Effects of some PGRs on seedling emergence and CAT and POD activity of maize under low temperature stress

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Abstract

Low soil temperature is one of the reasons for poor germination and establishment of maize. The aim of this study was to evaluate the possibility of improving the seedling emergence and performance of maize under low temperature stress. A pot experiment was conducted on *Zea maize* (single cross 704) at 14 ºC as cold stress and seed treatments were priming with 200, 300 and 400ppm of GA3; 100, 200 and 300ppm of salicylic acid (SA) and ascorbic acid (AA) with a hydro priming treatment and non-primed seed. Results showed that priming with SA 100ppm and AA 100ppm could strongly improve the emergence percentage and rate. SA100ppm and AA 100ppm improved root dry weight better than other treatments. The CAT activity in root was not affected by priming treatments but POD activity increased only by GA_3 300ppm. In leaf, CAT and POD activity increased by priming with GA_3, SA and AA. There was a negative correlation between CAT activity in root and seedling emergence percentage. There was no correlation between CAT activity in leaf and CAT in root. Meanwhile POD activity in leaf and root was positively correlated.

Keywords: chilling; emergence; maize; phytohormone; seedling.

Eisvand , H. R. , N. Fathi and **D. .Goudarzi.** 2015. ' Effects of some PGRs on seedling emergence and CAT and POD activity of maize under low temperature stress' .*Iranian Journal of Plant Physiology* 5 (3), 1393-1402.

Introduction

Wheat, rice and corn are the most important food suppliers in the world respectively (FAO, 2012). There is no doubt that climate affects crop production. Low temperature is one of the most important limiting factors in the productivity of plants. Maize (*Zea mays* L.) is a thermophile crop and low temperature frequently causes injuries to maize seed germination and seedling growth, thus it is detrimental to early spring planting (Parera and Cantliffe, 1994). Optimum temperature for germination of corn is 25-28 °C (Farooq et al., 2008). Temperature from 12 to 15 °C could induce chilling stress in the maize (Hola et al., 2003).

Plants have developed an antioxidant defense system that is including key enzymes such as CAT, POD and SOD (Ashraf, 2009). Many studies have linked chilling tolerance to antioxidant capacity in maize. Exposure to low temperatures causes an increase in CAT, GR and guaiacol peroxidase activities (Prasad, 1996, 1997). Similarly, decreased CAT, APX and MDHAR activities were found to be associated with chilling sensitivity during the early stages of development

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Received: January , 2014
Accepted: May, 2015

of four inbred lines of *Zea mays* (Hodges et al., 1997). Growing under low temperatures will increase ROS that may damage to membrane lipids, proteins and nucleic acids and result in cell death (Apel and Hirt, 2004).

ROS production in plants at a definite threshold is accounted for their adaptation to biotic and abiotic stresses (Dat et al., 2009). But high level of them will result in oxidative stress. Antioxidant enzymes protect cells against oxidative stress. Catalase and peroxidase are two antioxidant enzymes which have an important role in decomposition of H_2O_2 in the cells (Rahnama et al., 2011). Peroxidases generally catalysis reaction between H_2O_2 as an electron acceptor and many substrates such as phenols, ascorbic acid and aromatic amins; result in detoxifying ROS (Smirnoff and Wheeler, 2000).

Catalase and ascorbate peroxidase activity may induce chilling tolerance in early development stage in corn (Hodges et al., 1997). Also Aroca et al., (2001) showed that antioxidant activity increased in chilling resistant cultivar of maize when plants were growing under low temperature, but chilling sensitive plants did not show such results. Enzymes such as CAT, POD and SOD act against ROS in the cells and detoxify them. On the other hand, the production of the ROS causes an increase in the production and activity of these enzymes (Dat et al., 2009).

Partially hydrating seeds for a period of time followed by dehydration (seed priming) can accelerate their germination when they are subsequently planted (Bewley et al., 2013). Primed seed generally have more uniform germination and result in better seedling establishment, more power of competition with weeds, better performance under stress condition and finally more yield (Clark et al., 2004).

Priming and hormonal priming are useful for germination and establishment of many plants under normal and especially stressful conditions (Eisvand et al., 2010). Application of spermine as seed priming improved maize chilling tolerance under cold conditions at germination and seedling stage (Saeidnejad et al., 2012).

