The effect of the silicon and aluminum interaction on the physiological parameters of maize

Kourosh Delavar¹, Faezeh Ghanati¹*, Hassan Zare-Maivan¹ and Mehrdad Behmanesh²

¹. Department of Plant Biology, Faculty of Biological Science, Tarbiat Modares University (TMU), Tehran, Iran
². Department of Genetics, Faculty of Biological Science, Tarbiat Modares University (TMU), Tehran, Iran

Abstract

Aluminum and silicon are usually abundant in the soil and most plants are affected by them. Therefore, in the present study, the effect of the interaction of Si (0 and 2 mM) and low concentrations of Al (0, 50, 100, and 150 µM) on some physiological parameters of Zea mays var. Merit were investigated and were analyzed by cluster heatmaps for better interpretation of results. Results showed that application of Al increased the fresh and dry weight of maize, and plant growth rate was increased by increasing Al concentration in treatments. However adding Si to Al-treated plants had no effects on the maize plant biomass. Pigments content of plant increased and decreased in Al treatments and Al + Si treatments, respectively. Also, both Al and Si had negative effect on the activity of antioxidant enzyme and proline content of maize in general. Aluminum treatments mostly enhanced the K and Fe content of plants, but decreased Mg and Ca content. However, adding Si to Al-treated plants reversed this trend. Apparently, Al and Si have an antagonistic effect on the mineral content of the plant. Results of the present study suggested that increasing of the plant pigment content contributes to the enhancing of plant growth rate, and the K content of the shoot in maize has an important role in increasing the plant growth.

Key words: Aluminum; antioxidants; maize; mineral content; silicon


Introduction

Some minerals that are naturally present in the soil may be beneficial or toxic for plants. Excess amount of these elements may have some common or specific effects on plants (Rout et al., 2001). Aluminum and silicon are usually abundant in soils. The silicon availability for plant uptake is limited by low solubility; however, Al absorption is dependent on the soil pH (Osaki et al., 1997). Due to the abundance of these two elements in soils, most plants are usually affected by them. Moreover, Al and Si have various interactions both in plants or the soil (Liang et al., 2007). Therefore, in the present study simultaneous presences of both Al and Si in the plant nutrient solution were investigated.

Silicon (Si) is the second most abundant element after oxygen in soil and its concentration in most soil solution is between 100 to 500 µM in the form of monosilicic acid (Sommer et al., 2006). As a consequence, almost all plants grown in soil contain some Si in their tissues, and concentration of Si in the plant species range
between 0.1% and 10% in the dry weight (Hodson et al., 2005). Silicon is not considered as an essential element for plants; however, it plays many important roles in the growth and development of plant species (Epstein, 1999; Ma, 2004). The beneficial effects of Si in plants are particularly revealed under abiotic and biotic stress conditions such as disease, insects, drought, flood, frost, salinity, heavy metals toxicity, and nutritional stress (Guntzer et al., 2012; Corrales et al., 1997). The most important mechanisms of Si function in alleviation of abiotic stresses in plants are: (1) precipitation of heavy metals by create complex with them, (2) stimulation of plant antioxidant systems, (3) effects on the uptake process in roots, (4) inactivation of toxic metals in plant growth medium, and (5) compartmentation of heavy metals within the plant cell (Ma and Takahashi, 2002).

Aluminum toxicity to plants is a serious problem in low pH soils, and its toxic effects on plants have been demonstrated (Delhaize and Ryan, 1995). Aluminum uptake is very fast in both apoplastic and symplastic paths (Vazquez et al., 1999) and therefore it can affect a variety of intercellular and intracellular regions of the cell (Jones et al., 1998). Aluminum ions (Al³⁺) can bind to various organic and inorganic ligands such as PO₄³⁻, SO₄²⁻, lipids, organic acids, and proteins, and suppress cells division and development (Teresa, 2001). Although Al is not regarded as an essential nutrient, sometimes its low concentrations can increase plant growth or induce other desirable effects (Rout et al., 2001). For example, Al can contribute to the alleviation of H⁺ toxicity (Kinraide 1993; Llugany et al. 1995).

