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# Effects of Seed Priming on Antioxidant Activity and Germination Characteristics of Maize Seeds under Different Ageing Treatment

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## ABSTRACT

Seed ageing is a main problem of seed storage. Unsuitable storage conditions with high moisture and temperature increases seed ageing. Ageing induces seed deterioration expressed as the loss of seed vigour and/or viability. An experiment was conducted in order to investigate the activity of catalase and peroxidase during accelerated ageing and repair during priming treatment of maize (*Zea maize* L.) seeds. In order to improve germination characteristics in aged seeds seed priming with $KNO_3$ was performed at different concentration of (0.5, 1, 2.5 and 4%) and seeds were soaked for 8, 12 and 24 h in each individual concentration. Results of study showed that there is significant difference for duration of ageing treatment on germination characteristics of maize seeds. Increasing ageing duration resulted higher reduction in germination characteristics. $KNO_3$ had positive effects on seed germination of aged seed. This was higher in application of 0.5% $KNO_3$ for 8 h and 2.5% for 24 h. Antioxidant activity of aged seeds increased after seed priming treatments. Hormone seed priming is more effective than seed priming with $KNO_3$ in activation of antioxidant enzymes. It is suggested that using seed enhancement treatments like seed priming could improve aged and non-aged seed performance especially for high aged seeds.

**Key words:** Antioxidant, accelerated ageing, $KNO_3$, gibberlline, seed priming, maize

## INTRODUCTION

Seeds vigor and growth potentials of most crops severely decrease when stored under conditions of high humidity and temperature (Sveinsdottir *et al*., 2009). Delayed germination and slow post-germination growth are two main characteristics of aged seeds. Most of cereal grains can be stored for long time without any microbial infections; however, biochemical changes could occur during storage period. During seed storage, processes of seed deterioration processes may rapidly be started and these processes will be followed by starting respiration and consequently decrease in seed matter, functional and nutritional properties of the grain (Reed, 1992; Woltz and Tekrony, 2001). Grain storage quality have traditionally been intensified for seed producers. Application of accelerated aging treatment is used to assess storage quality, germination characteristics by simulating natural ageing conditions for different crops like (Galleschi *et al*., 2002) maize, (Nik and Tilebeni, 2011) and cotton (Miranda *et al*., 2001). Many factors are responsible for seed ageing such as genetics, seed water content, mechanical damage, relative humidity and

temperature of the storage environment. The reduction in seed viability is mainly a function of temperature and seed moisture content (McDonald, 1999, 2004). Mohammadi *et al.* (2011) reported that seed aging results in dramatic reduction in seedling growth which might happen due to decline in weight of mobilized seed reserve (seed reserve depletion percentage), not seed reserve utilization efficiency. It has been shown that the loss of seed germination ability by natural ageing or controlled deterioration is due to a series of metabolic defects which effects embryonic and non-embryonic parts of the seeds (Roberts, 1973; Osborne, 1983). Some Studies reveal that that the radicle and scutellum are possibly the primary sites of seed deterioration in monocots like wheat and corn (Bingham *et al.*, 1994).

Murthy *et al.* (2003) suggested that some physiological processes have been linked to seed ageing; for example, in aged seeds of *Vigna radiata* loss of seed viability is associated with Maillard reactions. They suggested that biochemical ageing and viability loss are highly inhibited in seeds stored below a high critical temperature. Sukesh and Chandrashekar (2011) reported that viability of seeds during storage could be influenced by decrease in total carbohydrate and increase in lipid peroxidation.

There are some reports that showed degradation and inactivation of enzymes due to changes in macromo lecular structures of seeds could causes of ageing in seeds (Bailly, 2004; Goela *et al.*, 2003; Lehner *et al.*, 2008). These studies suggested that decreases occur in the activity of enzymes such as superoxide dismutase, catalase, peroxidase and glutathione reductase in aged seeds.

Seed priming is known as technique of seed enhancements that improves germination or seedling growth in many crops such as Dry bean (*Phaseolus vulgaris* L.) cordia (*Cordia millennii*) (Adebisi, 2011), coffee (*Coffea arabica* L.) (Gebreselassie *et al.*, 2010), capsicum (*Capsicum annum*) chickpea (Ghassemi-Golezani *et al.*, 2008) and *Agropyron elongatum* (Tavili *et al.*, 2010).

There is little information available for effects of seed priming on antioxidant activity and germination characteristics of deteriorated Maize seeds. In this study, responses of deteriorated maize seeds to seed priming and antioxidant activity of primed seeds at different duration of accelerated ageing treatment were investigated.