It is well known that GA_3 plays a positive role in germination (Bewley et al., 2013). In addition GA_3 affects antioxidant enzymes via its effects on mineral uptake that are necessary cofactors for these enzymes (Panou-philtheou et al., 2002). Salicylic acid is a phenolic compound that acts as a plant growth regulator (Arteca, 1995). Also it is known as an important signal molecule in response of plants to environmental stresses (Senaratna et al., 2000). This plant hormone is water soluble and acts as a primary substrate in detoxifying cycle of hydrogen peroxide (Beltagi, 2008). Decrease in photosynthesis pigments and protein content are reported under low temperature in rice seedling. But seed priming with salicylic acid mitigated the chilling stress and improved photosynthetic pigments and protein content (Pouramir-Dashtmian et al., 2014).

The aim of this study was to evaluate the effects of priming with GA_3, salicylic acid and ascorbic acid on emergence percent and rate, dry matter accumulation in shoot and root parts of seedling in maize under low temperature stress. Also, the effects of applied treatments on two important antioxidant enzymes i.e. CAT and POD activity and relationship between seedling performance and enzymes activity were evaluated.

Materials and Methods

Seeds of maize (*Zea maize* L. Single Cross 704) were prepared from Moghan Agro Industry, Iran. Seed viability and moisture content were 94% and 12.5% respectively.

Seed treatments were 200, 300 and 400 ppm of GA3; 100, 200 and 300 ppm of salicylic acid, 100, 200 and 300 ppm of ascorbic acid and hydropriming. Each of these treatments was prepared by dissolving related material distilled water; the zero concentration of hormone considered as hydropriming. Seeds were primed in the priming solutions in dark conditions for 12 h at 20±2 °C and dried at 25±2 °C until their moisture content reach to the original level.

The primed seeds and non primed seeds (control) were planted in pots with 20 cm diameter and 30 cm height. Twenty five seeds planted in each pot. There were two pots per treatment. Three replications were allocated to each treatment. Then pots were transferred into a germinator at 14 °C and 12-12 h photoperiod. The pots were arranged in germinator as a

completely randomized design. The experiment period was longed 32 days.

The number of emerged seedling was recorded daily. A seedling was considered emerged when the first leaf appear from the soil. Seedling emergence was calculated according to the number of total planted seeds for each treatment and is reported in the text as percent. Emergence rate was calculated by the following formula (Agrawal et al., 2004); where n_i is number of emerged seedling in day *i* and *Di* is the day after planting.

$$\text{Emergence rate (seedling/day)} = \sum_{1}^{j} \frac{n_i}{Di}$$

Chlorophyll index was measured using SPAD instrument (Minolta Company, Japan). The measuring of chlorophyll index was done four times and their mean was used for data analysis.At the end of the experiment (32 d after sowing), ten seedlings were selected randomly in each pot. Root and shoot were separately oven-dried at 75 °C for 24 h. Dry weights of each sample were measured using a digital balance.

CAT and POD assay

Samples (2g) of fresh leaf and root were separately collected at the 25 d after sowing and kept at -80 °C. The samples were frozen by liquid nitrogen and powdered by pistil and mortar. 1.5ml of extraction buffer was added and tubes were centrifuged (15000 rpm for 20min). The supernatant was transferred to new 2ml tubes and kept at -15 °C. All of the extractions were done in a cool room at about 5 °C.

The extraction buffer for both antioxidant enzymes (CAT and POD) consisted of 0.1 M TRIS-HCl (pH 7.5), 0.23 M sucrose, 20% PVP (polyvinylpyrrolidone), 4 mM β-mercaptoethanol, 1 mM EDTA, 10 mM KCl, 10 mM MgCl2, and 5.1 mM Ascorbic acid. Also 2 g PEG 4000 and 100 µl Triton x-100 were added to total 200 ml buffer (Zaka *et al*., 2002). CAT activity was measured by spectrophotometer (MAPADA 1800 UV/Vis model, China) at 240nm (Pinhero et al., 1997) and POD at 510nm (Futami, 1990) at 25±2 °C. Enzyme activity was recorded as optical density (OD). All determinations were obtained from triple measurements on each sample.

Statistical analysis

Data were tested for normality and analyzed (ANOVA) by MSTATC software as completely randomized design. Duncan multiple range test was used for mean comparisons. Graphs were drawn by Excel software.

Results

Emergence percentage

The effect of priming was significant on seedling emergence (P≤0.05; Table 1). Ascorbic acid 100ppm and salicylic acid 100ppm significantly improved the emergence in comparison to hydro primed and also non-primed seeds. The lowest seedling emergence was observed in non-primed seed (Table 2).

