Alinteri J. of Agr. Sci. (2020) 35(1): 1-6
e-ISSN: 2587-2249
info@alinteridergisi.com
http://dergipark.gov.tr/alinterizbd
http://www.alinteridergisi.com/
DOI: 10.28955/alinterizbd.741556

Alinteri Journal of Agriculture Sciences

RESEARCH ARTICLE

The Silicon Effects on Antioxidant System of Wheat Cultivars under Pb Stress

Masoumeh Abedini* • Sakineh Hosseini • Gader Habibi

Payame Noor University, Faculty of Basic Sciences, Department of Biology, Tehran/Iran

ARTICLE INFO

Article History:
Received: 13.03.2019
Accepted: 12.03.2020
Available Online: 22.05.2020

Keywords:
Antioxidant enzymes
H_2O_2
Lead
MDA
Silicon
Wheat

ABSTRACT

Pb poses a major threat to plant growth and silicon can reduce its toxicity. This work was conducted hydroponically as a completely randomized factorial design to study the effect of Si (70 and 140 ppm) on *Triticum aestivum* cultivars Chamran and Shiroudi under Pb stress (150 ppm). Pb caused significant increases in the H_2O_2, free amino acids and proline contents of wheat cultivars and MDA content of cv. Chamran. Furthermore, Pb stimulated the activities of SOD and APX in cv. Chamran and POD in cv. Shiroudi. Si application significantly increased the free amino acid content of cultivars and proline content of cv. Chamran in absence of Pb. The protein content of wheat cultivars significantly increased at 70 ppm of Si in absence of Pb and at both levels in presence of Pb. In cv. Chamran, Si application significantly decreased the H_2O_2 content and the activities of SOD, POD and APX at both levels, free amino acids and proline contents at 70 ppm and MDA content at 140 ppm in presence of Pb. In cv. Shiroudi, Si application significantly decreased the proline content at both levels, H_2O_2 and free amino acids contents at 70 ppm and MDA content at 140 ppm.

Please cite this paper as follows:

Abedini, M., Hosseini, S. and Habibi, G. (2020). The Silicon Effects on Antioxidant System of Wheat Cultivars under Pb Stress. *Alinteri Journal of Agriculture Sciences*, *35*(1): 1-6. doi: 10.28955/alinterizbd.741556

Introduction

Silicon is an abundant element in soils and consists about 28% of the earth's crust (Emamverdian and Ding, 2017). Plants normally absorb silicon in the form of monosilicic and polysilicic acids. Even though, this element is not traditionally considered as an essential element for plants, but it has positive effects on plant's growth and could alleviate the environmental stresses by changing the extracellular matrix (apoplast), improving the water transport and water status of plant, affecting the ion transport, increasing the plant's antioxidant activities and reducing the lipid peroxidation (Liang et al., 2007; Emamverdian and Ding, 2017; Kim et al., 2017).

Lead (Pb) is an abundant and ubiquitous toxic element that is present in soils, seawaters, lakes and rivers. Due to low solubility and strong binding capacity with soil colloids, Pb has long residence time in soil, causing a large number of direct and indirect effects on plants growth and metabolism (Britto et al., 2011).

Wheat contributes more calories and proteins to the world diet than any other cereal crops and is considered as a good source of protein, minerals, B-group vitamins and dietary fiber (Kumar et al., 2011). The aim of this work was to study the antioxidant system responses in two wheat cultivars (Chamran and Shiroudi) to Si application under lead stress.

Materials and Methods

The seeds of wheat (*Triticum aestivum* L.) cultivars (cvs.) Shiroudi and Chamran were obtained from the Agricultural Research Center of Tabriz, Iran.

