

*Full Length Research Paper*

# Interactive effects of silicon and aluminum on the malondialdehyde (MDA), proline, protein and phenolic compounds in *Borago officinalis* L.

Gagoonani Shahnaz<sup>1\*</sup>, Enteshari Shekoofeh<sup>1</sup>, Delavar Kouros<sup>2</sup> and Behyar Moohamadbagher<sup>3</sup>

<sup>1</sup>Department of Biology, Faculty of Sciences, Payam Noor University of Najafabad, Isfahan, Iran.

<sup>2</sup>Azad University, Ashtian branch, Iran.

<sup>3</sup>Atmospheric Sciences and Metrological Research Center, Iran.

Accepted 17 August 2011

Aluminum stress is considered as a restricting factor for plant products. Therefore, many compounds have been applied to minimize the harmful effects of stress. Recently, the mitigating role of Silicon (Si) in Aluminum (Al) stress has received some attention. Si is known to increase tolerance in higher plants. Si in plants provides structural support and improves tolerance to diseases, drought and metal toxicity. We investigated the role of Si in alleviating Aluminum tolerance in *Borago officinalis*. Plants were grown in hydroponic culture and housegreen condition. When the seventh leaf appeared, plants were treated with five levels of Si: 0, 0.5, 1, 1.5 and 2 mM Si, after which they were treated with four levels of Al<sup>3+</sup>: 0, 20, 40 and 60 mM Al<sup>3+</sup>. Thereafter, the interaction effects of Silicon and Al stress on some physiological and biochemical parameters of *Borago* plants were investigated. Our results indicated that Al<sup>3+</sup> significantly increased malondialdehyde (MDA), other aldehydes, proline content and proteins. Al<sup>3+</sup> also caused a decrease in phenolic compounds. Silicon offset the negative impacts and increased tolerance of *Borago* to Al stress by enhancing phenolic compounds and proline contents compared to the control. Based on our observations that pretreatment with Si caused a decrease in peroxidation of lipids.

**Key words:** *Borago*, Silicon, Aluminum, malondialdehyde (MDA), proline, phenolic compounds, protein.

## INTRODUCTION

This research was designed to investigate the role of Si in alleviating Aluminum and in *Borago* grown hydroponically. This evidence amounts to a powerful case for the importance of Si in plant biology. Although Silicon (Si) is not usually listed in the list of essential elements, it is considered as one of the important beneficial nutrient for plant growth (Epstein, 1999; Liang et al., 1996, 2001). Si is the second most abundant element after oxygen in the soil. Si dioxide comprises 50 to 70% of the soil mass. As a result, all plants rooting in soil contain some Si in their tissues. Nevertheless, the role of Si in plant growth and development was overlooked until the beginning of the 20th century (Epstein,

1999; Ma, and Takahashi, 2002; Richmond, and Sussman, 2003). Because of the abundance of the element in nature and because visible symptoms of either Si deficiency or toxicity are not apparent, plant physiologists largely ignored it (Ma, 2004). This element can make up to 10% of the shoot dry weight, which is several-fold higher than those of essential macronutrients such as N, P and K (Ma, and Takahashi, 2002). The beneficial effects of this element on the growth, development, yield and disease resistance have been observed in a wide variety of plant species (Ma, 2004). The beneficial effect of Si is more evident under stress conditions (Ma and Takahashi, 2002), because Si is able to protect plants from multiple abiotic and biotic stresses (Jian and Naoki, 2006). Silicon alleviates many abiotic stresses including chemical stress (metal toxicity, salt, nutrient imbalance) and physical stress (drought, lodging, radiation, high temperature, freezing, UV) and many

\*Corresponding author. E-mail: Shgagoonani@yahoo.com.  
Tel: 09103110509. Fax: 03725222040.

**Table 1.** Twenty treatments on nutrient solution (in *Borago*).

Number	Name	Treatments	Number	Name	Treatments
1	Si <sub>0</sub> Al <sub>0</sub>	Control	11	Si <sub>1</sub> Al <sub>40</sub>	With 1 mM Si and With 40 mM Al
2	Si <sub>0</sub> Al <sub>20</sub>	With 20 mM Al	12	Si <sub>1</sub> Al <sub>60</sub>	With 1 mM Si and With 60 mM Al
3	Si <sub>0</sub> Al <sub>40</sub>	With 40 mM Al	13	Si <sub>1.5</sub> Al <sub>0</sub>	With 1.5 mM Si
4	Si <sub>0</sub> Al <sub>60</sub>	With 60 mM Al	14	Si <sub>1.5</sub> Al <sub>20</sub>	With 1.5 mM Si and With 20 mM Al
5	Si <sub>0.5</sub> Al <sub>0</sub>	With 0.5 mM Si	15	Si <sub>1.5</sub> Al <sub>40</sub>	With 1.5 mM Si and With 40 mM Al
6	Si <sub>0.5</sub> Al <sub>20</sub>	With 0.5 mM Si and With 20 mM Al	16	Si <sub>1.5</sub> Al <sub>60</sub>	With 1.5 mM Si and With 60 mM Al
7	Si <sub>0.5</sub> Al <sub>40</sub>	With 0.5 mM Si and With 40 mM Al	17	Si <sub>2</sub> Al <sub>0</sub>	With 2 mM Si
8	Si <sub>0.5</sub> Al <sub>60</sub>	With 0.5 mM Si and With 60 mM Al	18	Si <sub>2</sub> Al <sub>20</sub>	With 2 mM Si and With 20 mM Al
9	Si <sub>1</sub> Al <sub>0</sub>	With 1 mM Si	19	Si <sub>2</sub> Al <sub>40</sub>	With 2 mM Si and With 40 mM Al
10	Si <sub>1</sub> Al <sub>20</sub>	With 1 mM Si and With 20 mM Al	20	Si <sub>2</sub> Al <sub>60</sub>	With 2 mM Si and With 60 mM Al

others (Epstein, 1999; Ma and Takahashi, 2002; Richmond and Sussman, 2003; Ma, 2004). For instance; intra and extracellular deposition of aluminosilicates in roots and shoots is thought to protect some species from potential Al toxicity (for example, Hodson and Evans 1995; Hodson and Sangster, 1999; Britez et al., 2002; Jansen et al., 2003; Wang et al., 2004).

Aluminum (Al), a light metal, makes up 7% of the earth's crust and is the third most abundant element after oxygen and silicon (Ma et al., 2001). The primary Al toxicity symptom observed in plants is inhibition of root growth (Delhaize and Ryan, 1995). Al interferes with uptake, transport and utilization of essential nutrients including Ca, Mg, K, P, Cu, Fe, Mn and Zn (Foy, 1984; Guo et al., 2003).

