

Responses of germination characteristics and antioxidant enzymes activity to different levels of hydro-priming and seed ageing in three maize (*Zea mays* L.) hybrids

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Abstract

Seed storage at unfavorable conditions of high humidity and temperature is a difficulty which decreases seed vigor and/or seed viability. Current experiment was conducted to evaluate the activity of antioxidant enzymes and seed germination characteristics after ageing on maize seeds (*Zea mays* L.) in the research laboratory of Razi University, Kermanshah, Iran. A three-factorial experiment was performed with completely randomized design with three replications, using three maize hybrids (SC704, SC647, SC260), and three levels of accelerated ageing (non-aged, artificially aged for 3 and 6 days at 45 °C temperature and 98% relative humidity). The hydro-priming was performed at different durations (0, 12 and 24 hours). Results indicated that germination characteristics of maize seeds were considerably affected by priming time periods. The germination characteristics of deteriorated seeds were significantly enhanced by hydro-priming, especially in 24 h priming. The highest germination characteristics and seedling length were obtained in the control (non-aged) seeds which were primed for 24 h. The lowest germination performance was obtained for the non-primed seeds after 6 days of ageing. The catalase, peroxidase and glutathione reductase activity and malondialdehyde (MDA) concentration of aged maize seeds were restored partially by hydro-priming. In fact, ageing-associated MDA decreased by priming. The results suggested that hydro-priming could improve deteriorated seed performance.

Keywords: Biochemical traits; Maize hybrids; Pre-treatment; Seed deterioration; Seedling growth characteristics

Abbreviations: Malondialdehyde (MDA), Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD), Ascorbate peroxidase (APX), Glutathione reductase (GR), Active oxygen species (ROS), Superoxide (O₂⁻), Hydroxyl radicals (OH⁻), Hydrogen peroxide (H₂O₂), Singlet oxygen (¹O₂), Germination index (GI), Germination percentage (GP), Mean germination time (MGT), Seedling length (SL), Oxidized glutathione (GSSG), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Polyvinylpyrrolidone (PVP), Fresh weight (FW), Analysis of variance (ANOVA).

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Introduction

Warehouse and storage of seeds of crop plants in unfavorable conditions such as high humidity and temperature is a difficulty which reduces seed quality (Probert and Hay 2000; Hacisalihoglu 2008). Thus ageing is an ordinary phenomenon in seed storage. Seed quality at storage time affects seed longevity (Probert and Hey 2000).

Additionally, storage temperature and seed moisture content impact seed longevity (Steiner and Ruckenbauer 1995; Hampton *et al.* 2004).

Ageing has adverse effects on membranes (Tahmasebi *et al.* 2015), proteins (Nautiyal *et al.* 1985; Tahmasebi *et al.* 2015) and nucleic acids (Abdalla and Roberts 1968). One important reason for loss of storability is peroxidation of

unsaturated fatty acids. This can be due to reduced amounts of antioxidants and decreased activity of free radical and peroxidase scavenging enzymes (Hsu and Sung, 1997; Yeh and Sung, 2008). Shaban *et al.* (2018) reported that with increasing of ageing duration, germination percentage, lipid peroxidation, hydrogen peroxide production and electrolyte leakage increased. One of the earliest responses of plants to ageing condition is the accumulation of active oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^\cdot), superoxide (O_2^\cdot) and singlet oxygen (1O_2). It is well known that the activity of enzymes such as ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), peroxidase (POD) and superoxide dismutase (SOD) decreases in aged seeds (Khajeh *et al.* 2015; Mansouri-Far *et al.* 2015; Kong *et al.* 2015; Shaban *et al.* 2018).

Priming, a seed enhancement technique with water and/or soaked in different solutions with high osmotic potential, can be a useful tool for improving germination of crop seeds such as maize. Seed priming treatments such as hydro-priming, osmo-priming, bio-priming, hormonal-priming, magneto-priming, chemical priming and nutrient priming, are employed in crop plants under the broad range of environmental stresses (Farooq *et al.* 2005; Jisha *et al.* 2013; Paparella *et al.* 2015). Seed priming allows some of the metabolic processes necessary for germination to occur without germination take place (Parray *et al.* 2019). Furthermore, priming increases the activity of anti-oxidants in seeds (Hsu *et al.* 2003; Bosco de Oliveira *et al.* 2012; Ghasemi *et al.* 2014). Seed priming enhances the germination

and emergence of crop species such as corn (Siadat *et al.* 2012), rye (Ansari *et al.* 2013), wheat (Ghasemi *et al.* 2014), pepper (Aloui *et al.* 2014) and fennel (*Foeniculum vulgare* L.) (Abdoli 2014). Seed priming treatments have been accustomed to decrease aging and invigorate their performance in maize (Siadat *et al.* 2012).

It is well known that priming decreases aging and that some antioxidant enzymes are upregulated during this process. However, there is not much information about the effects of seed priming on antioxidant activity of aged maize seeds. The objective of the present study was to evaluate the impact of hydro-priming with different durations on activities of antioxidant enzymes and seed germination characteristics of maize (*Zea mays* L.) seeds under different levels of accelerated aging. In other words, the aim was to find out possible methods of improving storability of maize seeds with regard to storage conditions.

Material and Methods

Experimental site and method

The present study was conducted during 2016 at the research laboratory of the Razi University, Kermanshah, Iran. A factorial experiment was carried out with three factors using completely randomized design with three replications. The first factor was maize hybrids (SC704, SC647, SC260), the second factor was accelerated ageing (aged for 0 [non-aged or control], 3 and 6 days) and third factor was priming by distilled water for different time periods (0 [non-priming seed or control], 12 and 24 hours). The hydro-priming technique used in this study was easy to apply and

had a low cost. The maize hybrids had different growth periods (SC704: late, SC647: medium, SC260: early). The seeds of maize (*Zea mays* L.) hybrids were procured from Seed and Plant Improvement Institute (SPII), Karaj, Iran. For accelerated ageing, the seeds were exposed to a high temperature of 45 ± 1 °C and about 98% humidity in an airtight desiccator (ISTA 1999). The aged seeds were pre-treated by distilled water for 12 and 24 hours at 20 ± 2 °C under dark condition. After drying the seeds for 10 hours at room temperature, seeds were used for evaluating the germination characteristics and biochemical analyses.

Measurement of germination percentage and seedling traits

50 disinfected seeds were placed in a sterile Petri dish (12 cm diameter) on filter paper. The 5% (w/v) sodium hypochlorite solution (5 min) was used for disinfecting the seed surfaces. The Petri dishes were put in a growth chamber at 25 ± 2 °C for 7 days in darkness, and distilled water was added every 24 hours as required. At the final count (7th day), all seedlings that showed complete morphological parts with no lesions or defects (normal seedlings), were selected to measure germination characteristics. The normal and abnormal seedlings of maize were detected as follows: normal seedlings had healthy roots and well-developed green leaves, but abnormal seedlings were deformed and had damaged leaf and coleoptile tip, spiral splitting of coleoptile at the base and the coleoptile split more than 1/3 of the length from the tip (ISTA 2008).

