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The Effect of Silicon on Cadmium Stress in *Echium amoenum*

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***Abstract*—**The beneficial effects of Si are mainly associated with its high deposition in plant tissue and enhancing their strength and rigidity. We investigated the role of Si against cadmium stress in (*Echium C*) in house green condition. When the seventh leaves was be appeared, plants were pretreated with five levels of Si: 0, 0.2, 0.5, 0.7and 1.5 mM Si (as sodium trisilicate, $Na_2(SiO_2)_3$) and after that plants were treated with two levels of Cd (30 and 90 mM). The effects of Silicon and Cd were investigated on some physiological and biochemical parameters such as: lipid peroxidation (malondialdehyde (MDA) and other aldehydes, antocyanin and flavonoid content. Our results showed that Cd significantly increased MDA, other aldehydes, antocyanin and flavonoids content in Echium and silicon offset the negative effect and increased tolerance of Echium against Cd stress. From this results we concluded that Si increase membrane integrity and antioxidative ability in this plant against cd stress.

***Keywords*—**Silicon, Cadmium, *Echium*, MDA, antocyanin, flavonoid

I. Introduction

MANY trace metals, when present at high concentrations, cause toxicity in plants. This applies both to those trace metals that are considered essential for plant growth and those that have no known function in plants. Cadmium has not been shown to be an essential element for physiological processes, but high amounts of this element are absorbed either by root tissues or leaves of plants [1].

The absorption coefficient is higher for cadmium when compared with other heavy metals like zinc and copper [2, 3]. Soil can be polluted by cadmium from a variety of sources. These are mainly phosphate fertilizer and sewage sludge. Excess cadmium induces toxicity in plants, the critical level varying with species. Absorption of cadmium depends on the cadmium concentration in soil as well as the amount of cadmium ions available for absorption by plants [4]. Cation exchange capacity, pH values, fertilizer application, redox state of cadmium and cation competition is the main factor affecting the availability of cadmium [5], [6].

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Toxicity symptoms including chlorosis of the mature leaf and the necrotic appearance of leaf tissues, red or dark red appearance of the leaf margin, and root tips with a dark or red-brown color have been reported with high concentrations of cadmium. [7, 8]. It has been proposed that Cd2+ and other heavy metals can induce free oxygen radical generation and cause oxidative stress. However the exact mechanism of Cd2+ toxicity is not clearly known in the molecular level [10]. One possibly is that cadmium ions either inhibit the activity of some antioxidative enzymes or enhances their activity. The effects of cadmium on activities of some antioxidant enzymes like peroxidase, catalase, ascorbate peroxidase and glutathione reductase have been studied [4]. Lipid peroxidation is the major index of the increase in active free radicals, and malondealdehyde (MDA) is the main by-product of the lipid peroxidation process. It has also been reported that anthocyanin has a role in quenching free radicals. The reaction between anthocyanin and metals depends on the redox state of metal. This reaction is reversible. Marss and Walbot [3] reported that Cd2+ causes an increase in the antioxidative content of Zea mays. They also reported that Cd2+ significantly increased the expression of the Bronze2 gene. This gene is probably responsible for encoding the Glutathione-S-Transferase (GST) enzyme. The GST enzyme performs the last genetically defined step in anthocyanin biosynthesis, allowing for recognition and entry of anthocyanins into the vacuoles [14].

In this research the effects of different concentrations of cadmium on MDA as anindex of oxidative stress induced by cadmium flavonoid and anthocyanin content as detoxification compounds in (*Echium amoenum*)plant were studied.

In the other hand, silicon is the second most abundant element in soil, it is not considered to be anessential element for higher plants [15]. However, there is increasingevidence that it has a number of beneficial effects on plant growth under biotic and abiotic stresses [16]. In the aspect of abiotic stresses, afunction of Si on alleviating Cd toxicity has been observed in rice, maize and strawberry [17]. Cucumber is one of the important vegetables inthe world, it can actively absorb silicon [16], and exogenous silicon could increase cucumber tolerance to many abiotic and biotic stresses [18]. In this study the possibility of increased (*Echium amoenum*) resistance by siliconeagainst cadmium stress has been studied .

II. Material And Methods

A. Plant material and growth condition

(*Echium amoenum*) seeds were obtained from Research Neka (IRAN) and were grownunder greenhouse conditions.

Seeds with similar sizes, after disinfection, werechose and to plastic pots containing perlite (vermiculite) had been transferred. 10seeds were placed in each pot to maintain moisture in the pots were covered with nylon. . When the seventh leaves was be appeared, plants were pretreated with five levels of Si: 0, 0.2, 0.5, 0.7 and 1.5 mM Si (as sodium trisilicate, $Na_2(sio_2)_3$) and after that plants were treated with two levels of Cd (30 and 90 mM) For this purpose, plants were divided into 15 groups.