Also, there are some studies reporting the alleviative effect of silicon on the aluminum toxicity (Liang et al., 2007). These effects of silicon may be based on ex or in planta mechanisms. Silicon has a high affinity with Al (Dorneles et al., 2016) and can alleviate its toxicity by several mechanisms. Silicon can decrease the phytotoxic levels of Al in the medium by production of Al-Si subcloids complexes (Liang et al., 2007). Also, Kidd et al. (2001) indicated that silicon may stimulate phenolic exudation through roots and by chelated Al and thus Al absorption reduced by the corn roots. Moreover, Al can be detoxified either by forming of hydroxy aluminum silicates in the root apoplast (Wang et al., 2004; Ryder et al., 2003) or by a sequestration in phytoliths (Hodson and Sangster, 1993).

Beneficial effects of Si on the maize are well known, and maize has been recognized as a moderate sensitive plant to Al toxicity. On the other hand, some of our previous experiments (in press) revealed that Zea mays var. Merit is a tolerant plant to Aluminum toxicity. Therefore, in the present study, the interaction of Si and low concentrations of Al on the physiological parameters of Zea mays var. Merit were investigated. Moreover, the data were analyzed by cluster heat maps for better interpretation of results. Heat maps allow users to easily visualize changing patterns in metabolite concentrations across samples and across experimental conditions.

Materials and Methods

Plants materials

Seeds of maize (Zea mays var. Merit) were surface sterilized with 5% (w/v) sodium hypochlorite (15 min), and then rinsed with distilled water, sown in quartz sand bed and kept in the greenhouse (relative humidity of 65-75% with a 12h light period at a 25/15 °C day/night temperature, and a photosynthetic photon flux of 250 µmol m⁻² s⁻¹ (400–700 nm) at the plant level). The seedlings were irrigated with distilled water and after one week, uniform size of seedlings were selected and transferred to pots containing 1500 mL of full-strength Long Ashton nutrient solution (Hewitt, 1966) with or without 2 mM of Na₂SiO₃.5H₂O as silicon source. All hydroponic culture media were continuously aerated and maintained) under the above mentioned conditions. The pH of the nutrient solution was adjusted to 5.5 and the solution was renewed every 5 days. After two weeks, AlCl₃ was added to the nutrient solution at 0, 50, 100, and 150 µM concentrations and the pH was reduced to 4.5. The experiments were arranged based on a completely randomized design with three replications. Treatments included Si (0 and 2 mM), AlCl₃ (0, 50, 100, and 150 µM) and interaction of them. The concentrations of Si
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were decided based on preliminary studies. Two weeks after application of treatments, the plants were harvested, washed thoroughly, and then either immediately frozen in liquid nitrogen and stored at -80 °C, or dried at 70 °C for 48 h.

**Measurements**

The fresh weight of plants was measured immediately after harvesting and dry weight was measured after oven drying at 70 °C for 48 h, to give a constant weight. Plants extracts were provided by homogenizing plant samples (root or shoot) in 3 % sulfosalicylic acid and then by centrifuging at 10000 × g for 5 min. Free proline was determined in plant extracts by Bates et al. (1973) method. Total content of reduced sugars was detected by Nelson-Somogyi method using glucose as standard, and CuSO₄ and H₂PMO₁₂O₄₀ as reducing agent (Somogyi – Nelson, 1952). For measurement of chlorophylls and carotenoids contents, fresh leaf samples were ground and extracted with 80% acetone, centrifuged at 1500 × g for 5 min. The supernatant was collected and its absorbance was read at 663.6, 646.6 and 440.5 nm. The contents of chlorophyll a and b and carotenoids in collected supernatants were calculated using the equations of Porra et al. (1989) and Holm (1954), respectively.

For measurements of enzymes (CAT, SOD, and AOX) activity, leaf samples were homogenized with 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA (ethylenediaminetetraacetic acid) with pre-chilled pestle and mortar. The extract was centrifuged at 4 °C for 15 min at 15000 × g and supernatant was used for the assessment of enzymes activity. Superoxide dismutase activity (SOD) was measured as described by Gianopolitis and Reis (1977) method. In this way the inhibition of photochemical reduction of NBT (nitroblue tetrazolium) was determinate and the absorbance of solution was measured at 560 nm. For determination of APX activity, reaction mixture was prepared as a blend of sodium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H₂O₂, and appropriate amounts of the enzyme extract (Nakano and Asada, 1981). The absorbance at 290 nm was read (as kinetic mode) in the final extract. Catalase activity was determined by following the consumption of H₂O₂ at 240 nm for 1 min, as described by Aebi (1984).

