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The role of exogenous silicon to mitigate Al_2O_3 nanoparticle-induced toxicity in barley (*Hordeum vulgare* L.)

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The role of exogenous silicon to mitigate Al_2O_3 nanoparticle-induced toxicity in barley (*Hordeum vulgare* L.)

Abstract: In this study, we used silicon (Si, in the form of K_2SiO_3, 2 mM) to alleviate the toxicity of aluminum oxide (Al_2O_3) nanoparticles (NPs) in barley (*Hordeum vulgare* L.). Using Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) analyses, we showed that the Al_2O_3 NPs were taken up by barley plants. Barley growth was negatively affected by the addition of 3 g l^{-1} nano-Al_2O_3, whereas the diminishing effect of NPs on barley growth was not obvious when 1 g l^{-1} nano-Al_2O_3 was applied, indicating that the nano-Al_2O_3 action is dependent on nano-Al_2O_3 dose. Si pretreatment ameliorated toxic effects of high nano-Al_2O_3 on root growth. Si pretreatment did not decrease nano-Al_2O_3 entry into roots but reduced nano-Al_2O_3 accumulation in the shoot. The restriction of the root-to-shoot translocation of nano-Al_2O_3 was one of the important mechanisms for Si to mitigate high nano-Al_2O_3 toxicity. The occurrence of oxidative stress was found under 3 g l^1 nano-Al_2O_3 treatment, as evaluated by the accumulation of malondialdehyde (MDA). Exogenous addition of Si could alleviate toxicity symptoms induced by Al_2O_3 nanoparticles by reducing lipid peroxidation via enhancing antioxidant activity of catalase as well as by limiting the root-to-shoot translocation of nano-Al_2O_3. These data provide the first direct evidence that the Si pretreatment ameliorates nano Al_2O_3 phytotoxicity in plants.

Key words: *Hordeum vulgare* L.; malondialdehyde; nano-Al_2O_3; nanotoxicity; silicon

Vloga dodajanja silicija za preprečevanje strupenosti nano delcev Al_2O_3 pri ječmenu (*Hordeum vulgare* L.)

Izvleček: V raziskavi je bil uporabljen silicij (Si), v obliki 2 mM K_2SiO_3, za preprečevanje strupenosti nano delcev aluminijeva oksida (Al_2O_3;NPs) pri ječmeni (*Hordeum vulgare* L.). Analiza z ICP-MS je pokazala, da so bili nano delci Al_2O_3 privzeti v rastline. Na rast ječmena je negativno vplival dodatek 3 g l^{-1} nano delcev Al_2O_3, medtem, ko rast ječmena ni bila občutno zmanjšana pri dodatku 1 g l^{-1} nanodelcev Al_2O_3, kar kaže, da je učinek nano delcev Al_2O_3 odvisen od doze. Predhodno obravnavanje rastlin s silicijem je oblažilo toksičen učinek velikih koncentracij nano delcev Al_2O_3 na rast korenin. Predhodno obravnavanje s Si ni zmanjšalo privzema nano delcev Al_2O_3 v korenine ampak zmanjšalo njihovo kopičenje v poganjkih. Omejitev translokacije nano delcev Al_2O_3 iz korenin v poganjke se je izkazala kot pomemben mehanizem preprečevanja njihove toksičnosti s silicijem. Pojav oksidacijskega stresa pri obravnavanju z 3 g l^{-1} nano delci Al_2O_3 je bil ovrednoten s kopičenjem malondialdehida (MDA). Dodajanje silicija lahko prepreči nastanek toksičnih znakov, ki jih povzročajo nano delci Al_2O_3 preko zmanjšanja peroksidacije lipidov s povečevanjem aktivnost katalaze kot tudi z omejevanjem njihove translokacije iz korenin v poganjke. Ti izsledki so prvi neposreden dokaz, da predobravnavanje s silicijem zmanjšuje strupenost nano delcev Al_2O_3 pri rastlinah.