The uptake of Al into the apoplasm and symplasm is quick (Lazof et al., 1996; Vazquez et al., 1999) and therefore various inter and intracellular sites may be affected. Al accumulation in plants may be dangerous for animal life and human health because Al enters the food chains in this way (Rafia and Sehrish, 2008). Aluminum toxicity is a major factor in limiting growth in plants in most strongly acid soils. Toxic effects on plant growth have been attributed to several physiological and biochemical pathways (Roy et al., 1998). Si has been reported to alleviate Al toxicity in conifers (Ryder et al., 2003), barley (*Hordeum vulgare*; Hammond et al., 1995), soybean (*Glycine max*; Baylis et al., 1994), maize (*Zea mays*; Barcelo et al., 1993), and sorghum (*Sorghum bicolor*; Galvez et al., 1987). Little or no effect of Si on Al resistance has been found in wheat (*Triticum aestivum*), pea (*Pisum sativum*; Hodson, Evans, 1995), and cotton (*Gossypium hirsutum*; Li et al., 1989). Ma et al. (1997) suggested that the ameliorative effect of Si on Al toxicity resulted from decreasing the toxic Al<sup>3+</sup> concentration in solution by forming Al-Si complexes. The present study was performed in order to:

1. Provide incite into the over-arching hypothesis: that an increase of Al, or availability, causes toxicity to *Borago officinalis* and which concentration of Al<sup>3+</sup> applied is toxic

to plants.

2. As an approach to understand the physiological mechanism of Al toxicity in plants so as to induce production of tolerance traits that can overcome its deleterious effect.

3. Assess influence of silicon and Aluminum on physiological and Biochemical characterizes in *Borago*.

4. Si and Al have combined effects on some biochemical compounds such as: proline, protein, malondialdehyde (MDA) and phenolic content in this cured plant.

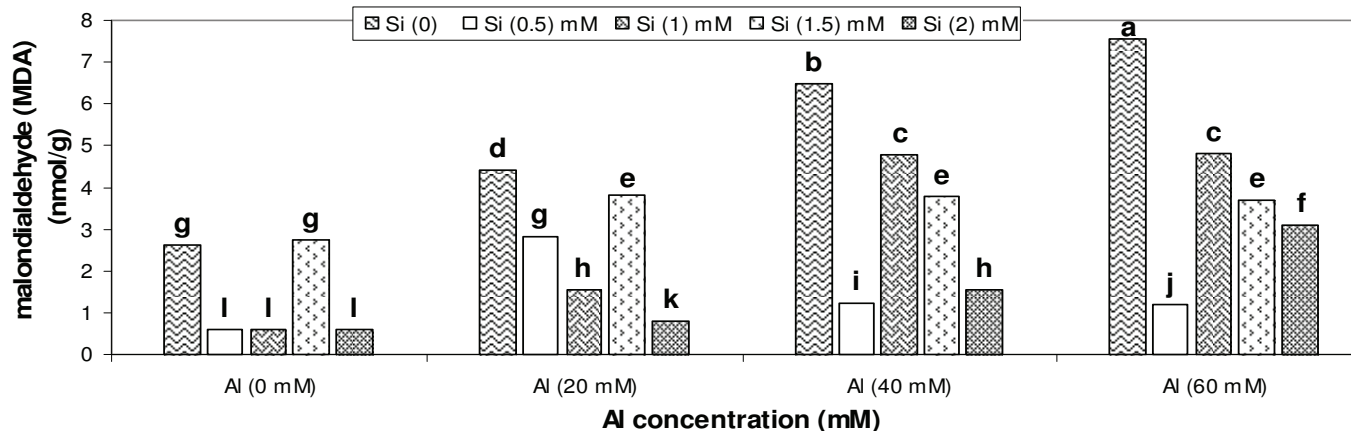
5. Compare these compounds activity levels together.

## MATERIALS AND METHODS

### Plant materials and treatments

The seeds of *B. officinalis* were obtained from the Isfahan Agriculture Research institute (Isfahan, Iran). After being sterilized with 5% sodium hypochloride for 5 min to prevent fungal attack and rinsed in several changes of sterile water. The seeds were allowed to germinate on filter paper saturated with sterile distilled water. Selected seedlings of equal size and vigor were transferred to plastic pots with a 20 cm diameter containing vermiculite (perlite) in a greenhouse. 14 seeds were sown in pots. The pots were incubated in a naturally light greenhouse at 6 to 20°C and relative humidity (RH) was 1.2 to 30.3%. The seedlings were irrigated with water once a day. At the same time, seedlings were also irrigated with Hoagland's solution (Hoagland and Arnon, 1950) (pH = 6.7) twice a week to prevent mineral deficiency. Hoagland's solution containing the following macronutrients: KNO<sub>3</sub>, Ca (NO<sub>3</sub>)<sub>2</sub> × 4H<sub>2</sub>O, MgSO<sub>4</sub> × 7H<sub>2</sub>O, NaH<sub>2</sub>PO<sub>4</sub> × 2H<sub>2</sub>O and micronutrients: FeCl<sub>3</sub>, H<sub>3</sub>BO<sub>3</sub>, MnSO<sub>4</sub> × 4H<sub>2</sub>O, ZnSO<sub>4</sub> × 7H<sub>2</sub>O, CuSO<sub>4</sub> × 5H<sub>2</sub>O, NaCl, (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> × 2H<sub>2</sub>O. The pH of the solution was adjusted to 7 daily, with dilute HCl and or NaOH. When the seventh leaf of *Borago* plants appeared, plants was treated with five levels of Si: 0, 0.5, 1, 1.5 and 2 mM Si (as sodium trisilicate, Na<sub>2</sub>(SiO<sub>2</sub>)<sub>3</sub>).

Archambault et al. (1996), Ishikawa and Wagatsuma (1998) and Lidon and Barreiro (1998) used Aluminum chloride (AlCl<sub>3</sub>·6H<sub>2</sub>O) for toxicity tests, and this form was therefore used in the present study. The pH was kept at or below 4 to maintain the dissolved Al<sup>3+</sup> (aq) form. Al<sup>3+</sup> was supplied at a rate of 0, 20, 40 and 60 mM nutrient solution (Table 1). Position of the pots was changed weekly to avoid a position effect in the growth environment. Then the plants were harvested for analysis. Samples were taken snap-frozen in liquid nitrogen and then kept at 70°C until measurements. Data



**Figure 1.** Changes in the malondialdehyde content (MDA) in the leaves of *Borago* under Al stress. Values are mean  $\pm$  SE based on three replicates ( $n = 3$ ).

were collected at four week intervals. Plants were analysed for estimating the proline, peroxidation of lipids, protein and phenolic compounds.

#### Malondialdehyde (MDA) and other aldehydes

A decomposition product of polyunsaturated fatty acids has been utilized as a biomarker for lipid peroxidation (Mittler, 2002). The level of lipid peroxidation in plant tissues was measured by determination of MDA (Heath and Packer, 1969) and other aldehydes (Propanal, Botanal, Hexanal, Heptanal and Propanal dimethylstall) (Meirs et al., 1992), breakdown products of lipid peroxidation.

#### Free proline content

Proline was extracted and its concentration determined by the method of Bates et al. (1973). Leaf tissues were homogenized in 3% sulfosalicylic acid and the homogenate was centrifuged at  $3,000 \times g$  for 20 min.