Average seedling length (SL) was measured

by the modified formula of Abdul Baki and Anderson (1973) and AOSA (1983). Germination index (GI) was calculated according to AOSA (1983). Germination percentage (GP) was obtained as the number of germinated seeds at the 7th day (ISTA 1999) using the following formula: $GP = (Ng / Nt) \times 100$, where Ng is the total number of germinated seeds and Nt is the total number of evaluated seeds.

Mean germination time (MGT) was calculated using the following formula (Bailly *et al.* 2000): $MGT = \sum Dn / \sum n$, where n is the number of germinated seeds on day D, and D is the number of days from the beginning of emergence.

To measure the biochemical characteristics such as antioxidant enzyme activity and malondialdehyde concentration in the embryo, 30 seeds were dehydrated for 6 h. After removing the seed coat, isolated embryos were rolled in a foil and frozen in liquid nitrogen and stored at -80 °C. For every characteristic, three replications were used.

Determination of antioxidant enzyme activity

10 mg of embryo was grinded in 5 ml of 10 mM potassium phosphate buffer (pH 7.0) containing 4% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 12,000 g for 30 min at 4 °C and the resultant supernatant was used as enzyme extract.

Catalase (CAT, EC 1.11.1.6) activity was determined by recording the decrease in absorbance at 240 nm in 50 mM phosphate buffer (pH 7.5) containing 20 mM H₂O₂. One unit of CAT activity is defined as the amount of enzyme that uses 1 μmol H₂O₂ per min (Sinha 1972).

Peroxidase (POD, EC 1.11.1.7) activity was determined by recording the increase in absorbance at 470 nm in 50 mM phosphate buffer (pH 7.0) which contained 1 mM guaiacol and 0.5 mM H₂O₂. One unit of POD activity is defined as the amount of enzyme that results in an increase in absorbance of 0.01 per min (Chance and Maehly 1995).

Glutathione reductase (GR, EC 1.6.4.2) activity was determined by the method of Foyer and Halliwell (1976). The assay medium contained 0.5 mM oxidized glutathione, 0.025 mM Na-phosphate buffer (pH 7.8), 0.12 mM NADPH Na₄ and 0.1 ml enzyme extract in a final assay volume of 1 ml. NADPH oxidation was monitored at 340 nm.

Measurement of malondialdehyde concentration

0.5 g of embryo was homogenized in 5 ml of 1% trichloroacetic acid (TCA) and centrifuged at 20,000 g for 15 min. Then, 4 ml of 5% (w/v) thiobarbituric acid in 20% (w/v) TCA was added to 1 ml of the homogenate. The extract was incubated at 95 °C for 25 min, cooled on ice and centrifuged at 16,000 g for 25 min. Malondialdehyde (MDA) concentration (mmol g/FW) was determined by spectrometer at 452 nm (Heath and Parker 1968).

Statistical analyses

After analysis of variance (ANOVA), means were compared by Duncan's multiple range test at $p \leq 0.05$ using SAS (Ver. 8.0) and MSTAT-C software. The figures were generated by Excel software (Ver. 10.0).

Results

Germination percentage and seedling traits

ANOVA indicated significant difference between priming treatments (Table 1). Accelerated ageing significantly delayed the onset of germination. Thus, the lowest GP was observed for the 6-day ageing treatment, and the highest GP was observed in the seeds without ageing (Figure 1).

The germination ability of the seeds after priming is shown in Figure 1. The priming of maize seeds by water affected seedling characteristics. The 24-h prime treatment significantly promoted germination of aged seeds in comparison with the control seeds. The maximum germinability was obtained for seeds of SC704 hybrid that were primed for 24 h (Figure 1). The priming for 12 h also enhanced the germination ability of the seeds, although the germinability was lower than the priming for 24 h (Figure 1).

Increase in the duration of accelerated ageing, considerably reduced GI of aged seeds (Figure 2). The lowest GI (Figure 2) and the highest MGT (Figure 3) were observed in the control seeds. However, priming improved germination of aged seeds and germination index in the studied maize hybrids. The highest GI was observed in the non-aged seeds of SC704 and SC647 maize hybrids which were treated for 24 h. The lowest GI was noticed in the non-primed six-day aged seeds in SC260 hybrid (Figure 2). However, the results obtained for MGT were opposite to that of GI. MGT of all maize hybrids under different priming times increased with accelerated ageing level (Figure 3).

In general, 6 days of accelerated ageing showed the lowest normal seedling percentage

Table 1. Analysis of variance for germination percentage (GP), germination index (GI), mean germination time (MGT), seedling length (SL) and normal seedling percentage (NSP) of maize hybrids under ageing and different priming conditions.

| SOV | df | Mean squares | | | | |
|-------------|----|--------------|----------|----------|---------|----------|
| | | GP | GI | MGT | SL | NSP |
| Hybrid (H) | 2 | 798 ** | 34.8 ** | 0.506 ** | 151 ** | 1497 ** |
| Ageing (A) | 2 | 25887 ** | 3937 ** | 2.270 ** | 1219 ** | 28054 ** |
| Priming (P) | 2 | 1298 ** | 161 ** | 0.319 ** | 8473 ** | 621 ** |
| H×A | 4 | 251 ** | 6.11 * | 0.199 ** | 8.70 ** | 188 ** |
| H×P | 4 | 20.9 ** | 1.14 ns | 0.119 ** | 5.55 * | 7.51 ** |
| A×P | 4 | 106 ** | 0.79 ns | 0.061 ** | 6.90 * | 9.99 ** |
| H×A×P | 8 | 15.0 ** | 0.803 ns | 0.096 ** | 4.42 * | 6.30 ** |
| Error | 54 | 2.70 | 2.19 | 0.009 | 2.00 | 1.41 |
| CV (%) | - | 2.47 | 7.21 | 3.58 | 6.8 | 2.18 |

ns, *, **not significant and significant at 5% and 1% probability levels, respectively.

