Reducing destructive effects of drought stress on cucumber through seed priming with silicic acid, pyridoxine, and ascorbic acid along with foliar spraying with silicic acid

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Summary

Cucumber is considered as a drought-sensitive plant so that a decrease in the soil moisture causes decreased yield and quality of cucumber. This study investigates the effects of seed priming and foliar application with silicic acid on biochemical traits of cucumber (*Cucumis sativus* L.) under drought stress through a split-split plot experiment with three replications. The main plot was allocated to different levels of drought stress including moderate drought stress (80-85% Field Capacity (FC)), severe drought stress (60-65% FC), and without stress – control (90-95% FC). The sub-plot was allocated to seed priming treatments at three levels: control (hydro-priming), ascorbic acid 150 mgL^{-1}, and pyridoxine 0.04%. The sub-sub plot was assigned to foliar spraying with silicic acid at three levels: 0, 100, and 200 mgL^{-1}. The results obtained from the evaluation of all traits showed that under free-stress condition, the best seed priming treatment belonged to pyridoxine 0.04% alone or along with foliar spraying of silicic acid at 100 mgL^{-1}. In moderate drought stress, the best seed priming treatment belonged to pyridoxine 0.04% and foliar spraying with silicic acid at 200 mgL^{-1}, and under severe drought stress, the best seed priming treatment belonged to pyridoxine 0.04% or ascorbic acid at 150 mgL^{-1}.

Key words

chlorophyll, antioxidant enzymes, seed priming, impress cultivar, drought stress

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INTRODUCTION

Cucumber (*Cucumis sativus* L.), as an important vegetable belonging to the family of Cucurbitaceae, is regarded to have great economic and dietary importance (Salunkhe and Kadam, 1998). Considering abiotic stresses, drought is among the major problems for producing successful yields in Iran and elsewhere in the world. It leads to the production of harmful products and disturbs the balance of forming Reactive Oxygen Species (ROS), (Arora et al., 2002).

Drought stress-induced free radicals cause lipid peroxidation and membrane deterioration in plants (Nair et al, 2008). Drought stress leads to an imbalance between antioxidant defenses and the amount of Reactive Oxygen Species (ROS) resulting in oxidative stress. ROS are necessary for intracellular signaling but at high concentration can cause damage at various levels of the organization including chloroplasts (Smirnoff, 1993). ROS have the capacity to initiate lipid peroxidation and degrade proteins, lipids and nucleic acids (Hendry, 2005). The mechanism of retardation of lipid peroxidation consists of free radical scavenging enzymes such as catalase, peroxidase, and superoxide dismutase (Fridovich et al., 2000). Several enzymatic and nonenzymatic antioxidants are present in chloroplasts that serve to prevent ROS accumulation (Srivalli et al., 2003). The underwater stress, the formation of ROS increased and the antioxidant system protect the cell by controlling the intracellular ROS concentration. One of the expected consequences of water stress-induced cellular buildup of ROS is an increase in lipid peroxidation. The peroxidation of lipids in the cell membrane is one of the most damaging cellular responses observed in response to water stress (Thankamani et al., 2003). The amount of lipid peroxidation has also long been considered as one of the factors indicating the severity of stress experienced by a plant (Chowdhury and Chowdhury, 1985).

Increasing the germination rate is of great importance in improving plant establishment. The use of priming methods is an approach to increase germination and seed emergence under stress conditions (Murungu, et al., 2003).

Silicium (Si) is one of the beneficial nutrients influencing the growth and health of plants (Cherif and Belanger, 1992). This element can increase production and quality of the product, reducing evaporation and transpiration, increasing resistance to salinity stress, drought stress, and toxicity of heavy metals, increasing production of some antioxidant enzymes and also decreasing the susceptibility to some fungal diseases in plants like cucumber (Zhu et al., 2004). Reduced oxidative damage due to the addition of Si under saline conditions has also been reported in barley (Liang et al., 2005) and cucumber (Zhu et al., 2004). Si increases the activity of anti-oxidative enzymes, thus mitigating the stress and improving the growth of the plants (Al-Aghabary et al. 2004). It is suggested that Si may affect membrane lipid composition and maintenance of optimal membrane fluidity in stressed plants (Zhu et al., 2004).

Pyridoxine (Vitamin B6) is involved in cell defense against oxidative stress caused by the production of reactive oxygen radicals and acts as an antioxidant. This vitamin protects the plant against non-biological stresses (Denslow et al., 2007). Sadeghi et al. (2013) in their study showed a significant effect of different levels of pyridoxine on the percentage of germination, dry weight of the plant, length of shoot and root, and amount of CAT enzyme activity in watermelon. They found that priming of seed by the pyridoxine could help the farmers in setting and producing suitable watermelon under drought stress. External application of ascorbic acid can increase stress resistance and reduce the effect of oxidative stress (Shalata and Newman, 2001).

Ascorbic acid increases the growth of plants tissues under stress conditions and elevates the number of the plants that can survive under these conditions (such as high salinity, dryness, etc.) (Gill and Tuteja, 2010).

Antioxidants act as electron donors, reducing ROS to less harmful molecules; the oxidized products formed in the process are not very reactive or harmful. Purely enzymatic defenses include a variety of scavengers (Racchi, 2013). Each antioxidant enzyme plays an important role in controlling physiological activities of the plants. Previous studies showed that major antioxidant enzymes in plants including *Polyphenol Oxidase* (PPO), *Catalase* (CAT), *Superoxide Dismutase* (SOD) and *ascorbate peroxidase* (APX) play important roles in regulating plant defense mechanisms against biotic and abiotic stresses (Wang, et al. 2009).

Superoxide dismutase enzyme is the first reactive oxygen species (ROS) destructive enzyme. It converts superoxide (O_2) to hydrogen peroxide (H_2O_2). The hydrogen peroxide is also eliminated by ascorbate peroxidase (Gill et al., 2013). SOD enzyme plays a protective role in the plants; SOD has been shown to stabilize the membrane of plant cells under dryness (Jose et al., 1999).

APX can be a good intracellular regulator and controller for maintaining ROS balance (Mittler, 2002). Peroxidase and Polyphenol Oxidase (PPO) are oxidative enzymes, involved in the production and use of reactive oxygen, formation of lignin, and other oxidized phenols as defense dams for strengthening of cell structure (Chen et al., 2000).

Accordingly, the present study aims to assess the effects of seed priming with pyridoxine, ascorbic acid, and silicic acid and foliar spraying with silicic acid on field cucumber under drought stress condition and the effect of the used treatments on the antioxidant system. Another goal of this research is to evaluate the antioxidant system.

MATERIALS AND METHODS

An experiment was conducted to investigate the effects of seed priming and foliar spraying with silicic acid on the activity of some enzymes in Impress cultivar of field cucumber under drought stress conditions. The experiment was done from late winter (mid-March) to late spring (mid-June) during 2016-2017 at the Research Farm of Department of Horticultural Sciences, Shahid Chamran University of Ahvaz (Khuzestan, Iran). The experiment was done as a split-split plot with three replications (8 plants per replicate).

Treatment and Priming

The main plot was allocated to different levels of drought stress including severe drought stress (60-65% FC), moderate drought stress (80-85% of Field Capacity (FC)), and without stress (control level), (90-95% FC).

Sub-plot was allocated to the priming of seed at three levels: control (hydro priming), ascorbic acid (AsA) at 150 mgL^{-1}, and pyridoxine 0.04%.

The sub-sub plot was assigned to foliar spraying with silicic acid (SiA) at 3 levels: 0, 100, and 200 mgL^{-1}. The seeds were treated for 16 hours at 25 °C with above-mentioned seed priming treatments and then cultivated directly to the main field.

Method of Planting

After preparation of the field (plowing and adding NPK fertilizer), the seeds were directly sown in the field, and furrowing was applied as a planting system. The width of the barrows was equal to 100 cm, and the distance between the seeds was equal to 50 cm, and they were planted as heap. After germination, numbers of germinated seeds were counted, and thinning was performed.

Evaluated Attributes and Method of Quantification

The activity of antioxidant enzymes in leaves and roots was measured at 8-leaf stage, and contents of *Malondialdehyde*, *Proline*, and total chlorophyll in leaves were also measured.

Quantification of the Antioxidant Enzymes Activity

To prepare the enzyme extract, 0.5 g (fresh weight) of leaves and for the roots sample powder was poured into microtube, 1.5 ml of phosphate buffer 0.1 M (pH=7) was added to the sample (At 18,000 rpm at 4 °C for 15 minutes) and centrifuged in a centrifuge equipped with a refrigerator (Sigma 2-16KL). After that the supernatant was used as an extract to measure enzyme activity.