The supernatant was treated with acetic acid and ninhydrin, boiled for 1 h, and then the absorbance was determined at 520 nm. Proline (Sigma) was used for a standard curve.

#### Free protein content

The protein concentration in cell extracts was measured by the modified Lowry et al. (1951) method, using bovine serum albumin as the standard. The concentration of Cu ions was measured with an atomic absorption spectrophotometer.

#### Phenolic compounds

Phenolic compounds were measured according to Sonald, and Laima (1999).

#### Statistical analysis

The experiment was completely randomized design consisting of twenty all treatments and three replication. Differences between the means were compared through Duncan's multiple range test

( $p < 0.05$ ) using the "MSTAT-C" computer program.

## RESULTS

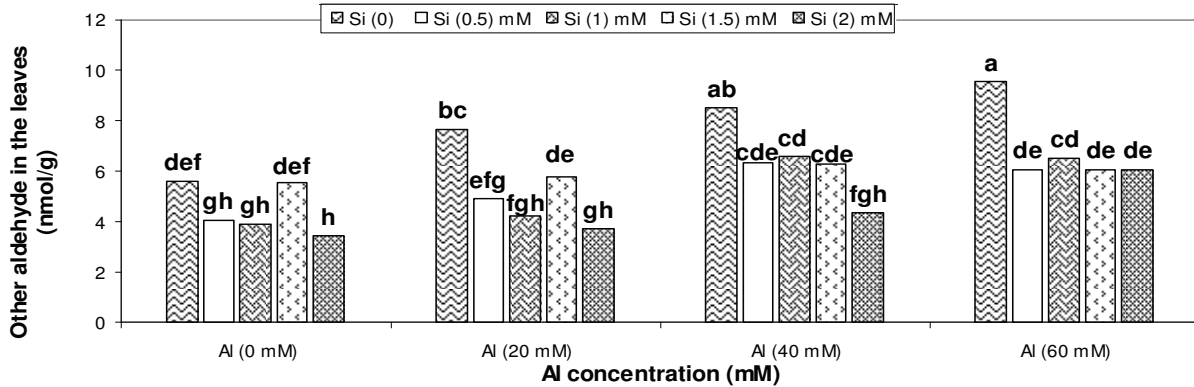
These are the first results that show the role of Si on some biochemical parameters under Al stress conditions in *B. officinalis*.

#### Interactive effects of silicon and aluminum on lipid peroxidation of *Borago*

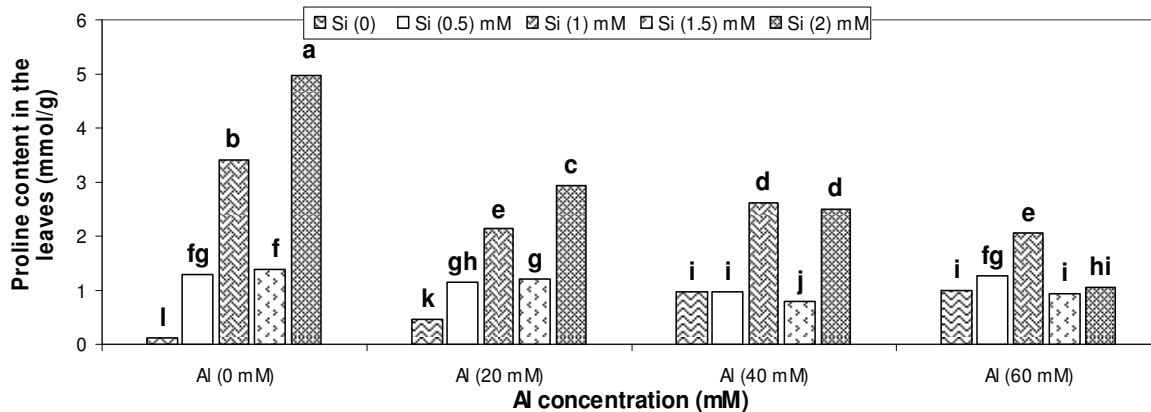
It may act to alleviates Al stress in *Borago* by increasing permeability of plasma membranes and membrane lipid peroxidation and not maintaining the membrane integrity and function. The results of the present study showed that Aluminum significantly altered the levels of MDA and other aldehyds in the leaves of this plant. It was obvious that Al stress increased lipid peroxidation in plants. Maximum MDA and other oldehydes contents were noticed in Al-stressed plants. The contents of MDA and other aldehydes were reduced in plants treated with Si under Al stress. The data recorded in Figures 1 and 2 reveal that the tolerance indexes of *Borago* were significantly increased by increasing the silicon and Al<sup>3+</sup> levels. Lipid peroxidation was low in all treatments in present of Si.

#### Interactive effects of silicon and aluminum on proline contents (in the leaves and roots) of *Borago*.

Proline accumulation is a plant resistance mechanism to various stress factors, such as Al stress. Proline may act as an osmoregulator compound to equilibrate the osmotic potential in plant cells. However, Proline has been considered as a carbon and nitrogen source for rapid recovery from stress and growth and some and also a free radical scavenger (Jain et al., 2001). The level of



**Figure 2.** Changes in the other aldehyde content in the leaves of *Borago* under Al stress. Values are mean  $\pm$  SE based on three replicates ( $n = 3$ ).



**Figure 3.** Free proline content in the leaves of *Borago* under Al stress. Values are mean  $\pm$  SE based on three replicates ( $n = 3$ ).

proline content in leaves increased significantly ( $p < 0.05$ ) with increasing Al stress in *Borago*.

Plants treated with Si alone showed an increase in proline content both in the roots and leaves (Figures 3 and 4).

The results given in Figures 3 and 4 indicated that in the plants increasing  $Al^{3+}$  level resulted in an increase of the proline concentrations either in the absence or the presence of Si as compared to control. Compared to the plants treated with Si alone, addition of Al decreased the level of proline content in Al-stressed plants in the leaves. Maximum proline content in the leaves was noticed in plants that applied whit 2 mM Si without any  $Al^{3+}$  stress but in the roots was noticed in plants that applied without any Si in present of 60 mM  $Al^{3+}$ .