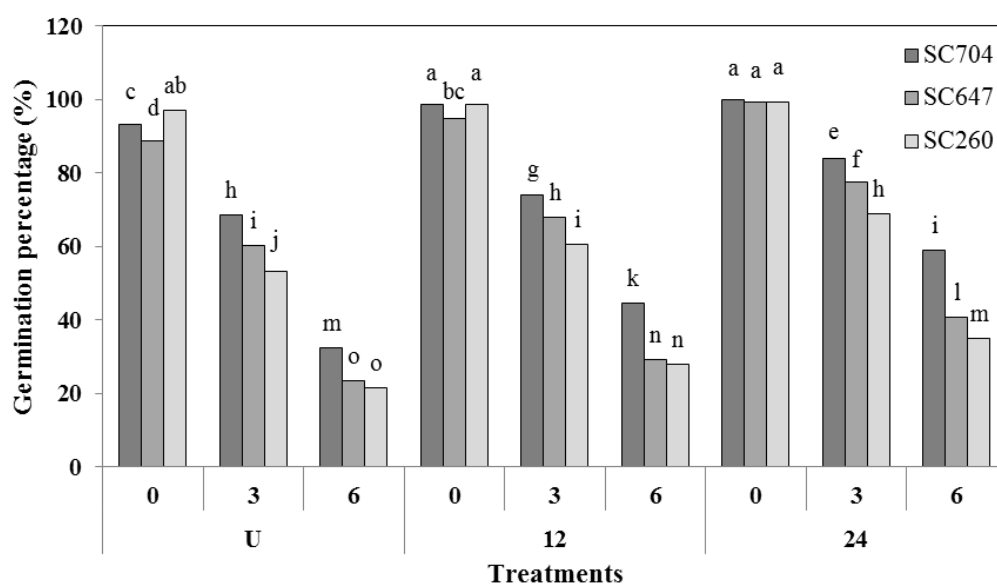


Figure 1. Effect of priming and ageing on germination percentage (%) in maize hybrids (SC704, SC647, SC260). Levels of aging treatments: 0, 3 and 6 days. Priming treatments: unprimed (U), primed by distilled water for 12 hours (12) and 24 hours (24), respectively. Different letters show significant differences among treatment combinations at 5% probability level using Duncan's multiple range test.

(NSP) and SL. The highest NSP and SL were observed in the non-ageing seeds (Figures 4 and 5). As shown in Figure 4, priming treatments (12 and 24 h) significantly influenced seedling growth and SL in all maize hybrids. However, priming of the seeds for 24 h caused the seeds to grow faster and accumulate more biomass as compared to 12 h priming and un-priming treatments.

The highest SL belonged to the control (non-aged) seeds of SC704, SC647 and SC260 maize hybrids which were primed for 24 h (34.8, 33.9 and 33.7 cm, respectively) and the lowest SL was observed in the 6- days aged SC260 seeds which were unprimed (8.1 cm) (Figure 4). As shown in Figure 5, 24 h water priming treatment significantly influenced NSP in all maize seeds.

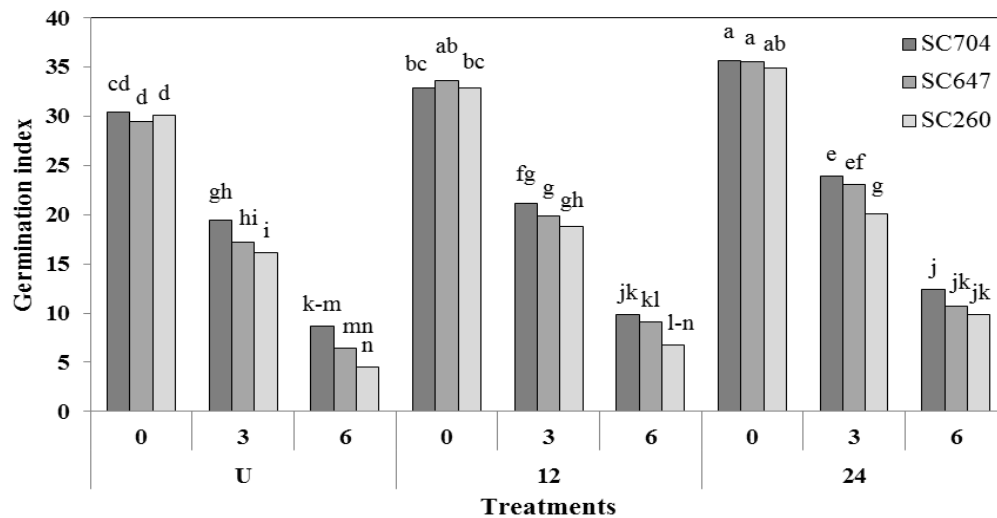


Figure 2. Effect of priming and ageing on germination index in maize hybrids (SC704, SC647, SC260). Levels of aging treatments: 0, 3 and 6 days. Priming treatments: unprimed (U), primed by distilled water for 12 hours (12) and 24 hours (24), respectively. Different letters show significant differences among treatment combinations at 5% probability level using Duncan's multiple range test.

The highest NSP was in the control (non-aged) SC704 seeds which were treated for 24 h (94.4%), and the lowest NSP was in 6-days aged seeds of

SC260 hybrid which were unprimed (12.4%) (Figure 5).

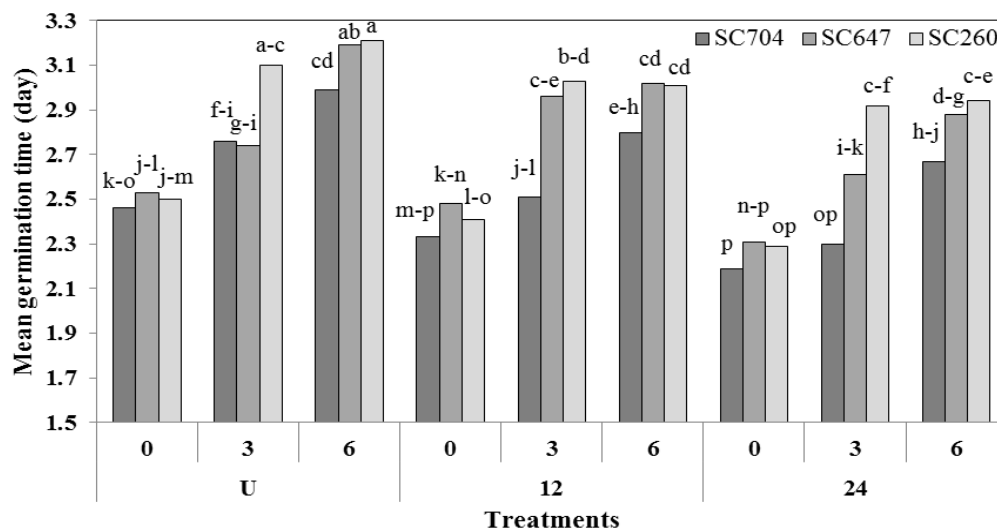


Figure 3. Effect of priming and ageing on mean germination time (day) in maize hybrids (SC704, SC647, SC260). Levels of aging treatments: 0, 3 and 6 days. Priming treatments: unprimed (U), primed by distilled water for 12 hours (12) and 24 hours (24), respectively. Different letters show significant differences among treatment combinations at 5% probability level using Duncan's multiple range test.