## MATERIALS AND METHODS

**Accelerated aging treatments:** Maize seeds (*Zea maize* L.) cv. Single cross 704 were obtained from Safi Abad Agricultural Research Center, Dezfuz, Iran in November, 2010. Three accelerated aging regimes were performed by placing the seeds in the incubator with temperature of 40°C and relative humidity of 90-95% for 0, 4 and 7 day periods (Modarresi *et al.*, 2002; Basra *et al.*, 2003). For each aging treatment, about 400 g of pure maize seeds were scattered within a vacuum container on wire screens; the floor of the container was covered by distilled water (70% of total container volume). The containers were placed in an incubator at a fixed temperature of 40°C.

**Seed treatment with gibberlline and $KNO_3$:** In order to evaluate the effect of seed priming on germination characteristics of aged and non aged seeds two factorial experiment were conducted in a completely randomized design with four replications. In Experiment one, seeds were divided into two group of aged and non-aged and both groups were subjected to soak in different gibberlline solutions. Seeds were soaked at gibberlline solutions for individual 8, 12 and 24 h. Gibberlline solutions were prepared at concentrations of 50, 100, 200, 400 and 800 ppm and 0 as control. In experiment two, aged seeds were subjected to seed priming with $KNO_3$ at different concentration of (0.5, 1, 2.5 and 4%) and seeds were soaked for 8, 12 and 24 h in each individual concentration.

Standard germination test was carried out by placing 25 seeds on top of two Watman No.1, filter paper in 120 mm petri dishes (ISTA, 1999). All petri dishes moistened with 12 mL of distilled water and covered with plastic bags in order to reduce the water evaporation then all petri dishes moved to germinator with 25°C temperature at dark condition (ISTA, 1999). Seeds were observed daily until day 7th and germinated seeds were recorded. Seeds were considered as germinated when the radicle length reached 2 mm long. Investigated parameters were the final germination percentage, Mean Daily Germination (MDG) which measured using Eq. 1, Peroxidase and Catalase activity in selected priming treatments:

$$MDG = \frac{FGP}{d} \quad (1)$$

where, FGP is final germination percentage and d is days to maximum germination percentage.

**Enzyme extraction and assay:** Most effective treatment of giberlline and $KNO_3$ were subjected to antioxidant activity assay. The seed samples, weighing about 200 mg, were homogenized with 10 mL of phosphate buffer pH 6.8 (0.1 M) and divided into two equal 5 mL portions. One 5 mL portion was centrifuged at 2 C for 15 min at 15,000 g in a refrigerated centrifuge. The clear supernatant was taken as the enzyme source. The other 5 mL portion was taken for the biochemical analysis.

**Catalase assay:** The activity of catalase as well as peroxidase was assayed after the method of Kar and Mishra (1976) with the slight modifications. Five milliliters of the assay mixture for the catalase activity comprised: 300 μmoles of phosphate buffer, pH 6.8, 100 μmoles of $H_2O_2$ and 1 mL of the twice diluted enzyme extracted. After incubation at 25 C for 1 min, the reaction was stopped by adding 10 mL of 2% (v/v) $H_2SO_4$ and the residual $H_2O_2$ was titrated against 0.01 N $KMnO_4$ until a faint purple color persisted for at least 15 sec. A control was run at the same time in which the enzyme activity was stopped at "zero" time. One unit of catalase activity is defined as that amount of enzyme which breaks down 1 μmol of $H_2O_2$ min under the assay conditions described.

**Peroxidase assay:** Five milliliters of the assay mixture for the peroxidase activity comprised: 125 μmoles of phosphate buffer, pH 6.8, 50 μmoles of pyrogallol, 50 μmoles of $H_2O_2$ and 1 mL of the 20 times-diluted enzyme extract. This was incubated for 5 min at 25°C after which the reaction was stopped by adding 0.5 mL of 5% (v/v) $H_2SO_4$. The amount of purpurogallin formed was determined by taking the absorbancy at 420 nm.

**Statistical analysis:** Data of germination percentage were subjected to data transformation (arcsine) before the statistical analysis in order to unify the variance of the data. Data of experiment were subjected to factorial analysis and Duncan's multiple comparison test was performed for mean comparison test. Statistical softwares used in this research for data analysis consisted of Minitab, 16, MSTAT-C and Microsoft Excel 2010 software, respectively.

## RESULTS AND DISCUSSION

Analysis of variance showed that there is a significant difference between primed and non-primed seeds.

