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ARTIGO ORIGINAL

MITIGAÇÃO DA TOXICIDADE DE ALUMÍNIO EM MUDAS DE *GLYCYRRHIZA GLABRA* L. USANDO SILÍCIO

MITIGATING ALUMINUM TOXICITY IN SEEDLINGS OF *GLYCYRRHIZA GLABRA* L. USING SILICON

بهبود سمیت آلومینیوم در گیاهچه‌های *GLYCYRRHIZA GLABRA* L. با استفاده از سیلیکون

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RESUMO

Introdução: O silício é um elemento benéfico para a planta, com papel primordial no aumento da resistência da planta à toxicidade de metais pesados e considerando a importância da fitorremediação para remover metais pesados de solos contaminados. Pode ser usado para a aplicação exógena para aliviar os efeitos nocivos dos metais pesados na planta. **Objetivo:** Este estudo teve como objetivo investigar o papel do silício no equilíbrio dos efeitos destrutivos do alumínio em *Glycyrrhiza Glabra* L. **Métodos:** as mudas foram cultivadas em sistema hidropônico com solução nutritiva Long Ashton; as mudas com 15 dias de idade foram expostas ao silício (0, 0,5, 1,5 mM) por 110 dias e, posteriormente, estressadas por interações de cloreto de alumínio ($AlCl_3.6H_2O$; 0, 100, 250 e 400 M). **Resultados e Discussão:** os efeitos interativos do silício melhoraram significativamente as consequências negativas da toxicidade do alumínio. A combinação de Si 1,5 mM e Al 400 µM produziu a maior biomassa na parte aérea (45,67 g). O simples efeito do Si 1,5 mM (12,14 g) proporcionou o maior peso seco da parte aérea. Por outro lado, a maior quantidade de massa fresca e seca de raiz (12,52 e 3,22 g, respectivamente) foi observada em Si 1,5 mM. Entre os tratamentos, Si 0,5 mM + Al 100 µM apresentou a maior altura do caule (38 cm) entre os tratamentos de interação. Da mesma forma, os pigmentos fotossintéticos afetados pelo silício, Al 250 µM + Si 1,5 mM apresentaram o maior teor de clorofila a (1,91 µg / g FW), enquanto Al 400 + 1,5 mM indicou o maior aumento na clorofila b (0,78 µg / g FW) entre os efeitos de interação. Este tratamento, ao produzir 0,663 µg/g FW, rendeu o maior teor de carotenóides. Os maiores teores de prolina na parte aérea e nas raízes (69,54 e 81,46 µg/g FW, respectivamente) foram observados na interação de Al 400 µM e Si 1,5 mM. Além disso, observou-se que esse tratamento apresentou a maior concentração de catalase (1,22 U/mg proteína). O menor conteúdo de malondialdeído foi marcado em Si 1,5 mM + Al 100 µM (0,702 nM/g FW). **Conclusões:** no geral, *Glycyrrhiza Glabra* L. parece ter alto potencial de fitorremediação de Al, que pode ser aprimorado com a aplicação exógena de um nível moderado de silício.

Palavras-chave: *estresse abiótico, MDA, prolina, alcaçuz, metais pesados.*

ABSTRACT

Background: Silicon is a beneficial element for the plant, with the primary role in increasing plant resistance to heavy metals' toxicity and considering the importance of phytoremediation to remove heavy metals from contaminated soils. It could be used for the exogenous application for alleviating the harmful effects of heavy metals on the plant. **Aim:** This study aimed to investigate the role of Silicon in balancing the destructive effects of aluminum on *Glycyrrhiza glabra* L. **Methods:** the seedlings were grown under a hydroponic system using Long Ashton nutrient solution; the 15-day-old seedlings were exposed to Silicon (0, 0.5, 1.5 mM) for 110 days and afterward stressed by interactions of aluminum chloride ($AlCl_3.6H_2O$; 0, 100, 250, and 400 M). **Result and Discussion:** the interactive effects of Silicon significantly ameliorated the negative consequences of aluminum toxicity. The combination of Si 1.5 mM and Al 400 µM produced the highest biomass in shoots (45.67 g). The simple effect of Si 1.5 mM (12.14 g) made the highest shoot dry weight. On the other hand, the highest quantity of root fresh and dry weight (12.52 and 3.22 g, respectively) was observed in Si 1.5 mM. Among the treatments, Si 0.5 mM + Al 100 µM had the most stem height (38 cm) among interaction treatments. Similarly, photosynthetic pigments affected by Silicon, Al 250 µM + Si 1.5 mM had the highest content of chlorophyll a (1.91 µg/g FW), while Al 400 + 1.5 mM indicated the most increase in chlorophyll b (0.78 µg/g FW) among interaction effects. This treatment by producing 0.663 µg/g FW yielded the highest carotenoid content. The highest proline content in shoots and roots (69.54 and 81.46 µg/g FW, respectively) were observed in the interaction of Al 400 µM and Si 1.5 mM. Additionally, this treatment was observed to have the highest concentration of catalase (1.22 U/mg protein). The lowest malondialdehyde content was marked in Si 1.5 mM + Al 100 µM (0.702 nM/g FW). **Conclusion:** overall, *Glycyrrhiza Glabra* L. seems to have high Al phytoremediation potential that can be enhanced with the exogenous application of a moderate Silicon level.

