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Full Length Research Paper

An assessment of the effects of seed ageing, application of phytohormone and KNO_3 on aged corn seeds

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Seed ageing is a main problem of seed storage. Unsuitable storage conditions with high moisture and temperature increases seed ageing. In fact, seed ageing results in the decrease of seed quality. Seed priming is known as a seed enhancement treatment which could improve seed germination and germination synchrony in many of crops. In this experiment we investigated effects of seed ageing, application of phytohormone and KNO_3 on aged corn seeds. In order to improve germination characteristics in aged seeds phytohormone treatment using different gibberlline concentration (50, 100, 200, 400 and 800 ppm) and KNO_3 (0.5, 1, 2.5 and 4%) were used. Results of study showed significant difference in aged seeds. It was also observed that with higher ageing duration, more germination characteristics decreased. Gibberlline at 400 ppm increased germination characteristics in aged seeds and had a positive effect on seed germination of aged corn seeds. KNO_3 also 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. Based on our results, it was suggested that seed ageing for higher duration could significantly decrease seed quality. Using seed enhancement treatments like seed priming or application of phytohormone could improve aged and non-aged seed performance especially for high aged seeds.

Key words: Seed, accelerated ageing, KNO_3, gibberlline, seed priming, maize.

INTRODUCTION

Most cereal grains can be stored for long duration without microbial infections, although biochemical changes could occur during storage. During seed storage, seed deterioration processes could be rapidly started and followed by respiration and loss of seed matter, conditions that lead to decrease in the functional and nutritional properties of the grain (Reed, 1992). Grain storage quality have traditionally main concentrated for seed producers. Application of accelerated aging treatment with high temperatures ranging from (30 to 45°C) and 100% relative humidity (RH) is used for many crops in order to appraise storage quality, germination characteristics and seed vigor by simulating natural ageing conditions for different crops (maize, Woltz and TeKrony, 2000; wheat, Galleschi et al., 2002; maize, Santipracha et al., 1997; sorghum, Miranda et al., 2001). Coin et al. (1995) reported that using high temperature and high humidity in accelerated storage treatment could be a good predictor of grain longevity and quality.

Many factors are responsible for seed ageing, and genetics, mechanical damage, relative humidity and temperature of the storage environment, seed water content, presence of microflora and seed maturity are most effective factors. The reduction in seed viability is mainly a function of interaction between temperature and seed moisture content (McDonald, 1999, 2004). Mohammadi et al. (2011) reported that seed aging results in seedling

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Abbreviations: GA, Gibberellic acid; **KNO_3,** potassium nitrate; **PPM,** part per million; **cm,** centimeter, **RH,** relative humidity.

growth reduction and this might happen due to decline in weight of mobilized seed reserve (seed reserve depletion percentage), which is not related to seed reserve utilization efficiency. They showed that the weight of mobilized seed is the most sensitive component of seedling growth. Various methods have been used by researchers in order to investigate seed ageing, for instance Dell'Aquila et al. (1998) used moderate or high temperature conditions at ambient (12%) moisture levels to assess seed vigor in wheat and barley.

It has been shown that the loss of seed germination ability following natural ageing or controlled deterioration is because of series of metabolic blemish that affect embryonic and non-embryonic parts of the seeds (Roberts, 1973; Osborne, 1983). Some studies revealed that in monocots like wheat and corn, the radicle and scutellum are possibly the primary sites of seed deterioration (Bingham et al., 1994). Bruggink et al. (1991) suggested that many physiological processes have been linked to seed ageing; for example in aged seeds of some species, phospholipid portion of cellular membrane decreased and fatty acids levels increased, although no extensive lipid peroxidation occurs.

There is little information available for effects of hormone priming on germination characteristics of deteriorated seeds. In this study we investigated the response of deteriorated maize seeds to application of gibberlline (hormone priming) and KNO_3 at different duration of accelerated ageing treatment.

MATERIALS AND METHODS

Accelerated aging treatments

Maize seeds *(Zea mays* L.) cv. Single cross704 were obtained from Safi Abad Agricultural Research Center, Dezful, Iran. Three accelerated aging regimes were performed by placing the seeds in the incubator with temperature of 40°C and relative humidity of 90 to 95% for 0, 4 and 7 days 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 investigate the effect of seed priming on germination characteristics of aged and non-aged seeds, two factorial experiments were conducted in a completely randomized design with four replications. In the first experiment, seeds were divided into two groups of aged and non-aged, and both groups were subjected to soak in different gibberlline solutions. Seeds were soaked in gibberlline solutions for 8, 12 and 24 h. Gibberlline solutions were prepared at concentrations of 50, 100, 200, 400 and 800 ppm and 0 as control.

Standard germination test was performed by placing 25 seeds on top of two Whatman no.1, filter papers in 120 mm petri dishes. All Petri dishes were moistened with 12 ml of distilled water and covered with plastic bags in order to reduce the water evaporation and then all petri dishes moved to germinator with 25°C, temperature at dark condition (ISTA, 1999). Seeds were observed daily until day 7, and seeds were considered as germinated when the radicle length reached 2 mm long. Investigated parameters were the final germination percentage, germination rate, root length, shoot length and seedling vigor (Dezfuli et al., 2008). Germination rate was calculated according to method of Elis and Roberts (1986).