* Corresponding author
E-mail address: ms_abedini@pnu.ac.ir

Plant Growth Condition

The experiment was conducted in a growth chamber with a temperature regime of 28/20°C, photoperiod of 16 h and relative humidity of 70%. After disinfection, seeds were germinated in petri-dishes and uniform seedlings were transferred to pots containing one liter of Hoagland solution. One week after transferring, Pb (150 ppm, as lead acetate) and Si (70 and 140 ppm, as potassium silicate) were applied through root medium. Plant's shoots were harvested two weeks after treatments and frozen in liquid nitrogen until assays.

Antioxidant Enzymes Assays

To obtain the crude extract, 0.5 g of leaves were homogenized in 5 mL of 50 mM potassium phosphate buffer (pH 7), containing 0.2% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12000 g at 4°C for 20 min. The resulting supernatant was used to measure the activities of antioxidant enzymes. Each enzyme assay was tested for linearity between the volume of crude extract and the measured activity. Changes in the absorbance of substrates or products were measured using spectrophotometer.

The activity of superoxide dismutase (SOD) was measured according to its capacity to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture contained 2.65 mL of 67 mM potassium phosphate buffer (pH 7.8), 0.2 mL of 0.1 mM EDTA solution containing 0.3 mM sodium cyanide, 0.1 mL of 1.5 mM NBT, 50 mL of 0.12 mM riboflavin and 0.5 µL of enzyme extract. One unit of SOD was defined as the amount of enzyme that caused 50% inhibition of NBT reduction (Winterbourn et al., 1976).

Guaiacol peroxidase (POD) assayed following the method of Chance and Maehly (1955). The reaction mixture contained 1.5 mL of 100 mM citrate-phosphate- borate buffer solution (pH 7.5), 50 µL of 15 mM guaiacol, 25 µL enzyme extract and 50 µL of 3.3 mM H_2O_2. The polymerization of guaiacol was initiated by adding H_2O_2 and an increase in absorbance at 470 nm was recorded for 3 min. POD activity was calculated using the extinction coefficient, 26.6 $mM^{-1}.cm^{-1}$, for guaiacol. The generation of 1 µM of tetraguaiacol per min was catalyzed by the amount of enzyme that was introduced as one unit of POD.

The activity of ascorbate peroxidase (APX) was measured according to Nakano and Asada (1987). The reaction mixture contained 25 µL of enzyme extract with 2.5 mL of phosphate buffer (pH 7) containing EDTA 0.1 mM, H_2O_2 1 mM and ascorbic acid 0.25 mM. The decrease in absorbance at 290 nm for 1 min was recorded and the amount of ascorbate oxidized was calculated using extinction coefficient of 2.8 $mM^{-1}\cdot cm^{-1}$. One unit of APX was defined as the amount of enzyme that oxidized 1µM of substrate.min^{-1}.

The catalase (CAT) activity was determined by monitoring the absorbance decrease due to H_2O_2 dismutation at 240 nm for 3 min. The reaction mixture contained 1.5 mL of 100 mM citrate-phosphate-borate buffer solution (pH 7.5), 50 µL enzyme extract and 13 µL of 10 mM H_2O_2. The amount of enzyme for dismutation of 1 µM H_2O_2 per min was expressed as one unit. Extinction coefficient for H_2O_2 was considered 39.4 mM^{-1} cm^{-1} (Obinger et al., 1997).

Proline Assay

The proline was extracted with 10 mL of 3% sulfosalicylic acid solution. 2 mL of liquid was reacted with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid for 1 h in 100°C and reaction was terminated at ice bath. The reaction mixture was extracted by 4 mL toluene. The absorbance of chromophore containing toluene was read at 520 nm (Bates et al., 1973). Proline concentration of samples was determined from a standard curve.

Free Amino Acid Assay

Free amino acids were extracted by 80% ethanol and centrifuged at 5000 g for 10 min. Supernatants were taken in to test tubes and 1 mL of ninhydrin reagent and 0.2 mL of citrate buffer added to them. The mixtures were incubated at 100°C in a water bath for 10 min. The absorbance of samples was measured at 570 nm and free amino acid contents were calculated using a standard curve of glycine (Yemm and Cocking, 1955).