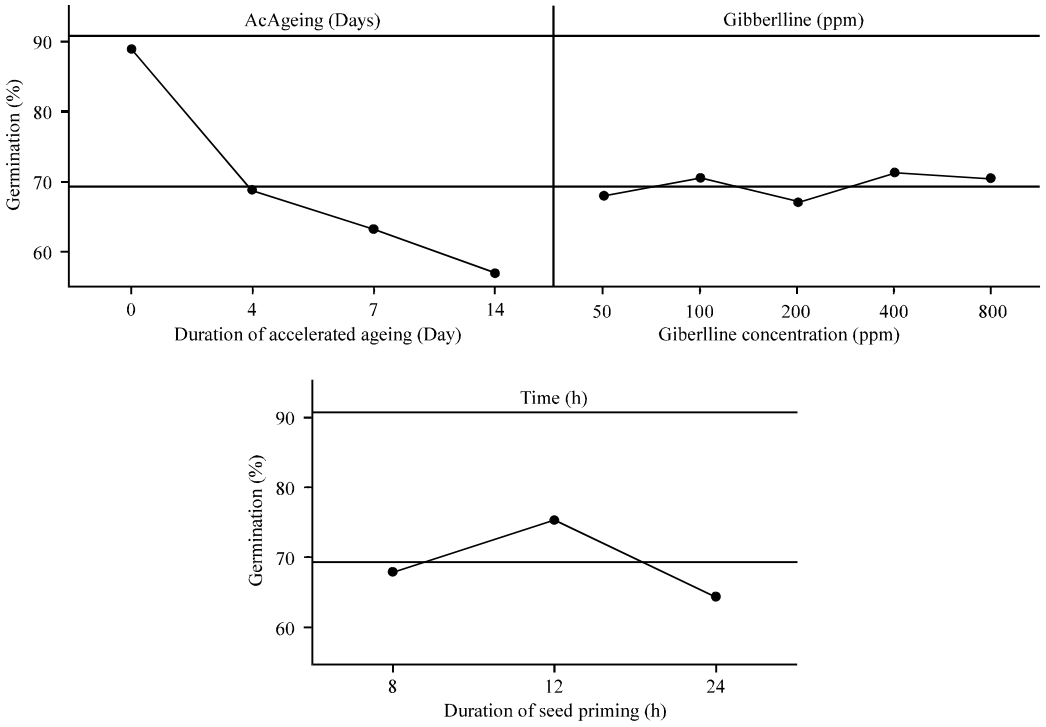


Fig. 1: Main effect plot of hormone priming on germination percentage of aged and control seeds

**Germination percentage:** Increase in the duration of accelerated ageing treatment, significantly reduced germination percentage of aged seeds. Hormone priming with gibberlline significantly improved germination percentage in aged seeds (Fig. 1).

Seed priming with $KNO_3$ significantly affected germination percentage at 0.5% but increasing concentration of $KNO_3$ produce no significant results. It is suggested that lower concentration of $KNO_3$ 0.5% is more effective than 1 and 2.5% (Fig. 2).

Germination percentage is highly depends on the duration of seed ageing treatment. 14 days of accelerated ageing treatment exhibited the lowest germination percentage and the highest germination percentage was observed in control seeds (Fig. 2).

Germination percentage increased by increasing the gibberlline concentrations. Soaking seeds for 12 h in gibberlline solutions showed good results for improvement of germination percentage of aged seeds. The Highest germination percentage was in the control (Non-aged) seeds which treated for 12 h in 50 ppm of gibberlline (Fig. 3). Otroshy *et al.* (2009) suggested that application of plant hormones like gibberlhc acid and 6-benzylaminopurine could improve seed germination in Asafoetida (*Ferula assafoerida* L.) seeds. Biabani *et al.* (2011) reported that, Chickpea seeds declined in germination and growth with increasing deterioration. There was no significant difference in application of 50-200 ppm gibberlline solutions in order to improve germination percentage of 4 and 7 days of ageing. Higher gibberlline concentration (400-800 ppm) resulted negative effect on germination percentage of aged seeds for 4days. Base on present results, optimum time for treating seeds with gibberlline solution is 12 h (Fig. 3). Two functions for gibberlline has been suggested in seed germination. First, gibberlline increases the growth potential

