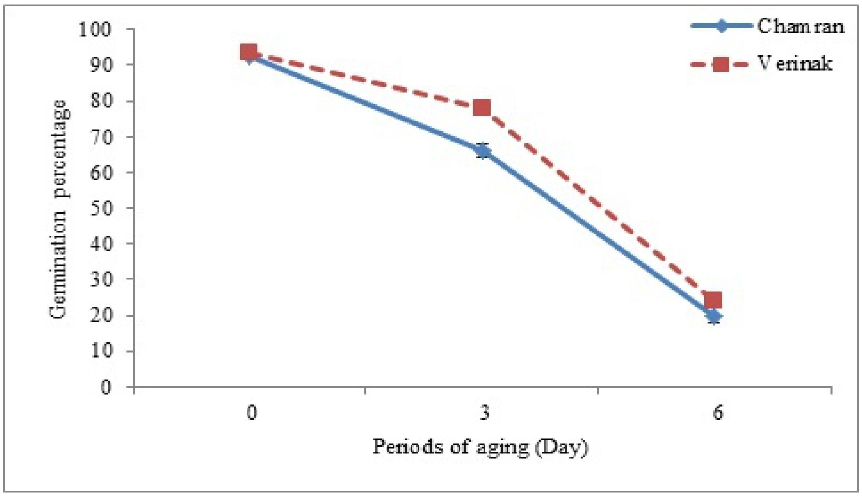

Figure 1. The effect of aging on germination percentage in two wheat cultivars.

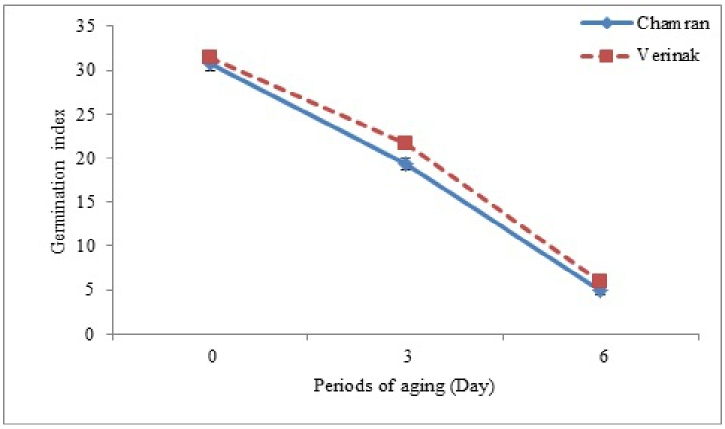

Figure 2. The effect of aging on germination index in two wheat cultivars.

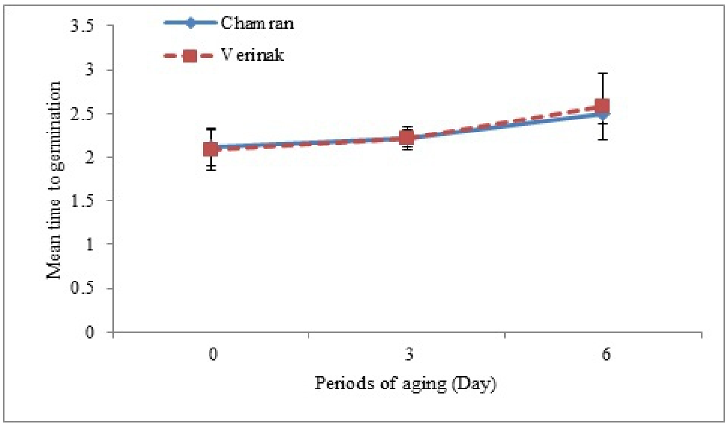

Figure 3. The effect of aging on mean time to germination in two wheat cultivars.

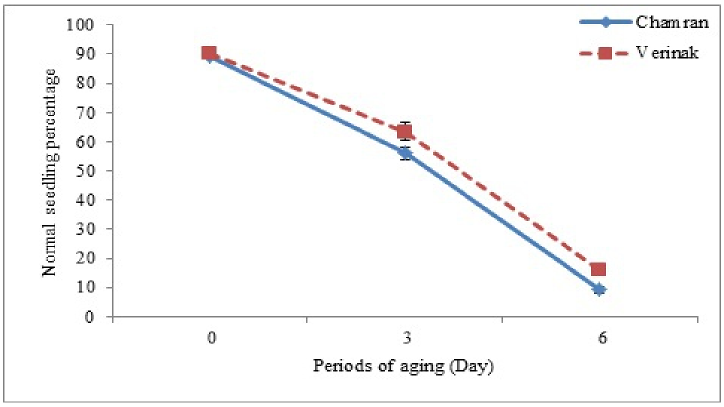

Figure 4. The effect of aging on normal seedling percentage in two wheat cultivars.