Statistical analysis

Data of germination percentage were subjected to data transformation (arcsine) before the statistical analyzes in order to unify the variance of the data. Data analysis was performed by using Minitab, 16, MSTAT-C and Microsoft Excel 2010 softwares.

RESULTS AND DISCUSSION

Analyses of variance showed that there was a significant difference between primed and non-primed 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 (Figure 1). Germination percentage highly depends on the duration of seed ageing treatment. Fourteen days of accelerated ageing treatment exhibited the lowest germination percentage and the highest germination percentage was observed in control seeds. 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 treated for 12 h in 400 ppm of gibberlline. 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 to 200 ppm gibberlline solutions in order to improve germination percentage of 4 and 7 days of ageing. Higher gibberlline concentration (400 to 800 ppm) resulted in negative effect on germination percentage of aged seeds for 4 days. Hence, based on our results, optimum time for treating seeds with gibberlline solution is 12 h (Figure 2.). Demir et al. (2004) reported that cucumber seed germination was reduced to 82 to 84% after 144 h of accelerated ageing at 40°C.

Germination rate

Accelerated ageing resulted in reduction of germination rate compared to control. Moreover, increasing the duration of ageing drastically decreased germination rate. The lowest germination rate was observed in 14 days of

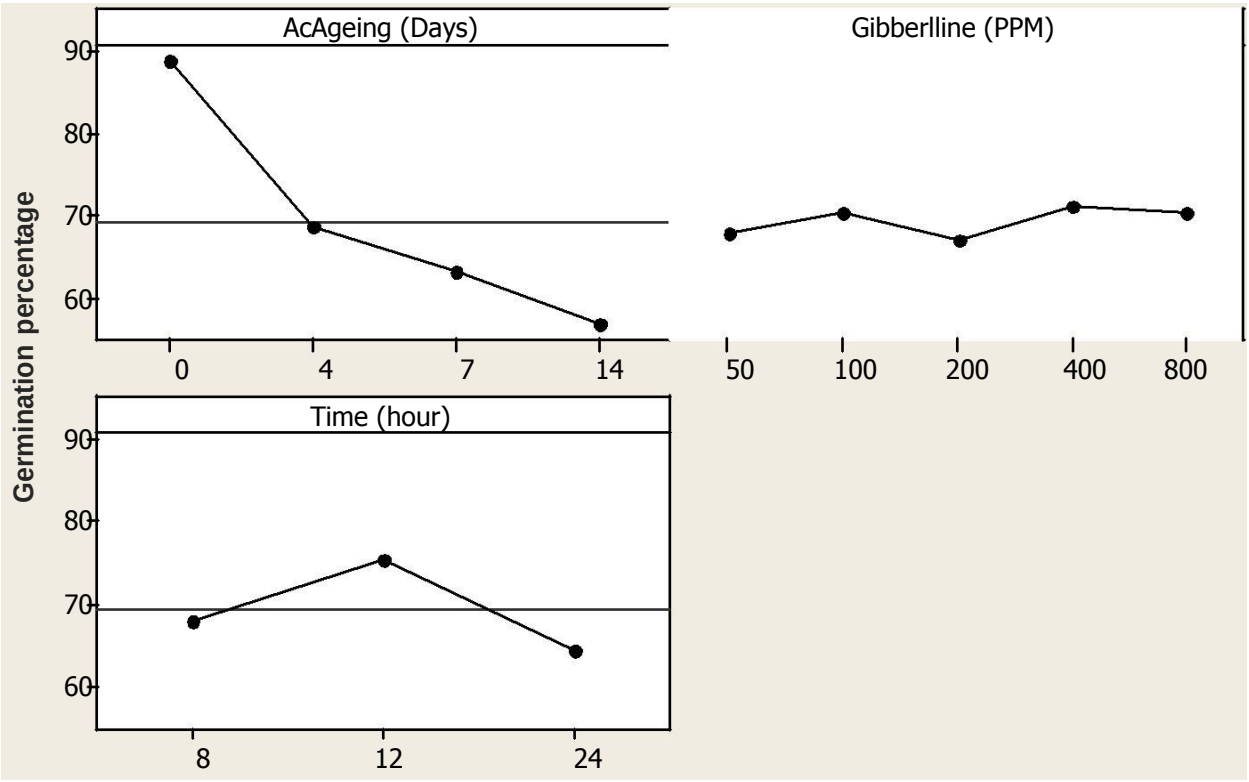

Figure 1. Main effect plot of treatments on germination percentage (%) of maize.