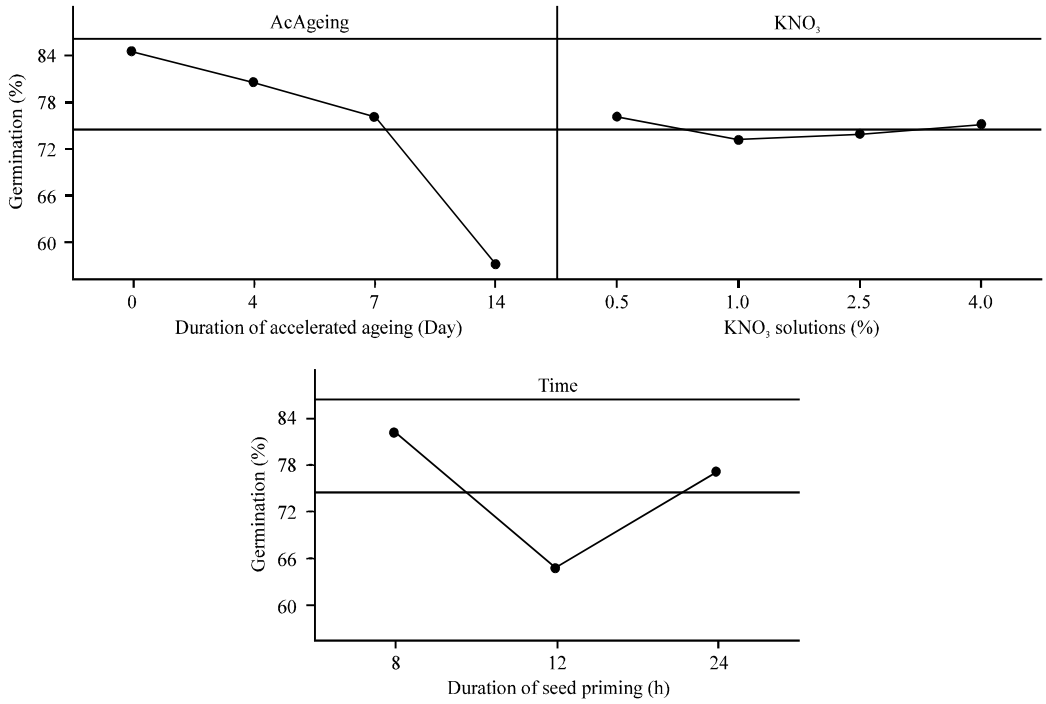


Fig. 2: Main Effects of seed priming with $KNO_3$ on germination percentage of aged and control seeds

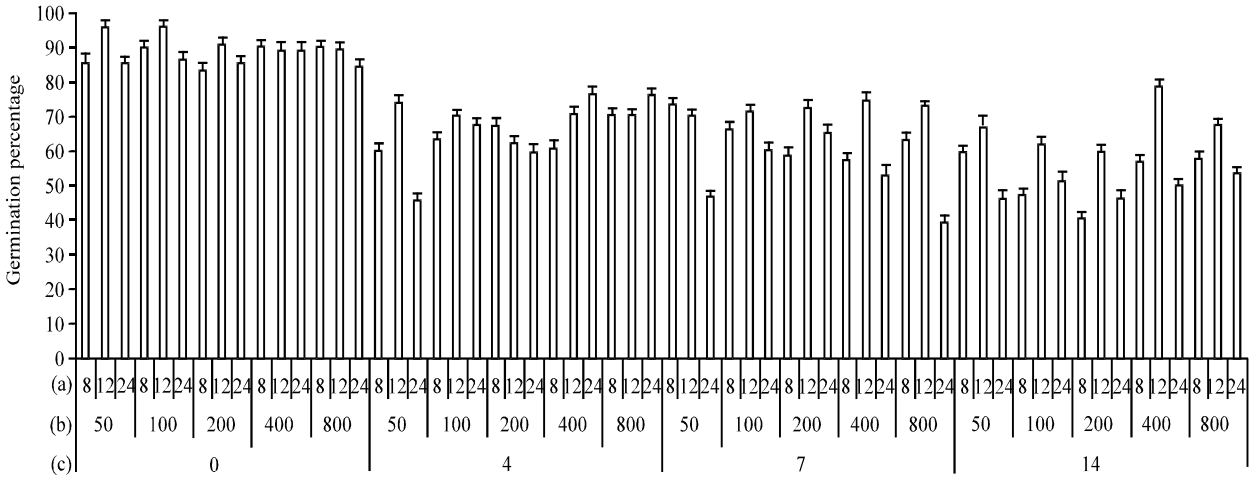


Fig. 3: Interaction plot of accelerated ageing, time of hormone priming and gibberlline concentrations on germination percentage. Vertical bars show SD. (a) Duration of priming (h), (b) Gilberlline concentration (ppm) and (c) Duration of agein (day)

of embryo and promotes germination. Secondly, gibberlline is needed to overcome the mechanical restraint of seed covering layers by weakening of the tissues surrounding the radicle (Finch-Savage and Leubner-Metzger, 2006).

For seed priming with $KNO_3$, 8 h of soaking seeds in $KNO_3$ solutions exhibited the most effective duration for seed priming. Present results showed that 8 h of soaking seeds in $KNO_3$ 0.5% will