Superoxide Dismutase Activity Quantification

The activity of SOD was measured according to the method proposed by Gianopolitis and Rice (1977), (Jinbo Li, 2019) from fresh leaf and root samples in micromoles per gram. The final reaction mixture was 3 ml containing 50 mM phosphate buffer. After exposing the cells containing the final mixture to a fluorescent lamp for 10 minutes, the absorption of the reaction mixture at 560 nm was read using a spectrophotometer Variyan 5/0 model. The amount of superoxide dismutase enzyme in leaf samples in terms of micromole per gram of fresh tissue was calculated from the following equation:

$$SOD_{activity} = \frac{A_{560}(control) - A_{560}(sample)}{A_{560}(control)} \quad (1)$$

where A_{560} (*control*) and A_{560} (*control*) are the values of light absorption of the control solution and the studied sample at 560 nm, respectively.

Ascorbate Peroxidase Activity Quantification

The activity of this enzyme was measured according to the method of Nakano and Asada (1981), and light absorption was read twice at 290 nm at 2-minute intervals. Ascorbate peroxidase enzyme levels were calculated based on µM of Ascorbate in mg of protein per minute.

Polyphenol Oxidase Activity Quantification

The measurement of this enzyme was done according to the method of Soliva et al (2001), and its absorbance was read at 410 nm by spectrophotometer (Variyan 5/0). The amount of PPO was calculated in terms of absorbance changes per mL of the enzyme extract.

Catalase Activity Quantification

The activity of this enzyme was measured based on the method introduced by Aebi (1983), and its absorbance was read twice at 240 nm by spectrophotometer (Variyan 5/0) at 2-minute intervals. It was measured based on mM of hydrogen peroxide in mg of protein per minute.

Proline Quantification

Bates et al. (1973) method was used to extract and measure proline level. Spectrophotometric absorption of the samples was measured at 520 nm by spectrophotometer (Variyan 5/0), and was quantified in micromoles per gram of leaf.

Malondialdehyde Quantification

MDA was measured according to the method proposed by Zhao et al (1994). Absorption intensity was read at 450, 532, and 600 nm by spectrophotometer (Variyan 5/0). Malondialdehyde content was calculated based on nano-mole per gram of fresh weight.

Measurement of Total Chlorophyll

Chlorophyll measurement was performed by the method of Leachtander and Welbom (1983). In order to extract and measure the total chlorophyll content, 0.5 g of leaves with 10 ml of 90% acetone was well ground in a mortar. The supernatants were immediately centrifuged in closed tubes at 5000 rpm for 15 minutes. Absorption rates were read at 663 and 646 nm using a spectrophotometer (Variyan 5/0). The total chlorophyll content was calculated in mgg-1of leaf fresh weight.

Statistical Analysis

The analysis of variance of the obtained data was done using SAS 9.1 software, and the means were compared using Duncan's test at $p<0.05$. Coefficients of correlation between germination traits and enzyme activity were determined using SPSS 0.16 software.

RESULTS

The results of ANOVA presented in Table 1 showed that drought stress had a significant effect on all studied traits except for PPO in leaves and SOD in roots. Seed priming also had a significant effect on all studied traits. A significant interaction was observed between drought stress and priming in all studied traits, except PPO in leaves. Foliar spraying also had a significant effect on all traits except MDA in leaves. Moreover, there was a significant interaction between drought stress and foliar spraying in all studied traits except SOD in roots, proline, and MDA in leaves.

Table 1. Analysis of variance in regards to the evaluated biochemical parameters in the leaves and roots of field cucumber Cucumis sativus Impress

S.V	D.F	Mean Square (M.S)										
		Leaf PPO	Root PPO	Leaf APX	Root APX	Leaf CAT	Root CAT	Leaf SOD	Root SOD	Leaf proline	Leaf MDA	Total chlorophyll
Replication	2	0.18^{ns}	0.140^{ns}	14.1^{ns}	6.6ns	5.9^{ns}	1.2^{ns}	113.6ns	2.9^{ns}	5.4**	0.055^{ns}	0.31^{ns}
Drought (A)	2	0.02^{ns}	0.996**	1693.3**	298.5**	661.1**	250.0**	5422.3**	0.5^{ns}	51.4**	0.880**	5.31**
Error a	4	0.08	0.124	3.1	3.5	2.2	1.0	55.4	1.2	3.2	0.055	0.26
Priming												
(B)	2	47.32**	5.4**	30225.8**	2281.7**	14283.9**	1405.9**	32886.6**	701.0**	9735.9**	8.090**	7.47**
AB	4	0.17^{ns}	1.687**	1428.5**	59.9**	605.7**	74.7**	2011.7**	13.1**	449.1**	0.465**	5.88**
Error b	12	0.11	0.070	12.4	4.2	4.6	0.7	64.8	1.0	1.1	0.017	0.14
Silicic acid												
(C)	2	2.32**	0.870**	3548.6**	51.4**	162.2**	20.1**	4119.5**	17.4**	3.4*	0.044^{ns}	4.31**
AC	4	2.39**	1.222**	1186.0**	366.5**	276.1**	56.0**	4837.9**	3.1^{ns}	0.4^{ns}	0.149^{ns}	15.64**
BC	4	1.54**	0.398**	5396.0**	441.8**	121.8**	10.3**	1959.7**	7.6**	0.9^{ns}	0.004^{ns}	7.77**
ABC	8	2.64**	0.283**	1280.8**	416.9**	355.4**	66.9**	201.4**	19.6**	0.4^{ns}	0.082**	3.63**
Error	36	0.10	0.093	11.2	3.8	4.0	2.0	48.9	2.097	0.5	0.005	0.122
C.V %	24.3	24.9	11	11.2	6.8	16.1	4.3	9.5	24.8	1.7	1.4	

^{ns} not significant, * and ** significant at $p<0.05$ and $p<0.01$, respectively

A significant interaction was observed between seed priming and foliar spraying in all studied traits except proline and MDA in leaf. Furthermore, the interaction of three factors of drought stress, seed priming, and foliar spraying was significant in all traits except MDA in leaf (Table 1).

PPO Enzyme Activity in Leaf and Roots

The use of pyridoxine in seed priming significantly influenced PPO content in leaf, so that amount of leaf PPO increased significantly (Fig. 1). Regarding the study on PPO changes in leaf at two watering levels, namely drought level 80-85% and control level (90-95%), seed priming with pyridoxine led to an increase in the amount of this enzyme compared to non-priming or priming with ascorbic acid. However, at these watering levels, foliar spraying with different amounts of silicic acid did not significantly influence PPO activity in leaves. A similar trend was observed under 65-60% of drought stress, with the exception that the use of silicic acid treatment significantly reduced PPO in leaf (Fig.1).

Regarding the amount of PPO in roots, the use of foliar spraying with silicic acid alone or along with seed priming by pyridoxine significantly influenced this parameter. It significantly increased PPO amount (Fig.2). The study on the changes in PPO content in roots at 60-60% of drought stress showed that seed priming with pyridoxine caused an increase in the amount of this enzyme in comparison with non- priming or with the use of ascorbic acid. At drought stress of 85-80%, there was no significant difference in the PPO changes regarding the use of different priming treatments. Non- priming and foliar spraying with SiA 200 mgL^{-1} significantly reduced the amount of PPO in the root. In addition, priming with pyridoxine and foliar spraying with SiA 200 mgL^{-1} significantly increased PPO in roots (Fig. 2).

APX Enzyme Activity in Leaf and Roots

The use of pyridoxine in seed priming significantly influenced APX activity in the leaf. As a result, the amount of APX significantly increased, and the highest activity of the enzyme was observed in seed priming with pyridoxine as well as with foliar spraying with SiA 200 mgL^{-1} (Fig. 3).

A study on APX changes in leaves and roots revealed that, at all the three levels of drought stress, seed priming with pyridoxine increased the amount of this enzyme in comparison with non-priming or the use of AS. In drought stress levels of 60-65% and 80-85%, seed priming with pyridoxine along with foliar spraying of SiA 200 mgL^{-1} remarkably increased APX in the leaf, although at the control level (90-90%), seed priming with pyridoxine and foliar spraying with SiA 100 mgL^{-1} significantly reduced APX in the leaf (Fig. 3).

Regarding APX activity in roots, using pyridoxine in seed priming significantly increased the APX (Fig. 4). In control level (90-95%), seed priming with pyridoxine together with foliar spraying with SiA 200 mgL^{-1} significantly reduced APX in the root.