***Keywords**: abiotic stress, MDA, proline, licorice, heavy metals*

چکیده

پیشنه تحقیق: سیلیکون عنصری مفید برای گیاه است که مهمترین نقش آن افزایش مقاومت گیاه به سمیت فلزات سنگین است. با توجه به اهمیت گیاه پالایی برای حذف فلزات سنگین از خاک‌های آلوده، این می‌تواند برای اعمال خارجی روی گیاهان و کاهش اثرات منفی فلزات سنگین استفاده گردد. **هدف:** این بررسی مطالعه نقش سیلیکون در توازن اثرات مخرب آلومینیوم بر نهال‌های *Glycyrrhiza glabra* L. را دنبال نمود. **روش‌ها:** نهال‌ها تحت سیستم هیدروپونیک و محلول تغذیه‌ای لانگ اشتون پرورش یافتند؛ نهال‌های پانزده روزه به مدت 110 روز در معرض سطوح مختلف سیلیکون (1/5، 0/5، 0 میلی مول) قرار گرفتند، سپس با اثرات منفرد و متقابل کلرید آلومینیوم (400، 250، 100، 0 میکرومول) مورد استرس قرار گرفتند. **نتایج و بحث:** اثرات متقابل سیلیکون به طور قابل توجهی تاثیرات منفی سمیت آلومینیوم را بهبود بخشید. اثر متقابل Al 400µM + Si 1/5 mM بیشترین میزان تولید توده زیستی را داشته است (67/45 گرم). در حالیکه اثر ساده Si 1/5 mM (12/14 گرم) بیشترین وزن خشک شاخساره را داشت. از سوی دیگر، بیشترین وزن تر و خشک ریشه (12/52 و 3/22 گرم) در Si 1/5 mM مشاهده گردید. نهال‌های تیمار شده با Al 100µM + Si 0/5 mM از بالاترین ارتفاع (38 سانتی متر) برخوردار بودند. به طور مشابه، رنگدانه های فتوسنتزی نیز تحت تأثیر سیلیکون قرار گرفتند که در آنها اثر متقابل Al 250µM + Si 1/5 mM با 1/91 µg/g FW بیشترین مقدار کلروفیل a را دارا بود، در حالیکه، Al 400µM + Si 1/5 mM بیشترین میزان افزایش میزان کلروفیل b را در میان اثرهای متقابل به همراه داشت (0/78 µg/g FW). این تیمار با تولید 0/663 µg/g FW، بیشترین میزان محتوای کارتنوئید را داشته است. بیشترین پرولین در ساقه و ریشه (به ترتیب 81/46 و 69/54 µg/g FW) در اثر متقابل Al 400µM + Si 1/5 mM مشاهده گردید، همچنین بیشترین میزان فعالیت آنزیم کاتالاز (1/22 U/mg protein) در این تیمار مشاهده گردید. کمترین میزان مالون دی آلدئید در همین تیمار متقابل Al 100µM + Si 1/5 mM مشاهده شد (0/702 nM/g FW). **نتیجه‌گیری:** به طور کلی، به نظر می رسد که *Glycyrrhiza glabra* L. دارای پتانسیل بالایی برای گیاه پالایی آلومینیوم برخوردار است که می تواند با اعمال خارجی سیلیکون افزایش یابد.

***کلیدواژه:** تنش غیرزیستی، پرولین، MDA، شیرین بیان، فلزات سنگین*

1. INTRODUCTION:

Various physical, chemical, and biological approaches have been proposed to refrain from contaminated areas with heavy metals and other pollutants, mostly cost-intensive and inefficient. One of the detoxification methods and the reduction of toxic substances, including heavy metals in the contaminated environments, is utilizing accumulating plants in so-called phytoremediation (Lombi *et al.*, 2001; Ali *et al.*, 2013; Mahar *et al.*, 2016). An approach for removing heavy metals and other pollutants from soil and water systems. This procedure utilizes plant species that can uptake and accumulate heavy metals in their tissues, and they can grow at a concentration of 10 to 100 times that of plants crop tolerant of accumulating. One of the benefits of phytoremediation in this way is the preservation of the soil building and fertility after harvesting heavy metals and finally is a reliable alternative solution to energy-intensive and cost-effective engineering methods (Ghosh and Singh, 2005; Chehregani *et al.*, 2009; Jabeen *et al.*, 2009; Mahar *et al.*, 2016). Phytoremediation is a cost-effective, environmental, and scientific technique, especially for developing countries. Unfortunately, despite this potential, some countries, such as Iran, have not received the attention it deserves as a commercial technology (Rafia and Sehrish, 2008). The effect and efficiency of hyperaccumulator plant species to a large extent depends on the plant characteristics, including growth rate, high biomass, tolerance range, and accumulation of heavy elements from the soil (Barceló and Poschenrieder, 1990). The application of ameliorating treatments can enhance biomass production in soils contaminated with heavy elements. Such treatments play an essential role in increasing phytoremediation efficiency (Das *et al.*, 1997). An ideal plant species for the phytoremediation process should have a high biomass production rate, high absorption capacity, high rate of reproduction, and fast-growing and resistance to unfavorable environmental conditions(Marrugo-Negrete *et al.*, 2016).

Aluminum (Al) is one of the critical factors limiting the growth and production of plants found in acidic soils around the world(Kochian *et al.*, 2004). Today, about 51 percent of the arable lands are occupied by acidic soils, and Al toxicity is one of the main problems in these soils (Singh *et al.*, 2017). Due to the high acidity of the subsoil, the toxicity of Al reduces the penetration depth of plant roots, increases sensitivity to drought and nutrient deficiency (Foy, 1988; Zuh *et al.*, 2009). Accumulation of Al at the cellular and ultrastructural parts leads to changes in leaves, heightens diffusion resistance, decreases in stomatal function, a decline in photosynthesis, reduces number and leaf size, and finally, reduction in aerial biomass (Shen *et al.*, 2014). Because stressful environmental conditions disrupt plants' biochemical processes, plant stress can be considered a tool to study and understand the mechanisms of tolerance in the plant (Rehmus *et al.*, 2014). The high growth rate and covering the ground under the aerial parts in the early stages of growth are advantageous for plant species growing in heavy metal polluted soils. Therefore any factor that delays or delays the germination of seeds or reduces the growth of roots and stems delays land cover uniformity, which eventually reduces plant yield (Nagy *et al.*, 2004; B. Ali *et al.*, 2008). Thus, the cultivation of plants for phytoremediation purposes in Al contaminated soils can be highly limiting. Treating plants with compounds such as Si to alleviate adverse effects of Al during critical growth stages as seedling stages can be helping plants in the successful establishment.