Emergence rate

Emergence rate was affected by seed priming treatments (P≤0.01; Table 1). The highest rate of emergence was observed in seeds which

Table 1

One-way ANOVA for some maize seedling characters affected by priming with GA_3 , salicylic acid and ascorbic acid

		(MS)								
S.O.V	df	Emergence percentage	Rate of emergence	Ch. index	Shoot dry weight	Root dry weight	Root CAT activity	Leaf CAT activity	Root POD activity	Leaf POD activity
Seed priming	10	0.012*	0.004*	0.341**	0.003**	0.001**	0.084**	0.037**	0.10**	0.094**
Error	22	0.001	0.0001	0.017	0.0002	0.0001	0.046	0.007	0.001	0.006
Total	32									
CV(%)		9.48	8.45	5.14	9.82	5.99	7.62	5.56	8.34	10.27

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

Table 2
The effect of seed priming treatments on some maize seedling characters grown under low temperature (14 °C).

Priming treatments	Seedling emergence (%)	Emergence Rate (seedling/day)	Chlorophyll index	Shoot dry weight (g/seedling)	Root dry weight (g/seedling)
GA_3 200ppm	24 de*	0.21 b	2.81 abcd	0.14 a	0.25 bcd
GA_3 300ppm	27 bcd	0.15 de	2.36 cde	0.11 bc	0.26 b
GA_3 400ppm	19 e	0.18 c	2.52 bcd	0.09 d	0.22 e
SA 100ppm	32 ab	0.23 a	3.12 a	0.13 ab	0.28 a
SA 200ppm	23 de	0.14 e	2.30 de	0.09 d	0.24 cd
SA 300ppm	24 de	0.11 f	1.92 e	0.093 cd	0.24 cd
AS 100ppm	35 a	0.21 b	2.85 abc	0.14 a	0.29 a
AS 200ppm	21 de	0.16 cd	2.42 bcd	0.093 cd	0.23 de
AS 300ppm	30 abc	0.17 c	2.53 bcd	0.12 b	0.28 a
Hydropriming	25 cd	0.21 b	2.91 ab	0.12 b	0.25 bc
Non-primed (control)	12 f	0.16 cd	2.44 bcd	0.08 d	0.17 f
LSD	0.053	0.017	0.4540	0.0170	0.0169

*Averages with different letters are different at 5% of probability according to Duncan's multiple range test.

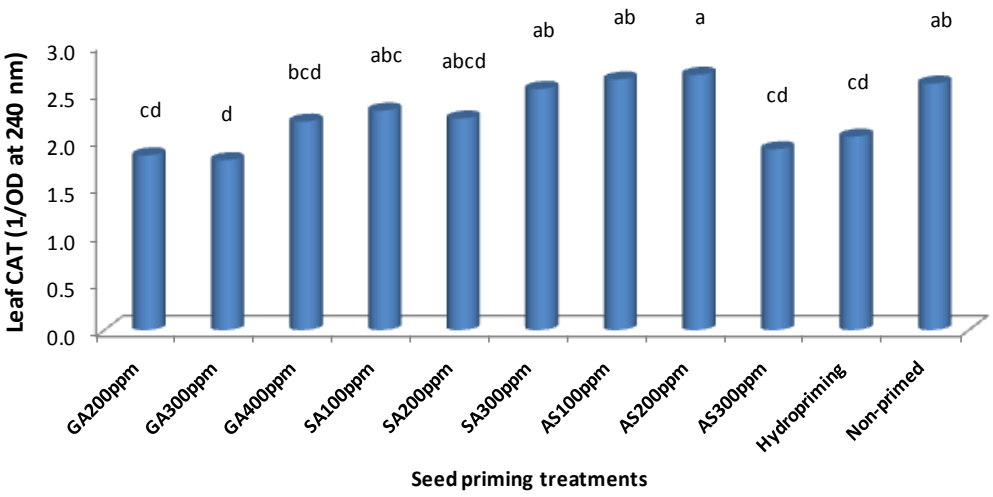

Fig. I. The effect of seed priming on leaf CAT activity in maize seedling at low temperature conditions (14°C).

were primed with 100ppm of salicylic acid. High concentration of SA decreased the rate of emergence, so the lowest of it was observed in seeds which were primed with 300ppm of salicylic acid (Table 2).

Chlorophyll index

The effect of seed priming was significant on chlorophyll index (P≤0.01; Table 1). The highest chlorophyll index was obtained by salicylic acid 100ppm (Table 2). But there was no significant difference between hydro priming treatment and non-primed seed in relation to chlorophyll index (Table 2).

Shoot dry weight

The effect of seed priming was significant on shoot dry weight (P≤0.01; Table 1). The highest shoot dry weight was observed in GA_3 200ppm and also ascorbic acid 100ppm. The lowest shoot dry weight observed in GA_3 400 pm, acid salicylic 200ppm and the non primed seeds (Table 2).