#### Interactive effects of silicon and aluminum on protein content of *Borago*

Added  $Al^{3+}$  increased the protein content under absent of

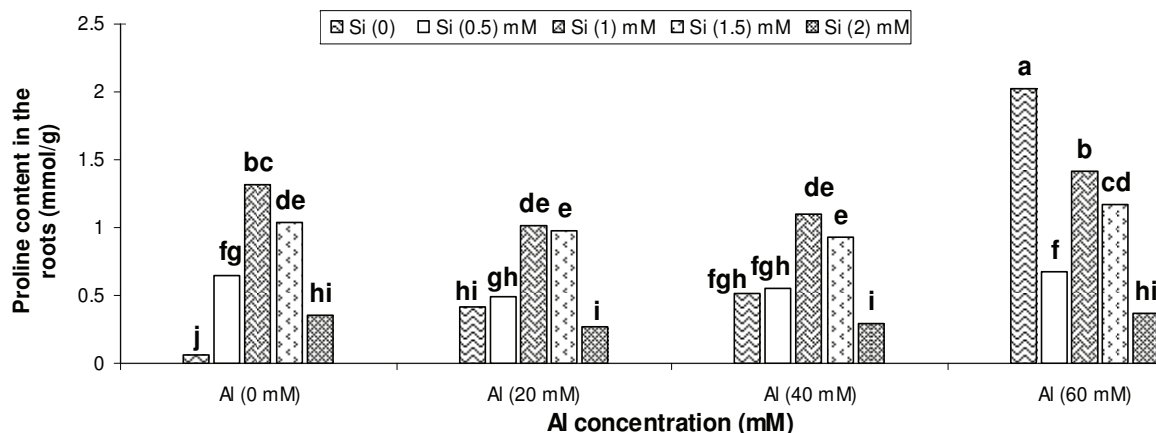
silicon as compared to the control. In Al-stressed plants addition of 0.5 and 1 mM Si increased the level of protein content under Al stress but addition of 1.5 and 2 mM Si decreased it (Figure 5).

Maximum protein content in the leaves was noticed in plants that applied whit 1.5 mM Si.

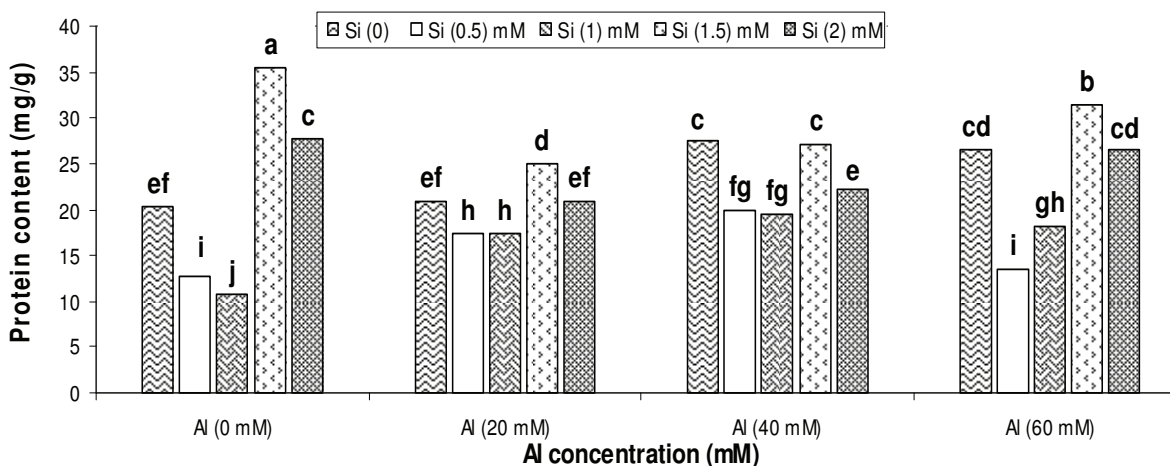
#### Interactive effects of silicon and aluminum on phenolic compounds of *Borago*

The results of the present study showed that Aluminum significantly altered the levels of phenolic compounds in the leaves of this plant. Plants treated with Si alone showed no remarkable increase in phenolic compounds except that of 1.5 mM as compared to the control.

Phenolic compounds of Al-stressed plants showed the same decrease under absent of silicon as compared to the control. Addition of 0.5, 1 and 2 mM Si decreased the level of phenolic compounds in plants grown in Al stress. However, no significant difference was observed between



**Figure 4.** Free proline content in the roots of *Borago* under Al stress. Values are mean  $\pm$  SE based on three replicates (n = 3).



**Figure 5.** Al stress-induced changes in soluble protein content in the leaves of *Borago* under Al stress. Values are mean  $\pm$  SE based on three replicates (n = 3).

1.5 mM Si accompanying with Al (Figure 6). Maximum phenolic compound in the leaves was noticed in plants that applied with 1.5 mM Si alone.

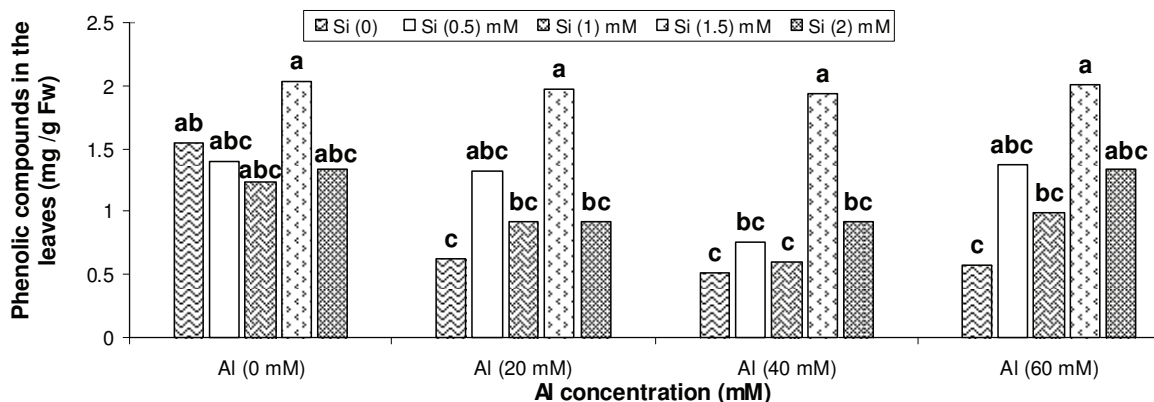
## DISCUSSION

The ameliorative effect of Si on Al toxicity in plants was attributed to a decreased availability of phytotoxic Al in the culture media by some authors (Baylis et al., 1994; Ma et al., 1997). A major problem in the investigation of Al and Si interactions in hydroponic culture over the last 15 years has been the uncertainties concerning the chemistry of Al and Si in the plants that were grown (Ryder et al., 2003). This decrease in Al concentration is suggested to be due to the formation of biologically inactive complexes of hydroxyl Aluminum silicates (HAS).

Although Si is considered as a non-essential element (Taiz and Zeiger, 2006), nevertheless, from some earlier studies it is evident that, exogenous application of Si can promote tolerance of most plant species. If whole plant growth parameters are useful for screening different species for (Al) tolerance, then biochemical parameters may be used to predict resistance variability in *Borago*.

Therefore the different changes of MDA, proline, protein, phenolic compounds observed in response to Al stress and enabled to distinguish the metabolic events caused by Al and variability of *Borago* in Al tolerance. Chemical compounds such as amino acids (proline) and proteins, are products of primary metabolism and are vital for the maintenance of life processes, while others like phenolics are products of secondary metabolism and have toxicological importance (for example, Bandaranayake, 2002). Moreover their synchronous





**Figure 6.** Phenolic compounds in the leaves of *Borago* under Al stress. Values are mean  $\pm$  SE based on three replicates (n = 3).

changes seem to be physiological traits associated with plant tolerant to Al stress.

We found in this literature the toxic [Al] for *Borago* was determined to be higher than any other species. Our results indicated that the cultivation of medicinal plants like *Borago officinalis* in Al stress increase its proline metabolisms.