In soil solution, Silicon exists as dissolved silica and absorbed by plants in the form of monosilicic acid (H_4SiO_4). This element composes 28% of the Earth crust as the second most abundant element after oxygen, 47%. Although Si is not an essential element for plant growth and development, the beneficial effects of Si on the plant under stress conditions have been reported (Liang *et al.*, 2005; Guntzer *et al.*, 2012; Emamverdian *et al.*, 2018). One of the benefits of Si application is the increased tolerance of some plant species to heavy metal toxicity is heavy (Samuels *et al.*, 1993). Si is deposited in the endoderm and reduces cadmium transport via apoplast or Intercellular open spaces (Abu-Muriefah, 2015). It is a mitigating agent of toxic effects caused by heavy metals and various types of environmental stresses as salinity, drought, and frost stress. Another positive influence of Si was found to the increase in light-receiving efficiency that with accumulation in the cell walls of the xylem increases plant resistance against heavy metal elements (Corrales *et al.*, 1997; Liang *et al.*, 2005; Kim *et al.*, 2014; Emamverdian *et al.*, 2018). This element stimulates the plant antioxidant system, the formation of complexes with heavy metals, and metal transferring of heavy ions to organs such as plant cell vacuoles reduces heavy metals' stress and toxicity. Si deficiency in the soil, photosynthetic pigments such as chlorophyll-a, reduces the photosynthesis rate (Wang *et al.*, 2004; Song *et al.*, 2011; Torabi *et al.*, 2015).

Glycyrrhiza glabra L. or licorice is one of the oldest medicinal plants with more than two thousand years of medicinal application of its roots(Wittschier *et al.*, 2009). Research on the healing properties of this plant has proven its impacts on subacute liver disorders, chronic hepatitis B and C, infectious hepatitis, and hemophilia. Preventing HIV replication and hindering immune system disorders in patients with AIDS are also among the therapeutic properties of this plant (Jalilzadeh-Amin *et al.*, 2015; Dastagir and Rizvi, 2016; Karkanis *et al.*, 2018). Expansion of the use of this plant and study strategies for its large-scale production is required. Considering the phytoremediation ability of licorice, studying possible approaches to improve seed germination and growth at early stages are critically in demand. Propagation via seed in this plant is an economically viable method.

This study aimed to investigate whether the application of Si can alleviate the toxic impacts of Al on seedlings of licorice further to evaluate its potentials as a phytoremediator of Al.

2. MATERIALS AND METHODS:

2.1. Medium preparation and sowing seeds

Healthy and vigor seeds of licorice were procured from PakanBazr Co. (Isfahan, Iran). Licorice seeds were first disinfected with 10% bleach (20 minutes) and 70% alcohol (60 seconds). After washing with distilled water, they were cultured in a soil mixture of perlite and irrigated with distilled water for one week. After germination and emergence of 2 to 3 leaves, seedlings were irrigated with Long Ashton nutrient solution (half concentration). After two weeks, healthy seedlings were selected and transferred to the hydroponic culture medium and fed with complete Long Ashton nutrient solution(Smith *et al.*, 1983). The hydroponic culture medium consisted of 1.5-liter dark plastic containers filled with Long Ashton nutrient solution and Si and Al treatments (Figure 1). The solution inside the containers was stirred continuously and changed every five days. The pH conditions of all nutrient solutions were considered to be 5.5. Each container contained two seeds, which were viewed as a total of one replicate (Figure 1). Plants were placed in the greenhouse controlled environment with a light period of 16 hours of light and 8 hours of darkness and day and night temperature 16±2 and 24±2, respectively. Light intensity of 250 µmol $m^{-2}s^{-1}$ was maintained at wavelengths of 400 to 700 nm. To prepare the Long Ashton nutrient solution, first stock solutions of macro and microelements were prepared then an appropriate amount was taken from them to make the nutrient solution.

2.2. Application of treatments

The 15-day-old licorice seedlings were exposed to different levels (0, 0.5, and 1.5 Mm) of sodium metasilicate ($Na_2SiO_3.5H_2O$) for 110 days, after which they stressed by simple and interaction effects of various concentrations of aluminum chloride ($AlCl_3.6H_2O$), zero, 100, 250, and 400 µM. At the end of each experiment, one of the two plants in each container was randomly selected to measure morphological traits. The second plant was immediately frozen in liquid nitrogen and transferred to a -20 °C freezer for assays that required fresh material.

2.3. Determining biomass production

Parameters of biomass production were recorded by measuring stem height and fresh shoot and root weight using digital calipers and scale. Then to obtain the dry weight of shoot and root, the samples placed in paper bags were put at 70 °C in the oven, where they dried for 48 hours.

2.4. Determination of photosynthetic pigments

To determine the amount of Chlorophyll-b, a, and carotenoids, the protocol previously detailed by Lichtenthaler (1987) was used, in which 0.5 g of green tissue of leaves with 10 ml of 80% acetone was pulverized. Then samples were centrifuged for 10 minutes at 6,000 rpm. The amount of chlorophyll-a in the absorption spectrum is 663, chlorophyll b at 645, and the carotenoid absorption spectrum at a wavelength of 470 nm with UV-Visible Spectrophotometer (Model -Cary50) analyzed. To set the device, acetone 80% was used. Concentrations of pigments using euqations 1, 2 and 3, in milligrams per gram of fresh weight (FW) of the sample, were calculated.

$$\text{Chlorophyll a} = (19.3 \times A663 - 0.886 \times A645)\ V / 100W\ \text{mg.g-1 FW} \quad \text{(Eq. 1)}$$

$$\text{Chlorophyll b} = (19.3 \times A645 - 3.6 \times A663)\ V / 100W\ \text{mg.g-1 FW} \quad \text{(Eq. 2)}$$

$$\text{Carotenoids} = 100\ (A470) - 3.27\ (\text{mg chl. a}) - 104\ (\text{mg chl. b}) /227\ \text{mg.g-1 FW} \quad \text{(Eq. 3)}$$

In the above equations, A is the wavelength (nm) read by the device, V volume (mL) of the filtered solution after centrifuge, and FW fresh weight of the sample per gram of fresh tissue.