Root dry weight

The effect of seed priming was significant on root dry weight (P≤0.01; Table 1). The most effective treatments on increasing root dry weight were salicylic acid 100 ppm, and ascorbic acid 100ppm and 300 ppm. The lowest root dry weight was observed in non-primed seed (Table 2).

Leaf CAT activity

The effect of seed priming was significant on the leaf CAT activity (P≤0.01; Table 1). Hydropriming, GA_3 (200 and 300ppm) and AA 300ppm decreased leaf CAT activity in comparison with non-primed seed. The other treatments had not significant difference with the non-primed seed (Fig I). But AA significantly increased the leaf CAT activity in comparison with hydropriming (Fig I).

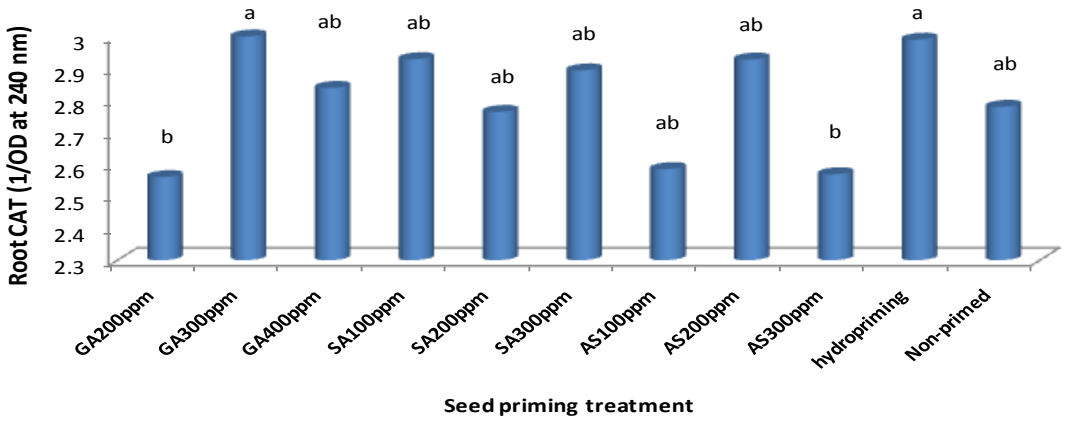

Fig. II. The effect of seed priming on root CAT activity in maize seedling at low temperature conditions (14°C).

Root CAT activity

The seed priming had significant effect on root CAT activity (P≤0.01; Table 1). Hydro priming had no significant effect on root CAT activity. In comparison to hydro priming and other priming treatments, GA_3 200ppm and also ascorbic acid 300ppm decreased root CAT activity (Fig.II).

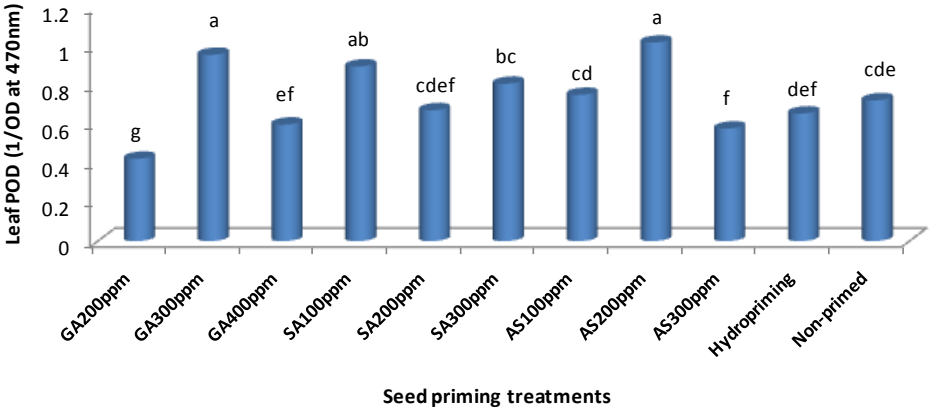

Fig. III. The effect of seed priming on leaf POD activity in maize seedling at low temperature conditions (14°C).

Leaf POD

Seed priming significantly affected leaf POD (P≤0.01; Table 1). The high leaf POD activity were observed in 300ppm of GA_3 and also 200ppm of ascorbic acid; however the lowest was observed in 200ppm of GA_3 (Fig.III).

Root POD activity

Seed priming significantly affected root POD activity (P≤0.01; Table 1). GA3 priming at 300ppm concentration significantly increased Root POD activity; meanwhile except hydropriming, other treatments had not significant effects (Fig. IV).