#### Interactive effects of silicon and aluminum on lipid peroxidation of *Borago*

Malondialdehyde is an oxidized product of membrane lipids, and its level can show the extent of oxidative stress (Guo et al., 2004). MDA content indicating that all indirectly produced superoxide radicals result in increased lipid peroxidative products and oxidative stress. Therefore, the concentration of MDA is commonly considered as a usual indicator of lipid peroxidation (Chaoui et al., 1997). Lipid peroxidation was useful in determining the effect of Al on *B. officinalis*. In the present study, lipid peroxidation (MDA and other aldehydes content) increased by increasing the Al stress (Figures 1 and 2). It was reported that Al induced enhancement of lipid peroxidation and antioxidative enzyme activities in soybean root apices (Cakmak and Horst, 1991). Our results are in agreement with those of Shamsi et al. (2008), who reported that Al stress treatments increased MDA content. Up till now, these phenomena have been reported in liposome (Oteiza, 1994), tobacco cell culture (Yamamoto et al., 2002), detached rice leaves (Kuo and Kao, 2003) and barley root apices (Tamas et al., 2003). Liu et al. (2008) reported that the MDA content increased significantly at all Al levels. Basu and Taylor (2001) reported that MDA content, an indicator of lipid peroxidation, increased significantly by two- and three-fold in roots in response to 100 and 200  $\mu$ M Al, respectively.

Liang et al. (1996, 1999) found that pretreatment with

Si decreased the accumulation of MDA contents under Al stress. Si has anti-stress effects on plants. Added Si decreased the permeability of plasma membrane of leaf cells. They suggested that Si may affect the structure, integrity and functions of plasma membranes by influencing the stress-dependent peroxidation of membrane lipids.

#### Interactive effects of silicon and aluminum on proline contents (in the leaves and roots) of *Borago*

Proline has been considered as a carbon and nitrogen source for rapid recovery from stress and growth and a stabilizer for membranes and some macromolecules (Jain et al., 2001). The content of proline in *Borago* plants which were under Al stress and Si treatments increased progressively with increasing Al level and concentration of Si (Figures 3 and 4). Different roles have been suggested for proline accumulation as an adaptive response. It accumulates in plant when exposed to wide variety of environmental stresses and provide stress tolerance (Alia and Mohanty, 1997). Proline also stabilizes cellular structures (Hare and Cress, 1997).

Proline plays important roles in osmoregulation (Ahmad and Hellebust, 1988; Laliberte and Hellebust, 1989; Alia and Mohanty, 1997), protection of enzymes (Nikolopoulos 1991; Laliberte and Hellebust, 1989; Paleg et al., 1984), stabilization of the folded protein structure (Low, 1985) and the machinery of protein synthesis (Kadpal and Rao, 1985), regulation of cytosolic acidity (Venekemp, 1989) and scavenging of free radicals (Smirnoff and Cumbes, 1989; Hare and Cress, 1997; Jain et al., 2001). It also acts as an effective singlet oxygen quencher (Alia et al., 2001) and plays a role in the regulation of cellular redox potentials (Saradhi and Saradhi, 1991).

Proline as a cytosolic osmoticum (Kavir et al., 2005) and a scavenger of OH<sup>•</sup> radical (Saradhi and Saradhi, 1991; Kavir et al., 2005) can interact with cellular

macromolecules such as DNA, protein and membranes and stabilize the structure and function of such macromolecules (Kavir et al., 2005). Proline has been shown *in vitro* to reduce enzyme denaturations caused due to heat, NaCl stress, etc (Hamilton, and Heckathorn, 2001).

The mechanism of accumulation of proline in plants or plant parts exposed to stress is still unknown. It is suspected to be due to a decrease in the activity of the electron transport system (Venekemp, 1989; Sawhney et al., 1990; Alia and Mohanty, 1993) that leading to accumulation of NADH and H<sup>+</sup>.

Proline accumulation (presumably through synthesis from glutamic acid) might be an adaptive mechanism for decreasing (a) the level of accumulated NADH and (b) the acidity; (2NADH + 2H<sup>+</sup>) is used for synthesizing each molecule of proline from glutamic acid (Venekemp et al., 1987). Binding with metal ions due to the chelating ability of proline (an amino acid) can also be a defense mechanism for survival.

#### **Interactive effects of silicon and aluminum on protein content of *Borago***

In the present work, protein content increased with increasing metal ion concentration. Increase in protein content under heavy metal stress in *S. platensis* indicates that it may contain a larger proportion of proline.

It was reported that promotion of growth in stressed seedlings of *Robinia Pseudoacacia* (Li et al., 2008), sorghum (Vardhini and Rao, 2003), wheat (Shahbaz et al., 2008) and rice (Anuradha and Rao, 2003) under stress conditions might be related to enhanced levels of nucleic acids, soluble proteins and photosynthesis. Basu et al. (1994) reported that Al<sup>3+</sup> could induce high molecular mass proteins.

#### **Interactive effects of silicon and aluminum on phenolic compounds (in the leaves and roots) of *Borago***

Plants have developed protection strategies against different pathogens and environmental stress. Phenols, phenolic acids, lignins, tannins and flavonoids as phenylpropanoids, are secondary metabolites and have several functions in plants. They functions as visual traps and antioxidants and play important roles on plant growth, enzymatic activities, fruit quality, graft compatibility and provide resistance against pathogenic microorganisms, UV radiation, herbivores, oxidative and thermal stress (Kuhnau, 1976; Sommer and Saedler, 1986; Winnard et al., 1986; Hahlbrock and Scheel, 1989; Treutter et al., 1990; Beier and Nigg, 1992; Seigler, 1998; Paolacci et al., 2001; Jaakola et al., 2002; Lorenc-Kukula et al., 2007). Kidd et al. (2001) suggested that an

enhanced exudation of phenolic compounds is responsible for the Si-induced Al resistance in maize.

Under their experimental conditions, phenol exudation was a major factor contributing to Si-enhanced Al resistance. Al and Si triggered the release of catechol and of the flavonoidtype phenols catechin and quercetin. In an Al-resistant variety, Si-pretreated plants exuded more phenols than plants not pretreated with Si.

In our short-term experiments, Si induced phenol exudation.

#### **Conclusion**

This report is the first documentation of Si uptake or accumulation in any species of the Boraginaceae family. Thus, *Borago* plants in which addition of Si to the culture solution prevented Al toxicity, in this experiment, also grew better.

However, Si is present in soil in different forms, but plants can easily absorb silicic acid (H<sub>4</sub>SiO<sub>4</sub>) from soil (Chen et al., 2000) and is taken up directly as silicic acid (Ma et al., 2001). Silicic acid is generally found in the range of 0.1 to 0.6 mM in soils (Epstein, 1994). It reduces micronutrient and metal toxicity (Horst and Marschner, 1978; Horiguchi and Morita, 1987; Cocker et al., 1998a; Britez et al., 2002). The effect of Si on many ornamental and greenhouse crop species has not been extensively studied. In greenhouse culture, plants are not exposed to Si from mineral soil, so Si should be added to the fertilizer solutions with mineral soil or soil amendments if there are benefits of Si. The most effective dose of Si under stress conditions was found to be 0.5 and 1.5 mM. In the plant, silicon reduces the phytotoxicity of Aluminum. Aluminum is a trace mineral that can be dangerous, even fatal, if excessive amounts are ingested. It has been found within the diseased brain cells of people with Alzheimer's disease.