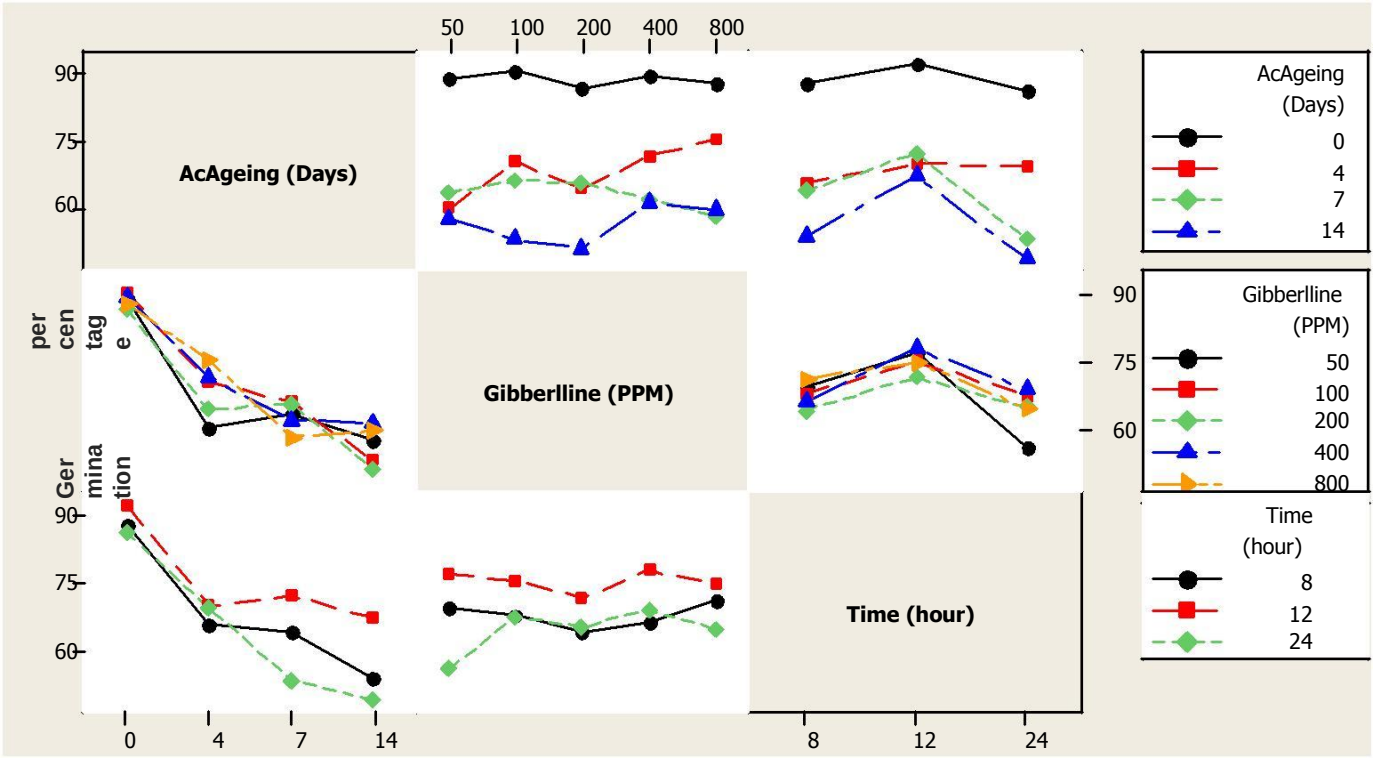

Figure 2. Interaction plot of accelerated ageing, time of hormone priming and gibberlline concentrations on germination percentage.

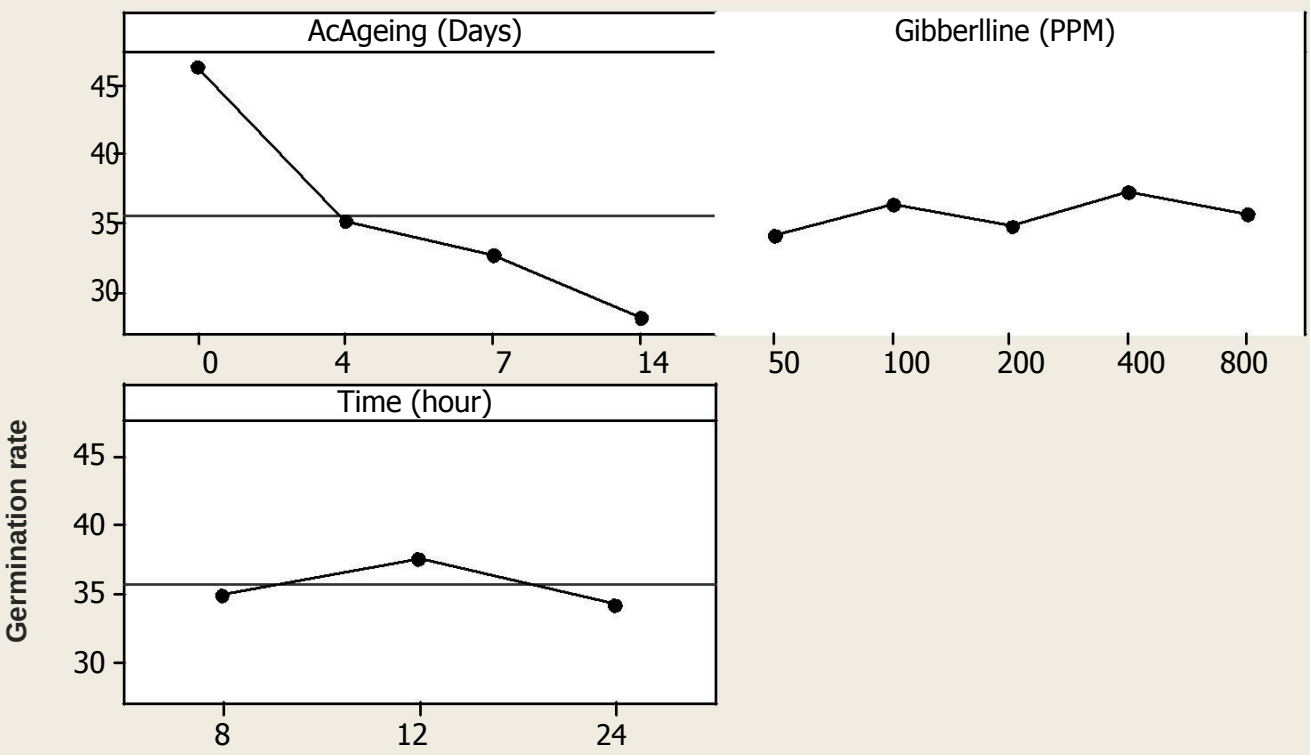

Figure 3. Main effect plot of treatments on germination rate of maize.