2.5. Extraction and measurement of proline

The proline content of stems was estimated by a method described by Bates *et al.* (1973). To prepare the reagent of Ninhydrin, 30 ml of glacial acetic acid mixed with 20 ml of 6 M phosphoric acid stir gently until completely dissolved. The solution was stable at 4 °C for 24 h. To measure the proline content, 0.5 g of fresh leaf tissue was homogenized in 10 ml of sulfosalicylic acid 3%. The obtained extract was filtered using Whatman No. 2, 2 ml of the extract was mixed with 2 ml of the Ninhydrin reagent and 2 ml of glacial acetic acid placed for one hour in a 100 ^{o}C hot water bath. Then the experiment ended by placing the tubes in an ice bath. Then 4 ml of toluene were added to the contentes of each tube and shaken for 30 seconds; Toluene is perfect to complete dissolve of proline. Therefore, there is no need for another centrifugation. The upper layer of the solution that includes toluene and proline was separated from the aqueous phase. The absorption of the remaining phase of the solution was measured by spectrophotometer at a wavelength of 520 nm and the proline concentration expressed in mg per gr fresh weight.

2.6. Malondialdehyde assay

To measure membrane lipid peroxidation, malondialdehyde (MDA) concentration was measured by Heath and Packer (1968) method. In this method, 0.1 g of leaf tissue was extracted with the help of 2 ml of 5% trichloroacetic acid (TCA) solution by sonicator for 30 seconds at four °C. The resulting extract was centrifuged at room temperature at 12000 rpm for 15 min. At 532 nm, the absorption of the supernatant was measured. The absorption of other non-specific pigments was determined at 600 nm and subtracted from the adsorption at 532 nm.

2.7. Catalase assay

The activity of this enzyme was measured using Nakano and Asada (1981) method. An amount of 0.1 g of the frozen leaf sample was extracted in 3 ml of 25 mM sodium phosphate buffer with pH = 6.8. The resulting homogenates were centrifuged at 15,000 rpm at four °C. The supernatant was used to measure catalase activity. The reaction mixture consisted of 2.5 mL of 25 mM sodium phosphate buffer at pH = 6.8, 0.5 mL of 10 mM H2O2, and 100 µl of enzyme extract, then read at 290 nm by spectrophotometer and expressed as per µg of protein of enzymatic activity.

2.8. Statistical analysis

The experiment was performed in a completely randomized factorial design with four replicates, and each replicated had two sub-samples. The main factors were Si (A), and Al (B) and the interaction of concentrations of factors A and B. Analysis of means carried out employing the SPSS package (version 26; IBM, US). Using the Duncan test, the differences among treatments were evaluated at the level of 5%.

3. RESULTS AND DISCUSSION:

The most expected effect of heavy metals on plants often involves growth inhibition(Gajewska and SkŁodowska, 2010). Exogenous application of Si limits the harmful effects of Al (Singh *et al.*, 2011; Emamverdian *et al.*, 2018). Measuring growth parameters has often been critical in showing heavy metals' influence on biomass production(Wang and Zhou, 2005; Gajewska and SkŁodowska, 2010). According to Figure 3, where means are compared at the probability level of 5% of the Duncan test, application of Si treatment, particularly at 1.5 mM, can effectively limit the adverse effects of Al toxicity. In plants exposed to various levels of Al stress and moderate or high levels of Si managed to increase fresh stem weight (Figure 2 and Figure 3a). However, the interaction of Si mM 1.5 and Al 400 µM produced 45.67 g biomass in shoots but was not statistically significant compared to the other treated plants. But plants under Al 400 µM treatment indicated the lowest quantity of fresh shoot weight (36.08 g) statistically significant at 5% of the Duncan test. The simple effect of Si 1.5 mM (12.14 g) produced higher shoot dry weight, which had no statistically significant difference with Si 0.5 mM and control (Figure 3b). The shoot dry weight followed a pattern in which the values declined as the concentration of Al increased. Si 1.5 mM had the highest quantity of fresh root weight (12.52 g) while Al 400 µM alone with 5.98 g showed the lowest root fresh weight, which both produced a significant difference in control. The interaction of Al 100 and Si 1.5 with 10.58 g had the highest root fresh weight among the interactions (Figure 3c; 2.61g). When it came to root dry weight, a similar pattern to root fresh weight was observed (Figure 3d). In the case of the shoot fresh weight/dry weight ratio, in general, the simple effect of Al in all concentrations had the higher ratios where Al 400 µM with 11.44 showed the highest ratio (Figure 3e), with a significant difference with control. An analogous paradigm was observed for root fresh weight/dry weight ratio in which the single effect of Al had a higher ration, however, the combination of Al 400 µM and Si 0.5 Mm indicated a high ratio (Figure 3f). Among the treatments, Si 0.5 mM and Al 100 µM, the stem height of 38 cm found to be the highest among interaction treatments, a similar pattern was observed in other interaction effects (Figure 3g).

The fundamentals of the physiological impact of Al toxicity still is an open question (Ryder *et al.*, 2003). Prabagar *et al.* (2011) studied the effect of Al toxicity on cell suspension cultures of Norway spruce observed growth inhibition due to necrosis of cells resulting from deformation of subcellular like creating several small size vacuoles most likely from the Golgi body. Enhanced aggregation of Al through the root system and consequently generating adverse physiological modification have often been associated with Al toxicity (Corrales *et al.*, 1997; Prabagar *et al.*, 2011). This previous evidence can explain the decrease of fresh and dry weight in stem and root. Additionally, Si application and its adverse effects on Al uptake have been proposed in several other studies (Prabagar *et al.*, 2011; Shahnaz *et al.*, 2011; Singh *et al.*, 2011; Pontigo *et al.*, 2017). In agreement with the results of this study, Singh *et al.* (2011) similarly indicated the positive impacts on Al-stressed seedlings of rice. Possible mechanisms suggested for ameliorating the influence of Si on Al toxicity involve lowering the chance of availability of sites for Al to bind in the cellular wall, decreased possibility of formation of Al-plasma member bonds. Finally, discharging exudates by roots makes apoplastic presence Al difficult (Cocker *et al.*, 1998). Thus, the application of Si along with Al possibility positively impacted one of those above mechanisms. Additionally, the mitigation influence of Si on Al-stressed plant can be associated with decrementing the quantity of available phytotoxic Al in nutrition media. For instance, reducing Al in the media due to adding Si has been correlated with the development and formation of nonfunctional biological compounds known as hydroxyl aluminum silicate (Wang *et al.*, 2004).