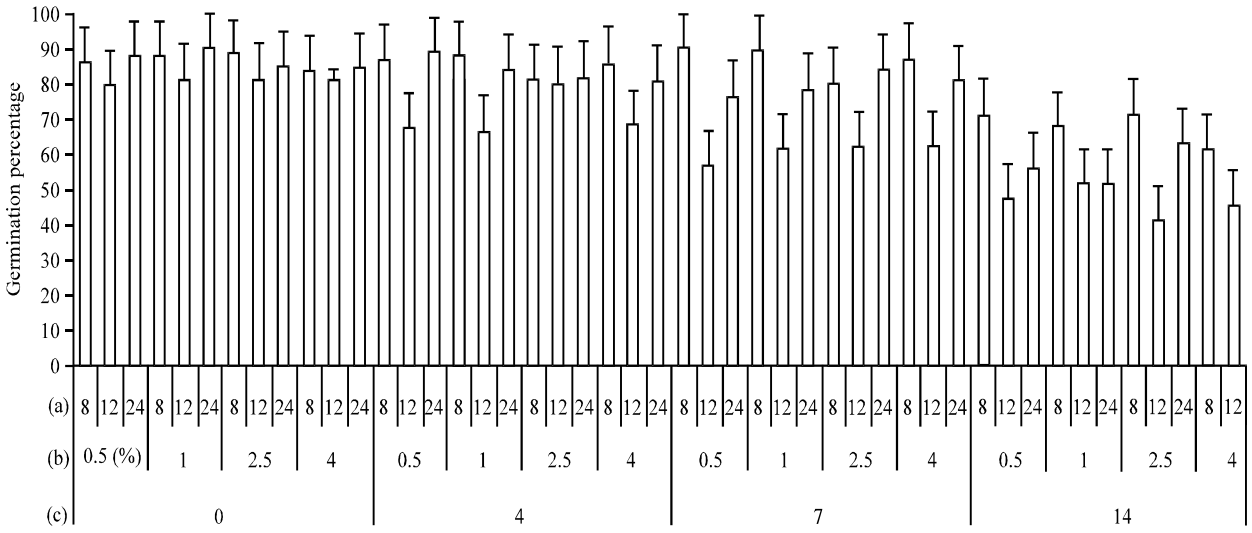


Fig. 4: Interaction plot of accelerated ageing, time of seed priming and $KNO_3$ concentrations on germination percentage. Vertical bars show SD. (a) Duratrion of seed priming, (b) Concentration of $KNO_3$ and (c) Duration of agein (day)

significantly improved germination percentage of aged seeds especially for seeds which were aged for 14 days (Fig. 4).

Demir *et al.* (2004), reported that cucumber seed germination was reduced to 82-84% after 144 h of accelerated ageing at 40°C.

**Mean daily germination (MDG):** Accelerated ageing resulted in reduction of MDG comparing to control. Increasing the duration of ageing drastically decreases germination rate. The lowest MDG value was observed in 14 days of ageing treatment Fig. 3. 100,400 and 800 ppm of Gibberlline concentrations improved MDG and 12 hour of treatment with gibberlline exhibited the most effective treatment (Fig. 5).

The highest MDG value was observed at control seeds while the lowest germination index was observed at 14 days of seed ageing treatment. Application of 100 ppm gibbelline exhibited highest germination rate. Using 400 ppm gibberlline significantly improve germination of 14 days aged seeds. Base on present results, soaking seeds for 12 h of in gibberlline solution considered as the optimum time for seeds treatment (Fig. 5, 6).

Analyze of variance showed significant difference for seed priming with $KNO_3$ on accelerated aged seeds comparing to control. Soaking seeds in $KNO_3$ solutions significantly improved MDG value of aged seeds which means more seeds can germinate after seed priming with $KNO_3$. Germination percentage and speed of germination decreased in the accelerated aged seeds but these effects were alleviated by seed priming (Fig. 7, 8). Seeds which were aged for 14 days, exhibited lower MDG value comparing to control seeds. Soaking seeds for 8 h in solution of 0.5% $KNO_3$ significantly increased MDG value in aged seeds which mean that seeds could germinate faster.

**Enzyme changes after seed priming:** Selected treatments were subjected to determination of antioxidant activity. Seed priming with 0.5% $KNO_3$ for 8 h increased catalase activity especially in