ageing treatment (Figure 3). Treatment with 100, 400 and 800 ppm of gibberlline concentrations improved germination rate and 12 h of treatment with gibberlline exhibited the most effective treatment. Furthermore, the highest germination rate was observed at control seeds, while the lowest germination index was observed at 14 days of seed ageing treatment. Application of 100 ppm gibberlline exhibited highest germination rate. Using 400 ppm gibberlline significantly improved germination of 14 days aged seeds. Base on our results, soaking seeds for 12 h in gibberlline solution was considered as the optimum time for seeds treatment Figure 4.

Root length

Root length significantly decreased by increasing ageing duration. The lowest root length was observed at 14 days of ageing (12.2 mm), while controls seeds exhibited 14.4 mm root length (Figure 5). Root length drastically started to decrease on the 4^{th} and 14^{th} days of ageing. However, there was no significant difference for root length in 4 and 7 days of ageing. Hence, from our results, we suggested that root length is very sensitive to accelerated ageing treatment. Interaction between ageing treatments and soaking duration of seeds into gibberlline solutions was significant. Root length was increased by application of gibberlline. This increase was clearer in 12 h of treatment with gibberlline solutions. Seed aged for four days exhibited longer root by application of gibberlline in 8 hour of soaking (Figure 6).

Shoot length

Analyses of variance showed significant difference for main effect of gibberlline solution. Shoot length significantly increased by soaking seeds in the gibberlline solutions (Figure 7). Soaking seeds in 200 ppm gibberlline resulted in higher shoot length comparing to other concentrations.

Experiment 2

Analyses of variance showed significant difference for seed priming with KNO_3 on accelerated aged seeds. Soaking seeds in KNO_3 significantly improved germination characteristics of aged seeds. Germination percentage and speed of germination decreased for accelerated aged seeds, but these effects were alleviated by priming (Figures 8 and 9). Main effect of duration of seed priming was significant for aged and non aged seeds. Increase in duration of soaking seeds in KNO3 solutions significantly improved root length of aged and control seeds (Figure 10).