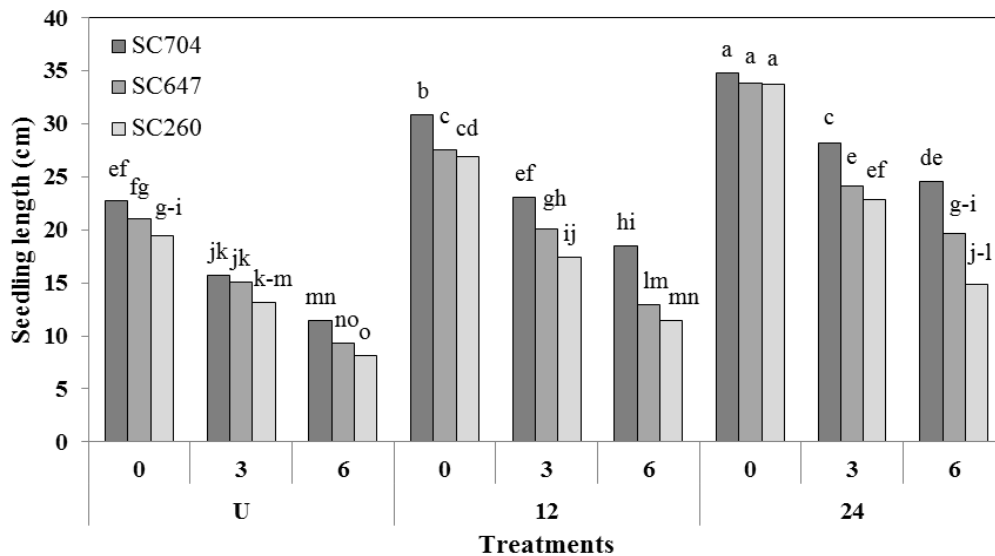


Figure 4. Effect of priming and ageing on seedling length (cm) in maize hybrids (SC704, SC647, SC260). Levels of ageing treatments: 0, 3 and 6 days. Priming treatments: unprimed (U), primed by distilled water for 12 hours (12) and 24 hours (24), respectively. Different letters show significant differences among treatment combinations at 5% probability level using Duncan's multiple range test.

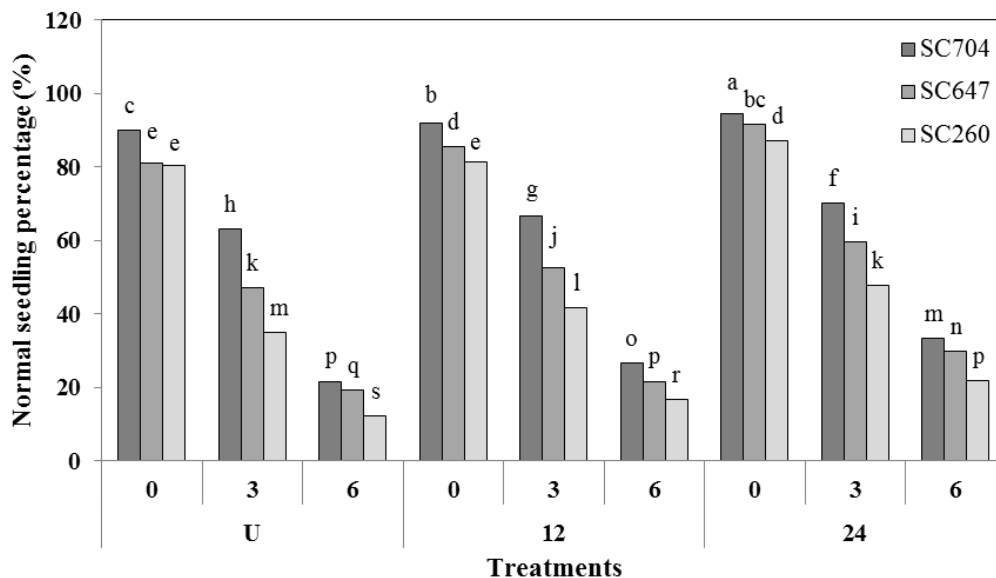


Figure 5. Effect of priming and ageing on normal seedling percentage (%) in maize hybrids (SC704, SC647, SC260). Levels of ageing treatments: 0, 3 and 6 days. Priming treatments: unprimed (U), primed by distilled water for 12 hours (12) and 24 hours (24), respectively. Different letters show significant differences among treatment combinations at 5% probability level using Duncan's multiple range test.

Biochemical and antioxidant enzymes activity

Table 2 shows the significant effects of ageing and priming on the activity of CAT, POD and GR and MDA concentration. The effect of hybrid and hybrid \times ageing interaction was also significant

for POD and GR activity and MDA concentration. Interactions of hybrid \times priming, ageing \times priming and hybrid \times ageing \times priming were only significant for Gr. In general, accelerated ageing reduced CAT, POD and GR activities (Figures 6-

8). After priming, CAT activity strongly changed in aged seeds. Priming of seeds for 24 h decreased CAT activity which were aged for 6 days. These results showed that seed priming with water altered the antioxidant activity (Figure 6). CAT

activity decreased during priming and had 14.2 and 34.0% lower activity at 12 and 24 h priming, respectively than in unprimed seeds.

POD and GR activity was higher in water primed seed than in non-primed seed (Figures 7

Table 2. Analysis of variance for catalase (CAT), peroxidase (POD) and glutathione reductase (GR) activities and malondialdehyde (MDA) concentration of maize hybrids under different ageing and priming conditions.

| SOV | df | Mean squares | | | |
|-------------|----|--------------|---------|--------|---------|
| | | CAT | POD | GR | MDA |
| Hybrid (H) | 2 | 0.31ns | 4332** | 406** | 0.884** |
| Ageing (A) | 2 | 669** | 97716** | 3181** | 15.0** |
| Priming (P) | 2 | 12372** | 6983** | 1946** | 5.51** |
| H×A | 4 | 1.27ns | 694** | 114** | 0.216* |
| H×P | 4 | 1.66ns | 40.0ns | 17.5** | 0.037ns |
| A×P | 4 | 191.6ns | 36.5ns | 31.8** | 0.061ns |
| H×A×P | 8 | 0.846ns | 23.6ns | 12.6** | 0.013ns |
| Error | 54 | 89.9 | 164.2 | 4.07 | 0.06 |
| CV (%) | - | 9.02 | 6.79 | 5.45 | 7.29 |

ns, *, **not significant and significant at 5% and 1% probability levels, respectively.

and 8). POD and GR activity had 10.4 and 30.1% as well as 18.7 and 59.6% higher activity in 12 and 24 h priming, respectively, as compared to unprimed seeds (Figures 7 and 8). Therefore, priming significantly improved the studied enzymes activity. The highest POD and GR activity was observed in un-aged SC704, SC647 and SC260 hybrids at 24 h priming and the lowest POD and GR activity was obtained in 6-days aged SC260 seeds in the non-priming condition (Figures 7 and 8). Our findings showed that CAT, POD and GR activity reduced by increasing of the aging period (Figures 6 to 8).

As shown in Figure 9. Accelerated ageing resulted in a marked increase in the MDA levels, whereas priming of aged seeds decreased the levels of MDA, indicating a fall in lipid peroxidation processes. The MDA concentration

decreased slightly with increased level of priming, so that the soaking of seeds for 24 h in water exhibited the highest effective duration for seed priming (Figure 9).

The combination of ageing and priming on maize hybrid seed showed the minimum MDA concentration in the non-aged seeds which were treated for 24 h, independent of hybrid varieties. The highest MDA concentration was observed in the 6-days ageing seeds in the unprimed SC260 maize hybrid (Figure 9).