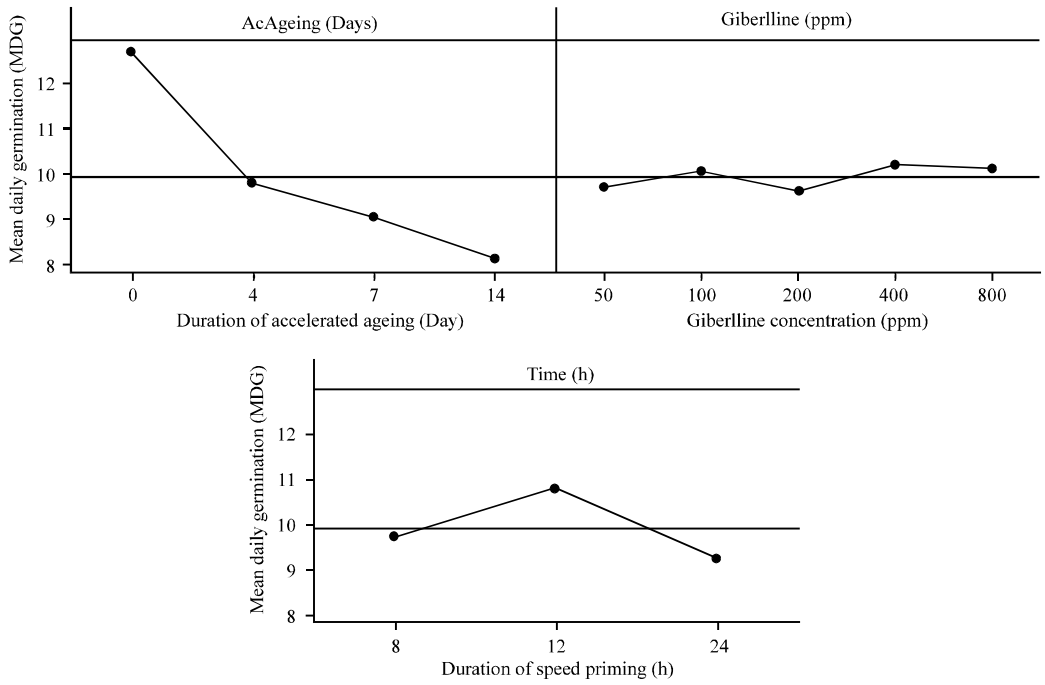


Fig. 5: Main effect plot of hormone priming on MDG value of aged and control seeds

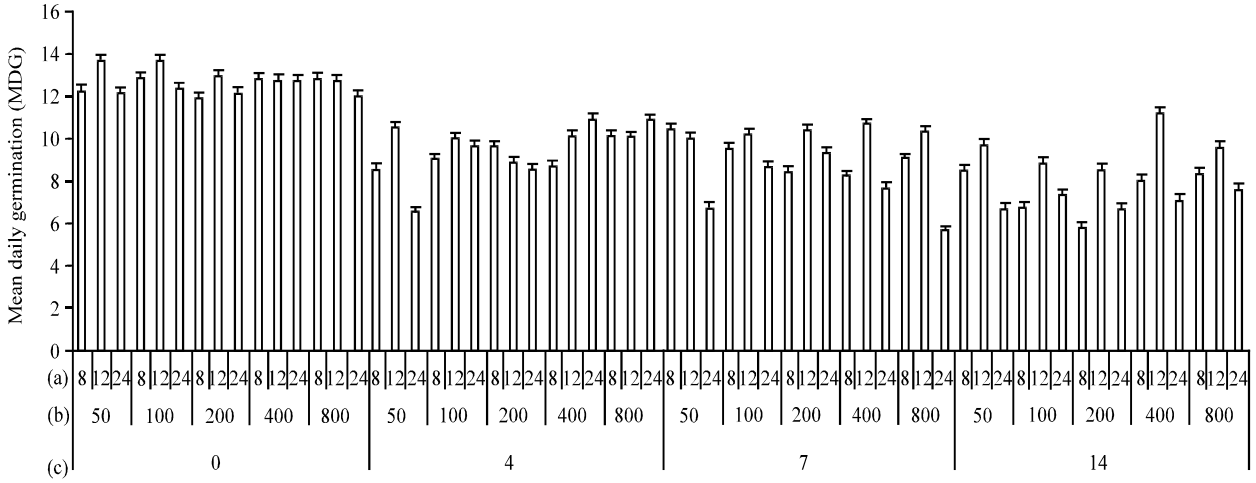


Fig. 6: Interaction plot of accelerated ageing, gibberlline and time of hormone priming on MDG value of maize. Vertical bars show SD. (a) Duration of priming (h), (b) Giberlline concentration (ppm) and (c) Duration of agein (day)

seeds which were aged for 14 days. Using 100 ppm Gibberlline significantly increased catalase activity. The highest catalase activity was achieved in 100 ppm gibberlline comparing to other treatments. This results showed that hormone seed priming is more effective on antioxidant activity than seed priming with KNO3 (Fig. 9).

Peroxidase activity was higher in hormone seed priming than seed priming with $KNO_3$. The highest peoxidase activity was observed at 200 ppm gibberlline and 24 h of soaking duration. 8 h of Seed treatment with 0.5% $KNO_3$ alsosignificantly increased peroxidase activity in seeds which were aged for 7days (Fig. 10).