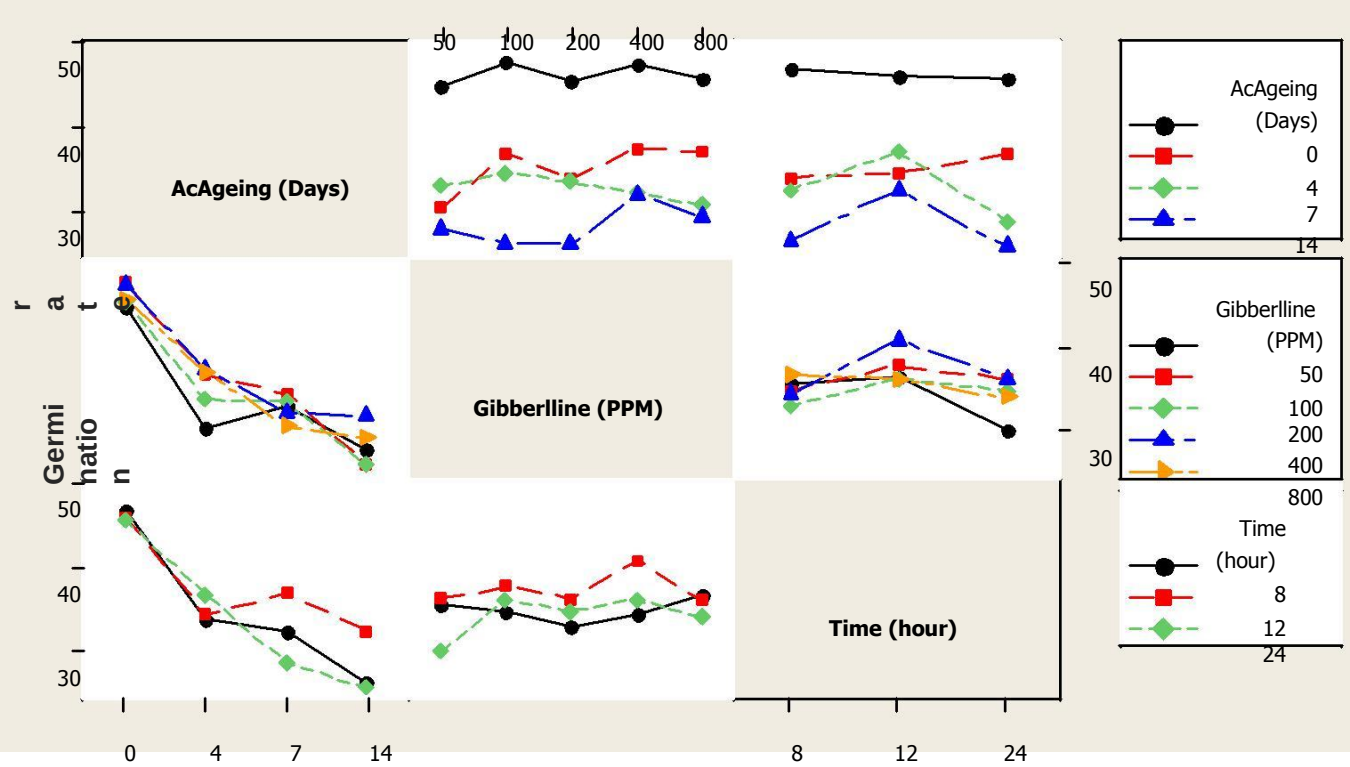

Figure 4. Interaction plot of Accelerated ageing, gibberlline and time of hormone priming on germination rate of maize.

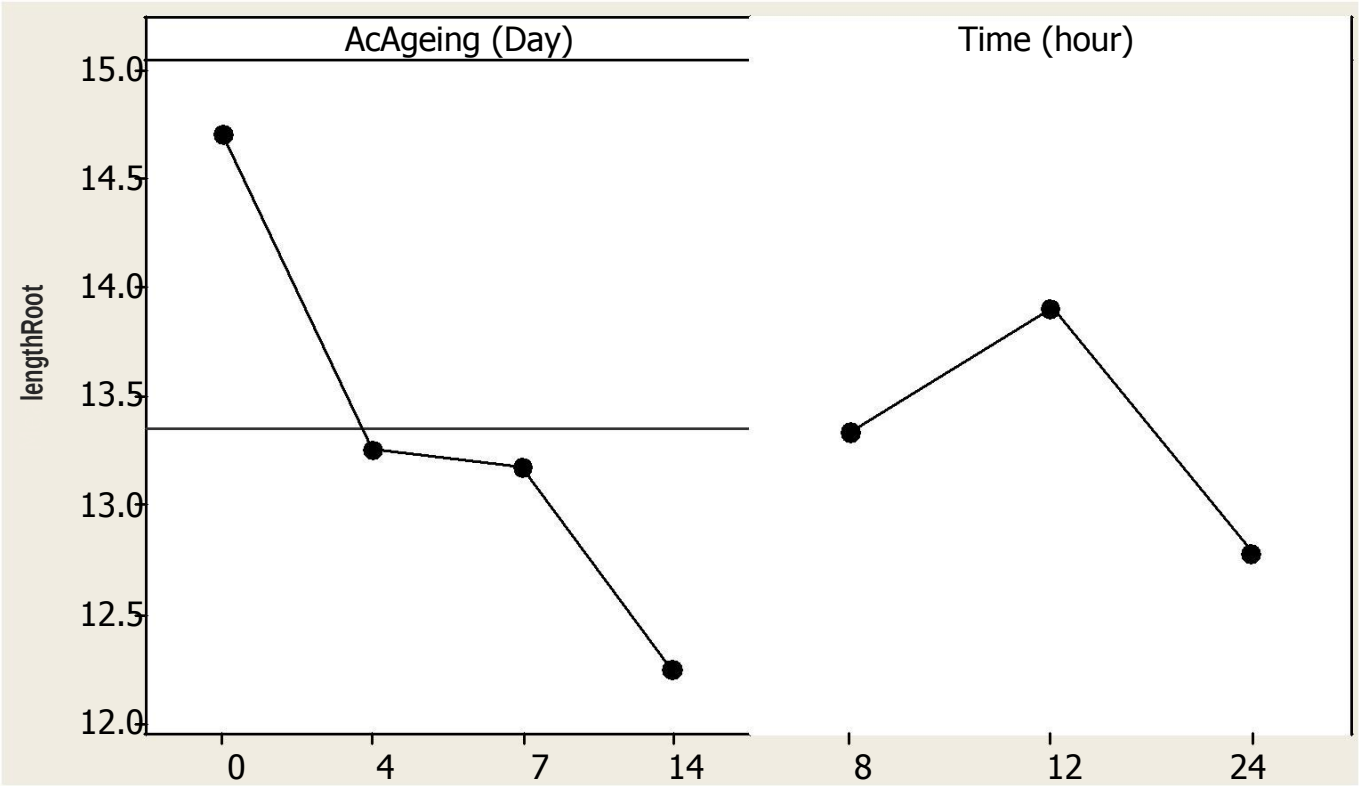

Figure 5. Main effect plot of treatments on root length (cm).