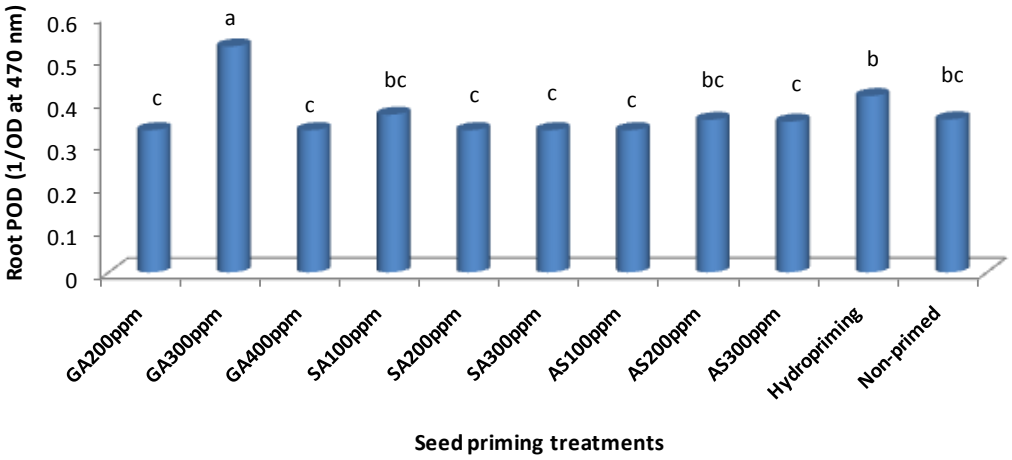

Fig. IV. The effect of seed priming on root POD activity in maize seedling at low temperature conditions (14°C).

Correlations

The rate of emergence and chlorophyll index had positive correlation with the seedling emergence. However root CAT activity and seedling emergence was negatively correlated. There was no significant correlation with emergence and leaf CAT, leaf POD and root POD activities (Table 3). Chlorophyll index, root and shoot dry weights were positively correlated with the rate of emergence. But the enzymes activity had no correlation with the rate of emergence. Chlorophyll index had a positive correlation with root and shoot dry weights and there was no significant correlation between it and CAT and POD activity.

Shoot dry weight was positively correlated with seedling emergence, rate of emergence, chlorophyll index and root dry weight but its correlation with leaf and root CAT activity were negative (Table 3). There was no correlation between shoot dry weight and leaf and root POD activities. Root dry weight had a negative correlation with root CAT activity.

There was no significant correlation between leaf and root CAT activities. But there was a positive correlation between POD activity in leaf and root (P≤0.05; Table 3). Also there was a positive correlation between the CAT and POD activities in leaf (P≤0.05; Table 3); however in the root, these enzymes had no significant correlation with each other. Leaf CAT activity and root POD activity were negatively correlated (Table 3).

Discussion

In spite of high seed viability (95%), seed and seedling performance were low under chilling stress (14 °C). This reflects the damage of chilling temperature because this temperature is not suitable for maize germination and seedling emergence as a warm season crop. Growth under low temperatures may increase ROS which have deteriorative effects via oxidative stress on cellular structure and cell metabolism (Wang et al., 2009). Li et al., (2011) reported that chilling stress at +1 °C for 5 days leads to 70% of maize seedlings mortality. Also low temperature (12 and 8 °C) stress at germination and seedling stages has harmful effects on seed and seedling performance and physiological processes in maize (Hola et al., 2003,

Table 3
Pearson's correlation coefficients between some maize seedling characters affected by priming and growing at low temperature (14 °C).

	Emergence	Emergence Rate	Chlorophyll index	Shoot DW	Root DW	Leaf CAT	Root CAT	Leaf POD	Root POD
Emergence	1								
Emergence Rate	0.979**	1							
Chlorophyll index	0.429*	0.979**	1						
Shoot dw	0.780**	0.644**	0.635**	1					
Root dw	0.954**	0.393*	0.411**	0.785**	1				
Leaf CAT	-0.241^{ns}	-0.175^{ns}	-0.208^{ns}	-0.356*	-0.264^{ns}	1			
Root CAT	-0.352*	-0.333^{ns}	-0.300^{ns}	-0.516**	-0.360*	0.198^{ns}	1		
Leaf POD	0.088^{ns}	-0.257^{ns}	-0.196^{ns}	-0.197^{ns}	0.039^{ns}	0.384*	0.421*	1	
Root POD	0.113^{ns}	-0.089^{ns}	0.005^{ns}	0.072^{ns}	0.149^{ns}	-0.345*	0.265^{ns}	0.390*	1

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

Saeidnejad et al., 2012). So low seedling emergence and totally seedling performance under low temperature (14 °C) is consistent with similar works that are mentioned above and in the introduction.