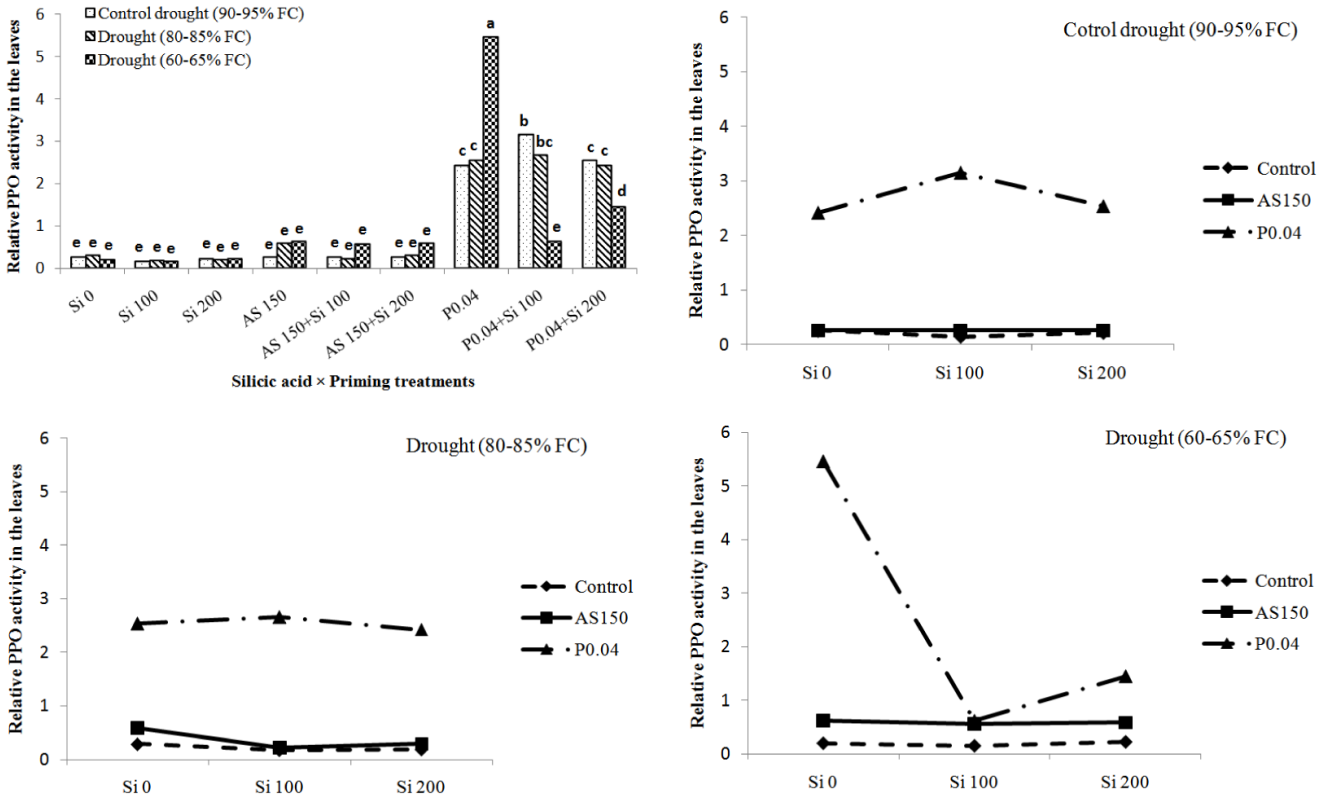

Figure 1. Leaf PPO activity in different watering levels (90-95%, 80-85% and 60-65%, FC) by changing priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%) and concentration of silicium (0, 100 and 200 mgL^{-1}) as foliar spraying

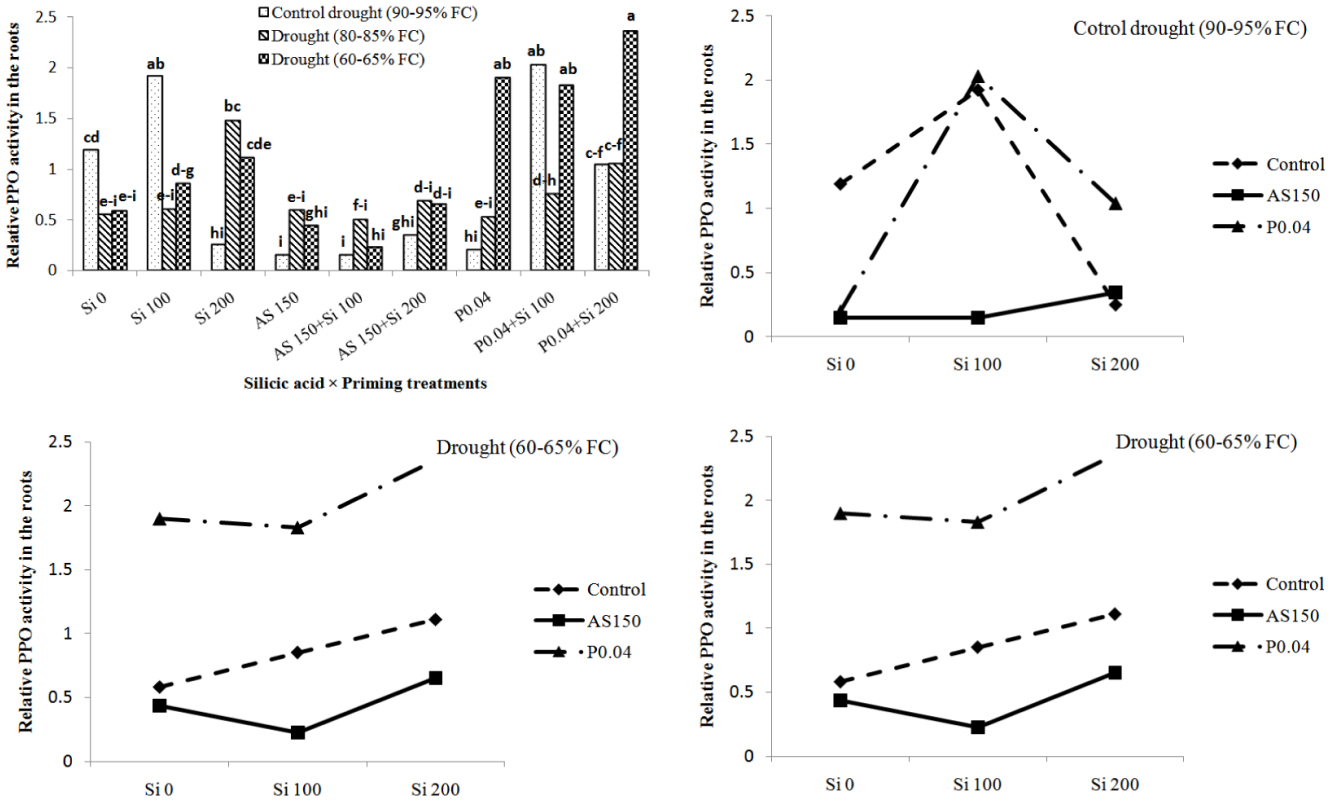

Figure 2. Root PPO activity in different watering levels (90-95%, 80-85% and 60-65%, FC) by changing priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%) and concentration of silicium (0, 100 and 200 mgL^{-1}) as foliar spraying