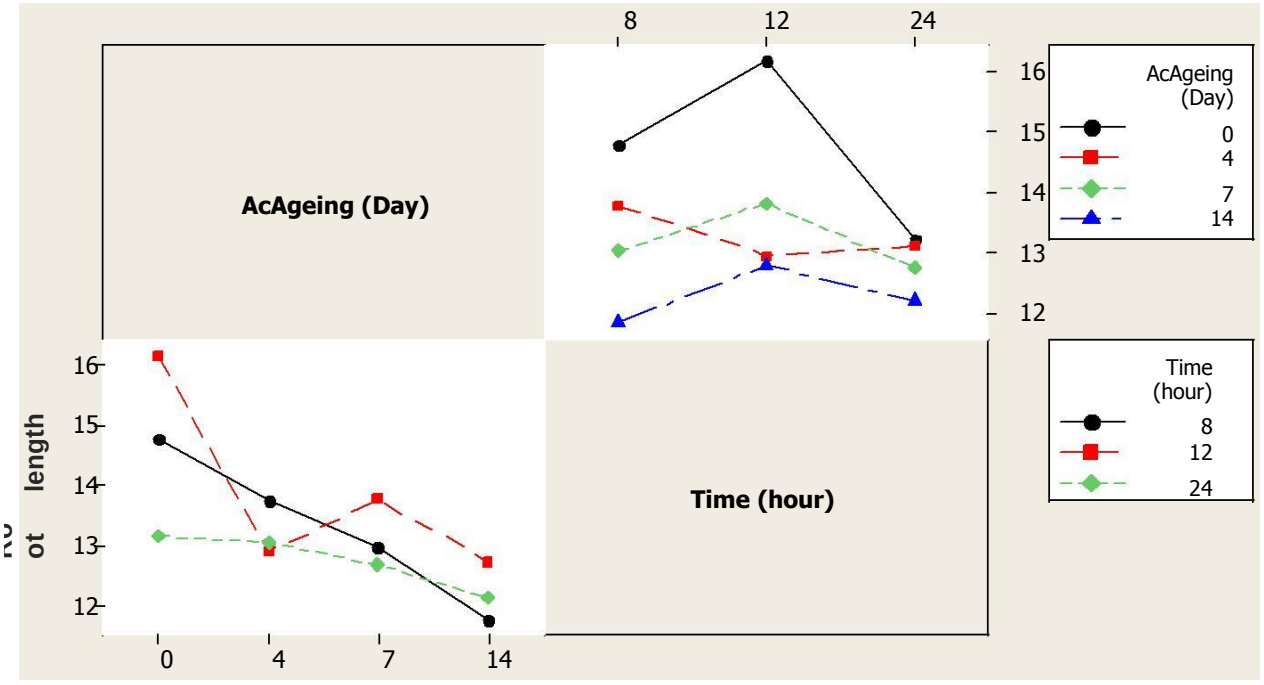

Figure 6. Interaction plot of accelerated ageing, Time of hormone priming and gibberlline concentrations on root length (cm).

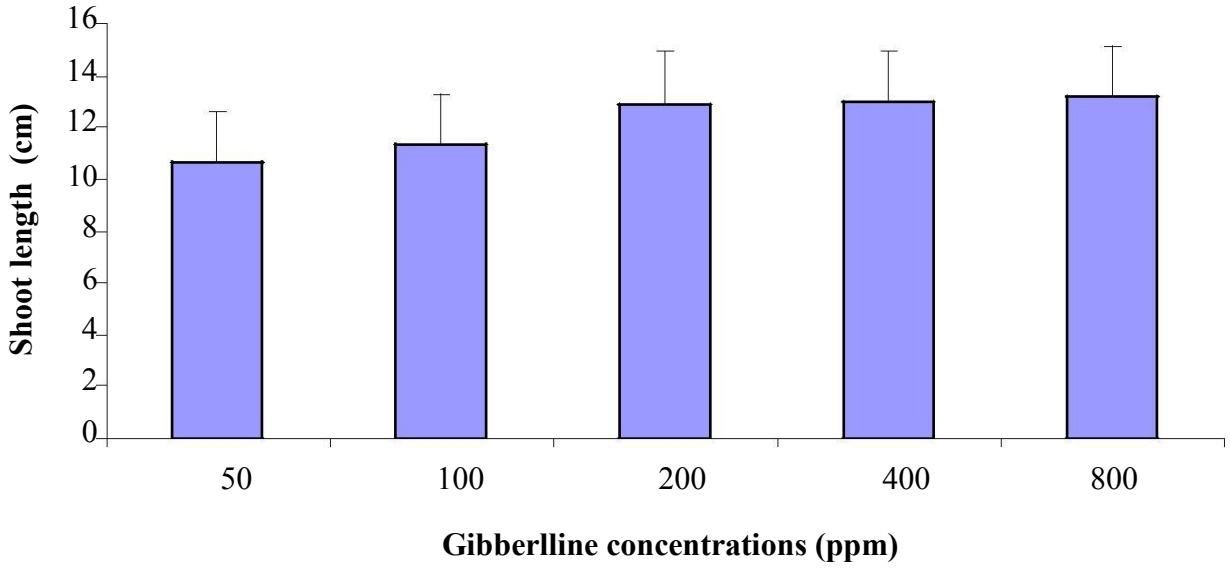

Figure 7. Effects of gibberlline solutions on shoot length of maize seedlings.

Shoot length

Soaking seed for 24 h in different KNO_3 solutions showed higher shoot length, except in 4 days of ageing. Soaking seed in 2.5 % KNO_3 solution for 12 h produced higher shoot length compared to other priming treat-ments. Two functions for gibberlline have been suggested in seed germination. First, gibberlline increases the growth potential of embryo and promotes germination. Secondly, gibberlline is needed to overcome the mecha-nical restraint of seed covering layers by weakening of the tissues surrounding the radicle (Finch-Savage and Leubner, 2006).