Hydropriming, and priming with GA3, SA and AA could improve seedling emergence and emergence rate. It was interesting that AA and SA were more effective on emergence than GA_3. This implies that emergence may be related not only to GA_3, but also to potential of coleoptiles emergence from soil. The defense mechanisms which are affected by AA and SA have an important role in seedling emergence. Ijaz et al., (2012) reported that maize seed priming with ascorbic acid, salicylic acid and hydrogen peroxide improved germination rate and help to seedling growth under low temperature.

The SA was able to mitigate the negative effect of chilling stress on chlorophyll index. It can be concluded that, SA via improving the seedling antioxidant system decreased the ROS negative effect on photosynthesis pigments. Salicylic acid is a growth regulator that affects several processes in plants including germination, ion uptake, membrane permeability, photosynthesis, antioxidant activity, signal transduction and also protective effects for many environmental stresses (Pouramir et al., 2014). Similarly, Türkyılmaz et al. (2005) mentioned that in the field condition SA solution spray improved photosynthesis pigments (chlorophyll a, b and carotenoid) concentration in bean (*Phaseolus vulgaris* L.).

Salicylic acid mitigates the impacts of stresses probably via increasing some phytohormones such as auxins and cytokinins. Also salicylic acid may induce resistance to environmental stress in plants by effect on ethylene biosynthesis (Sharikova et al., 2003). It has been found that SA plays a role during the plant response to abiotic stresses such as chilling (Rivas-San Vicente and Plasencia, 2011). As low temperature may cause oxidative stress, salicylic acid can act as electron donor for the CAT and POD (Smirnoff, 2000; Doulatabadian et al., 2008) and help to scavenging process of O_2 and H_2O_2.

Using the hormones (GA_3, SA) and vitamin (AA) in priming solution increased seedling shoot and root dry matters. This may due to this virtue that these are growth stimulant (GA_3) and stress tolerance inducer substances in plants (SA and AA) which at different specific concentration mitigate chilling stress. GA3 can promote cell division and elongation (Arteca, 1995). Antioxidant enzymes activity under chilling stress is correlated with chilling tolerance (Cui and Zhou, 2013).Wang and Li (2006) concluded that exogenous application of SA led to chilling resistance via increase in antioxidant activity and also improved physiological and biochemical processes in grape (Wang and Li, 2006). Farooq et al. (2008) also showed that maize seed

priming by SA solution improved germination characteristics and seedling root and shoot growth under both normal and low temperature conditions. They mentioned that the main reason of increase in maize seedling growth under chilling stress condition was activation of antioxidant enzymes system, including CAT, SOD and ascourbate peroxides.

Ascorbate is an antioxidant and, in association with other components of the antioxidant system, protects plants against oxidative damage. There is evidence for a key role of the ascorbate-glutathione cycle in protecting plants against oxidative stress. Ascorbate is also a cofactor for violaxanthin de-epoxidase. This enzyme links ascorbate to the photoprotective xanthophyll cycle. A role in regulating photosynthetic electron transport has been proposed. Ascorbate has also been implicated in regulation of cell division by influencing the progression from G1 to S phase of the cell cycle (Smirnoff, 1996).

SA is perceived by NPR1, as a transcriptional activator that regulates gene expression that might have a role in seed germination, flowering, and/or senescence regulation. In addition, SA is a key regulator of plant cell redox status by inhibiting catalase and peroxidase activity, and thus modulating ROS levels. The positive effect of SA on photosynthesis contributes to electron acceptor availability and redox status (Rivas-San Vicente and Plasencia, 2011). Rajjou et al., (2006) reported that two superoxide dismutases are induced by SA in Arabidopsis germinating seeds, which might contribute to an enhanced antioxidant capacity. SA treatment (0.5 mM for 24 h) also causes a strong up-regulation of translation initiation and elongation factors, proteases, and two subunits of the 20S proteasome, supporting the hypothesis that SA improves seed germination by promoting the synthesis of proteins that are essential for germination, and the mobilization or degradation of seed proteins accumulated during seed maturation (Rajjou et al., 2006).

Except root CAT activity, that had a weak negative correlation with seedling emergence ($P \leq 0.05$; Table 3), there was no significant relation between emergence and leaf CAT, leaf POD and root POD. The presence of negative correlation between shoot and root dry weight with antioxidant enzymes activity (CAT and POD) may indicate using GA3, SA and AA preventing ROS production and so oxidative stress has not been occurred. By refer to table 2, we see high dry matter is belonging to priming with GA3, SA and AS. These substances may be able to regulate metabolic process at different manners so ROS has not been produced. As we know one of reasons for ROS production is stopping growth while photosynthesis is continuing (Taiz and Zeiger, 2010).