B. MDA measurement

For calculating the content of per oxidation in membrane fats, the concentration ofmalon- dialdehyde (MDA) from this reaction was measured. The measurement ofMDA concentration was done in Heat& Packer[19] method (1969). According to this method, 0.2 grams of leaf fresh tissue was weighted, and wasabraded in a china mortar of 5 ml trichloroacetic acid (TCA) of 0.1%. The achieved juice was centrifuged by a centrifuge device in 10000 ×g for 5 min. About 5.4 milliliter TCA solution which is 20% and which contains 0.5% thiobarbituric acid (TBA) was added to 1 ml of the solution from the centrifuge.

The achieved compound was heated in hot bath at 95°C for 30 min. Then, it became cool in ice immediately and again the compound was centrifuged in 10000×g for 10 min. The intensity absorption of this solution was read by spectrophotometer in a wavelength of 532 nm. The specified compound for absorption of this wavelength is red complex (MDA-TBA). The absorption of other nonspecific pigments was measured in 600 nm and it reduced in this content. For calculating MDA concentration, the silence coefficient was used, and the results from the measurement was calculated and presented according to mgr on fresh weight gram. For measuring the other aldehydes (Propanal, Botanal, Hegzanal, Heptanal and Propanal Dimethylestal), the Meirs method in 1992 wasused. The obtained results from the measurement were presented on the basis of mg/g.

C. Anthocyanins measurement

For measuring anthocyanin,0.2 grams of leaf fresh tissue was weighted, and was abraded in a china mortar with 10ml of methanol: HCl (99:1) and placed in a vial . The mixture was stirred for1–2 min with a hand held stirrer. The supernatant was then placed in a screw-cap vial and placed in a cold room (2◦C) overnight to facilitate extraction. An aliquot of the filtered extract was used the next day to determine total anthocyanins. The intensity absorption of this solution was read by spectrophotometer in a wavelength of 550 nm.

D. flavonoids measurement

We used Krizek et al., (1998) method for measuring flavonoids. According to this method, 0.2 grams of leaf fresh tissue was weighted, and was abraded in a china mortar of with 10 ml of ethanol: *Acetic acid, glacial* (99:1) and after that centrifuged then put it in banmari 80 c0 for 10 minutes . The intensity absorption of this solution was read by spectro-photometer in a wavelength of 300 nm&330nm. Collected data with SAS computer software and "MSTAT-C" were analyzed. Tested in a randomized complete block design with three replications.

III. Results

MDA content increased in plants which were treated with30and 90mM cadmium, but si with 0.2µM density decreased that,significantly. Other Aldehid increased in plants which were treated with30and 90 mM cadmium, but si with 0.2 & 0.5µM density decreased that, significantly(Fig. 1)

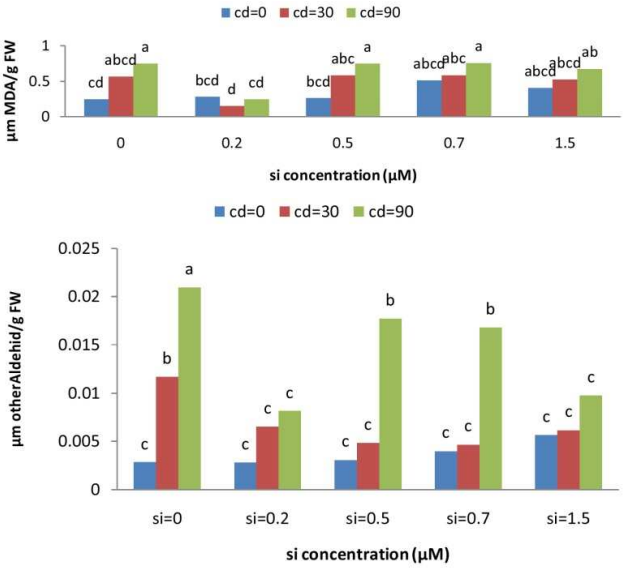

Fig. 1 The effect of different concentrations of Si and Ca on TBA-MDA content in Root & other aldehid, LSD= 37.11. P= 5%. Each value is mean ± std of three replicates

Anthocianin content increased in plants which were treated with 30 and 90 mM cadmium when compared with the control group and silicon in 1.5µM concentration increased those but this increase doesn't significantly in other silicon concentration (Fig. 2)

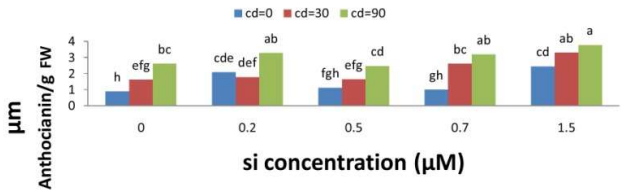

Fig. 2 The effect of different cadmium and silis concentrations on Anthocianin content in P= 5%. Each value is mean ± std of three replicates

plants which were treated with 30 and 90 mM cadmium when compared with the control group and silicon in 0.2µM concentration increased those but this increase doesn't significantly in other silicon concentration (Fig. 3).