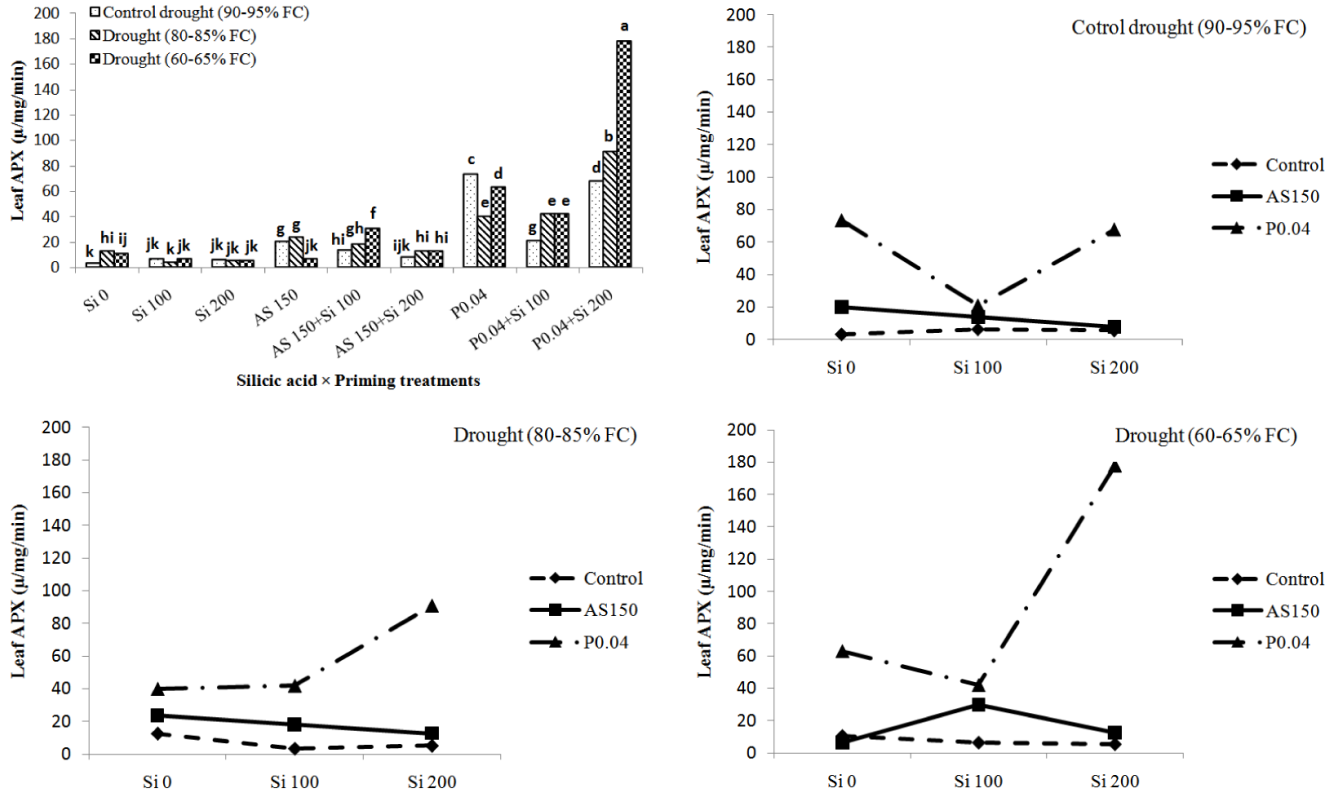

Figure 3. Leaf APX activity in different watering levels (90-95%, 80-85% and 60-65%, FC) by changing priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%) and concentration of silicium (0, 100 and 200 mgL^{-1}) as foliar spraying

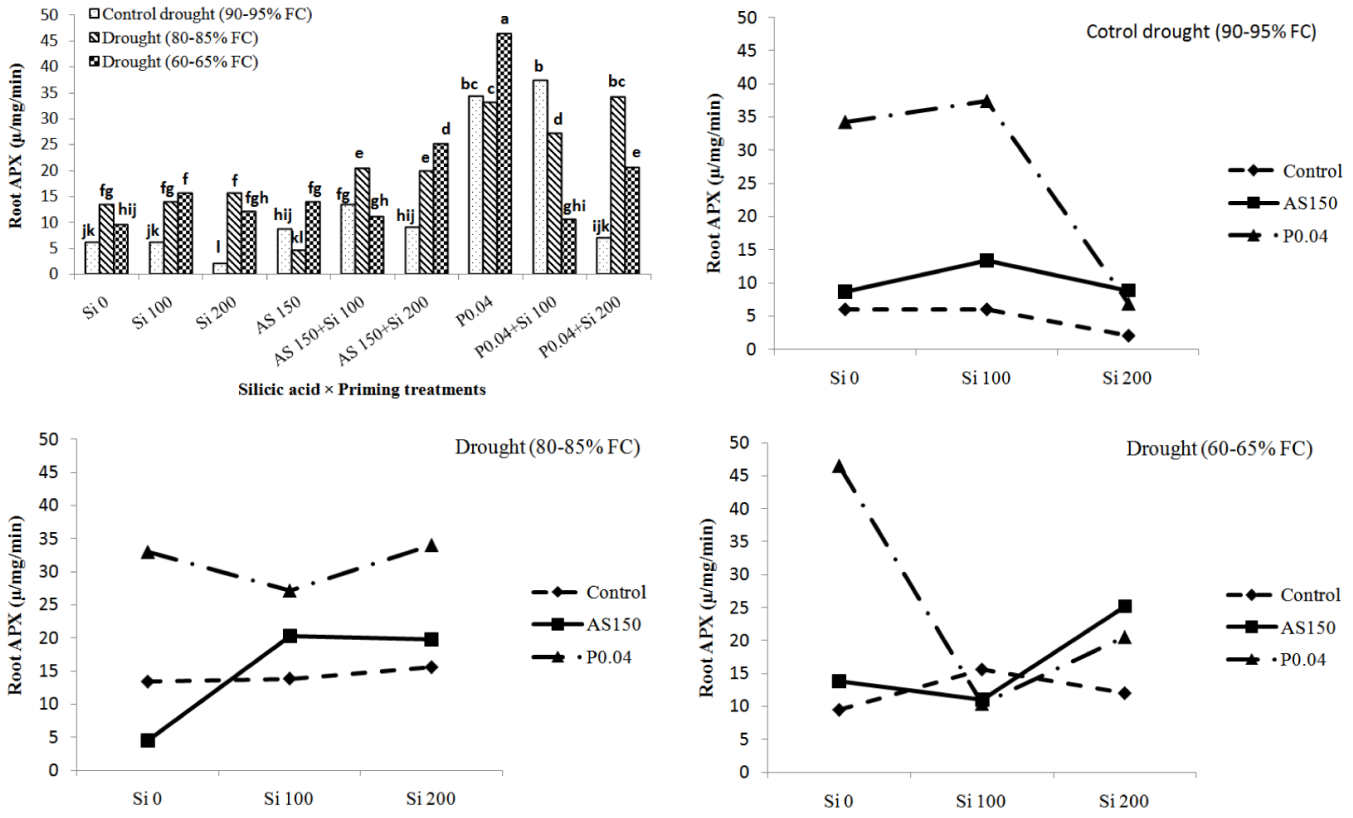

Figure 4. Root APX activity in different watering levels (90-95%, 80-85% and 60-65%, FC) by changing priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%) and concentration of silicium (0, 100 and 200 mgL^{-1}) as foliar spraying

In 60-65% of drought stress, seed priming with pyridoxine and foliar spraying with SiA 100 mgL-1 significantly reduced APX; however, seed priming with AS150 plus leaf spraying with SiA 200 mgL^{-1} increased its amount. In 85-80% of drought stress, seed priming with AS150 plus foliar spraying with SiA significantly increased APX. The use of AS and the lack of priming did not affect amount of APX in the root at different levels of SiA (Fig. 4).

CAT Enzyme Activity in Leaves and Roots

With regards to the study on CAT changes in the leaf, at control level (90-95%) and drought stress level 60-65%, seed priming with pyridoxine caused an increase in the amount of this enzyme in comparison with non- priming or the use of AsA. At the control level (90-95%) and seed priming with AsA, the use of SiA reduced CAT activity in the leaf, and in the case of pyridoxine, foliar spraying with SiA 100 mgL^{-1} significantly increased CAT in the leaf. Opposite results were obtained at 60-65% of drought stress. In 80-85% of drought stress, priming with AsA as well as foliar spraying with SiA 200 mgL^{-1} and priming with pyridoxine plus foliar spraying with SiA 100 mgL^{-1} significantly increased CAT in the leaves (Fig. 5).

Regarding CAT rate in roots, the use of AsA and pyridoxine in seed priming significantly influenced CAT, so that CAT value increased significantly (Fig. 6). A study on CAT changes in roots showed that, at control level (90-95%), seed priming with pyridoxine increased the amount of this enzyme in comparison with non-priming or the use of AsA. Seed priming with pyridoxine along with foliar spraying with SiA 200 mgL^{-1} resulted in a significant decrease in the enzyme activity (Fig. 6). In 80-85% of drought stress, seed priming with AsA and pyridoxine plus foliar spraying with SiA increased CAT in the roots. However, in 60-65% of drought stress, priming with AsA and foliar spraying with SiA 200 mgL-1 reduced CAT in the root, and seed priming with pyridoxine together with foliar spraying with SiA 200 mgL^{-1} also increased CAT in the root. It was found that at control level (90-90%), seed priming with AsA and foliar spraying with SiA 100 mgL^{-1} increased CAT rate and priming with pyridoxine and foliar spraying with SiA 200 mgL^{-1} reduced it significantly (Fig. 6).

SOD Enzyme Activity in Leaf and Roots

A study on the interaction of the three factors showed that when SiA was used alone in all drought stress levels, SOD value was higher in the leaf. However, when AsA and pyrodoxine were used in seed priming, SOD value was not high. SOD was also found to be increased in the leaf (Fig. 7). A study on SOD changes in the leaf indicated that at all drought stress levels non- priming resulted in an increase in the amount of this enzyme compared to seed priming with AsA and pyridoxine. In control level (90-90%), non- priming and seed priming with pyridoxine and foliar spraying with SiA increased SOD content in the leaves. However, in 80-85% of drought stress, priming with AsA, foliar spraying with SiA 100 mgL^{-1} and non-priming, and foliar spraying with SiA 200 mgL^{-1} significantly increased SOD in the leaf (Fig. 7).