Aluminum toxicity is a serious problem for plants growing on acid soils, whether the acidity is caused naturally, as in many tropical soils, or is due to acid rain. Silicon may be involved in Aluminum tolerance. In contrast little work in Aluminum-silicon interaction in higher plants has been carried out, and the results so far are somewhat contradictory. It is now clear that silicon ameliorate the effects of Aluminum toxicity in sorghum, but not in cotton (Li et al., 1989).

The reason for this difference is unclear, but the much more works remain to be done in Aluminum or silicon interaction in higher plants and more species and cultivars should be investigated. Recently there has been considerable interest in the interaction between Aluminum and Silicon in biology (Birchall, 1992). Some workers have shown that Si can decrease the toxic effects of Al in hydroponics culture in several species (Ma et al., 1997; Cocker et al., 1998b; Zsoldos et al., 2000). Galvez et al. (1987) investigated Aluminum-silicon

interaction in sorghum plants growing in water culture. They claimed that silicon ameliorate the toxic effects of Aluminum.

The mechanism of the amelioration is unclear, but three suggestions have been put forward: Si-induced increase in solution pH during the preparation of hydroponic solutions; decreased availability of Al due to the formation of hydroxyaluminosilicate (HAS) species in those solutions during plant growth; or in detoxification.

Although the beneficial effects of Si on plant tolerance to metal have been accumulating in recent years, its mechanism remains unclear.

However, the present study indicated that Si caused a number of physiological and biochemical changes in the seedlings, including increased free proline content, phenolic compounds. In contrast, treatment with Si decreased peroxidation of lipids (MDA and other aldehydes). The results of the present study indicate that proline may exert some action on the cell surface and suggest that one function of accumulated proline is to reduce the uptake of metal ions. However, the function of proline accumulation in response to metal stress is still unknown. However, it is still unknown whether proline accumulation is related to metal tolerance. Understanding the biosynthesis, degradation, transport, roles of proline during stress and the events that regulate stress-induced accumulation is vital in developing plants for stress-tolerance.

Dramatic accumulation of proline due to increased synthesis and decreased degradation under a variety of stress conditions such as salt, drought and metal has been documented in many plants.

Numerous previous studies showed that Si was inert in plants and not involved in the physiological metabolism (Epstein 1994, 1999). However, further studies by using new techniques should be carried out to reach at more certain realistic results.

However, the roles of Si in plant metabolism have received increasing attention and it has been proposed that Si should be included routinely in the formulation of nutrient solutions (Epstein, 1994, 1999; Rafi et al. (1997).

## REFERENCES

- Ahmad I, Hellebust A (1988). The relationship between inorganic nitrogen metabolism and proline accumulation in osmoregulatory responses of two euryhaline microalgae. *Plant Physiol.*, 88: 348-354.
- Alia SPP, Mohanty P (1993). Proline in relation to free radical production in seedlings of *Brassica juncea* raised under sodium chloride stress. *Plant Soil*, 155/156: 497-500.
- Alia SPP, Mohanty P (1997). Involvement of proline in protecting thylakoid membranes against free radical induced photodamage. *J. Photochem. Photobiol. B*, 38: 253-257.
- Alia SPP, Mohanty P, Matysik J (2001). Effect of proline on the production of singlet oxygen. *Amino Acid*, 21: 195-200.
- Anuradha S, Rao SSR (2003). Application of brassinosteroids to rice seeds (*Oryza sativa* L.) reduced the impact of salt stress on growth, prevented photosynthetic pigment loss and increased nitrate reductase activity. *Plant Growth Regul.*, 40: 29-32.
- Archambault DJ, Zhang G, Taylor GJ (1996). A comparison of the kinetics of Aluminum (Al) uptake and distribution in roots of wheat (*Triticum aestivum*) using different Aluminum sources. A revision of the operational definition of symplastic Al. *Physiol. Planetarium*, 98: 578-586.
- Bandaranayake WM (2002). Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecol. Manag.*, 10: 421-452.
- Barcelo J, Guevara P, Poschenrieder C (1993). Silicon amelioration of aluminium toxicity in teosinte (*Zea mays* L. ssp. *Mexicana*). *Plant Soil*, 154: 249-255.
- Basu U, Basu A, Taylor GJ (1994a). Differential exudation of polypeptides by roots of Aluminum-resistant and Aluminum-sensitive cultivars of *Triticum aestivum* L. in response to Aluminum stress. *Plant Physiol.*, 106: 151-158.
- Basu GAG, Taylor GJ (2001). Transgenic *Brassica napus* plants overexpressing aluminium-induced mitochondrial manganese superoxide dismutase cDNA are resistant to aluminium. *Plant Cell Environ.*, 24: 1269-1278.
- Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free enzyme activities in tomato plants under salt stress. *J. Plant Nutr.*, 27: 2101-2115.
- Baylis AD, Gragopoulou C, Davidson KJ, Birchall JD (1994). Effect of Silicon on the toxicity of aluminium to soybean. *Commun. Soil Sci. Plant Anal.*, 25: 537-546.
- Beier RC, Nigg HN (1992). Natural toxicants in foods. In Nigg HN, and Seigler D (eds) *Phytochemical Resources for Medicine and Agriculture*. Plenum Press, New York, pp. 247-367.
- Birchall JD (1992). The inter relationship between Silicon and Aluminum in the biological effects of Aluminum. *Ciba found. Symp.*, 169: 50-68.
- Britez RM, Watanabe T, Jansen S, Reissmann CB, Osaki M (2002). The relationship between aluminium and Silicon accumulation in leaves of *Faramea marginata* (Rubiaceae). *New Phytol.*, 156: 437-444.
- Cakmak I, Horst WJ (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol. Plant*, 93: 463-468.
- Chaoui A, Mazhoudi S, Ghorbal MH, Ferjani EL (1997). Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant Sci.*, 127: 139-147.
- Chen J, Russell DC, Cynthia AR, Robert S (2000). Silicon: The estranged medium element. Florida Cooperative extension service, Institute of Food and Agricultural Sciences, University of Florida, USA. Bulletin 341, series of Envi. Hort. Dept.
- Cocker KM, Evans DE, Hodson MJ (1998). The amelioration of Aluminum toxicity in Higher Plants: Solution Chemistry or an in Planta Mechanism. *Physiol. Plant*, 104: 608-614.
- Delhaize E, Tyan PR (1995). Aluminum toxicity and tolerance in plants. *Plant Physiol.*, 107: 315-321.
- Epstein E (1994). The anomaly of Silicon in plant biology. *Proc. Natl. Acad. Sci. U.S.A.*, 91: 11-17.
- Epstein E (1999). Silicon. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 50: 641-664.
- Foy CD (1984). Physiological effects of hydrogen, Aluminum and manganese toxicities in acid soil. In: Pearson RW, Adams F (ed.): *Soil Acidity and Liming*. Wisconsin, 1984. *Am. Soc. Agron.* pp. 57-97.
- Galvez L, Clark RB, Gourley LM, Maranville JW (1987). Silicon interaction with Manganese and Aluminum toxicity In *Sorghum*. *J. Plant Nutr.*, 10: 1139-1147.
- Guo TR, Zhang GP, Zhou Mx, Wu FB, Chen JX (2004). Effects of Aluminum and cadmium toxicity on growth and antioxidant enzyme activities of two barley genotypes With Different Al Resistance. *Springer Science Business. Media Rev.*, 258.1: 241-248.
- Guo TR, Zhang GP, Lu WY, Wu HP, Wu FB, Chen JX, Zhou MX (2003). Effect of Al on dry matter accumulation and Al and nutrients in barleys differing in Al tolerance. *Plant Nutr. Fert. Sci.*, 9: 324-330.
- Hahlbrock K, Scheel D (1989). Physiology and Molecular Biology of Phenylpropanoid Metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 40: 347-369.
- Hamilton III EW, Heckathorn SA (2001). Mitochondrial adaptations to NaCl. Complex I is protected by antioxidants and small heat shock proteins, whereas complex II is protected by proline and betaine. *Plant Physiol.*, 126: 1266-1274.