For determination of mineral contents, dry matters of the plant samples were digested with nitric acid containing perchloric acid (1%) and the ion content was determined by inductively coupled plasma optical emission spectrometry (ICP- Optima 7300dv) (Basitas et al., 2004).

**Statistical Analysis**

Data from quantitative parameters were analyzed using ANOVA (SAS version 8.1) and means were compared by Duncan’s multiple range tests at the 5% level. To design cluster heat maps, data files were prepared as comma separated values (.csv) format and uploaded in MetaboAnalyst web server (www.metaboanalyst.ca). After processing of uploaded data files by the MetaboAnalyst software, results were downloaded. All data were transformed as Log Normalization to make features more comparable.

**Results**

Results of the present study indicated that application of Al (50, 100 and, 150 µM) increased the fresh and dry weight of maize, and plant growth rate increased by the increasing Al concentration in treatments. Also, the biomass of Si 2 mM treated plants significantly increased compared with control group. However, simultaneous application of Si and Al had no significant effect on the plants biomass compared with Al treatments (Fig. I. a). Although the low concentration of Al (50 µM) decreased the pigments contents of plants, in Al 150 µM treatments the pigments content of plants significantly increased compared with control group. Adding Si to Al treated plants also reduced the pigments contents of maize plants (Fig. I. b).

Results showed that the catalase activity increased in Al treatments while it significantly decreased when Si was added to Al treatments plants. Also, the SOD and APX activities reduced in Al treatments and application of Si to Al treatments decreased their activity (Fig. II. a).
Results showed that the proline contents of shoots decreased in Al treatments, and addition of Si to Al treatments decreased it further. On the other hand, application of Si, Al or Si + Al treatments had no effects on the proline contents of roots (Fig. II. b).

Application of the Al or Si treatments increased the K content of shoot, but in Si + Al treatments its contents decreased compared with Al treatments. Unlike the shoot, application of Al treatments often decreased K content of roots, and adding Si to Al treated plants enhanced its contents (Fig. III). Magnesium contents in Al treated plants decreased in general, and adding Si to them enhanced its concentration (Fig. III). Similar to the Mg, Ca contents of plants decreased in Al treatments. Application of Si in Al treated plants enhanced the Ca concentration of shoot, but it had no effects on roots (Fig. III). Also, the application of Al treatments increased the Fe concentration of shoot, but adding Si to Al treated plants decreased its content. Finally, both Al and Al + Si treatments had no effects on the iron content of roots (Fig. III).

Discussion

Plant growth and pigment contents
Maize is recognized as a sensitive plant to Al stress while in some studies it is known as a plant with moderate tolerance to Al stress (Poschenrieder et al., 2008). However, according to the results of present study, the Zea mays var. Merit can be introduced as a tolerant plant to Al stress. Corrales et al. (1997) showed that application of Al treatments (20 and 50 µM) in a sensitive var. of barley to Al stress decreased dry weight of roots; however, it had no effects on shoot dry weights. Interestingly, Corrales et al. (1997) found that the application of Si (1 mM) under Al stress condition significantly decreased the dry weight of roots and shoots compared with Al stressed plants. Similarly, Liang et al. (2001) observed that the dry weight of barley plants decreased under Al stress (50 and 150 µM) condition and adding Si to Al treated plant decreased its dry weight. Also, unlike this study, there are some reports about the beneficial effects of Si on plants under Al stress (Liang et al., 2007).

Pigments contents of maize in 150 µM Al treatments significantly increased, and adding Si to Al treated plants also reduced its contents. Similarly, Singh et al. (2011) indicated that application of 50 µM Al decreased the carotenoids and total chlorophylls of rice seedlings, and application of Si in Al stressed plants increased chlorophylls content of plants, but had no effects on the carotenoids contents. Also, Malekzade et al., (2015) found that the application of different concentrations of Al (25-100 mM) in Zea mays seedlings significantly decreased its pigment content.