H_2O_2 and MDA assays

To obtain the crude extract, 0.1 g of leaves were homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12000 g at 4°C for 15 min.

The H_2O_2 content was assayed according to the Harinasut et al. (2003). To 0.5 mL of the supernatant, 0.5 mL of 10 mM phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide was added. The mixture was incubated at 25°C for 15 min. The absorbance was measured at 390 nm. The H_2O_2 content was calculated using a standard curve of H_2O_2.

For malondialdehyde (MDA) assay to 1 mL of the supernatant, 4 mL of 20% TCA containing 0.5% thiobarbituric acid was added. The mixture was incubated at 95 °C in a water bath for 30 min, and then quickly cooled on ice. The mixture was centrifuged at 10000 g for 15 min and the absorbance was measured at 532 nm. MDA levels were calculated from 1,1′,3,3′-tetraethoxypropane standard curve (Heath and packer 1968).

Total Protein Assay

Total protein content was measured by the method of Bradford (1976) using bovine serum albumin as a standard.

Statistical Analysis

All assays were carried out in triplicate and the results were presented as mean values ± SD. Statistical analyses were performed using a one-way analysis of variance test and the significance of the differences between means was determined by Student's multiple range test. The InStat (3.0) software was used to perform statistical analysis.

Results and Discussion

Antioxidant Enzymes

In cv. Chamran, Pb induced the significant ($p<0.05$) increases in the activities of SOD (10.87%) and APX (46.19%). Si application had not significant effect on the activities of antioxidant enzymes in absence of Pb in this cultivar, but significantly decreased the SOD (43.42% and 32.44%), POD (49.11% and 35.76%) and APX (28.47% and 30.9%) activities at 70 and 140 ppm in presence of Pb (Table1).

In cv. Shiroudi, Pb significantly increased the activity of POD (24.06%). Si application could not change the activities of antioxidant enzymes in absence of Pb, while its application at 140 ppm significantly decreased the activities of SOD (27.49%), POD (54.23%) and APX (28.84%) in presence of Pb (Table 1).

Table 1. Effect of Si on the activities of antioxidant enzymes of wheat cultivars under Pb stress.

Cultivar	Treatments	CAT (U g^{-1}FW)	SOD (U g^{-1}FW)	POD (U g^{-1}FW)	APX (U g^{-1}FW)
Chamran	Control	0.14 ± 0.02^{bc}	287.09 ± 4.37^{b}	3.37 ± 0.47^{a}	1.97 ± 0.03^{b}
	Pb 150	0.15 ± 0.02^{b}	318.32 ± 2.21^{a}	3.97 ± 0.12^{a}	2.88 ± 0.13^{a}
	Si 70	0.13 ± 0.03^{bc}	276.7 ± 6.21^{b}	3.64 ± 0.21^{a}	2.03 ± 0.11^{b}
	Si 140	0.16 ± 0.03^{b}	299.4 ± 9.11^{ab}	2.94 ± 0.71^{ab}	2.11 ± 0.04^{b}
	Pb150, Si 70	0.13 ± 0.01^{bc}	180.08 ± 7.73^{c}	2.02 ± 0.11^{b}	2.06 ± 0.02^{b}
	Pb 150, Si 140	0.16 ± 0.05^{b}	215.05 ± 7.29^{bc}	2.55 ± 0.15^{b}	1.99 ± 0.14^{b}
Shiroudi	Control	0.16 ± 0.01^{A}	238.16 ± 7.37^{AB}	4.28 ± 1.00^{B}	2.22 ± 0.29^{A}
	Pb 150	0.14 ± 0.02^{AB}	281.72 ± 9.70^{A}	5.31 ± 0.29^{A}	2.60 ± 0.47^{A}
	Si 70	0.17 ± 0.03^{A}	232.11 ± 6.14^{AB}	4.22 ± 1.1^{B}	2.32 ± 0.33^{A}
	Si 140	0.15 ± 0.02^{A}	229.17 ± 8.36^{AB}	3.98 ± 0.96^{B}	2.29 ± 0.49^{A}
	Pb 150, Si 70	0.12 ± 0.01^{B}	221.28 ± 9.45^{AB}	3.64 ± 1.16^{AB}	2.48 ± 0.12^{A}
	Pb 150, Si 140	0.17 ± 0.02^{A}	204.26 ± 16.4^{B}	2.43 ± 0.87^{B}	1.85 ± 0.08^{A}