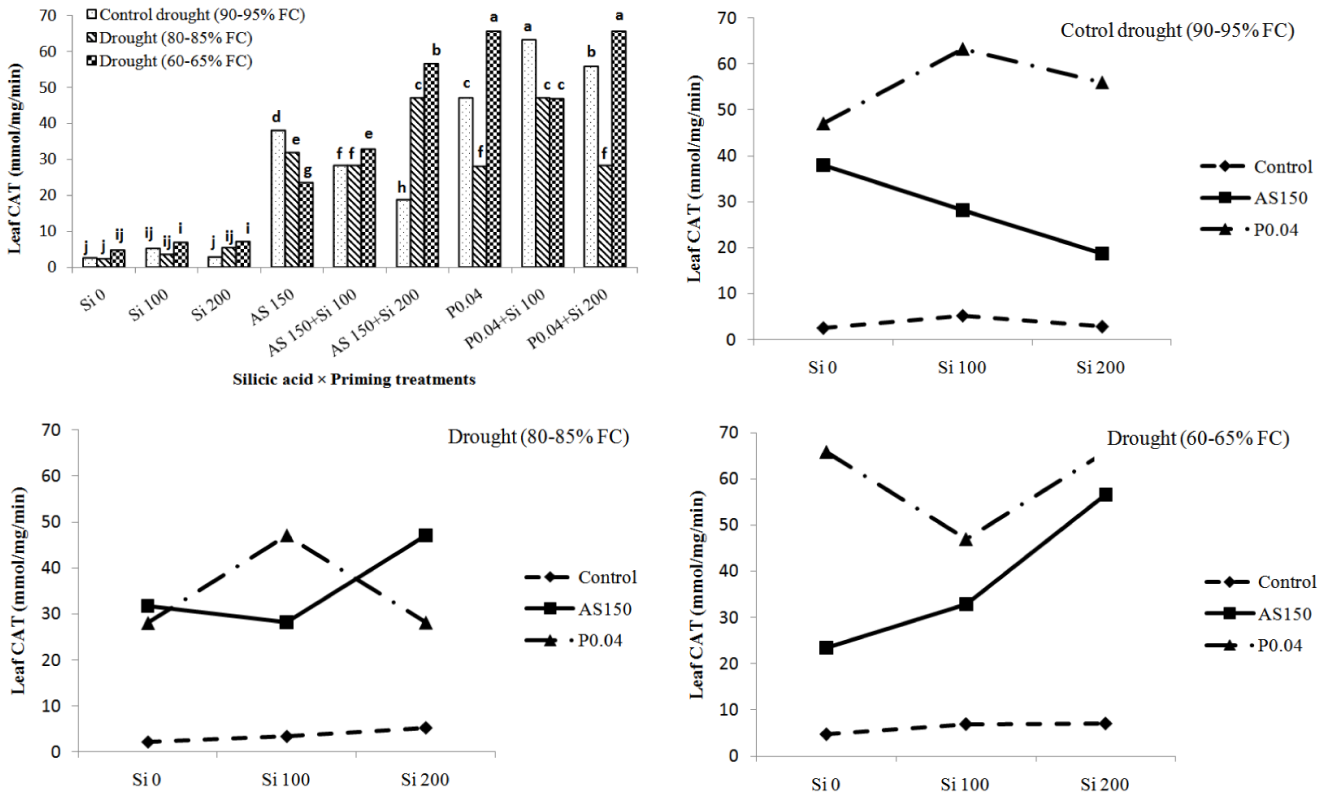

Figure 5. Leaf CAT activity in different watering levels (90-95%, 80-85% and 60-65%, FC) by changing priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%) and concentration of silicium (0, 100 and 200 mgL^{-1}) as foliar spraying

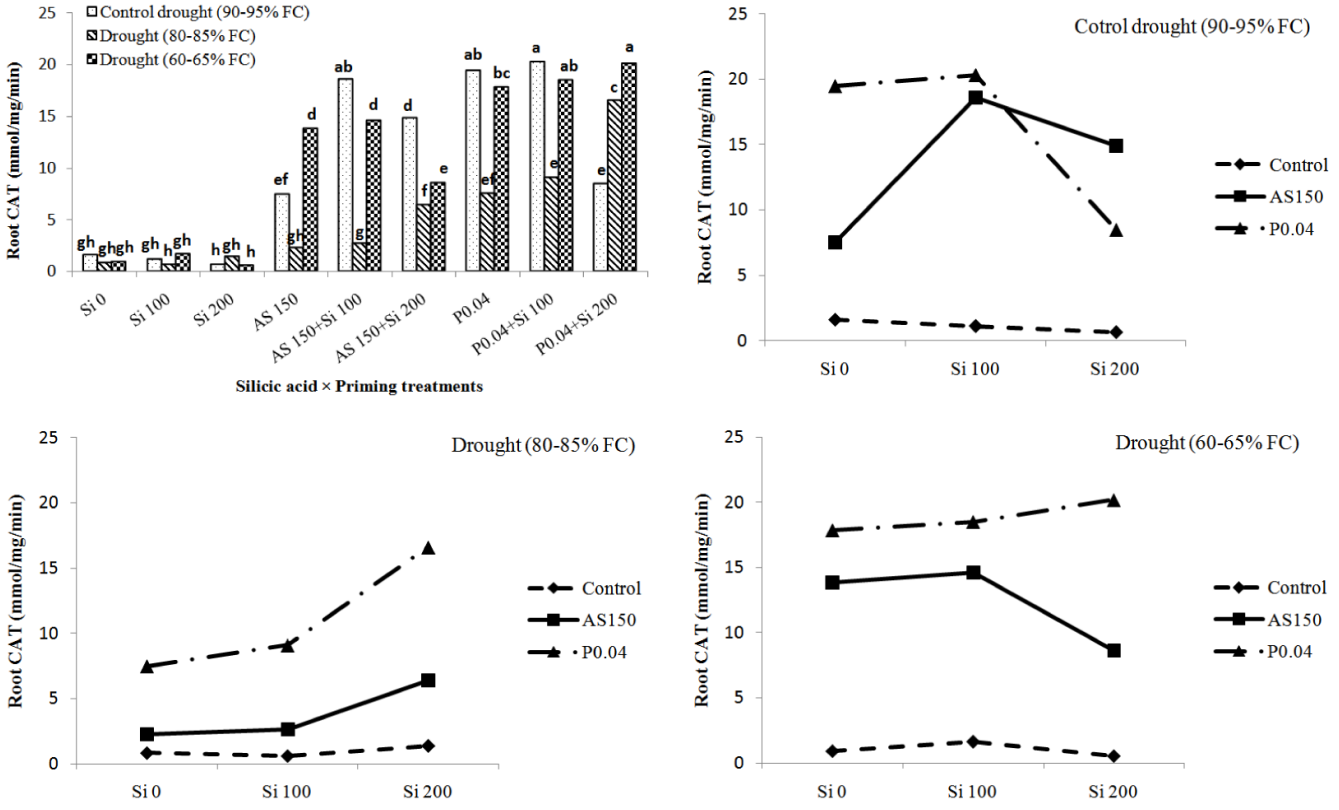

Figure 6. Root CAT activity in different watering levels (90-95%, 80-85% and 60-65%, FC) by changing priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%) and concentration of silicium (0, 100 and 200 mgL^{-1}) as foliar spraying