Discussion

The results showed that seed ageing additionally affected all traits under investigation (Figures 1 to 9). Also hydro-priming significantly improved the germination characteristics of aged maize seeds. These results are in concordance with

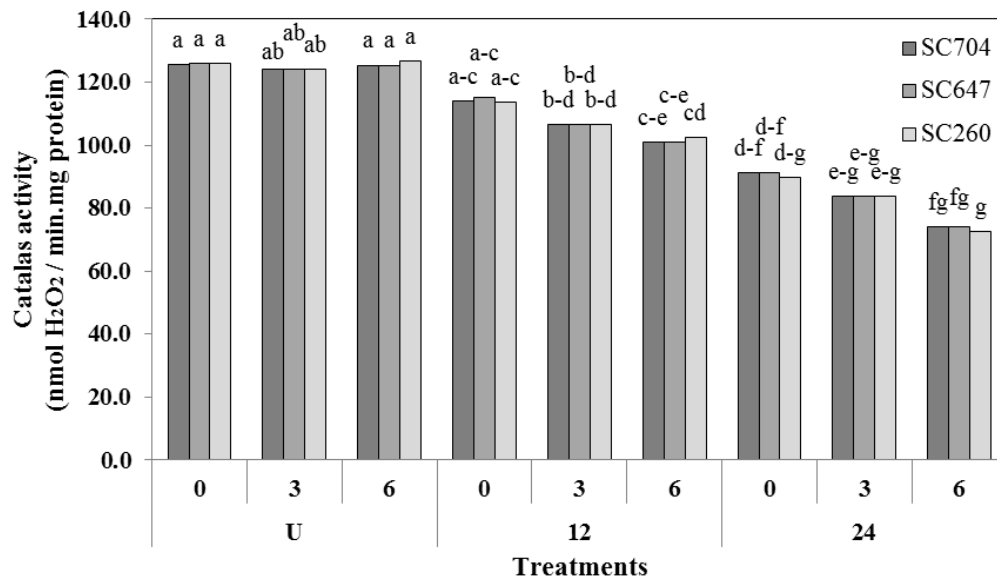


Figure 6. Effect of priming and ageing on catalase (CAT) activity in maize hybrids (SC704, SC647, SC260). Levels of ageing treatments: 0, 3 and 6 days. Priming treatments: unprimed (U), primed by distilled water for 12 hours (12) and 24 hours (24), respectively. Different letters show significant differences among treatment combinations at 5% probability level using Duncan's multiple range test.

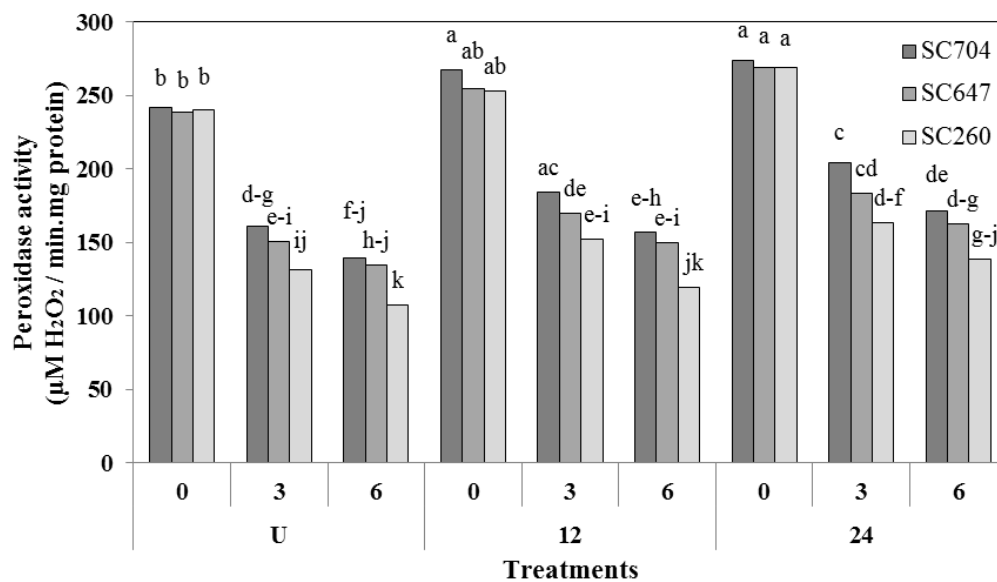


Figure 7. Effect of priming and ageing on peroxidase (POD) activity in maize hybrids (SC704, SC647, SC260). Levels of ageing treatments: 0, 3 and 6 days. Priming treatments: unprimed (U), primed by distilled water for 12 hours (12) and 24 hours (24), respectively. Different letters show significant differences among treatment combinations at 5% probability level using Duncan's multiple range test.

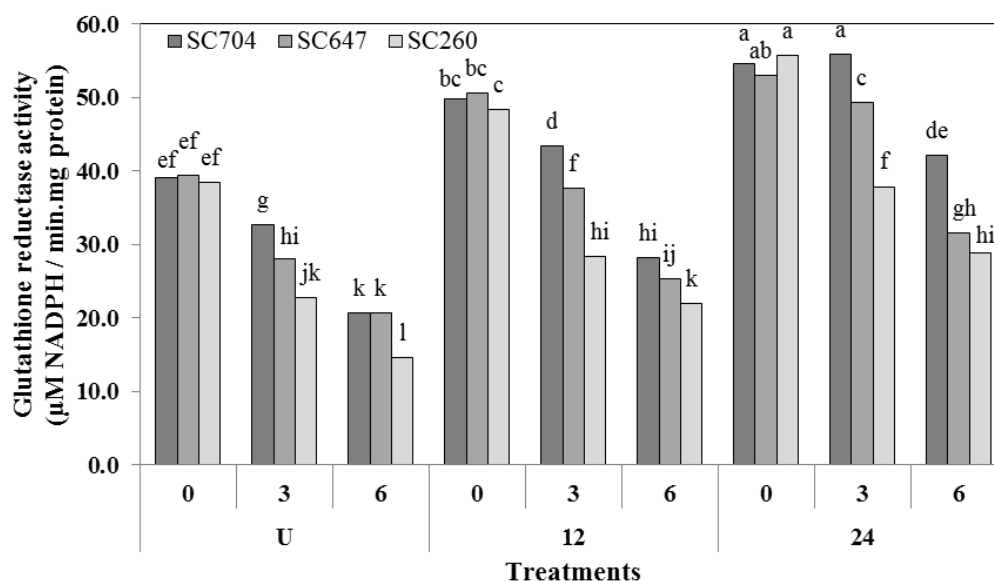


Figure 8. Effect of priming and ageing on glutathione reductase (GR) activity in maize hybrids (SC704, SC647, SC260). Levels of aging treatments: 0, 3 and 6 days. Priming treatments: unprimed (U), primed by distilled water for 12 hours (12) and 24 hours (24), respectively. Different letters show significant differences among treatment combinations at 5% probability level using Duncan's multiple range test.