Photosynthetic pigments are highly critical for photosynthesis apparatuses. In comparison, they are significantly vulnerable to the stress imposed by toxic doses of heavy metal ions (Ozyigit *et al.*, 2013; Paunov *et al.*, 2018). In this study, exogenous application of Si 0.5 and 1.5 mM improved the chlorophyll content in the plant exposed to Al stress (Figure 4a). In the interaction of Al 250 µM and Si 1.5 mM, stressed plants had the highest quantity of chlorophyll a by 1.91 µg/g FW among the interaction effect, which did not significantly differ from the other treatments at $p < 0.05$ level. The combination and single effects of Al 400 µM treatment generated the lowest values for chlorophyll a where Al 400 µM concentration with 0.836 indicated to be the lowest. Again, an exact similar trend was observed for chlorophyll b except that the interaction and simple effects of Al 400 µM yielded higher values (Figure 4b). Among the consequences of Si and Al, the interaction of Al 400 µM with Si 1.5 mM could increase chlorophyll b content (0.78 µg/g FW) to a statistically significant level ($p < 0.05$) (Figure 4b). In treatments that plants exposed to Al only, by increasing Al concentration, the content of chlorophyll a and chlorophyll b experienced a significant reduction. Similar patterns reflect the importance of Si against toxic ions of heavy metals, especially Al, as its possibly due to the preventative effects of Si on Al uptake (Guntzer *et al.*, 2012; Pontigo *et al.*, 2017; Liu *et al.*, 2018). An interesting pattern was observed in the reaction of carotenoid accumulation to Al stress and Si application. Al 400 µM interaction with Si 1.5 by producing 0.663 µg/g FW carotenoid had a notable difference in compared with other interaction effects and was statistically significant ($p < 0.05$). However, Al 400 µM indicated the lowest carotenoid 0.263 µg/g FW among all treatments (Figure 4c).

The enzymatic and non-enzymatic antioxidants such as catalase (CAT), α-tocopherol, carotenoids, and proline collectively play a significant role in plants stressed by heavy metals(Anjum *et al.*, 2016; Rao *et al.*, 2016). Besides being a nosmolyte to protect plant cells against osmotic stress imposed by a toxic heavy metal ion, proline is a vital energy source for plants that they can rely on to swift recovery from stress (Jain et al., 2001). Enzymatic and non-enzymatic antioxidants preserve plant cells from the negative consequences of heavy metal stress, mainly by scavenging reactive oxygen species (ROS). This process is through intercellular mechanisms in different organs, including cytosol, mitochondria, chloroplast, apoplast, and peroxisomes (Nwugo and Huerta, 2008; Foyer and Noctor, 2011; Hasanuzzaman *et al.*, 2012; Emamverdian *et al.*, 2018). In the present study, the amount of proline in the stem and roots under $AlCl_3.6H_2O$ treatment was stressed, which positively affected the concentration of $AlCl_3.6H_2O$ and Si treatment used (1.5 mM). Interaction of Al 250 and 400 µM and Si 1.5 mM in stem by 72.14 and 81.46 µg/g FW and had the highest quantity of proline content (Figure 5a) with statistically significant differences with the rest of the treatments at $p < 0.05$. The proline content of roots also had a similar trend in which Al 400 µM and Si 0.5 and 1.5 mM in the root by 69.22 and 69.54 µg/g FW produced the highest statistically significant values (Figure 5b). Proline, known to have cytosolic activities and quenching ROSs, has enabled this compound to increase almost in all the possible conditions that plant faces abiotic and biotic stress (Hayat *et al.*, 2012; X. Liang *et al.*, 2013; Kavi and Sreenivasulu, 2014). This is possibly why the proline content significantly increased by increasing the concentration of Al, and the positive influence of Si also can be witnessed. These results indicate the importance of proline biosynthesis in plants under Al stress and the importance of Si to intensify its production. The ratio of proline in shoot/root was mainly increased by enhancement of Al concentrations. Additionally, the highest ratios were found in treatments; Al 250 and 400 µM in combination with 1.5 mM (1.35 and 1.17, respectively, Figure 5c).

Catalase is localized in a specific cellular organ, peroxisomes. Its primary responsibility is to eradicate the H2O2 generated by the SOD reaction; therefore, it is crucial for survival plants when exposed to Al stress (Racchi, 2013). The stress-induced by Al increased enzymatic antioxidant content, CAT in both stem and root (Figure 5d). It seems the addition of both Si (0.5 and 1.5 Mm) and Al (100, 250, and 400 µM) combined or alone increased CAT by increasing in concentration. Si 1.5 mM alone with 1.61 U/mg protein had the highest value among all treatments, also amongst the interaction effects, Al 400 µM and Si 1.5 were observed to have the highest concentration of CAT (1.22 U/mg protein). Simultaneously, the simple impact of Al 250 µM by having a value of 0.44 U/mg protein showed the lowest concentration of CAT.

Accumulation of ROS is highly expected in plants exposed to heavy metal stress because of the important role of ROS in signaling Al stress to initiate defense mechanisms. Plants also have potent antioxidant mechanisms to scavenge the ROS when it reaches an excessive level(Yamamoto *et al.*, 2003; Achary *et al.*, 2012; Huang *et al.*, 2014); in this study, significant enhancement in CAT by increasing the concentration of Al which means that *G.glabra* has a robust antioxidant system. However, the introduction of Si to Al treated plants caused a notable reduction in CAT, as Torabi *et al.* (2015) analogously reported a decrease in CAT activity of *Borago officinalis* L. when exposed to salinity. Similarly, Kim *et al.* (2014)observed a reduction in CAT activity in salt-stressed rice plants. The effect mechanisms of Si on the antioxidant defense system is poorly understood. But the addition of Si is often reported to increase antioxidant enzymes, particularly CAT (Kachout *et al.*, 2009; Shi *et al.*, 2010; Hajiboland and Cheraghvareh, 2014; Adrees *et al.*, 2015).