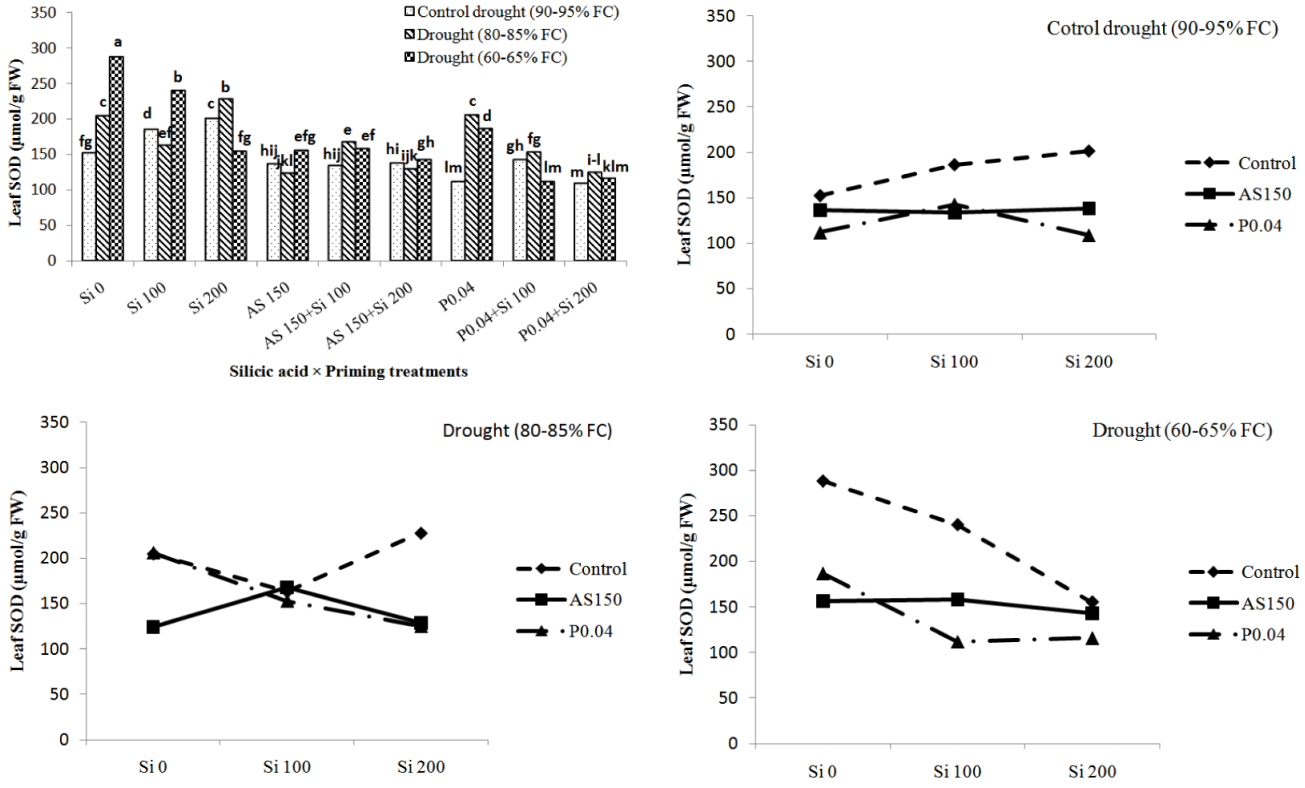

Figure 7. Leaf SOD activity in different watering levels (90-95%, 80-85% and 60-65%, FC) by changing priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%) and concentration of silicium (0, 100 and 200 mgL^{-1}) as foliar spraying

The evaluation of SOD changes in the root showed that the use of AsA and pyridoxine in seed priming significantly increased SOD in the root (Fig. 8). The study on SOD changes in the roots indicated that at three levels of drought stress seed priming with AsA and pyridoxine increased amount of this enzyme compared to non-priming; this increase was higher in case of using pyridoxine (Fig. 8). In drought stress levels of 80-85% and 60-65%, non-priming and foliar spraying with SiA increased SOD in the root (Fig. 8).

Leaf Proline Content

The comparison of the results obtained regarding individual effect of foliar spraying showed that by increasing SiA concentration, leaf proline increased significantly (Fig. 9). The assessment of the interaction between drought stress and seed priming showed that at all three levels of drought stress, seed priming with AsA and pyridoxine, the proline amount increased in comparison with control, and this increase was higher at all drought stress levels in case of using pyridoxine 0.04%. Seed priming with pyridoxine caused an increase in the amount of proline with the increase in the level of drought stress; however, seed priming with AsA increased the amount of proline until 80-85% of drought stress level, and then with the increase in the drought stress level, it decreased significantly. Drought stress did not influence the amount of proline in non-priming plants (Fig. 10).

Leaf MDA Content

A study on the effects of the three factors showed that foliar spraying with SiA alone caused higher MDA levels than using AsA and pyridoxine in seed priming at all levels of drought stress (Fig. 11). Evaluating MDA changes in leaves showed that at three levels of drought stress non-priming resulted in an increase in the amount of MDA compared to seed priming with AsA and pyridoxine (Fig. 11).

Total Chlorophyll

The evaluation the interaction of the three factors indicated that seed priming with AsA without foliar spraying in drought stress levels of 80-85% and 60-65%, and seed priming with AsA and foliar spraying with SiA 200 mgL^{-1} in 80-85%of drought stress could prevent a significant reduction in chlorophyll content caused by dryness (Fig. 12). Analysis of changes in total chlorophyll content in three levels of drought stress showed that in 80-85% of drought stress, seed priming with pyridoxine and AsA and foliar spraying with SiA 200 mgL^{-1} resulted in increased chlorophyll content. In 60-65% of drought stress, seed priming with pyridoxine increased chlorophyll content (Fig. 12).

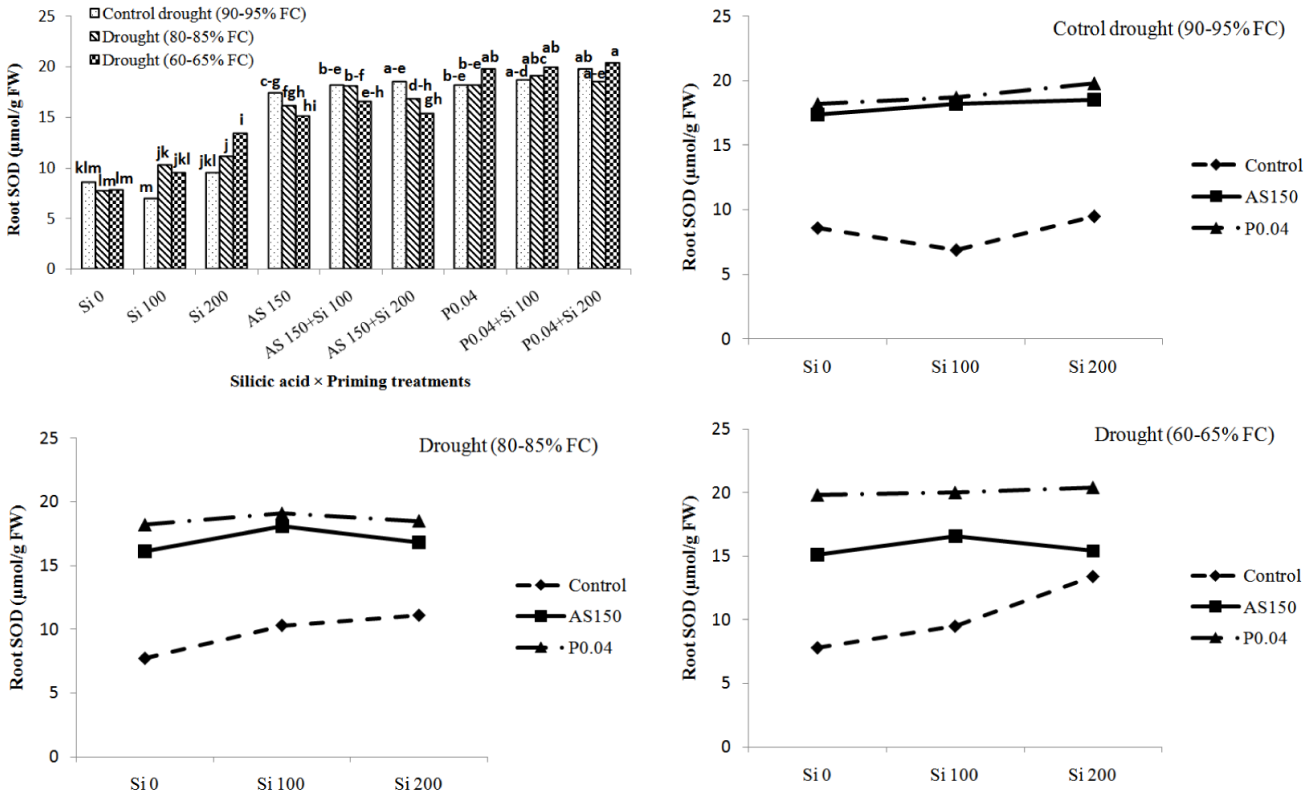

Figure 8. Root SOD activity in different watering levels (90-95%, 80-85% and 60-65%, FC) by changing priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%) and concentration of silicium (0, 100 and 200 mgL^{-1}) as foliar spraying