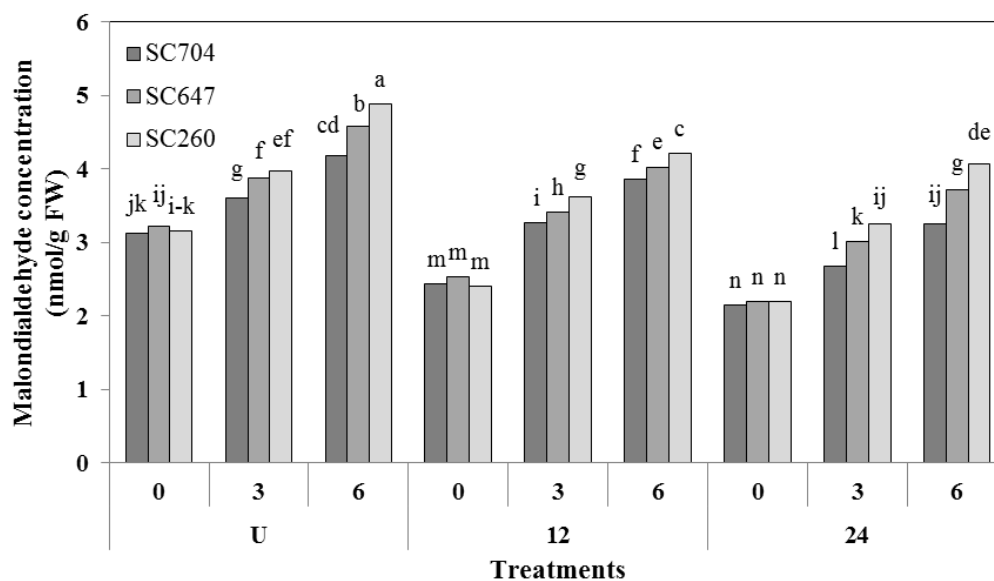


Figure 9. Effect of priming and ageing on malondialdehyde (MDA) concentration in maize hybrids (SC704, SC647, SC260). Levels of aging treatments: 0, 3 and 6 days. Priming treatments: unprimed (U), primed by distilled water for 12 hours (12) and 24 hours (24), respectively. Different letters show significant differences among treatment combinations at 5% probability level using Duncan's multiple range test.

those of Lehner *et al.* (2008), Sedghi *et al.* (2010) and Ansari *et al.* (2013). Woltz and Tekrony (2001) and Goodarzian Ghahfarokhi *et al.* (2014) showed that the accelerated aging may foretell

seed vigor better than regular germination test. In maize, the low shoot and root growth of aged seeds was found to be the result of reduced cell expansion. Bingham and Merritt (1999) showed

that ageing decreased cell expansion higher than cell division. Sveinsdottir *et al.* (2009) stated that germination of maize seeds was reduced from 100 to 70% and 40% after 24 and 72 h of ageing, respectively. According to McDonough *et al.* (2004), ageing reduced the hardness and density of maize seeds due to the development of voids and cracks. They also reported a reduction in soluble proteins due to the increase of insoluble proteins during ageing process.

Our results showed that priming stimulated seedling growth (Figures 1 to 5), possibly by accelerating several developmental stages. Basra *et al.* (2003), Farooq *et al.* (2006), Aloui *et al.* (2014) and Yan (2015) suggested that priming had positive impact on germination characteristics. Van Pijlen *et al.* (1995) reported that osmo-priming partially alleviated the adverse effect of ageing on the quality of seedlings in tomato seeds.

The improvement in seedling growth of aged seeds by priming was related to higher GR and POD and lower CAT activities (Figures 6 and 8). Increasing the activity of antioxidant enzymes (POD, GR, etc.) leads to the removal of ROS and reduces damage to cell membranes. Similar results were also reported by Lehner *et al.* (2008), Sedghi *et al.* (2010), Siadat *et al.* (2012), Ansari *et al.* (2013) and Yan (2015). Wattanakulpakin *et al.* (2012) and Ghasemi *et al.* (2014) reported that hydro-priming improved seed germination in aged maize and wheat seeds and possibly was linked to increased activity of SOD, POD and APX enzymes. Shaban *et al.* (2018) showed that ageing changed activity of CAT, POD, APX, SOD and GR enzymes. Seeds with higher CAT activity during aging performed better than the seeds with

lower CAT activity. The activity of this enzyme is a good indicator of seed storability condition. However, in our study, accelerated aging reduced the activity of CAT enzyme and activity of this enzyme did not improve by priming (Figure 6). This is probably due to the aggravated and damaging effects of accelerated aging on the structure of protein and ultimately the deformation of structure and function of antioxidant enzymes. Khamadi *et al.* (2017) stated that the effect of hydro-priming with different durations was not significant on the activity of catalase enzyme, but increased the activity of APX enzyme in the seeds.

MDA concentration enhanced significantly with increased ageing level (Figure 9). MDA is produced by peroxidation of cell membrane. Changes in lipid peroxidation are regarded as an important sign of oxidative damage in plants (Del Rio *et al.* 2006). Tahmasbi *et al.* (2015) suggested that increased levels of fatty acids and lipid hydroperoxides during seed deterioration indicated damage to cellular membranes witnessed by increased MDA concentration and electrical conductivity. Several researchers have also reported that seed ageing increased cell membrane damage and instability (Ghassemi-Golezani *et al.* 2010; Siadat *et al.* 2012; Ansari *et al.* 2013; Ghasemi *et al.* 2014; Shaban *et al.* 2018). On the other hand, priming of aged seeds restored somehow the initial germination potential and resulted in a marked decrease in the MDA level, indicating a fall in lipid peroxidation processes (Figure 9). It has been demo that the reduction of MDA content due to seed priming may be associated with a further increase of

antioxidant enzymes (Chiu *et al.* 2006). Demirkaya *et al.* (2010) indicated that reduction in enzyme activity in the seed decreases the respiratory potential of seeds and consequently lowers the energy and assimilates supply of the germination seed. Therefore, we may state that changes in the enzyme activity might decrease the germination ability of the seeds. Damage to the constructive enzymes, cell membranes injury, and loss of membrane integrity could be the result of unfavorable changes in protein synthesis during the germination process (Gidrol *et al.* 1990), which might contribute to abnormal growth, delayed germination and loss of germination ability (Ellis and Roberts 1981; Yeh and Sung 2008; Yan 2015).

Improvement in the uniformity and rate of germination has been reported after seed priming (Roberts 1973; Narayana Murthy *et al.* 2003; Bosco de Oliveira *et al.* 2012). Priming duration had a significant influence on the growth characteristics, with the highest improvement achieved with 24 hours of priming (Figures 1 to 9). Davison and Bray (1991) reported the change in protein pattern of the primed seeds. These

results suggest that priming is an efficient approach for imparting tolerance in maize against ageing.

Conclusions

In conclusion, the hydro-priming may considerably improve the growth ability of aged seeds. Seed soaking of maize by water might reduce the damage imposed by storage conditions. In general, the best germination characteristics and antioxidant enzyme activity were obtained in the non-aged seeds which were treated for 24 h, and the worst condition was observed after six days of ageing in the nonprime seeds. The results suggest that the antioxidant defense system may have an important impact on seed vigor. Therefore, soaking of the seeds for 12 and 24 hours in water can be regarded as a good hydro-priming treatment to improve germination and growth ability of aged maize seeds under unfavorable storage conditions.