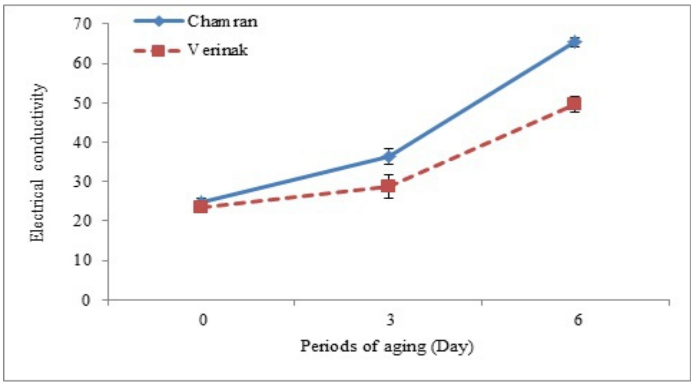

Figure 5. The effect of aging on electrical conductivity in two wheat cultivars.

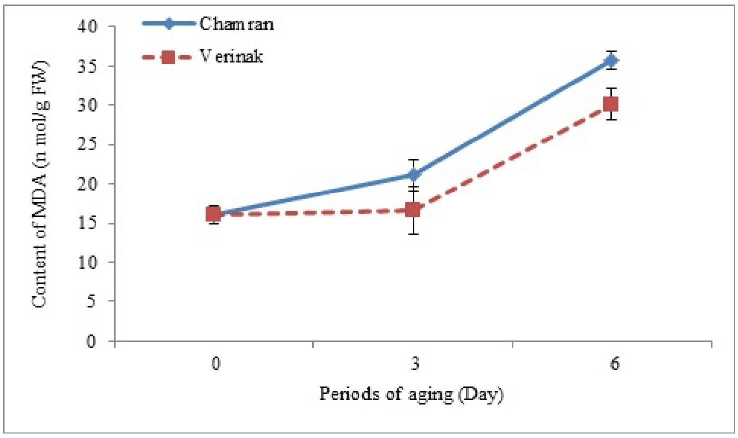

Figure 6. The effect of aging on content of malondialdehyde (MDA) in two wheat cultivars.

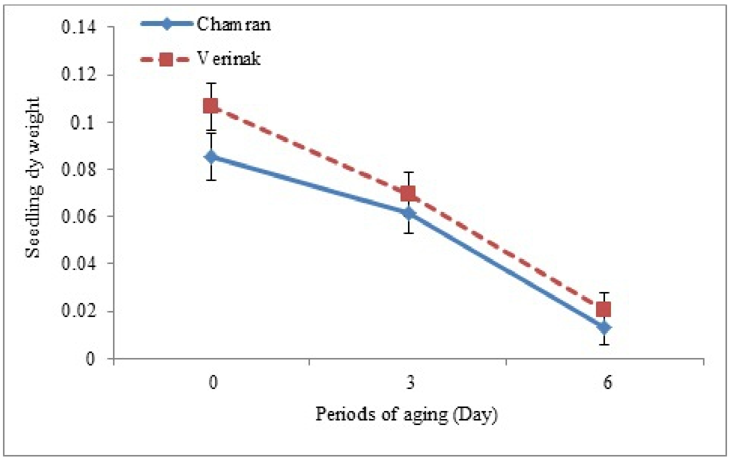

Figure 7. The effect of aging on seedling dry weight in two wheat cultivars.

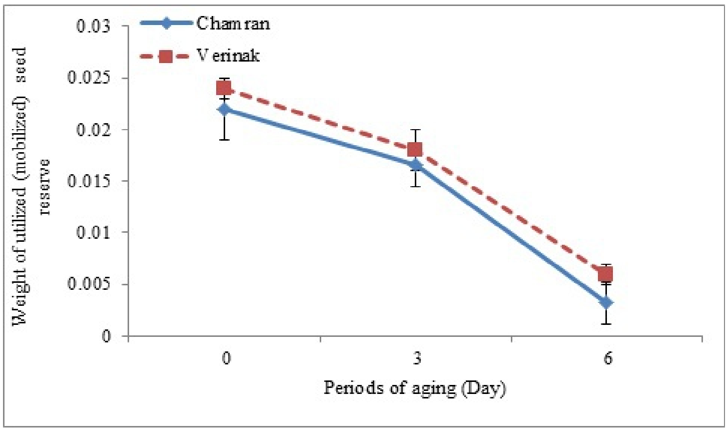

Figure 8. The effect of aging on weight of utilized seed reserve in two wheat cultivars.

CONCLUSIONS

Results indicates that germination percentage (Fig. 1), germination index (Fig. 2), normal seedling percentage (Fig. 4), seedling dry weight (Fig. 7), and weight of utilized (mobilized) seed reserve (Fig. 8) decreased significantly as seed aging progressed. In other hand, mean time to germination (Fig. 3), electrical conductivity (Fig. 5) and malondialdehyde content (Fig. 6) increased significantly as seed aging progress. Also, the decrease in seed reserve mobilization rate was the cause of decreased other traits.

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