

THE EFFECT OF HYDRO-PRIMING ON GERMINATION CHARACTERISTICS, SEEDLING GROWTH AND ANTIOXIDANT ACTIVITY OF ACCELERATED AGING WHEAT SEEDS

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ABSTRACT. Seed aging is an important problem in developing countries which seeds stored in inappropriate condition. Delayed germination, reduced normal seedling percentage and changed antioxidant enzymes activity are some indications of aged seeds. Priming is a technique applied before planting and can improve seed characteristics especially under abiotic stress conditions. The main objective of the study was to evaluate aging effect on seed quality and to study the interaction between seed aging and seed priming. A factorial experiment based on completely randomized design with three replicates was conducted. The experimental treatments included cultivar, priming and aging. Results showed that seed aging reduced germination percentage, germination index, seedling length, normal seedling percentage, seedling dry weight,

catalase and ascorbate peroxidase activity and increased the germination mean time and electrical conductivity of seeds. The highest germination percentage, germination index, seedling length, normal seedling percentage, seedling dry weight, catalase and ascorbate peroxidase activity and the minimum germination mean time and electrical conductivity of seeds were attained from hydro-priming treatment under non aged condition. Hydro-priming improved aged seeds quality and increased enzymes activity. Therefore, priming is a technique can be applied to improve aged seeds germination and seedling characteristics.

Key words: Wheat; Accelerated aging; Hydro-priming; Germination; Catalase; Ascorbate peroxidase.

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INTRODUCTION

Seed aging is an important problem in developing countries where seeds are stored in places usually without appropriate humidity and temperature. Temperature and seed moisture content are two main factors influencing seed viability in storage (Barton, 1964; James, 1967; Roberts, 1972).

Delayed germination, slow growth, reduced normal seedling number and changed antioxidant enzymes activity are some indications of aged seeds (Walters, 1998). Oxidative damages are responsible for deterioration in aged seeds. Free radical oxidations and protein enzymatic dehydrogenation and aldehyde oxidation might reasonably contribute to seed quality reduction (Ghassemi-Golezani *et al.*, 2010). Reductions in the activity of enzymes such as superoxide dismutase, catalase, peroxidase and glutathione reductase in aged seeds have been suggested by most of researches. Reduced enzyme activity in aged seed decreases the respiratory capacity, which in turn lowers both the energy (ATP) and assimilates supply of the germinating seed. Therefore, several changes in the enzyme macromolecular structure may contribute to lowered germination efficiency.

Vigorous seeds can increase crop yield in two ways: firstly, optimum density made by higher seedling percentage even under abiotic stress conditions and secondly, increased

growth and higher emergence rate (Ghassemi-Golezani *et al.*, 2010). Many seed priming and post priming treatments have been used to improve the performance of invigorate and damaged seeds of many crops (Basra *et al.*, 2003; Farooq *et al.*, 2006). Priming is a technique applied before planting and can improve germination characteristics especially under abiotic stress conditions like drought, cold, salinity and high temperature stresses (Sedghi *et al.*, 2010). Also most of researches reported that priming improved the quality of aged seeds by increasing enzymes activity such as antioxidant enzymes and amylases (Ansari *et al.*, 2013; Seiadat *et al.*, 2012). In present study the effect of hydro-priming on germination characteristics, seedling growth and antioxidant activity of aged wheat seeds has been investigated.

MATERIALS AND METHODS

Freshly harvested seeds of Chamran and Verinak cultivars of wheat were obtained from the Seed and Plant Improvement Institute (SPII), Karaj, Iran. During the accelerated aging, seeds were subjected to 100% relative humidity and 41°C for 0, 3 and 6 days in sealed aging boxes. The aged seeds were pretreated with 10°C water for 12 and 24 h.

Germination

To determine the germination percentage, seeds were placed in glass Petri dishes containing 15 mL water. The imbibed seeds were then washed four times with tap water, dried on filter paper at 15±1°C for 24 h (Ansari *et al.*, 2012)

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and kept in germination chamber at 20°C in three replicates of 50 seeds. Then the germination tests were evaluated for 8 days, according to the rules of the International Seed Testing Association (ISTA, 1985).

Measurements

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, germination mean time, seedling dry weight and seedling length were determined.