As it was showed in the results, some of priming treatments were able to improve seed and seedling performance when temperature was below the optimum level for maize. Such treatments are recommended for region with cool spring that soil temperature usually is under the optimum level. It seems that the role of improvement of these treatments is due to priming advantages, as well as the useful role of GA_3, SA, AA in germination, growth and stress tolerance.

We suggest SA 100ppm and AA 100ppm for increasing period of growth season in regions with cool spring. However, it needs field experiments for final decision. Also we suggest further study in this case specifically in the application of the combination treatments such as AS+AA and GA_3+AS+AA.

References

Agrawal, R.L. 2004. 'Seed technology'. New Delhi: Oxford and IBH Publishing Co. Ltd. 829 pages.

Apel K. and **H. Hirt.** 2004.'Reactive oxygen species: Metabolism, Oxidative Stress, and Signal Transduction'. *Annual Review of Plant Biology,* 55: 373–99.

Aroca, R., J. J. Irigoyen and **M. Sanchez-Diaz.** 2001. 'Photosynthetic characteristics and protective mechanisms against oxidative stress during chilling and subsequent recovery in two maize varieties differing in chilling sensitivity'. *Plant Science*, 161:719-726.

Arteca, R. N. 1995. 'Plant Growth Substances: Principles and Applications'. Chapman and Hall, 288 pages.

Ashraf, M. 2009. 'Biotechnological approach of improving plant salt tolerance using antioxidants as markers'. *Biotechnology Advances*, 27: 84-93.

Beltagi, M. S. 2008. 'Exogenous ascorbic acid (Vitamin C) induced an aboulic changes for salt tolerance in chick pea (*Cicer arietinum* L.)'. *African Journal of Plant Science* ,2(10):118-123.

Bewley, J.D., K. Bradford, H.W.M. Hilhorst and **H. Nonogaki.** 2013. 'Seeds: Physiology of Development, Germination and Dormancy', 3rd Edition, New York, Springer, 392 pages.

Clark, L.J, W.E. Finch-Savage and **K.C. Dent.** 2004. 'Soak conditions and temperature following sowing influence the response of maize (*Zea mays* L.) seed to on-farm priming (pre-sowing seed soak)'. *Field Crop Research* 90: 361-374.

Cui, C. and **Q.Y. Zhou.** 2013.' Antioxidant enzyme and morphological characteristics of roots of three *Nicotiana tabacum* L. genotype seedlings under chilling stress'. *African Journal of Agriculture Research* ,8(1):64-69.

Dat, J., S. Vandenabeele, E. Vranova, M. Van Mmontago, D. Inze and **F. Van Breusegem.** 2009. 'Dual action of active oxygen species during plant stress responses. *Cellular Molecular Life Science,* 57:779-795.

Eisvand, H.R., R. Tavakkol-Afshari, F. Sharifzadeh, H. Maddah Arefi and **M. Hesamzadeh Hejazi.** 2010.' Effects of hormonal priming and drought stress on activity and isozyme profiles of antioxidant enzymes in deteriorated seed of tall wheatgrass (*Agropyron elongatum* Host)'. *Seed Science and Technology,* 38:280-297

Doulatabadian, A., S.A.M. Modarres Sanavy and **F. Etemadi.** 2008. 'Effect of pre-treatment of salicylic acid on Wheat (*Triticum aestivum* L.) seed germination under salt stress'. *Iranian Journal of Biology*,21(4):692-702.

FAO. 2012. 'Food and agriculture organization year book, world food and agriculture'. http://www.fao.org/docrep/015/i2490e/i2490e00.htm.

Farooq, M., T. Aziz, S.M.A. Basra, M.A. Cheema and **H. Rehman.** 2008.' Chilling tolerance in hybrid maize induced by seed priming with salicylic acid'. *Journal of Agronomy Crop Science* ,194(2):161-168.

Futami, K. 1990.' Method of peroxidase activity'. In methods of plant nutrition analysis, 50-52, Hakuyu-sha,Tokyo, Japan.

Li, H.Y., C.G. Li. and **M. Gong.** 2011.' Short-term cold-shock at 1 °C induced chilling tolerance in maize seedlings'. *International Conference on Biology, Environment and Chemistry*, 1:346–349.

Hodges, D.M., C.J. Andrews, D.A. Johnson and **R.I. Hamilton. 1997.** 'Antioxidant enzyme responses to chilling stress in differentially sensitive inbred maize lines'. *Journal of Experimental Botany* ,48:1105-1113.