Ključne besede: *Hordeum vulgare* L.; malondialdehid; nano-Al_2O_3; nanotoksičnost; silicij

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1 INTRODUCTION

Aluminum (Al) toxicity is one of the main stress factors limiting plant growth and crop yields in acid soils (Wang et al., 2004), which now account for ~40 % of the earth's arable land (Ma et al., 2001; Rahman et al., 2018). To alleviate damaging effect of Al toxicity as well as to prevent root growth inhibition, some plant species used diverse mechanisms such as releasing organic acids that chelate Al, transporting of organic acid anions out of the root cells and forming complexes with organic acids in their leaves, that enable them to grow on acid soils (Ma et al., 2001). Barley is considered to be most sensitive to Al toxicity among cereal species (Wang et al., 2006). Because of the fact that the yield of barley was reduced in acid soils (Fujii et al., 2012), the understanding of the physiological and biochemical mechanisms improving Al tolerance of this species is very important.

Nowadays aluminum oxide (Al_2O_3) nanoparticles (NPs) are one of the most used NPs and developed for applications in cosmetic fillers producing, materials packaging, tools cutting, glass and metal production, etc (Hanemann and Szabó, 2010). Thus these NPs can enter to the waste water streams and may predominantly be applied to agricultural fields (Colvin 2003; Navarro et al. 2008). Regarding to nanotoxicology, many studies have been published concerning the different cytotoxic effects of such nanoparticles on mammalian, animals and bacteria (Wiesner et al., 2006; Lin and Xing, 2007), and only a few studies have focused on the toxicity of NPs to plants (Lee et al. 2008, 2010). Recently, root growth inhibition by 2 g l^{-1} nano-Al_2O_3 was reported for soybean, cabbage, and carrot (Yang and Watts, 2005), tobacco (Burklew et al., 2012) and wheat (Yanik and Vardar, 2015). Since quantitative methods for determining nanoparticles in plant tissues have not been considered, in this study, uptake and accumulation of nano-Al_2O_3 nanoparticles were quantified by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) analyses. In this study, we used exogenous Silicon (Si) to mitigate toxicity symptoms induced by Al_2O_3 NPs in barley plants.

Silicon is a beneficial mineral element for plants. A number of studies have been demonstrated that Si is beneficial for the growth of plants, especially those belonging to the family Poaceae (Broadley et al., 2012). Si can mitigate the effects of various environmental stresses such as salinity, drought, chilling, UV radiation (Collin et al., 2014; Zhu and Gong, 2014; Habibi, 2016) and Al and Mn toxicity (Zargar et al., 2019). Exogenous application of Si exhibits the capacity to enhance the plant growth and yield as well as stress tolerance under metal toxicity by reducing the metal uptake and transport in plants (Adrees et al., 2015), formation of silicon bodies in the cell wall (Prabagar et al., 2011) and enhancing the activities of antioxidant enzymes (Habibi, 2014; Shen et al., 2014; Dorneles et al., 2019).

This research was conducted to study the effects of Si application on the amelioration of nano-Al_2O_3 toxicity. According to the best of our knowledge, there is no information in literature regarding the ameliorating effect of Si on nano-Al_2O_3 toxicity in plants. To address this issue, we examined in some detail the biochemical mechanisms by which nano-Al_2O_3 influences the growth, photosynthetic pigments and antioxidant capacity in barley plants. Since the Si alleviates elemental aluminum-induced damages resulting in better plant growth under aluminum toxicity, we hypothesize that Si can also mitigate nano-Al_2O_3 toxicity damages.

2 MATERIALS AND METHODS

2.1 CHARACTERIZATION AND PREPARATION OF NANOPARTICLE SUSPENSION FOR TREATMENT

Aluminum oxide nanoparticles (Al_2O_3 Nanopowder, alpha, 99 %, 80 nm, Hydrophilic) were obtained from US Research Nanomaterials, Inc. The morphology and diameter of Al_2O_3 NPs were also evidenced by scanning electron microscope (SEM, Seron Technology, AIS2100 model) as shown in the Figure 1. After dispersing NPs in distilled water, the solution was sonicated through ultrasonication (230 V/50–60 Hz) for 30 min in order to obtain a homogeneous mixture.