Membrane permeability

Fifty seeds from each accelerated aging treatment were soaked in 30 mL deionized water and kept at 20°C for 24 h (Ansari *et al.*, 2012). The electrical conductivities of the seed leachates were recorded using a direct reading conductivity meter and expressed in $\mu\text{S}\cdot\text{cm}^{-1}$.

Extraction and estimation of enzymes

The determination of catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.7) was performed using the extract of 0.3 g of seeds and 3 mL Tris-HCL buffer at pH 7.8. The obtained homogenate was centrifuged at 20000 $\text{r}\cdot\text{min}^{-1}$ and 4°C for 20 min. The supernatant was used for the determination of enzymes activity. Catalase activity was determined spectrophotometrically based on H_2O_2 consumption at 240 nm (Chiu *et al.*, 1995). Ascorbate peroxidase activity was determined using the procedure described by Johnson and Cunningham (1972). 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.

Statistics

All data were analyzed statistically by analysis of variance using SAS 9.2 Software. Data for germination were subjected to arcsine transformation before analysis of variance was carried out with SAS software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ($P < 0.01$) test.

RESULTS AND DISCUSSION

There was a significant ($P < 0.05$) interaction between cultivar, priming and aging for the germination percentage, germination index, normal seedling percentage, germination mean time, seedling dry weight, seedling length, electrical conductivity of the seed leachates, catalase and ascorbate peroxidase activity (Table 1).

Germination percentage and germination index of both cultivars seed decreased as the aging treatment period progressed (Fig. 1). In favor of this finding, Ansari *et al.* (2013) and Seiadat *et al.* (2012) reported that priming improves germination characteristics in many crops. Decreased germination in aged seeds can be due to the reduction of α -amylase activity and carbohydrate contents (Bailly *et al.*, 2002), or denaturation of proteins (Nautiyal *et al.*, 1985). According to Abdalla and Roberts (1968), accelerated aging of barley and pea seeds caused genetic damage and loss of viability.

Under each level of aging period (0, 3 or 6 days), germination percentage and index of both cultivars

seed was increased by increment of priming time (Fig. 1).

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

S.O.V.	df	GP	GI	SL	NSP	MTG	SDW	EC	CAT	APX
Cultivar (A)	1	394.74**	58.21**	3.89**	574.85**	0.009**	0.004**	165.62**	43.38**	26.74**
Treatment (B)	2	501.85**	113.34**	4.62**	251.63**	0.34**	0.005**	153.01**	121.26**	73.85**
Aging (C)	2	21458.74**	2777.6**	329.81**	25782.3**	0.5**	0.02**	707.73**	533.68**	203.63**
A x B	2	3.63 ^{ns}	1.05*	0.05 ^{ns}	3.63 ^{ns}	0.08**	0.0001 ^{ns}	26.16**	1.7*	0.52 ^{ns}
A x C	2	65.85**	1.83*	0.25*	53.41**	0.001 ^{ns}	0.0001 ^{ns}	14.75**	2.06*	0.07 ^{ns}
B x C	4	35.29**	1.45**	0.06 ^{ns}	13.3*	0.04 ^{ns}	0.0001 ^{ns}	21.91**	6.56**	3.46**
A x B x C	4	5.74**	0.86*	0.17*	7.51*	0.06*	0.0001	2.43**	2.13**	1.02**
Error	36	77.33	11.21	2.16	93.33	0.002	0.002	18.44	14.91	12
C.V%	-	2.21	2.62	2.81	2.81	6.	6.82	3.69	5.11	7.35

** and * indicates significant difference at 1% and 5% probability level, respectively; GP, GI, SL, NSP, MTG, SDW, EC, CAT and APX indicate: germination percentage, germination index, seedling length, normal seedling percentage, mean time to germination, seedling dry weight, electrical conductivity, catalase activity and ascorbate peroxidase, respectively.