- Hammond KE, Evans DE, Hodson MJ (1995). Aluminium/Silicon interactions in barley (*Hordeum vulgare* L.) seedlings. *Plant Soil*, 173: 89-95.
- Hare PD, Cress WA (1997). Metabolic implications of stress induced proline accumulation in plants. *Plant Growth Regul.*, 21: 79-102.
- Heath RL, Pacher L (1969). Photoperoxidation in Isolated Chloroplast. Kinetics and Stoichiometry of Fatty Acid Peroxidation. *Arch. Biochem. Biophys.*, 125: 189-198.
- Hoagland DR, Arnon DI (1950). The water culture method for growing plants without soil. *Calif. Agric. Exp. Stat. Circ.*, p. 347.
- Hodson MJ, Evans DE (1995). Aluminum/Silicon interactions in higher plants. *J. Exp. Bot.*, 46: 161-171.
- Hodson MJ, Sangster AG (1999). Aluminum/Silicon interactions in conifers. *J. Inorg. Biochem.*, 76: 89-98.
- Horiguchi T, Morita S (1987). Mechanism of manganese toxicity and tolerance of plants VI. Effect of Silicon on alleviation of manganese toxicity of barley. *J. Plant Nutr.*, 10: 2299-2310.
- Horst WJ, Marschner H (1978). Effect of Silicon on manganese tolerance of bean plants (*Phaseolus vulgaris* L.) *Plant Soil*, 50: 287-303.
- Ishikawa S, Wagatsuma J (1998). Plasma membrane permeability of root tip cells following temporary exposure to Al ions is a rapid measure of Al tolerance among plant species. *Plant Cell Physiol.*, 39: 516-525.
- Jaakola L, Maatta K, Pirttila AM, Torronen R, Karenlampi S, Hohtola A (2002). Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during Bilberry fruit development. *Plant Physiol.*, 130: 729-739.
- Jain M, Mathur G, Koul S, Sarin NB (2001). Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogea* L.). *Plant Cell Rep.*, 20: 463-468.
- Jansen S, Watanabe T, Dessein S, Smets E, Robbrecht E (2003). A comparative study of metal levels in leaves of some Al-accumulating Rubiaceae. *Ann. Bot.*, 91: 657-663.
- Jian MF, Yamaji N (2006). Silicon uptake and accumulation in higher plants, *Trends in Plant Science* Vol. 11 No. 8 Jones, DL, LV Kochain, and Gilary S., 1998. *Plant Physiol.*, 116: 81-89.
- Kadpal RP, Rao NA (1985). Alteration in the biosynthesis of proteins and nucleic acid in finger millet (*Eleusine coracana*) seedling during water stress and the effect of proline on protein biosynthesis. *Plant Sci.*, 40: 73-79.
- Kavir KPB, Sangam S, Amrutha RN, Laxmi PS and NKR (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Current sci.*, 88(3): 424-438.
- Kidd PS, Llugany M, Poschenrieder C, Gunse B, Barcelo J (2001). The role of root exudates in Aluminum resistance and Silicon-induced amelioration of Aluminum toxicity in three varieties of maize (*Zea mays* L.). *J. Exp. Bot.*, 52: 1339-1352.
- Kuhnau J (1976). The flavonoids: a class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet*, 24: 117-191.
- Kuo MC, Kao CH (2003). Aluminum effects on lipid peroxidation and antioxidative enzyme activities in rice leaves. *Biol. Plant*, 46: 149-152.
- Laliberte G, Hellebust JA (1989). Regulation of proline content of *Chlorella autotopica* in response to change in salinity. *Can. J. Bot.*, 67: 1959-1965.
- Lazof DB, Goldsmith JG, Ruffy TW, Linton RW (1996). The early entry of Al into cells of intact soybean roots. A comparison of three developmental root regions using secondary ion mass spectrometry imaging. *Plant Physiol.*, 112: 1289-1300.
- Li YC, Alva AK, Sumner ME (1989). Response of cotton cultivars to Aluminum in solutions with varying Silicon concentrations. *J. Plant Nutr.*, 12: 881-892.
- Li KR, Wang HH, Han G, Wang QJ, Fan J (2008). Effects of brassinolide on the survival, growth and drought resistance of *Robinia pseudoacacia* seedlings under water-stress. *New Forests*, 35: 255-266.
- Liang YC, Shen QR, Shen ZG, Ma TS (1996). Effects of Silicon on salinity tolerance of two barley cultivars. *J. Plant Nutr.*, 19: 173-183.
- Liang YC, Ding RX, Liu Q (1999). Effects of Silicon on salt tolerance of barley and its mechanism. *Sci. Agric. Sin.*, 32(6): 75-83.
- Liang YC, Yang CG, Shi HH (2001). Effects of Silicon on growth and mineral composition of barley grown under toxic levels of aluminium. *J. Plant Nutr.*, 24: 229-243.
- Lidon FC, Barreiro MG (1998). Threshold Aluminum toxicity in maize. *J. Plant Nutr.*, 21: 413-419.
- Liu Q, Yang JL, He LS, Li YY, Zheng SJ (2008). Effect of Aluminum on cell wall, plasma membrane, antioxidants and root elongation in triticale. *Biol. Plant.*, 52 (1): 87-92.
- Lorenc-Kukula K, Wrobel-Kwiatkowska M, Starzycki M, Jan SJ (2007). Engineering flax with increased flavonoid content and thus *Fusarium* resistance. <http://rss.sciencedirect.com/getMessage?RegistrationId=EBFJECFJFHFKMBGGBFQCFRFFJRJFJLHTRHFGONL>.
- Low PS (1985). Molecular basis of the biological compatibility of nature's osmolytes. In Gilles R and Gilles-Bailien M [Eds.] *Transport Processes, Iono- and Osmoregulation*. Springer-Verlag, Berlin, pp. 469-477.
- Lowry OH, Roscberough NJ, Farr AL, Randall RJ (1951). Protein mesurement whit the folin-phenol reagent. *Biol. Chem. J.*, 193: 265-275.
- Ma JF, Sasaki M, Matsumoto H (1997). Al-induced inhibition of root elongation in corn, *Zea mays* L., is overcome by Si addition. *Plant Soil*, 188: 171-176.
- Ma JF, Ryan PR, Delhaize E (2001). Aluminum tolerance in plants and the complexing role of organic acids. *Trends Plant Sci.*, 6: 273-278.
- Ma JF, Miyake Y, Takahashi E (2001). Silicon as a beneficial element for crop plants. In: *Silicon in Agriculture*. (Eds.): Datnoff LE, Snyder GH, Korndorfer GH. Elsevier Sci., Amsterdam, pp.17-39.
- Ma JF, Takahashi E (2002). Soil, fertilizer, and plant Silicon research in Japan. Elsevier Sci., Amsterdam.
- Ma JF (2004). Role of Silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.*, 50: 11-18.
- Meirs S, Philosoph-Hadu S, Aharoni N (1992). Ethylene increased accumulation of fluorescent lipid-peroxidation Products detected during parsley by a newly developed method. *J. Am. Soc. Hort. Sci.*, 117: 128-132.
- Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.*, 7: 405-410.
- Nikolopoulos D (1991). Compatible solutes and *in vitro* stability of *Salsola soda* enzyme: proline incompatibility. *Phytochemistry*, 30: 411-413.
- Oteiza PL (1994). A mechanism for the stimulatory effect of Aluminum on iron- induced lipid peroxidation. *Arch. Biochem. Biophys.*, 308: 374-379.
- Paleg LG, Steward GR, Bradbeer JW (1984). Proline and glycine betaine influence proline salvation. *Plant Physiol.*, 75: 974-978.
- Paolacci AR, D'ovidio R, Marabottini R, Nali LG, Abanavoli MR, Badiani M (2001). Ozone induces a differential accumulation of phenylalanine ammonia-lyase, chalcone synthase and chalcone isomerase RNA transcripts in sensitive and resistant bean cultivars. *Aust. J. Plant Physiol.*, 28: 425-428.
- Rafi MM, Epstein E, Falk RH (1997). Silicon deprivation causes physical abnormalities in wheat (*Triticum aestivum* L.). *J. Plant Physiol.*, 151: 497-501.
- Rafia A, Sehrish H (2008). Photochemistry of light harvesting pigments and some biochemical changes under Aluminum stress. *Pak. J. Bot.*, 40(2): 779-784.
- Richmond KE, Sussman M (2003). Got Silicon? The non-essential beneficial plant nutrient. *Curr. Opin. Plant Boil.*, 6: 268-272.
- Roy AK, Sharma A, Talukder G (1998). Some aspects of Aluminum toxicity. *Bot. Rev.*, 54: 145-178.
- Ryder M, Gerard F, Evans DE, Hodson MJ (2003). The use of root growth and modeling data to investigate amelioration of aluminium toxicity by Silicon in *Picea abies* seedlings. *J. Inorg. Biochem.*, 97: 52-58.
- Saradhi A, Saradhi PP (1991). Proline accumulation under heavy metal stress. *J. Plant Physiol.*, 138: 554-558.
- Seigler DS (1998). Flavonoids. In Seigler DS (ed) *Plant secondary metabolism*, Kluwer Academic Publisher, Dordrecht, pp. 151-193.
- Shahbaz M, Ashraf M, Athar H (2008). Dose exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.). *Plant Growth Regul.*, 55: 51-64.
- Shamsi IH, Wei K, Zhang GP, Jilani GH, Hassan MJ (2008). Interactive