2.2 GROWTH CONDITIONS AND EXPOSURE OF NANOPARTICLES TO PLANT

Seeds of barley (*Hordeum vulgare* 'Bahman') were germinated on filter paper moistened with distilled water. Ten-day-old seedlings were transported to modified Hoagland nutrient solution (Johnson et al., 1957) containing 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $NH_4H_2PO_4$, 1 mM $MgSO_4$, 50 µM H_3BO_3, 2 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$, 0.5 µM H_2MoO_4 and 0.02 mM $FeSO_4$-EDTA for 25 days prior to the treatment procedure. At 25 days after germination, Al_2O_3 nanoparticles (0, 1 and 3 g l^{-1}) and Si (K_2SiO_3, 2 mM) were applied together with the nutrient solution described above. Plants were grown under a temperature regime of 22-25/17-19 °C, relative humidity of 60-65 % and daily pho-

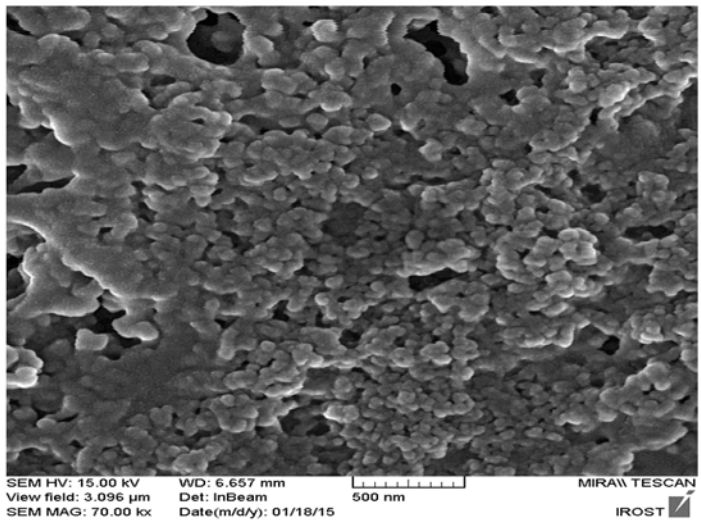

Figure 1: Scanning electron microscopy image of Al_2O_3 nanoparticles when nano-Al_2O_3 was mixed with hydroponic solutions.

ton flux density of about 300-350 µmol m^{-2} s^{-1} throughout the experimental period.

2.3 HARVEST PLANTS

Plants were harvested 14 days after applying the nanoparticles. Fully expanded and mature leaves were utilized for measurement of enzymatic analysis. Shoots and roots were washed with distilled water, blotted dry on filter paper and after determination of fresh mass (FM) they were dried for 48 h at 70 °C for determination of dry mass (DM). Plants height and tap root length were measured using a ruler.

2.4 DETERMINATION OF ALUMINUM

According to Yanik and Vardar (2015), shoot and root samples were oven-dried at 80 °C for 24 h, and mixed with 8 ml 65 % HNO_3 at 175 °C. To quantify Al_2O_3 NPs concentration in shoot and roots, we used Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) analyses; and the measured concentration of elemental Se in shoot and root samples was normalized by the dried mass of the shoot and root.

2.5 DETERMINATION OF TOTAL CAROTENOIDS AND CHLOROPHYLLS *a* AND *b*

Leaf concentration of chlorophyll and carotenoids was measured according to Lichtenthaler and Wellburn (1985). After extraction of fresh pigments in the cold acetone, the samples stand for 24 h in the dark at 4 °C.

2.6 ASSAY OF ANTIOXIDATIVE ENZYMES AND MALONDIALDEHYDE (MDA) CONTENT

The activities of superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) were determined according to methods described elsewhere (Habibi and Hajiboland, 2012). Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid according to methods described elsewhere (Habibi and Hajiboland, 2012).

2.7 STATISTICAL ANALYSES

Experiment was done in complete randomized block design with 4 independent replications. Statistical analysis was carried out using Sigma Stat (3.5) with Tukey test. Results were given as mean ± standard deviation (SD). Differences between treatments were considered to be significant, when a p value was less than 0.05 ($p < 0.05$).