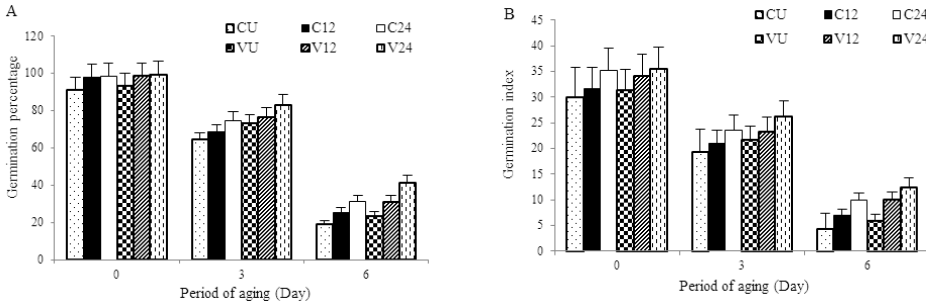


Figure 1 - Effect of cultivar x priming x aging interaction on germination percentage (A) and index (B); C: Chamran cultivar, V: Verinak cultivar, 0, 3 and 6: unprimed, primed for 12 and 24 h, respectively

Increased period of aging (3 or 6 days) significantly increased germination mean time, but this trait did not differ significantly among the levels of aging period (Fig. 2). Germination mean time of unprimed seeds of Chamran cultivar which had aged for 6 days was the highest and the 6 days aged seeds of this cultivar

which had primed for 24 h had the lowest germination mean time. Therefore, priming had accelerated germination of aged seeds.

Seedling length and normal seedling percentage was significantly reduced by increased period of aging (Fig. 3). Under each level of aging period (0, 3 or 6 days), increased

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priming time had a positive effect on seedling length and normal seedling percentage (Fig. 2).

Seedling dry weight was significantly reduced by increased

period of aging (Fig. 4). Under each level of aging period (0, 3 or 6 days), increased priming time significantly increased seedling dry weight (Fig. 4).

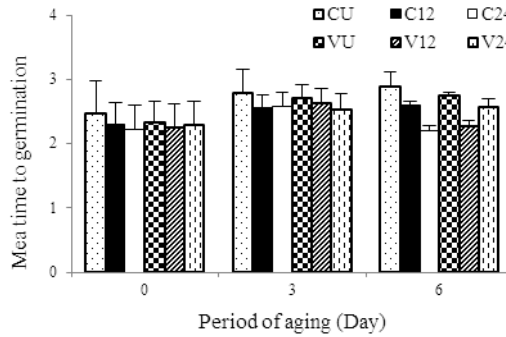


Figure 2 - Effect of cultivar × priming × aging interaction on mean time to germination; C: Chamran cultivar, V: Verinak cultivar, U: unprimed, primed for 12 and 24 h, respectively.

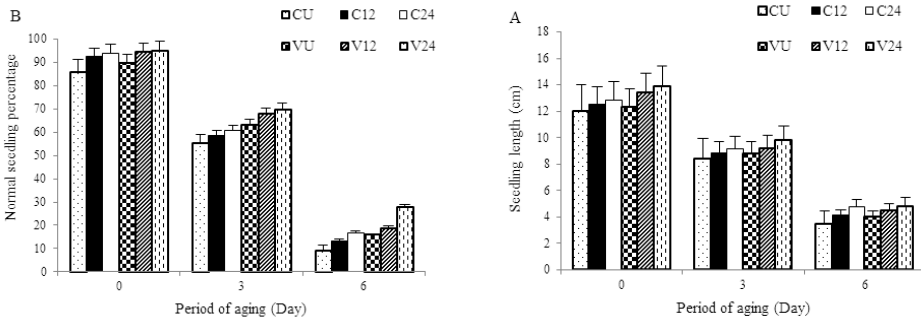


Figure 3 - Effect of cultivar × priming × aging interaction on seedling length (A) and normal seedling percentage (B); C: Chamran cultivar, V: Verinak cultivar, U: unprimed, primed for 12 and 24 h, respectively.

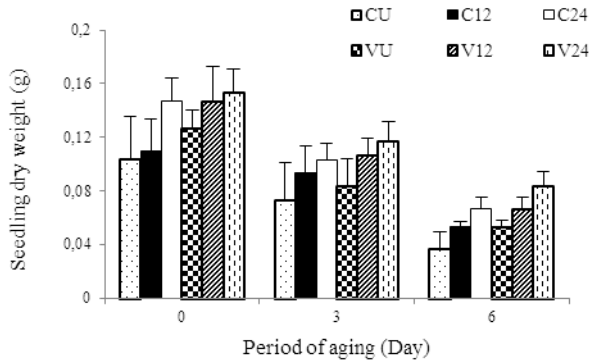


Figure 4 - Effect of cultivar × priming × aging interaction on seedling dry weight; C: Chamran cultivar, V: Verinak cultivar, U: unprimed, primed for 12 and 24 h, respectively.