Coin et al. (1995) reported that in covered or uncovered

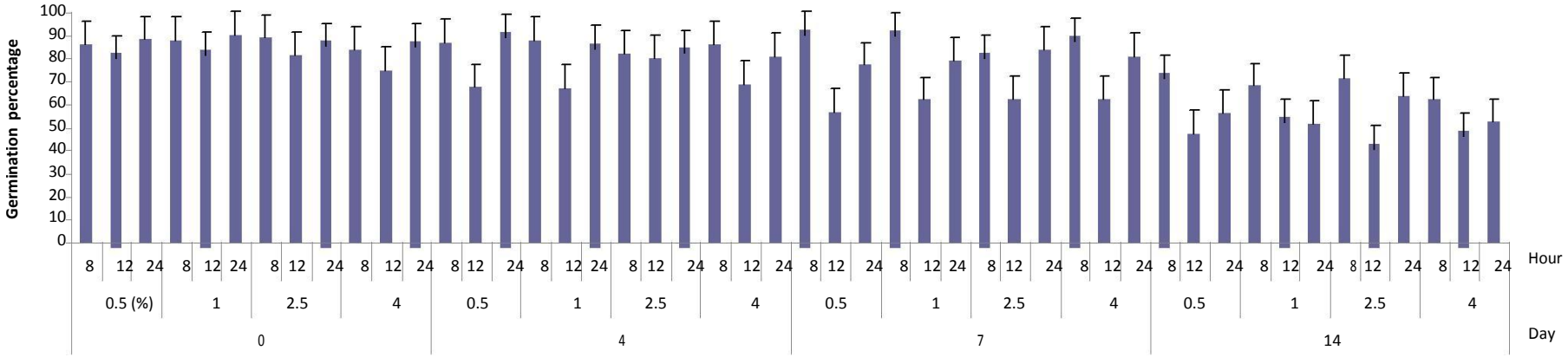

Figure 8. Effect of KNO_3 on germination percentage of aged and control seeds.

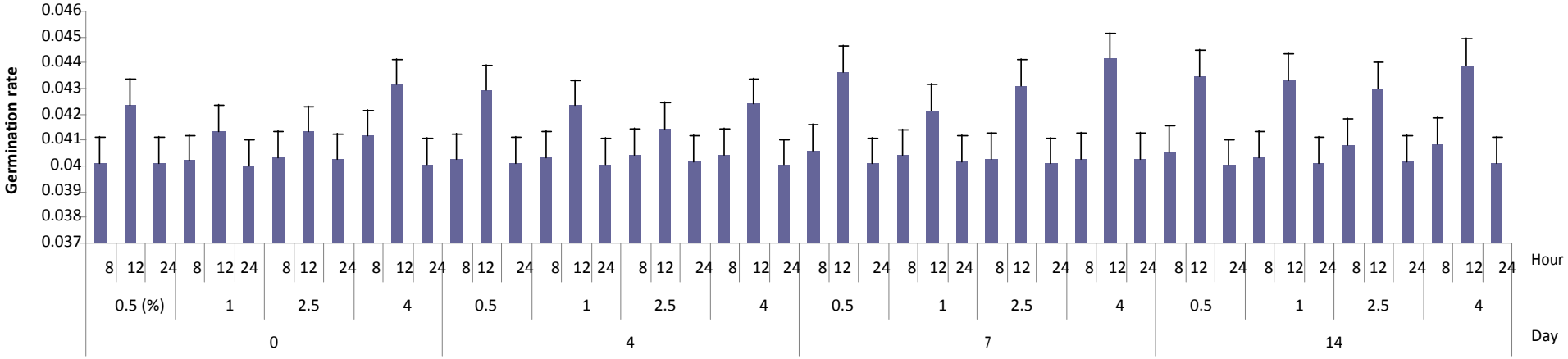

Figure 9. Effect of KNO_3 on germination rate of aged and control seeds.

barley, due to autooxidative properties, different response to aging existed. Woltz and TeKrony (2001) suggested that the accelerated aging could predict seed vigor better than standard germina-tion test. Genetic damage and loss of membrane integrity could lead to changes in protein synthesis during seed germination (Gidrol et al., 1990) and result in delayed germination, abnormal seedling growth and loss of germinability potential of seeds (Ellis and Roberts, 1981). However, some studies reviled that improvement in rate and uniformity of germination has occurred after osmoconditioning (priming) of seeds under normal and stress conditions (Moosavi et al., 2009; Rouhi et al., 2010). Improved seed performance and

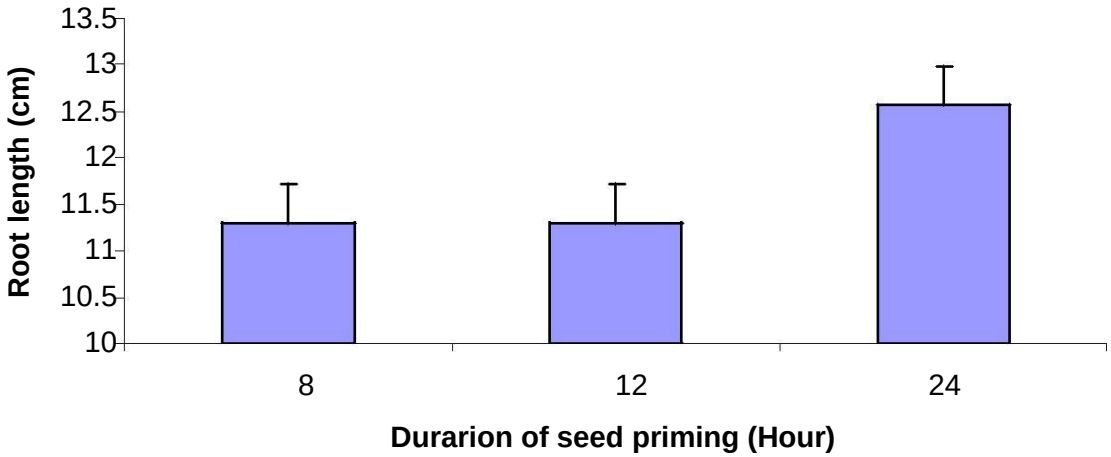

Figure 10. Main effect of seed priming duration on root length of maize seedlings.