Hola, D., K. Langrova, M. Kocova and **O. Rothova.** 2003. 'Photosynthetic parameters of maize (*Zea mays* L.) inbred line and F-1 hybrids: their different response to, and recovery from rapid or gradual onset of low-temperature stress'. *Photosynthetica* ,41: 429-442.

Ijaz, A., K. Tasneem, A. Ashfaq, S.M.A. Basra, H. Zuhair and **A. Amjed. 2012.'** Effect of seed priming with ascorbic acid, salicylic acid and hydrogen peroxide on emergence, vigor and antioxidant activities of maize'. *Journal of Biotechnology* 11(5):1127-1137.

Panou-philtheou, H., M. Koukourikiu-petridou, A. Bosabalidis and **S. Kavataglis.** 2002. 'Relation of endogenous and applied gibberellins to growth and accumulation of essential elements in oregano plants grown in copper rich soils'. *Advances in Horticulture Science,* 16:63-71.

Parera, .CA. and **D.J. Cantliffe.** 1994. 'Presowing seed priming'. *Horticulture Review,* 16(16):109-141.

Pinhero, R.G., M.V. Rao, G. Paliyath, D.P. Murr and **R.A. Fletcher.** 1997.' Changes in activities of antioxidant enzymes and their relationship to genetic and paclobutrazol induced chilling tolerance of maize seedlings'. *Plant Physiology* 114: 695-704.

Pouramir-Dashtmian, F., M. Khajeh-Hosseini and **M. Esfahani.** 2014. 'mproving rice seedling physiological and biochemical processes under low temperature by seed priming with salicylic acid'. *International Journal of Plant, Animal and Environmental Science* ,4(2):565-572.

Prasad, T.K. 1996.' Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids, and protease activities'. *The Plant Journal* , 10:1017–1026.

Prasad, T.K. 1997.'Role of catalase in inducing chilling tolerance in pre-emergence maize seedlings'. *Plant Physiology*, 114:1369–1376.

Rahnama, A., K. Poustini, R. Tavakol Afshari and **A. Rasoulnia.** 2011. 'Study of antioxidant enzymes and lipid peroxidation in flag leaf of sensitive and tolerant wheat (*Triticum aestivum* L.) cultivars to salt stress'. *Iran Journal of Field Crop Science* 42(2): 359-371.

Rajjou, L. and **M. Belghazi** 2006. 'Proteomic investigation of the effect of salicylic acid on Arabidopsis seed germination and establishment of early defense mechanisms'. *Plant Physiology* , 141:910–923.

Rivas-San Vicente, M. and **J. Plasencia.** 2011. 'Salicylic acid beyond defence: its role in plant growth and development'. *Journal of Experimental Botany,* 62(10): 3321–3338.

Saeidnejad, A.M., F. Pouramir and **M. Naghizadeh.** 2012.' Improving chilling tolerance of maize seedling under cold conditions by spermine application'. *Notulae Scientia Biolgica,* 4(3): 110-117.

Senaratna T., D. Touchel, E. Bumm and **K. Dixon.** 2000. 'Acetyl salicylic acid induces multiple stress tolerance in bean and tomato plants'. *Plant Growth Regulation,* 30:157-161.

Smirnoff, N. 1996.' The function and metabolism of ascorbic acid in plant'. *Annual of Botany*,78: 661-667.

Smirnoff, N. and **G.L. Wheeler.** 2000. 'Ascorbic acid in plants: biosynthesis and function'. *CRC Critical Review Plant Science* ,19**:**267-290.

Taiz, L. and **E. Zeiger.** 2010. 'Plant physiology. 5th edition'. Sinauer Associates, Inc. Publishers, Sunderland, MA. 782 pages.

Türkyılmaz, B., L.Y. Aktaş and **A. AGüven.** 2005. 'Salicylic acid induced some biochemical and physiological changes in *Phaseolus vulgaris* L.' *Journal Agricultural Science* 17: 319-326.

Wang, L.J. and **S.H. Li.** 2006.' Salicylic acid-induced heat or cold tolerance in relation to Ca^{2+} homeostasis and antioxidant systems in young grape plants'. *Plant Science* 170:685-694.

Wang, W.B., Y.H. Kim, H.S. Lee, X.P. Deng and **S.S. Kwak.** 2009. 'Differential antioxidation activities in two alfalfa cultivars under chilling stress'. *Plant Biotechnology Reports* ,3:301-307.

Zaka, R., C.M. Vandecasteele and **M.T. Misset.** 2002. 'Effects of low chronic doses of ionizing radiation on antioxidant enzymes and G_6PDH activities in *Stipa capillata* (Poaceae)'. *Journal of Experimental Botany* ,53: 1979-1987.