Therefore, our findings showed that priming improved aged seeds germination and seedling characteristics in both studied cultivars. Also Basra *et al.* (2003) and Farooq *et al.* (2006) suggested that priming had positive effect on germination characteristics.

Electrical conductivity of seed leachates found to be increased by period of aging increment (*Fig. 5*). In favor our findings, many researchers has reported that seed aging caused increased electrical conductivity of leachates (Seiadat *et al.*, 2012; Ghassemi-Golezani *et al.*, 2010; Sedghi *et al.*, 2010).

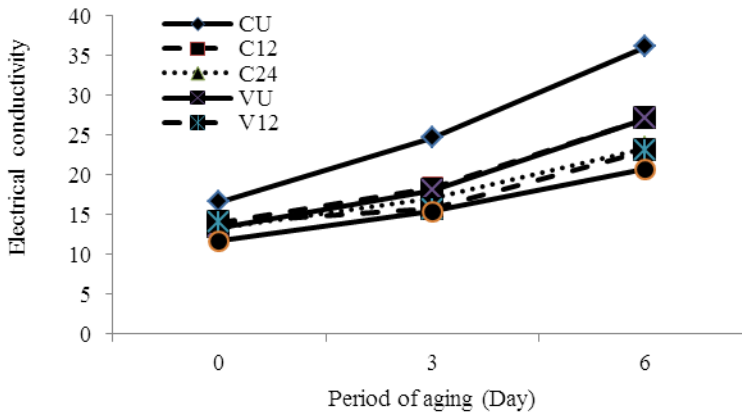


Figure 5 - Effect of cultivar × priming × aging interaction on electrical conductivity of seed leachates; C: Chamran cultivar, V: Verinak cultivar, 0, 3 and 6: unprimed, primed for 12 and 24 h, respectively.

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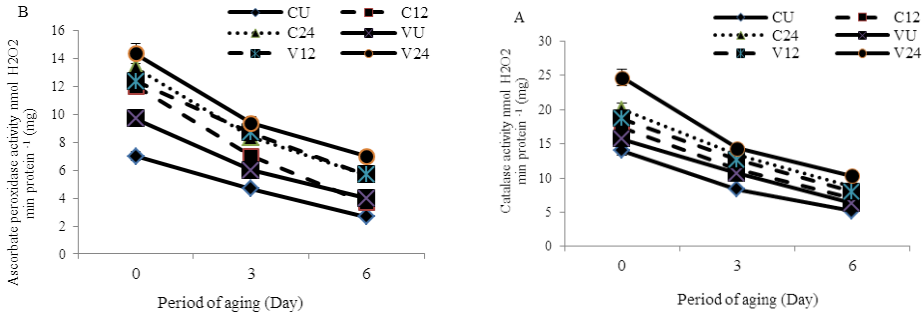


Figure 6 - Effect of cultivar × priming × aging interaction on catalase (A) and ascorbate peroxidase (B) activity; C: Chamran cultivar, V: Verinak cultivar, U: unprimed, primed for 12 and 24 h, respectively.

As shown in *Fig. 5*, electrical conductivity of unprimed and primed Verinak seeds was the highest and lowest, respectively, therefore, priming significantly improved seeds quality by the reduction of seed leachates.

Our findings showed that catalase and ascorbate peroxidase activity was reduced by increment of period of aging (*Fig. 6*). As shown in the figure, enzymes activity of primed and unprimed Verinak seeds was the highest and lowest, respectively. Therefore, priming significantly improved studied enzymes activity. These results are parallel to those of the Seiadat *et al.* (2012), Ghassemi-Golezani *et al.* (2010) and Sedghi *et al.* (2010).

CONCLUSION

Consequently, our results support the hypothesis of seed quality reduction during ageing. Moreover, our results provide convincing evidence that priming can improve reduced activity of catalase and

ascorbate peroxidase in aged seeds. Also seed priming reduced the electrical conductivity of aged seed leachates.

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