Values are means ± SD of three replicates; different letters in each column indicate significant differences at *p<0.05*.

Exposure to heavy metals enhances the ROS production and promotes oxidative stress in plants. The induction of antioxidant enzymes activities is necessary for protection the plants tissues and organelles against the produced ROS species under stressful conditions. The responses of antioxidant enzymes to heavy metals is varying among plant species, cultivars and tissues (Shu et al., 2012). SOD is considered as the first defense against oxidative stress and catalysis the transformation of free superoxide radicals to H_2O_2 and O_2 (Pourrut et al., 2011). The product of SOD, H_2O_2, is the substrate for peroxidases and catalase (Fendereski et al., 2015). In this study, Pb induced the notable increases in the activities of SOD and APX in cv. Chamran and POD in cv. Shiroudi. Catalase is an antioxidant enzyme that isn't active in low concentrations of H_2O_2 (Shu et al., 2012). Therefore, the concentration of produced H_2O_2 in wheat cultivars is not adequate to up-regulation the catalase activity under Pb stress. It seems that the alleviated activity of APX in cv. Chamran and POD in cv. Shiroudi are sufficient to remove the produced H_2O_2.

Silicon application under Pb stress could moderate the activities of up-regulated antioxidant enzymes in wheat cultivars. The stimulatory and also inhibitory effects of Si on antioxidant enzymes activities in different plant species have been reported by authors under heavy metals stresses (Shi et al., 2010; Tripathi et al., 2015). For example, similar to results of this study, Pontigo et al. (2017) reported the significant reductions in the activities of antioxidant enzymes by Si application in Al- treated plants of ryegrass. It seems that there are obvious differences between plants species and varieties from the viewpoint of antioxidant enzymes activities in response to Si application under various environmental stresses.

MDA and H_2O_2

The MDA content of cv. Chamran increased significantly ($p<0.05$) in response to Pb, but did not affect significantly in cv. Shiroudi. Si application had not notable effect on MDA content of cultivars in absence of Pb, but at140 ppm significantly decreased this metabolite content in presence of Pb (Figure 1).

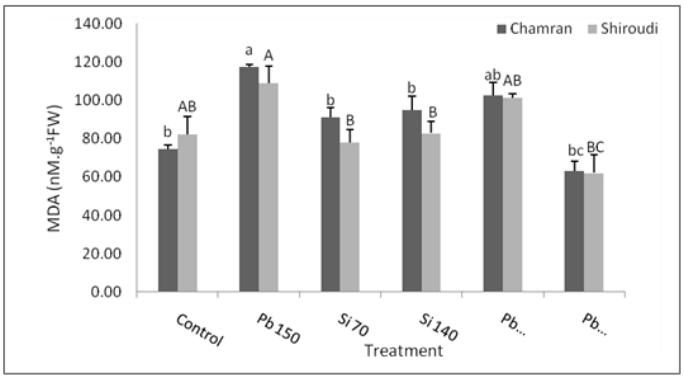

Figure 1. Effect of Si on MDA content of wheat cultivars under Pb stress

The H_2O_2 content of wheat cultivars significantly increased in response to Pb stress. Application of Si in absence of Pb, hadn't significant effect on H_2O_2 content of cultivars, but its application at both levels in cv. Chamran and at 140 ppm in cv. Shiroudi considerably reduced this metabolite content in presence of Pb (Figure 2).