Malondialdehyde is a damaging product of peroxidation of lipids of the cellular membrane. It is a reliable index of the degree of oxidative stress caused by heavy metal ions (Guo *et al.*, 2004). A dose-dependent behavior was observed in the effect of Si on MDA concentration (Figure 5e). In general, Si showed a notable influence on reducing lipid peroxidation rate. Still, in the interaction of Al (100, 250, and 400 µM) with Si treatments (0.5 and 1.5 Mm), the dominant effect of 1.5 mM in all Al concentration was vivid. The lowest lipid peroxidation was found to be in Si 1.5 (0.574 nM/g FW), which had a significant difference with the highest concentration of Al used in this study, 400 µM (1.31 nM/g FW) at $p < 0.05$. Among the interaction effects, Al 100 µM and Si 1.5 mM (0.702 nM/g FW) indicated the lowest MAD concentration. In a study conducted on rice, exposure to Al and the addition of Si led to a decrease in MDA content (Song et al., 2011), which later found out considerable enhancement in the quantity of proline and CAT due to Si addition. This possible can significant reduction in MDA by increasing the concentration of Si in our study.

Additionally, the result of this study is consistent with those of Shamsi *et al.* (2008), who observed a notable increase in MDA by rising in Al concentration. The positive effect of Si in interaction with Al on reducing MDA was observed in Borago officinalis L. (Shahnaz *et al.*, 2011). Similar results also have been reported in barley (Tamás *et al.*, 2003), tea (Ghanati *et al.*, 2005), and *Stipagrandis* and *Leymuschinensis* (X. Song *et al.*, 2016). Exogenous application of Si in rice by controlling metal transport prevents the uptake of Al, therefore, prevents the lipid peroxidation and ultimately reduces MDA content(Kim *et al.*, 2014).

4. CONCLUSIONS:

The results indicated that silicon-treated plants were to a significant extent protected from AlCl3.6H2O toxicity and produced higher biomass, suggesting that Silicon may increase plants' resistance to environmental stress (toxic ions). Improving the stressing situation for seedlings seems to be via increasing the proline content as a universal osmoprotectant and preventing lipids' oxidation. Finally, Silicon has reduced cell membrane vulnerability and improved the structure to deal with $AlCl_3.6H_2O$ stress in licorice and revealed some of the capability of Silicon to control the toxicity of $AlCl_3.6H_2O$. More comprehensive studies are required, exposing licorice to other heavy metal stresses and employing other ameliorating chemicals such as salicylic acid are recommended.

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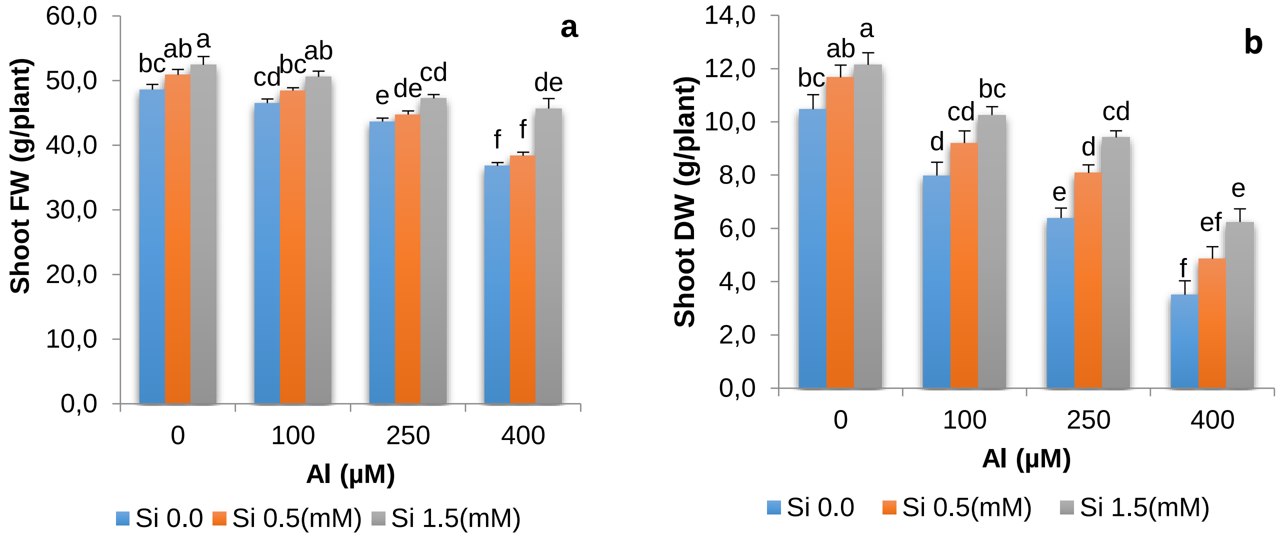
Figure 1. *15 and 30-day-old seedlings (left and right, respectively) of G. glabra in containers containing Long Ashton media.*

Figure 2. *Effect of different levels of Al (100, 250, and 400 µM in combination with Si (1.5 Mm) on G. glabra seedlings.*