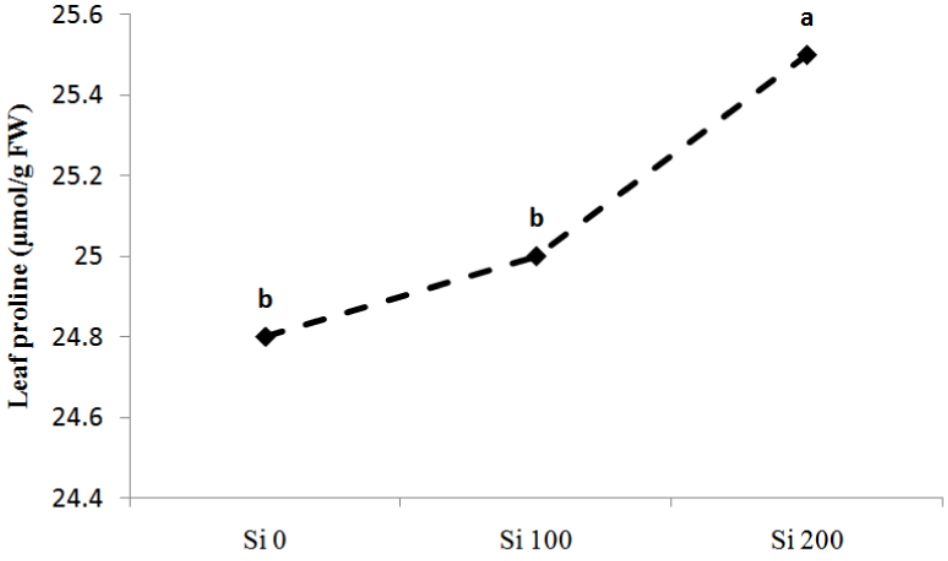

Figure 9. Proline amounts in leaves under the influence of different levels silicium (0, 100 and 200 mgL^{-1}) spraying

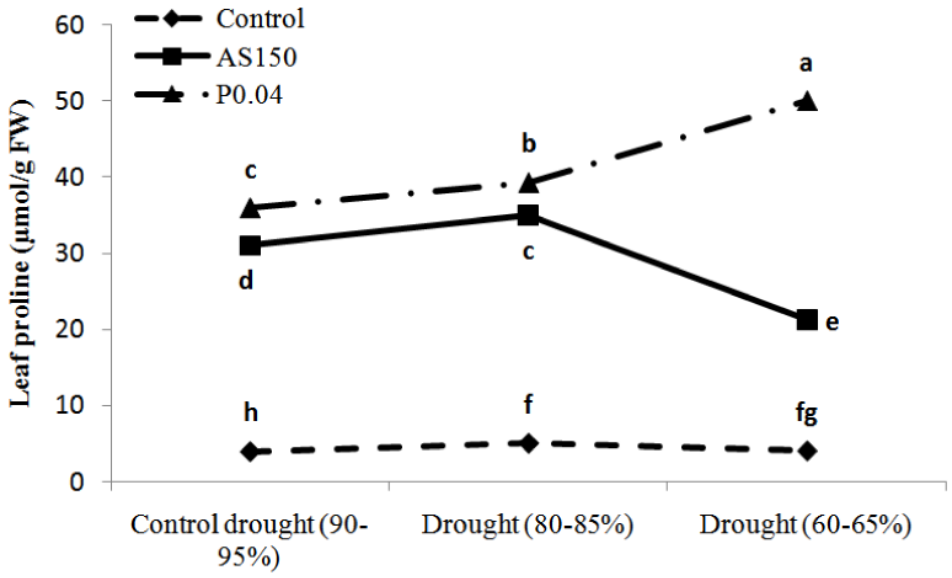

Figure 10. Proline amounts in leaves under the influence of different watering levels (90-95%, 80-85% and 60-65%, FC) by changing priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%)

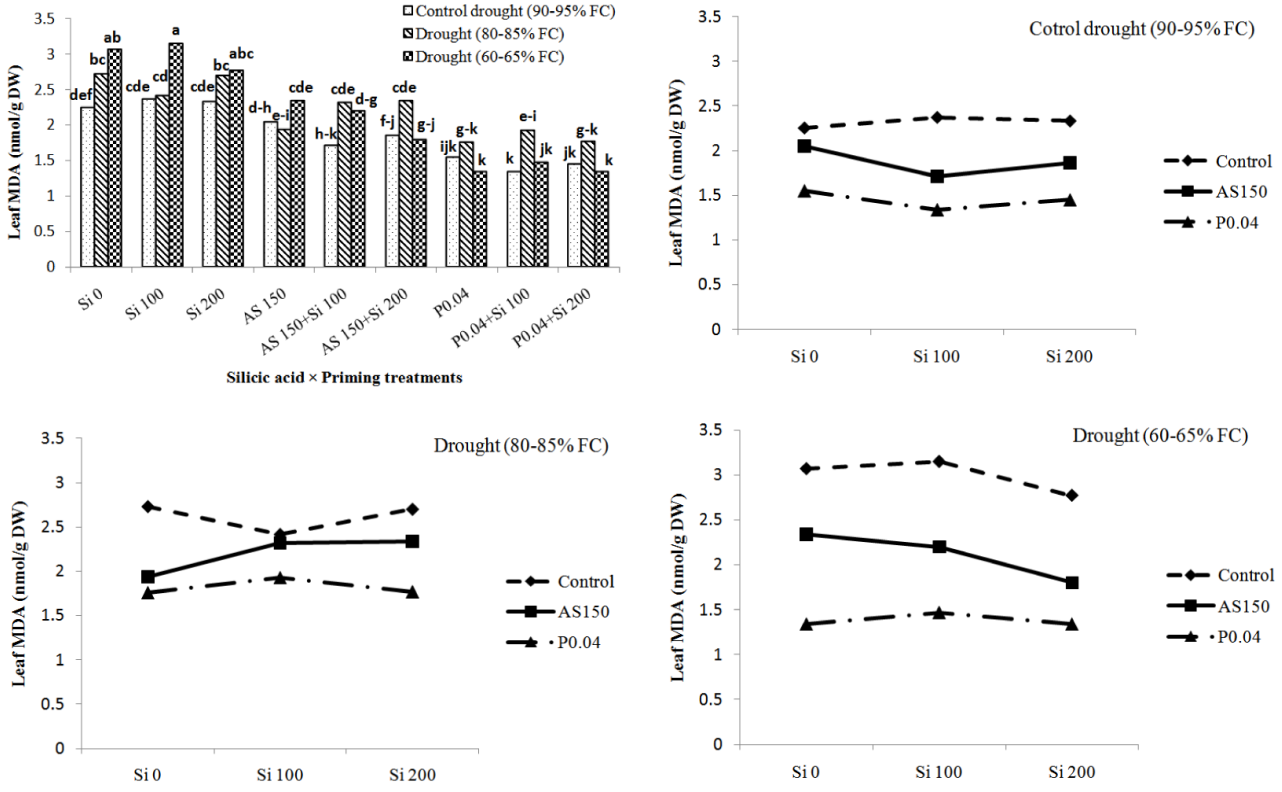

Figure 11. MDA contents at different watering levels (90-95%, 80-85% and 60-65%, FC) by changing priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%).and concentration of silicium (0, 100 and 200 mgL^{-1}) in foliar spraying