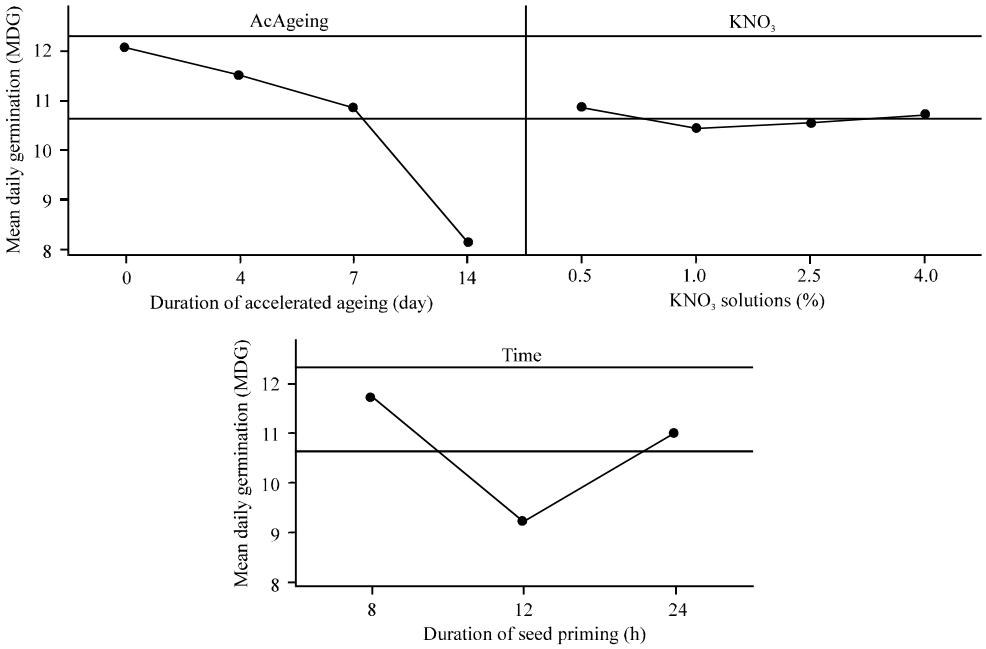


Fig. 7: Effect of priming treatment with $KNO_3$ on MDG value of aged and control seeds

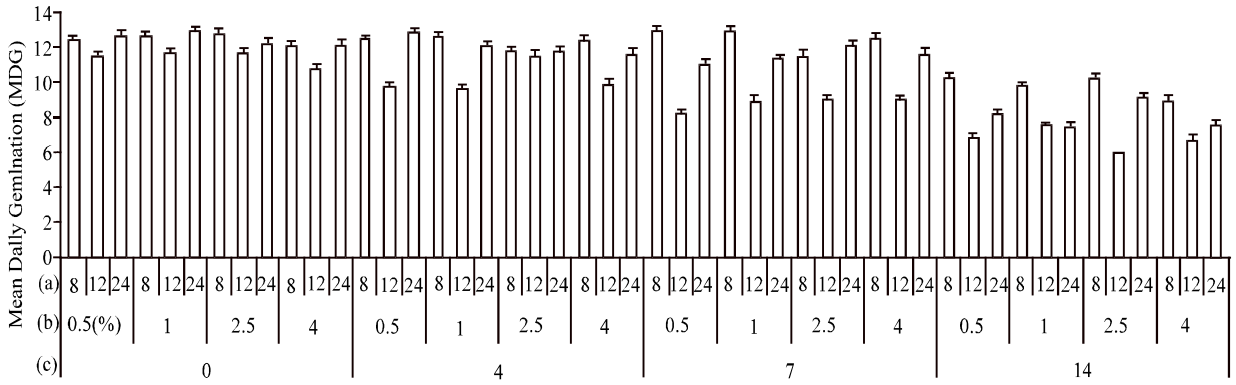


Fig. 8: Interaction plot of Accelerated ageing, $KNO_3$ solutions and time of seed priming on MDG value of maize. Vertical bars show SD. (a) Duration of seed priming, (b) Concentration of $KNO_3$ (%) and (c) Duration of ageing (day)

Demirkaya *et al.* (2010) suggested that decrease in enzyme activity in the seed lowers the respiratory potential of seeds, which causes lowering both the energy (ATP) and assimilates supply of the germinating seed. So it is concluded that several changes in the enzyme acromolecular structure may contribute to their lowered germination ability of seeds.

Coin *et al.* (1995) reported that due to the autooxidative properties in covered and uncovered barley different response to aging could be observed. Woltz and TeKrony (2001) suggested that the accelerated aging could predict seed vigor better than standard germination test. Genetic damage and loss of membrane integrity could resulted in undesirable changes in protein synthesis during germination (Gidrol *et al.*, 1990), which possibly responsible in delayed germination, abnormal growth and, finally, loss of germinability (Ellis and Roberts, 1981).

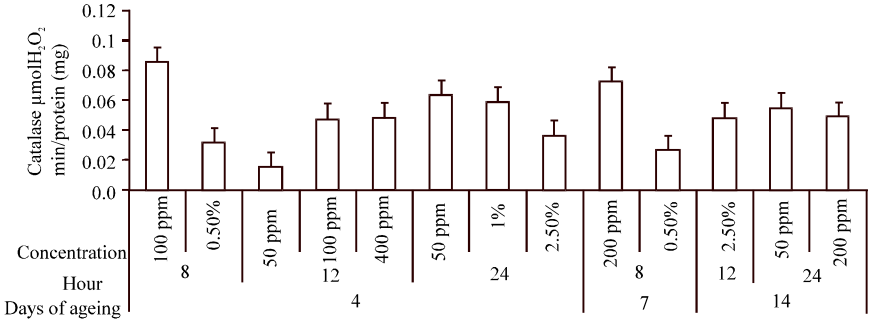


Fig. 9: Effect of seed priming on catalase activity at different ageing durations. Vertical bars show SD