Conflicts of interest

The authors declare no conflicts of interest.

References

- Abdalla FH and Roberts EH, 1968. Effects of temperature, moisture and oxygen on the induction of chromosome damage in seeds of barley, broad beans and peas during storage. *Annals of Botany* 32: 119-136.
- Abdoli M, 2014. Effect of seed priming on seed dormancy, vigor and seedling characteristics of fennel (*Foeniculum vulgare* L.). *Advances in Agricultural Science* 2(8): 18-24.
- Abdul Baki AA and Anderson JD, 1973. Vigor determination in soybean seed by multiple criteria. *Crop Science* 13(6): 630-633.
- Aloui H, Souguir M and Hannachi C, 2014. Determination of an optimal priming duration and concentration protocol for pepper seeds (*Capsicum annuum* L.). *Acta Agriculturae Slovenica* 103(2): 213-221.
- Ansari O, Sharif-Zadeh F, Moradi A, Azadi MS and Younesi E, 2013. Heat shock treatment can improve some seed germination indexes and enzyme activity in primed seeds with gibberellin of Mountain Rye (*Secale montanum*) under accelerated aging conditions. *Cercetări Agronomice în Moldova* 155: 21-30.
- AOSA, 1983. Seed Vigor Testing Handbook. Contribution No. 32 to the Handbook on Seed Testing. Association of Official Seed Analysis. Springfield, IL, USA.

- Bailly C, Benamar A, Corbineau F and Come D, 2000. Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. *Seed Science Research* 10: 35-42.
- Basra SMA, Ullah E, Warriach EA, Cheema MA and Afzal I, 2003. Effect of storage on growth and yield of primed Canola (*Brassica napus* L.) seeds. *International Journal of Agriculture and Biology* 5: 117-120.
- Bingham IJ and Merritt DJ, 1999. Effects of seed aging on early post-germination root extension in maize: a spatial and histological analysis of the growth-zone. *Seed Science and Technology* 27: 151-162.
- Bosco de Oliveira A, Gomes-Filho E, Ene'as-Filho J, Prisco JT and Mendes Alencar NL, 2012. Seed priming effects on growth, lipid peroxidation and activity of ROS scavenging enzymes in NaCl-stressed sorghum seedlings from aged seeds. *Journal of Plant Interactions* 7(2): 151-159.
- Chance B and Maehly AC, 1955. Assay of catalases and peroxidases. *Methods in Enzymology* 2: 764-775.
- Chiu KY, Chuang SJ and Sung JM, 2006. Both anti-oxidation and lipid-carbohydrate conversion enhancements are involved in priming-improved emergence of *Echinacea purpurea* seeds that differ in size. *Scientia Horticulturae* 108: 220-226.
- Davison PA and Bray CM, 1991. Protein synthesis during osmopriming of leek (*Allium porrum* L.) seeds. *Seed Science Research* 1: 29-35.
- Del Rio LA, Sandalio LM, Corpas FJ, Palma JM and Barroso JB, 2006. Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging and role in cell signaling. *Plant Physiology* 141(2): 330-335.
- Demirkaya M, Dietz KJ and Sivritepe HO, 2010. Changes in antioxidant enzymes during ageing of onion seeds. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 38: 49-52.
- Ellis RH and Roberts EH, 1981. The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology* 9: 373-409.
- Farooq M, Basra SMA and Hafeez K, 2006. Seed invigoration by osmohardening in coarse and fine rice. *Seed Science and Technology* 34(1): 181-187.
- Farooq M, Basra SMA, Hafeez K and Ahmad N, 2005. Thermal hardening: a new seed vigor enhancement tool in rice. *Acta Botanica Sinica* 47: 187-193.
- Foyer CH and Halliwell B, 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* 133: 21-25.
- Ghasemi E, Goodarzian Ghahfarokhi M, Darvishi B and Heidari Kazafi Z, 2014. The effect of hydro-priming on germination characteristics, seedling growth and antioxidant activity of accelerated aging wheat seeds. *Cercetări Agronomice în Moldova* 160(4): 41-48.
- Ghassemi-Golezani K, Khomari S, Dalil B, Hosseinzadeh-Mahootchy A and Chadordooz-Jeddi A, 2010. Effects of seed aging on field performance of winter oilseed rape. *Journal of Food, Agriculture and Environment* 8: 175-178.
- Gidrol X, Noubhani A and Pradet A, 1990. Biochemical changes induced by accelerated aging in sunflower seeds. *Physiologia Plantarum* 80(4): 598-604.
- Goodarzian Ghahfarokhi M, Ghasemi E, Saeidi M and Heidari Kazafi Z, 2014. The effect of accelerated aging on germination characteristics, seed reserve utilization and malondialdehyde content of two wheat cultivars. *Journal of Stress Physiology and Biochemistry* 10(2): 15-23.
- Hacisalihoglu G, 2008. Responses of three switchgrass (*Panicum virgatum* L.) cultivars to seed priming and differential aging conditions. *Acta Agriculturae Scandinavica, Section B- Soil and Plant Science* 58(3): 280-284.
- Hampton JG, Brunton BJ, Pemberton GM and Rowarth JS, 2004. Temperature and time variables for accelerated ageing vigour testing of pea (*Pisum sativum* L.) seed. *Seed Science and Technology* 32(1): 261-264.
- Heath RL and Parker L, 1968. Peroxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* 125(1): 189-198.
- Hsu CC, Chen CL, Chen JJ and Sung JM, 2003. Accelerated aging enhanced lipid peroxidation in bitter melon seeds and effects of priming and hot water soaking treatments. *Scientia Horticulturae* 98(3): 201-212.
- Hsu JL and Sung JM, 1997. Antioxidant role of glutathione associated with accelerated aging and hydration of triploid watermelon seeds. *Physiologia Plantarum* 111: 9-16.
- ISTA, 1999. International rules for seed testing. *Seed Science and Technology* 27(1): 1-333.