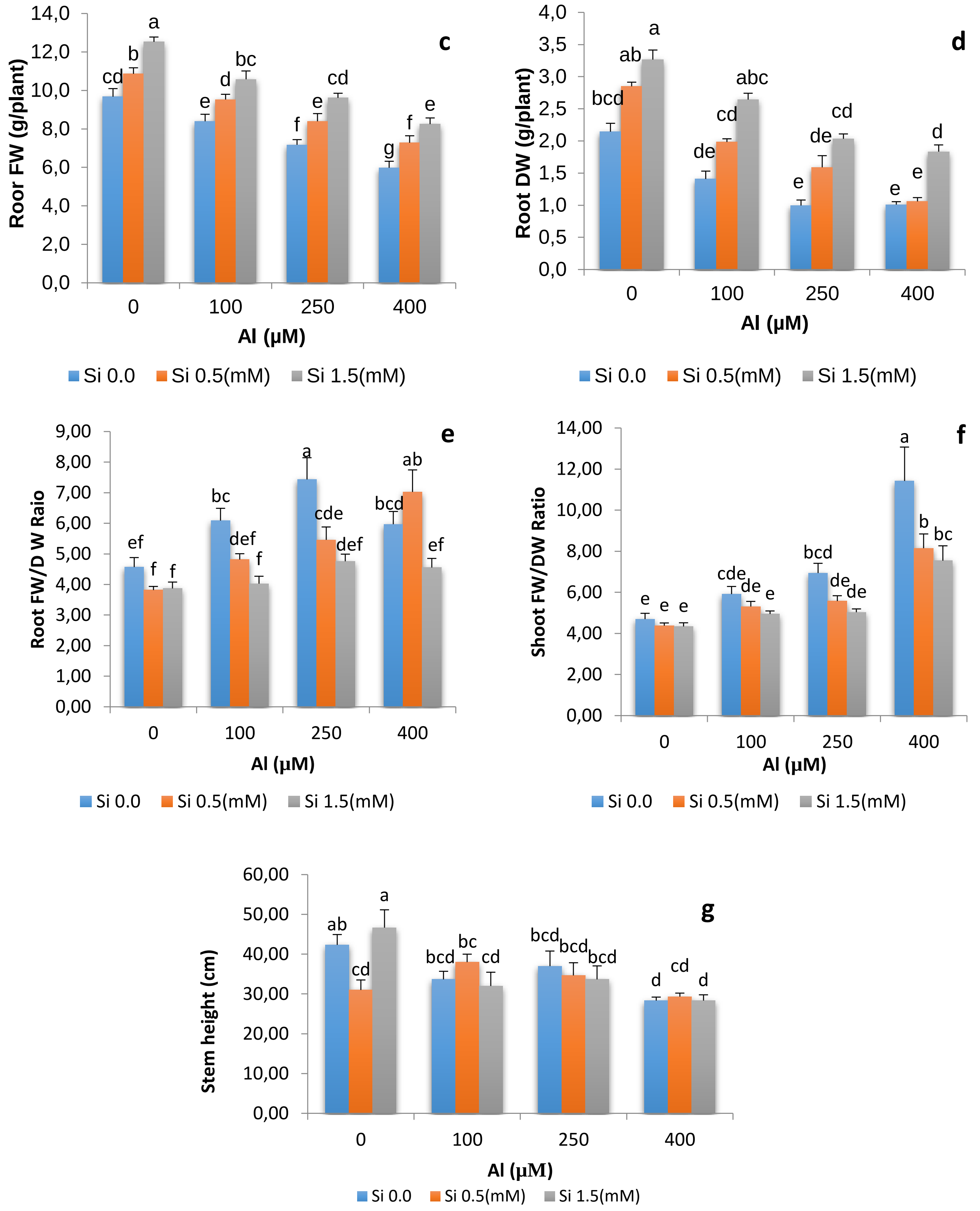


Figure 3. *Effect of different concentrations of Al (0, 100, 250 and 400 µM) and Si (0, 0.5 and 1.5 Mm) and their interaction on shoot fresh weight (a), shoot dry weight (b), root fresh weight (c), root dry weight (d), root FW/DW (e), shoot FW/DW (f), and stem height (g). Columns with non-common letters indicate a significant difference between treatments based on the Duncan test (p <0.05).*