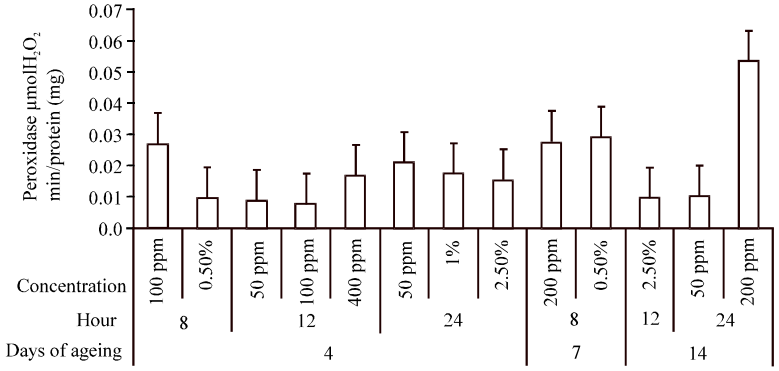


Fig. 10: Effect of seed priming on peroxidase activity at different ageing durations. Vertical bars show SD

On the other hand, improvement in rate and uniformity of germination has been observed after osmoconditioning (priming) of seeds under normal and stress conditions (Moosavi *et al*., 2009; Rouhi *et al*., 2010a). Improved seed performance and quality after priming could because of completion of DNA repair during priming (Osborne, 1983) and activation of many enzymatic processes and starting of endosperm weakening in primed seeds at the start of germination in water (Dell'Aquila *et al*., 1998; Moosavi *et al*., 2009). During the priming process some changes in protein pattern in the primed seeds have also been reported by Davison and Bray (1991). In corn, the low root growth of aged seeds was found to be resulted of decreased cell division and cell expansion parts of roots.

Studies showed that cell expansion was reduced by ageing to a greater extent comparing to cell division (Bingham and Merritt, 1999). Sveinsdottir *et al*. (2009) reported that the germination of maize seeds started to decrease due to ageing at 45°C for more than 48 h. Germination decreased from nearly 100% to 70 and 40% after 24 and 72 h of ageing treatment respectively. No germination was observed when seeds had been aged for 168 h. They also showed that the plasma membrane H+-ATPase may play an important role in the elongation growth of roots and maize seed germination. McDonough *et al*. (2004) reported that physical, structural and chemical changes occurred during accelerated aging treatment. Physical changes like, hardness and density of maize seeds decreased because of voids and cracks developed during the aging process. Interactions among starch, protein and cell walls increased within the endosperm. Amounts of soluble proteins decreased due to some changes in solubility of many protein and therefore and insoluble proteins increased because of increased protein interactions.

Study of gibberlline roles in seed germination showed that during the germination process, gibberellic acid is released from the embryo and activates some responsible genes of alpha-amylase mRNA transcription (Taiz and Zeiger, 1991) so application of exogenous gibberllic acid ($GA_3$) might lead to activation of such genes inside the seeds. Andreoli and Khan (1999) reported that treating Tomato and sweet pepper seeds using plant growth regulators germinate faster than controls. Using moist-chilling treatment for 6 or 4-week followed by soaking seeds in 500 ppm $GA_3$ solution could improve growth characteristics and the subsequent seedlings of *Ferula ovina* seeds. (Amooaghaie, 2009). They showed that the combination between $GA_3$ and moist-chilling treatments produced higher seedlings vigor than those of $GA_3$ treatment alone. Gibberellins, which generated endogenously by process of after-ripening or supplied exogenously, first act on the embryo, activates series of reactions essential to embryo growth. Potassium nitrate and thiourea are largely used to breaking seed dormancy but physiological role is not clear (Agrawal and Dadlani, 1995). Cetinbas and Koyuncu (2006) reported that in *P. avium* seeds, exogenous $GA_3$ application has been successful in breaking dormancy with 500 ppm for seeds with coat and without coat and increase final germination percentage. Rouhi *et al.* (2010b), suggested that applying 500 ppm concentration of $GA_3$ and 0.1% of $KNO_3$ after performing stratification treatment could be the best treatment to break seed dormancy of waterlily seeds.

## CONCLUSION

Ageing is a common phenomenon in seed storage. Humidity, Temperature and duration of exposing seeds in ageing conditions could significantly influence the seed quality. With increase in duration of ageing highly decreased seedling growth potentials. Gibberlline could significantly increase growth potential of aged seeds. Soaking aged seed of maize in gibberlline solution could cover damages of storage condition. Soaking seeds for 12 at 100 ppm gibberlline is a good hormone priming treatment to improve germination characteristics and growth potential of aged and low quality maize seeds.

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