IV. Discussion

Malondealdehyde, which is an indication of oxidative stress, increased as Cd2+ concentration increased in plants (Fig. 1). One of the main mechanisms of the metal toxicity in plants is free-oxygen radical generation and oxidative stress.

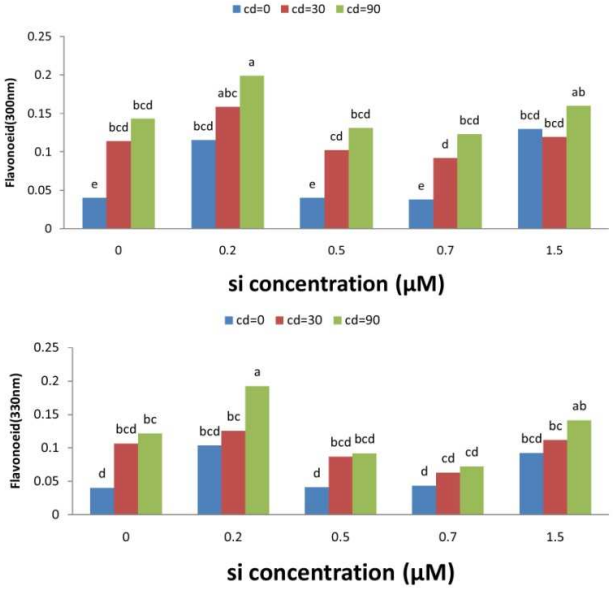

Fig. 3 The effect of different cadmium and silis concentrations on flavonoeid content in P= 5%. Each value is mean ± std of three replicates

Oxidative stress may occur because of a disruption of detoxification mechanisms for removing free oxygen radical. There are many observations that are shown when free radicals are increased, lipid peroxidation and MDA, which is the by-product of such peroxidation, enhanced [20, 21, and 11]. Our results confirm these observations (Fig. 1). There is report that increase in MDA content could be for the disruption of protein-lipid interaction in cell membranes. It has been reported that increase in H_2O_2 causes loss of membrane integrity and lipid peroxidation in the presence of heavy metals [22], [23]. The results of the present experiment werein line with the findings of Liang (1999) [24] in barley which showed that added Si decreased the permeability of theplasma membrane of root cells and decreased LPO level. It was reported that Si enhanced the stability of lipids incell membranes of rice plants exposed to drought and heat stresses, suggesting that Si prevented the structural and functional deterioration of cell membranes when rice plants were exposed to environmental stress [25]. The evidence suggests that Si decreases the permeability of plasma membranes and membrane lipid peroxidation and maintains the membrane integrity and functions of cd-stressed Echium, thus mitigating againstheavy metal toxicity and improving the growth of plants. There are many documents which show that anthocyanins and flavonoids are able to quench the oxygen radicals[13]. Because flavonoids have antioxidant role, directly Into inreducing reactions or Indirectly By making ironShlath to prevent oxidative stress [26].According to Verstraeten et al. [27], in addition to known protein-binding capacity of flavanols and procyanidins, they can interact with membrane phospholipids through hydrogen bonding to the polar head groups of phospholipids. As a consequence, these compounds can be accumulated at the membranes' surface, both outside and inside the cells. Through this kind of interaction, as they suggest, selected flavonoids help maintain membranes' integrity by preventing the access of deleterious molecules to the hydrophobic region of the bilayer, including those that can affect membrane rheology and those that induce oxidative damage to the membrane components.Anthocyanins have been reported to increase in response to many stresses including heavy metal stress [13]. However the role of anthocyanins is not well documented in response to heavy metals, butthere is a possibility that this compound serves as a transporter of heavy metals to vacuoles. In thisresearch we observed that when Cd2+ concentration increased, anthocyanin content of leaves increased (Fig. 2). It is also reported that when molybdenum (Mo) concentration increased in Brassica rapa, anthocyanin content increased [14]. Marrs & Walbot reported that Cd2+ can stimulatethe synthesis of the gluthathion-S-transferase (GST) enzyme, and therefore enhance the anthocyanin synthesis [3]. This enzyme is a key enzyme which catalyzes the last step in anthocyanin biosynthesis [1].The increase in anthocyanin and flavonoid, which we observed in leaves of plants treated with 30 and 90 µMCd2+, could possibly help plants to cope with stress either by compartmentation of Cd2+ to less sensitive parts of a cell (i.e. vacuole) or removing the free oxygen radical by quenching and detoxification of the free radicals.

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