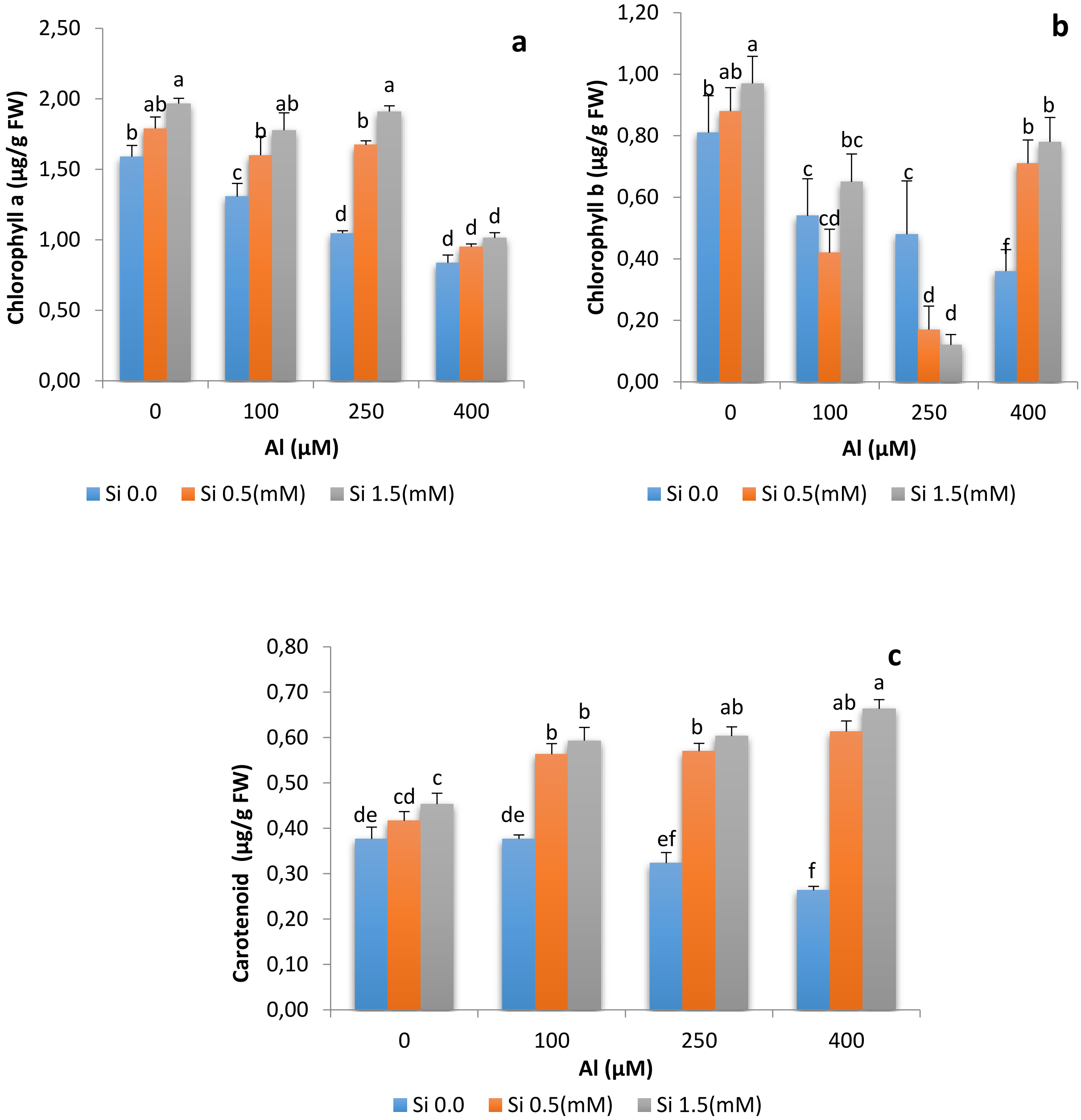


Figure 4. *Effect of different concentrations of Al (0, 100, 250 and 400 µM) and Si (0, 0.5 and 1.5 Mm) and their interaction on contents of Chlorophyll a (a), Chlorophyll b (b) and Carotenoid (c). Columns with non-common letters indicate a significant difference between treatments based on the Duncan test (p <0.05).*

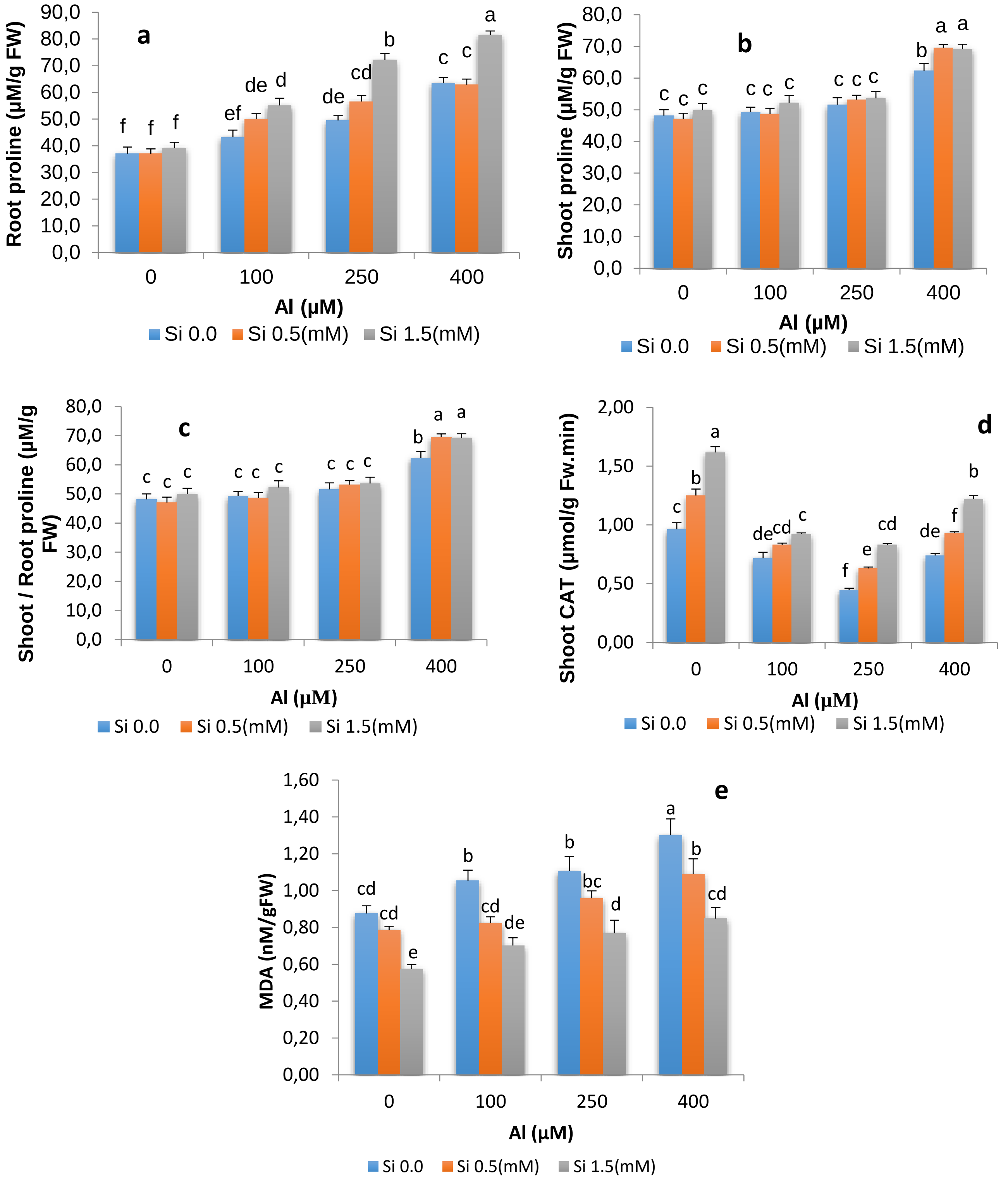


Figure 5. *Effect of different concentrations of Al (0, 100, 250 and 400 µM) and Si (0, 0.5 and 1.5 Mm) and their interaction on contents of proline in root (a), and stem (b), root/shoot proline ration (c) CAT enzyme content in root (d) and MDA (e). Columns with non-common letters indicate a significant difference between treatments based on the Duncan test (p <0.05).*