Antioxidants enzymes

Based on the results of the present study, it can be concluded that both Al and Si have
negative effect on the activity of antioxidant enzymes of maize in general. Shen et al. (2014) observed that the antioxidant enzymes (SOD, POD, and CAT) activity in roots and leaves of peanut decreased after Al exposure (160 mg/L) and the application of silicon enhanced these antioxidant activities under Al stress condition. Mandal et al. (2013) exposed Salvinia to different concentrations of Al (240, 360, and 480 µM) and observed that the SOD and APX activity increased by increasing the Al concentration, but the CAT activity decreased. Enhancing root CAT activity under Al stress condition was reported by Malekjadeh et al., (2015) in maize seedlings. Therefore, some of the above results are inconsistent with the results of the present study while others support them.

**Adjustment of osmolytes**
Apparently Si and Al have a reducing effect only on the proline contents of the shoot. Unlike this finding, decrease in the proline content of leaves and roots of borage was reported by Gagoonani et al. (2011) when they exposed the plant to Al or Si or both treatments. Reduced sugar content of shoot was affected by different Al, Si or Al + Si treatments, but increased in the roots in 150 µM Al treated plants indicated that application of Si under NaCl stress in borage significantly increased reduced sugar content of plant. Generally, it can be concluded that Si, Al or Si + Al treatments have little impact in on the adjustment of osmolytes content of maize.

Mineral content
Results showed that apparently Al and Si have different effects on the K content of roots and shoots in maize. Similar decreases in K absorption under Al stress condition was observed by Gerzabek and Edelbauer (1986) in *Zea mays*. Also, Liang et al. (2001) indicated that K contents of barley decreased under Al stress condition (100 and 150 μM). However, adding Si greatly reduced root K concentration and the uptake of K by barley plants exposed to 75 μmol L⁻¹ Al and beyond. These results suggest that at higher levels of Al, Si caused mineral nutritional imbalance, which in turn adversely affected the growth of plants (Liang et al. 2001). Aluminum competes with K for root absorption site and depresses K uptake (Alam, 1981). Decreases in K content of root in different cultivars of rice under Al stress condition was also reported by Macedo and Jan (2008). Therefore, according to the above investigations and the results of the present study it can be concluded that Al has a decreasing effect on the K content of plants, and adding of Si somewhat eliminated this effect.

Results showed that application of Al decreased Mg contents of plants in general, and adding Si enhanced its concentration (Fig. III). Similarly, Singh et al. (2011) found that application of Al 50 μM in rice seedlings decreased Mg content of plant, and using Si in Al treated plant increased Mg concentration. There are many reports demonstrating that Mg contents of plants reduced under Al stress condition (Roy et al., 1988). Also, some study indicated that low concentrations of Al elevated Mg in some plants such as Acer (Thornton et al., 1986a) and Gleditsia (Thornton et al., 1986b), but higher concentrations reduced Mg level (Thornton et al., 1986b).

Based on the results of the current study it is suggested that Si can alleviate the Ca decreasing effect of Al in plants. The Al³⁺ affects cell membrane structure and permeability by blocking the Ca²⁺ channels and thereby decreases Ca uptake (Ryan and Kochian, 1993; Plieth, 2005). Elevation of the Ca content by application of Si in Al treated plants may be due to the interaction of Si and Al in plant and formation of aluminosilicate complex. Probably, formation of Al-Si complex by reducing the Al content in the plant eliminated its reducing effects on the Ca concentration.

Results indicated that application of different treatments of Al or Si has effects on the iron content of shoots and not that of roots. Similarly, accumulation of Fe with increasing Al levels (up to 10 ppm) was observed in rice (Alam, 1983) and potato (Lee, 1971). However, some investigations indicated that treatment with Al reduced Fe in leaves and roots of *Zea mays* (Gerzabek & Edelbauer, 1986), and in tomato cultivars, Al exposure decreased the content of Fe in roots, stems, and leaves (Simon et al., 1994).

### Correlation analyses

Results indicated that there is a positive correlation between plants growth (dry and fresh weight) and its pigments contents. According to the results of the present study, a similar pattern was observed between plants growth and plant pigment content charts (Fig. IV). Therefore, it is suggested that increasing plants pigment content contributes to the enhancing of plants growth rate. Also, a positive correlation was observed between K contents of shoot and plants’ growth rate. Apparently, the K content of shoot in maize has an important role in increasing plant growth. A negative correlation also was observed between pigment and calcium content of shoots, which suggests that Ca may have an unfavorable effect on the pigment content of maize leaves.

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