- effects of cadmium and Aluminum on growth and antioxidative enzymes in soybean. *Biol. Plant.*, 52(1): 165-169.
- Sawhney V, Shearan IS, Singh R (1990). Nitrogen fixation photosynthesis and enzymes of ammonia assimilation and ureide biogenesis in nodules of mungbean (*Vigna radiata*) grown in presence of cadmium. *Indian J. Exp. Biol.*, 28: 883-886.
- Smirnof N, Cumbes QJ (1989). Hydroxyl radical scavenging activity of compatible solute. *Phytochemistry*, 28: 1057-1060.
- Sommer H, Saedler H (1986). Structure of the chalcone synthase gene of *Antirrhinum majus*. *Mol. Gen. Genet.*, 202: 429-434.
- Sonald SF, Laima SK (1999). Phenolics and cold tolerance of *Brassica napus*. *Plant Agric.*, 1: 1-5.
- Tamas L, Huttova J, Mistrik I (2003). Inhibition of Al-induced root elongation and enhancement of Al-induced peroxidase activity in Al-sensitive and Al-resistant barley cultivars are positively correlated. *Plant Soil*, 250: 193-200.
- Treutter P, Schmid PPS, Feucht W (1990). Q wallbound phenols and peroxidase activity in shoots of *Prunus*. *Gartenbauwissenschaft*. 55(2): 69-72.
- Vardhini BV, Rao SSR (2003). Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. *Plant Growth Regul.*, 41: 25-31.
- Vazquez MD, Poschenrieder C, Corrales I, Barcelo J (1999). Change in apoplastic Aluminum during the initial growth response to Aluminum by roots of a tolerant maize variety. *Plant Physiol.*, 119: 435-444.
- Taiz L, Zeiger E (2006). *Plant Physiology*. 4th edn. Sinauer Associates, Inc., Sunderland, MA, USA.
- Venekemp JH, Lampe JE, Kout TM (1987). Organic acid as a source of drought-induced proline synthesis in field bean plant *Vicia faba*, L. *J. Plant Physiol.*, 133: 654-659.
- Venekemp JH (1989). Regulation of cytosolic acidity in plants under condition of drought. *Plant Physiol.*, 76: 112-117.
- Wang YX, Stass A, Horst WJ (2004). Apoplastic binding of Aluminum is involved in Silicon-induced amelioration of Aluminum toxicity in maize. *Plant Physiol.*, 136: 3762-3770.
- Winnard U, Weydemann U, Niesbach U, Peterson PA, Saedler H (1986). Molecular cloning of the c2 locus of zeamays the gene coding for chalcone synthase. *Mol. Gen. Genet.*, 203: 202-207.
- Yamamoto Y, Kobayashi Y, Devi SR, Rikiishi S, Matsumoto H (2002). Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. *Plant Physiol.*, 128: 63-72.
- Zsoldos F, Vashegyi A, Bona L, Pecsvaradi A, Szegletes ZS (2000). Growth and potassium transport of winter wheat and durum wheat as affected by various Aluminum exposure times. *J. Plant Nutr.*, 23: 913-926.