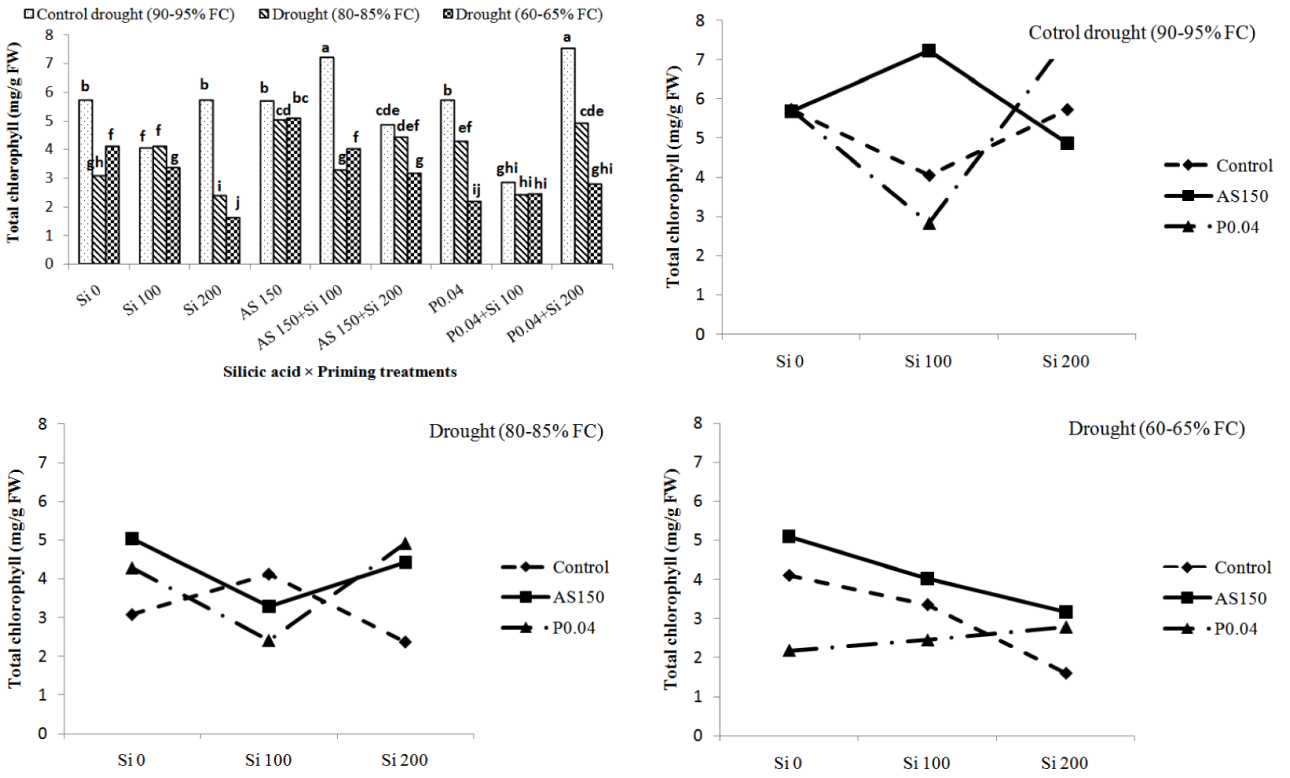

Figure 12. Total chlorophyll content in different watering levels (90-95%, 80-85% and 60-65%, FC) with changes in priming treatments (Control, AsA 150 mgL^{-1} and P 0.04%) and concentration of silicium (0, 100 and 200 mgL^{-1}) as foliar spraying

DISCUSSION

A study on PPO, APX, and CAT changes revealed that, in different drought stress levels, seed priming with pyridoxine caused an increase in the amount of these enzymes compared to non-priming or ascorbic acid treatment.

Results of experiments in the studies by Denslow et al (2005) showed that pyridoxine is effective on osmotic stress resistance and oxidation resistance. A gene, called PDX1, is responsible for this effect located in the root cells of the plant, (Chen and Xiong, 2005). In fact, these results correspond with the results of the present research.

Ascorbic acid (vitamin C) can be oxidized in direct reaction with reactive oxygen species such as superoxide, mono oxygen, or hydroxyl radicals. Moreover, ascorbic acid can be used as a regenerative agent in alpha-tocopherol reconstruction (membrane-bound antioxidant) protecting membrane against oxidative stress (Jithesh, 2006; Prida, 2005).

Silicone promotes the growth of the plant. In addition, in many cases stimulating growth, altering the activity of antioxidant enzymes, and reducing ROS levels in plant cells leads to the protection of the plant against environmental stresses. Silicon in plants induces resistance to biotic and abiotic stresses (Epstein et al., 1994).

Drought stress results in closure of the stomata and subsequently a decrease in photosynthesis rate. Silicon can reduce drought stress by reducing transpiration. The transpiration of the leaves is largely done through the stomata and to some extent through the cuticle. Deposited Si beneath the leaf cuticle forms two layers of silicon-cuticle and also decreases the transpiration (Ma, 2004).

The activity of some antioxidant enzymes changes under stress conditions. These changes also occur under drought stress. Under the conditions of our study, assessment of PPO enzyme activity under drought stress showed that application of pyridoxine as seed priming and foliar spraying of Si on the plants in the main field increased activity of PPO enzyme. An increased activity of PPO enzyme under drought stress has also been reported in beans (Demir and Kocacaliskan, 2001). In this regard, Fazeli et al. (2007) showed that a drought-tolerant sesame cultivar had more membrane stability and more PPO activity. In a previous study on PPO activity during the vegetative stage in the plants under drought stress assigned to treatment or control groups, it was shown that, under normal condition, a significant amount of PPO enzyme was produced in the plant in order to perform natural physiological processes of the plant; however, under stress condition, the activity of this enzyme increased (Siosemardeh, et al., 2014). Ahmadpourdehkordi and Baluchi (2012) found that the activity of PPO enzyme increased by increasing salinity and drought stress, and it further increased by implementing the priming.

Mafakheri, et al (2016) in their study implemented a 7 –day 65% stress and showed that APX activity decreased; however, by increasing stress (35%) APX activity also increased. Increased

activity of APX under drought stress has also been reported in several studies (Terzi et al., 2010; Turkan et al., 2005; Seglam et al., 2011).

Catalase is also one of the most important enzymes in the antioxidant system, which increases with the increase in the drought stress, but applying seed priming technique can increase this enzyme in the plants exposed to higher stress (Moosavi et al., 2009). For instance, seedlings obtained from primed melon seeds (*Cucumis melo* L.) showed higher catalase activity in comparison with seedlings grown from non-primed seeds (Farhoudi, et al., 2011).

Some previous studies have attributed the positive effect of osmopriming on germination of seeds to different mechanisms such as enhancement of the activity of reactive oxygen species destructive enzymes and activation of ATPase, acid phosphatase, and RNA synthetase (Jie et al., 2002).

Investigations on pea (Ataei-Sheikh, 2004) sorghum (Saei et al., 2005), and sunflower (Rafiei et al., 2005) have shown that activity of this enzyme is higher under drought stress conditions than normal conditions and SOD enzyme can be used to determine drought-resistant cultivars.

Proline is a free amino acid, which as a soluble substance is naturally accumulated in plant cells in response to stress. The essential role of proline is protecting the cells from negative effects of salt accumulation, osmotic exchange, and stability of cellular structure such as membranes and proteins (Morot-Guadry et al., 2001). Malondialdehyde (MDA) is peroxidation product of unsaturated fatty acids in phospholipids. Lipid peroxidation level is used as an indicator for evaluating harmful free radicals under stress conditions (Rukui, et al., 2006). At all three levels of drought stress, seed priming with ascorbic acid and pyridoxine increased proline content compared to the control, which was found to be more in pyrodoxin 0.04% treatment. Seed priming with pyridoxine increased proline content along with the increase in drought stress. However, priming with ascorbic acid increased proline content up to drought stress level of 80-85%, and then with the increase in drought intensity, it decreased significantly.

In a study on *Lotus japonicas*, proline enhancement was confirmed under salinity and drought stress conditions (Diaz et al., 2005), attributing to an increase in the activity of the pyrolin-5-carboxylic acid synthetase enzyme (which plays a role in the biosynthesis of the proline) and a decrease in the activity of the proline dehydrogenase enzyme (which plays a role in the breakdown of the proline). Results of the studies by Najafi et al., (2010); Hosseinzadeh et al. (2015); Mansorifar et al. (2012) also confirmed the increase in proline content in pea genotypes under drought stress conditions.

At different drought stress levels, non-priming increased MDA content compared to seed priming with ascorbic acid and pyridoxine. The production of reactive oxygen species in oxidative stress caused by drought stress leads to the destruction of lipid structure of the membrane and thereby increases the MDA level (Eraslan et al. 2007). In research on the pea (Hosseinzadeh et al., 2015), MDA accumulation significantly increased under drought stress conditions compared to non-stress conditions, which is inconsistent with the results of our research.

Seed priming with ascorbic acid without foliar spraying and along with foliar spraying by silicic acid at different drought stress levels prevented a significant decrease in chlorophyll content. Moreover, at different levels of drought stress, seed priming with ascorbic acid and pyridoxine and foliar spraying with silicic acid increased leaf chlorophyll content.

Leaf chlorophyll content is one of the most important indicators of environmental stresses on the plants, (Khayatnezhad et al, 2011). The reduction of chlorophyll content under stress conditions is due to the destruction of chloroplast under these conditions and also the reduction of pigments. It seems that chlorophyll reduction under drought stress conditions occurred due to the increase in the destruction of these pigments or decline in their production, and also disorder in the activity of the enzymes responsible for synthesis of photosynthesis pigments. The results from this study are consistent with findings of a study on wheat under drought stress conditions (Cober et al., 2000).

CONCLUSION

The findings of this study revealed that the use of pyridoxine for seed priming as well as foliar spraying with SiA 100 mgL^{-1} or SiA 200 mgL^{-1} resulted in higher activity of the antioxidant enzymes which are involved in the induction of resistance to drought stress. Under stress-free conditions (normal irrigation), the evaluation of all traits showed that pyridoxine 0.04% alone or along with foliar spraying of plants with SiA 100 mgL^{-1} was the best seed priming treatment.Under moderate drought stress conditions, pyridoxine 0.04% together with foliar spraying of plants with SiA 200 mgL^{-1} was the best seed priming treatment. Under severe drought stress conditions, pyridoxine 0.04% or ascorbic acid at 150 mgl^{-1} was the best seed priming treatment.

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