Increasing in the MDA and H_2O_2 contents of plants under Pb stress is common among plant species (Britto et al., 2011; Hasanuzzaman et al., 2017). Heavy metals are able to induce the overproduction of ROS, which can react with macromolecules and cause lipid peroxidation and oxidative

stress (Abu-Muriefah, 2015). In studied wheat cultivars, Si could alleviate the Pb-induced accumulation of MDA and H_2O_2. This could be partially related to the Si ability in improving the plant's defense capacity against oxidative damages. It has been demonstrated that Si could protect the plant from oxidation damages by regulating the general mechanisms of cellular redox cycle and decreasing the permeability of membranes (Abu-Muriefah, 2015; Hasanuzzaman et al., 2017). Results obtained from this study showed that, in both cultivars, the MDA contents of Pb-treated plants had the lowest values at 140 ppm of Si. Vice versa, the application of 70 ppm of Si was more effective in controlling the H_2O_2 contents in Pb-treated plants.

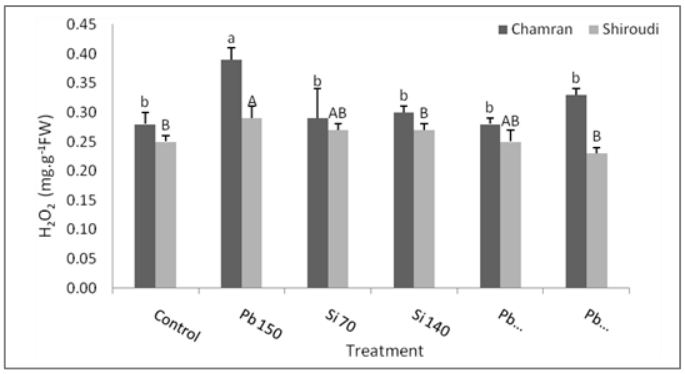

Figure 2. Effect of Si on H_2O_2 content of wheat cultivars under Pb stress

Proline

Pb caused a significant ($p<0.05$) increase in the proline content of wheat cultivars. In cv. Chamran, Si application at both levels significantly increased the proline content of plants in absence of Pb, but significantly decreased this metabolite in presence of Pb at 70 ppm.

In cv. Shiroudi, Si application had not significant effect on the proline content of plants in absence of Pb, but significantly decreased this metabolite at both levels in presence of Pb (Figure 3).

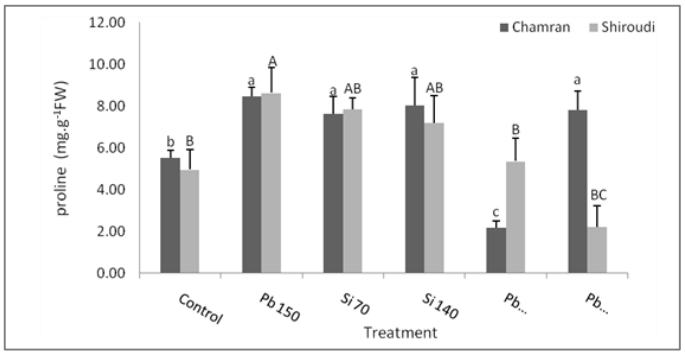

Figure 3. Effect of Si on proline content of wheat cultivars under Pb stress

Proline is a compatible osmolyte and is known to accumulate in response to various abiotic stresses (Abu-Muriefah, 2015). Accumulation of proline in plant tissues has been suggested to result from: (a) a decrease in proline degradation, (b) an increase in proline biosynthesis, (c) a decrease in protein synthesis or proline utilization, and (d) hydrolysis of proteins (Britto et al., 2011). Proline had a protective role in lipid peroxidation and possesses the ability to mediate osmotic adjustment, stabilize subcellular structures and scavenge free radicals. Silicon application reduced the proline content in wheat cultivars. This may be due to increased activities of proline degradation enzymes or incorporation of proline in the protein structure in plants received Si. Application of 70 ppm of Si in cv. Chamran and 140 ppm of Si in cv. Shiroudi were more efficient in moderating the proline level.