- ISTA, 2008. Method Validation Reports on Proposed Changes to the ISTA International Rules for Seed Testing 2008, Document 06-2008-OM, 68 p.
- Jisha KC, Vijayakumari K and Puthur JT, 2013. Seed priming for abiotic stress tolerance: an overview. *Acta Physiologiae Plantarum* 35: 1381-1396.
- Khajeh M, Tabatabaei SA, Ansari O and Sharif Zadeh F, 2015. Improvement of germination characteristics and enhancement of antioxidant enzymes activity of safflower (*Carthamus tinctorius* L.) aged seeds by used of gibberellin. *Cercetări Agronomice în Moldova* 163(3): 33-41.
- Khamadi N, Nabipour M, Roshanfekar H and Rahnema A, 2017. Effect of seed priming on seedling growth and grain yield of bread wheat (*Triticum aestivum* L.) cultivars in sowing date treatments. *Iranian Journal of Crop Sciences* 19(2): 116-131 (In Persian with English abstract).
- Kong L, Huo H and Moa P, 2015. Antioxidant response and related gene expression in aged oat seed. *Frontiers in Plant Science* 6: 1-9.
- Lehner A, Mamadou N, Poels P, Come D, Bailly C and Corbineau F, 2008. Change in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during aging in wheat grains. *Journal of Cereal Science* 47(3): 555-565.
- Mansouri-Far C, Goodarzian-Ghahfarokhi M, Saeidi M and Abdoli M, 2015. Antioxidant enzyme activity and germination characteristics of different maize hybrid seeds during ageing. *Environmental and Experimental Biology* 13: 177-182.
- McDonough CM, Floyd CD, Waniska RD and Rooney LW, 2004. Effect of accelerated aging on maize, sorghum and sorghum meal. *Journal of Cereal Science* 39(3): 351-361.
- Narayana Murthy UM, Kumar PP and Sun WQ, 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. *Journal of Experimental Botany* 54: 1057-1067.
- Nautiyal AR, Thapliyal AP and Purohit AN, 1985. Seed viability. IV. Protein changes: accompanying loss of viability in *Shorea robusta*. *Seed Science and Technology* 13: 83-86.
- Paparella S, Araújo SS, Rossi G, Wijayasinghe M, Carbonera D and Balestrazzi A, 2015. Seed priming: state of the art and new perspectives. *Plant Cell Reports* 34: 1281-1293.
- Parray JA, Mir MY and Shameem N, 2019. Sustainable Agriculture: Biotechniques in Plant Biology. Springer Nature Singapore Pte Ltd, Singapore.
- Probert RJ and Hay FR, 2000. Keeping seeds alive. In: Black M and Bewley JD (eds.). *Seed Technology and Its Biological Basis*. Pp. 375-410. Sheffield Academic Press, Sheffield, UK.
- Roberts EH, 1973. Loss of seed viability: chromosomal and genetic aspects. *Seed Science and Technology* 1: 515-527.
- Sedghi M, Nemati A and Esmailpour B, 2010. Effect of seed priming on germination and seedling growth of two medicinal plants under salinity. *Emirates Journal of Food and Agriculture* 22: 130-139.
- Shaban M, Ghaderi-Far F, Sadeghipour HR and Yamchi V, 2018. Study on germination and key enzymatic and non-enzymatic antioxidants involved in chickpea seed ageing during natural storage and accelerate ageing. *Journal of Crop Production* 11(1): 51-71 (In Persian with English abstract).
- Siadat SA, Moosavi A and Sharafizadeh M, 2012. Effect of seed priming on antioxidant activity and germination characteristics of maize seeds under different aging treatments. *Research Journal of Seed Science* 5(2): 51-62.
- Sinha AK, 1972. Colorimetric assay of catalase. *Analytical Biochemistry* 47(2): 389-394.
- Steiner AM and Ruckebauer P, 1995. Germination of 110-year-old cereal and weed seeds, the Vienna sample of 1877; verification of effective ultra-dry storage at ambient temperature. *Seed Science Research* 5: 195-199.
- Sveinsdottir H, Yan F, Zhu Y, Peiter-Volk T and Schubert S, 2009. Seed ageing-induced inhibition of germination and post-germination root growth is related to lower activity of plasma membrane H(+)-ATPase in maize roots. *Journal of Plant Physiology* 166(2): 128-135.
- Tahmasebi B, Ghaderi-Far F, Sadeghipour HR and Galeshi S, 2015. Enhanced accumulation of fatty acids and lipid hydroperoxides during ageing of sunflower seeds. *Journal of Plant Process and Function* 4(12): 73-83.
- Van Pijlen JG, Kraak HL, Bino RJ and De Vos CHR, 1995. Effects of ageing and osmopriming on germination characteristics and chromosome aberration of tomato (*Lycopersicon esculentum* Mill.) seeds. *Seed Science and Technology* 23: 823-830.

- Wattanakupakin P, Photchanachai S, Miyagawa S and Ratanakhanokchai K, 2012. Loss of maize seed vigor as affected by biochemical changes during hydropriming. *Crop Science* 52(6): 2783-2793.
- Woltz JM and Tekrony DM, 2001. Accelerated ageing test for corn seed. *Seed Technology* 23: 21-34.
- Yan M, 2015. Hydropriming promotes germination of aged napa cabbage seeds. *Seed Science and Technology* 42(2): 303-307.
- Yeh YM and Sung JM, 2008. Priming slows deterioration of artificially aged bitter melon seeds by enhancing anti-oxidative activities. *Seed Science and Technology* 36(2): 350-359.

پاسخ خصوصیات جوانه زنی و فعالیت آنزیم‌های آنتی‌اکسیدان به سطوح مختلف هیدروپرایم و پیری بذر در سه هیبرید ذرت (*Zea mays L.*)

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چکیده

نگهداری بذر در شرایط نامطلوب رطوبت و دمای بالا بسیار دشوار است که باعث کاهش بنیه بذر و یا زنده مانی بذر می‌شود. آزمایش حاضر به منظور بررسی فعالیت آنزیم‌های آنتی‌اکسیدان و خصوصیات جوانه زنی پس از پیری روی بذور ذرت (*Zea mays L.*) در آزمایشگاه تحقیقاتی دانشگاه رازی، کرمانشاه، انجام شد. آزمایش به صورت سه فاکتوره در قالب طرح کاملاً تصادفی با سه تکرار انجام شد که در آن سه هیبرید ذرت (SC260، SC647، SC704) و سه سطح زوال تسریع شده بذر (بدون زوال (سالم)، زوال مصنوعی بذر به مدت ۳ و ۶ روز در دمای ۴۵ درجه سانتیگراد و رطوبت نسبی ۹۸ درصد) استفاده شد. هیدروپرایمینگ با مدت زمان مختلف (صفر، ۱۲ و ۲۴ ساعت) اعمال شد. نتایج نشان داد که خصوصیات جوانه زنی بذر ذرت به طور معنی‌داری تحت تأثیر مدت زمان پرایمینگ قرار گرفت. معیارهای جوانه زنی بذور زوال یافته به طور معنی‌داری در اثر هیدروپرایمینگ به خصوص به مدت ۲۴ ساعت افزایش یافت. بیشترین میزان خصوصیات جوانه زنی و طول گیاهچه در بذور سالم (بدون زوال) که به مدت ۲۴ ساعت پرایم شده بود به دست آمد. کمترین میزان جوانه زنی پس از ۶ روز اعمال پیری در حالت بدون پرایمینگ حاصل شد. فعالیت کاتالاز، پراکسیداز و گلوکاتایون ردوکتاز و غلظت مالون دی آلدئید (MDA) در بذور زوال یافته با هیدروپرایمینگ بهبود یافت. در واقع میزان MDA که وابسته به پیری است با اعمال پرایم کاهش یافت. نتایج نشان می‌دهد که هیدروپرایمینگ می‌تواند زوال بذر را بهبود بخشد.

واژه‌های کلیدی: پیش تیمار؛ خصوصیات رشدی گیاهچه؛ زوال بذر؛ صفات بیوشیمیایی؛ هیبریدهای ذرت