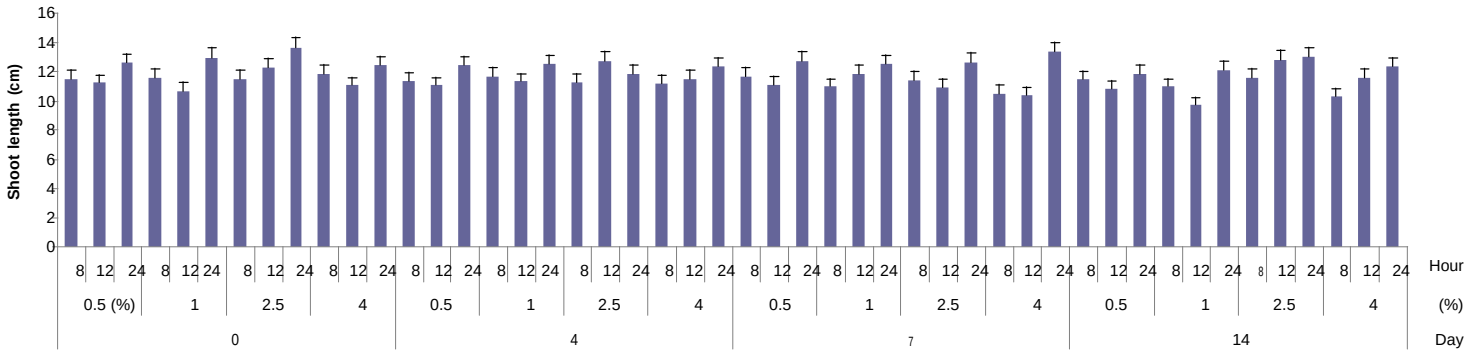

Figure 11. Effects of seed priming and seed ageing on shoot length of maize seedling.

quality after priming might be the results of completion of DNA repair during seed priming (Osborne, 1983) and activation of many enzymatic processes which may be followed by initiation of endosperm weakening in primed seeds at the start of germination in water (Dell'Aquila et al., 1978; Moosavi et al., 2009).

Furthermore, Davison and Bray (1991) suggested that during the priming process, protein pattern of primed seeds could have some changes. In corn, decreased cell division and cell expansion parts of roots lead to low root growth in aged seeds. Studies showed that cell expansion is reduced by ageing to a greater extent compared to cell division (Bingham and Merritt, 1999). Mc Donough et al. (2004) reported that physical, structural and chemical changes occurred during accelerated aging treatment. Physical changes like, hardness and density of maize decreased, and this was due to the voids and cracks developed during the aging process. Sveinsdo et al. (2009) also reported that the germination of maize seeds started to decrease when seeds were imposed in ageing condition 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. In ageing conditions, interactions among starch, protein and cell walls increased within the endosperm. Moreover, amounts of soluble proteins decreased due to some changes in solubility of many protein and therefore insoluble proteins content increased because of higher protein interactions.

Study of the role of gibberlline in germination showed that during the germination process, gibberellic acid is released from the embryo and activates some responsible genes of α-amylase mRNA transcription (Taiz and Zeiger, 1991). Hence, it was suggested that application of exogenous gibberellic acid (GA_3) could activate some of these genes inside the seeds. Andreoli and khan (1999) reported that treating tomato and sweet pepper seeds with plant growth regulators exhibited

faster germination than controls. Amooaghae (2009) reported that treating *Ferula ovina* seeds with moist-chilling treatment for 6 or 4 weeks followed by soaking seeds in 500 ppm GA3 solution, could improve growth characteristics and the subsequent seedlings of *F. ovina* seeds. They showed that the combination of gibberellic acid (GA_3) and moist-chilling treatments produced more vigorous seedling than those of seeds treated with GA_3 only. Exogenous gibberellic acid (GA_3) could affect cytokinins on transport across membranes and also play a key role in initiation of the biochemical processes necessary for germination to occur (Chen et al., 2008). Some seed treatments like after ripening could generate endogenous gibberlline, which first acts on the embryo then activates series of essential reactions for embryo growth. Rouhi et al. (2010) reported that application of 500 ppm of GA_3 and 0.1% of KNO_3 after performing stratification treatment could be the most effective treatment to break seed dormancy of water lily seeds.

Potassium nitrate (KNO_3) and thiourea are two main compounds largely used to break seed dormancy, but physiological role is not clear (Agrawal and Dadlani, 1995). Cetinbas and Koyuncu (2006) also reported that treating *Prunus avium* seeds, with 500 ppm of exogenous gibberlline (GA_3) successfully disrupted seed dormancy in seeds with coat and without coat and total germination percentage increased.

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 decreasing seedling growth potentials. However, 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 400 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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