Total Protein and Free Amino Acids

Lead stress reduced the soluble protein content of plants significantly in cv. Chamran and non-significantly in cv. Shiroudi. Si application at 70 ppm in absence of Pb and at both levels in presence of Pb significantly increased the soluble protein content of wheat cultivars (Figure 4).

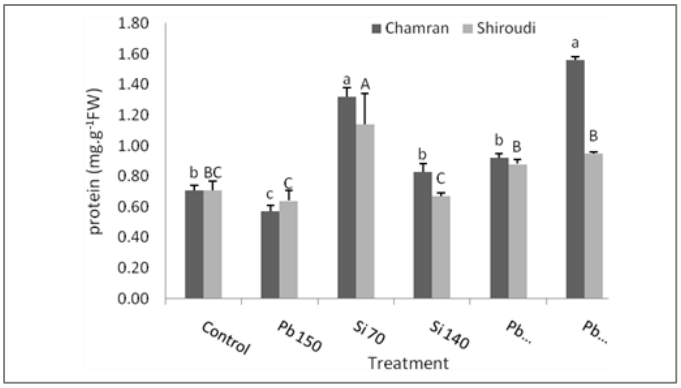

Figure 4. Effect of Si on total protein content of wheat cultivars under Pb stress

Free amino acid content of wheat cultivars significantly increased in response to Pb and Si application in absence of Pb. While Si application significantly decreased the free amino acid content of plants in presence of Pb (Figure 5).

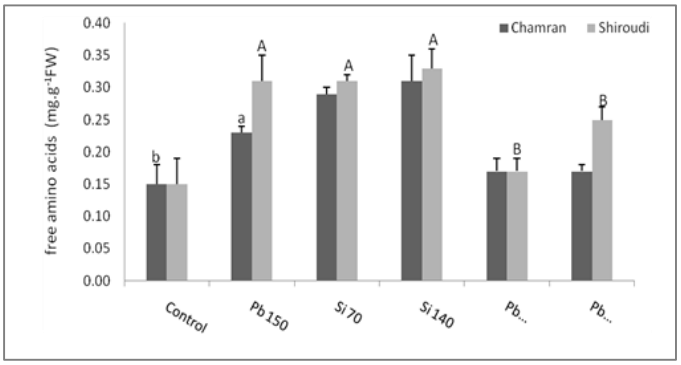

Figure 5. Effect of Si on free amino acid content of wheat cultivars under Pb stress

There is a consensus that proteins are key targets of heavy metals and heavy metals can bind to native proteins and inhibit their biological activity (Tamas et al., 2014). The quantitative decreases in the total protein content could be attribute to several Pb effects such as oxidative stress, modification in gene expression, increased ribonuclease activity, protein utilization by plants for the purposes of its detoxification and increased hydrolysis of protein (Alia et al., 2015). In this study,

silicon application improved the protein contents of cultivars, especially in cv. Chamran, that was accompanied with obvious decreases in the free amino acids and proline contents. It has been proposed that Si has a positive effect on protein synthesis from free amino acids precursors and inhibits the protein hydrolysis under stress conditions (Soundararajan et al., 2017).

Conclusion

From this study, it could be concluded that the antioxidant response of wheat plants to lead stress was relatively different between studied cultivars. Silicon application in plants that were not under Pb stress could not affect the antioxidant enzymes activities, but caused to increase in the protein and free amino acid contents of plants. Silicon application in plants that were under Pb stress could control the free radical formation and moderate the up-regulated activities of antioxidant enzymes more effectively in cv. Chamran.

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