3 RESULTS AND DISCUSSION

3.1 EFFECT OF DIFFERENT NANO-AL_2O_3 CONCENTRATIONS ON ITS UPTAKE AND ACCUMULATION USING ICP-MS

Exogenous Al_2O_3 NPs application increased en-

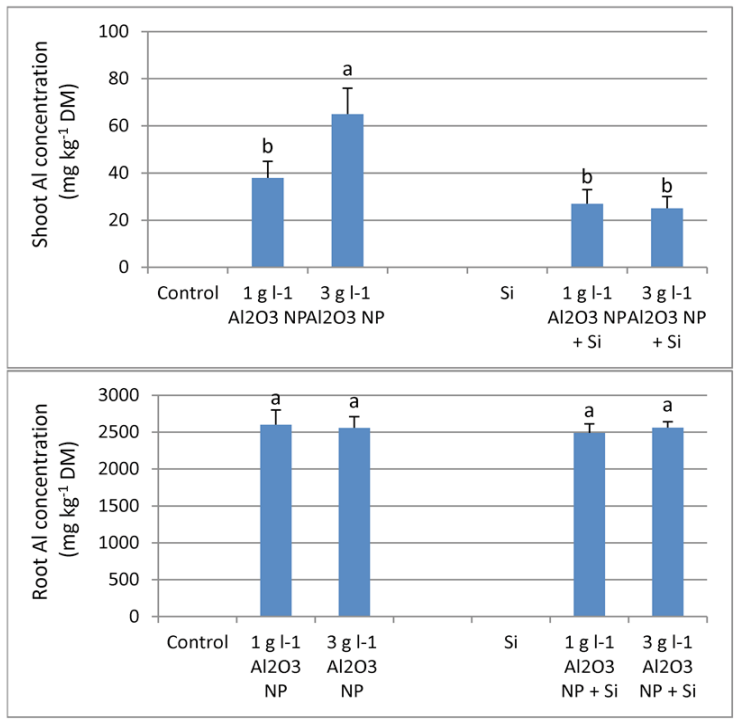

Figure 2: Al_2O_3 nanoparticles accumulation in barley that was recovered by ICP-MS in shoot and root sections of treated plants. Error bars indicate the standard deviation. Bars indicated with the same letter are not significantly different ($p < 0.05$).

dogenous Al_2O_3 NPs contents in shoot and roots of barley plants (Figure 2). Al_2O_3 NPs concentration in plants grown without NP addition was under the analyzing limits. The highest content of Al was found in roots of plants. The most effective uptake and transport of Al was observed for 3 g l^{-1} Al_2O_3 NPs. These results agreed with Asztemborska et al. (2015) who reported that Al_2O_3 NPs was taken up by *Zea mays* L. plants. Indeed, most research have mainly focused on assessing the nature of the safety and toxicity of these nanoparticles (Yanik and Vardar, 2015), but the uptake and entry of nanoparticles into plant systems is still poorly comprehended (Li et al., 2015). In this study, the relatively high concentrations of Al found in shoots grown in the presence of Al_2O_3 NPs strongly indicated the transport of intact particles of Al_2O_3 from the root to the shoot in barley plants.

3.2 Si PRETREATMENT REDUCED NANO-AL_2O_3 ACCUMULATION IN THE SHOOT

Si pretreatment did not reduce nano-Al_2O_3 entry into roots but decreased nano-Al_2O_3 accumulation in the shoot (Figure 3). Recently, authors proposed the formation of aluminosilicate complexes in the cell wall (Prabagar et al., 2011; Adrees et al., 2015). Similarly, possible retention of aluminum in the cell wall has been studied in relation to ameliorating effect of Si on aluminum toxicity in maize (Wang et al., 2004). These authors reported that Si causes higher aluminum tolerance in plants via binding of aluminum to the cell wall. Our results indicated significant effect of supplemental Si on nano-Al_2O_3 concentration in shoots. They are in agreement with the findings of Dorneles et al. (2016) who reported a decrease in aluminum concentration by Si application in the shoots of potato plants. However, there is no information in literature regarding the ameliorating effect of Si on nano-Al_2O_3 toxicity in plants.

3.3 NANO-AL_2O_3 ACTION IS DEPENDENT ON NANO-AL_2O_3 DOSE

Barley growth was negatively affected by nano-Al_2O_3 levels up to 1 g l^{-1}. Although no change was observed in 1 g l^{-1}, NP treatment at 3 g l^{-1} decreased the shoot and root fresh mass, and root elongation with regard to controls (Figure 3). Moreover, shoot and root dry mass was affected negatively by high concentration of nano-Al_2O_3 during the experiment (Figure 4). In this study, the highest applied concentration of Al_2O_3 was about 1.5 times higher than that reported to be toxic (2 g l^{-1}) for

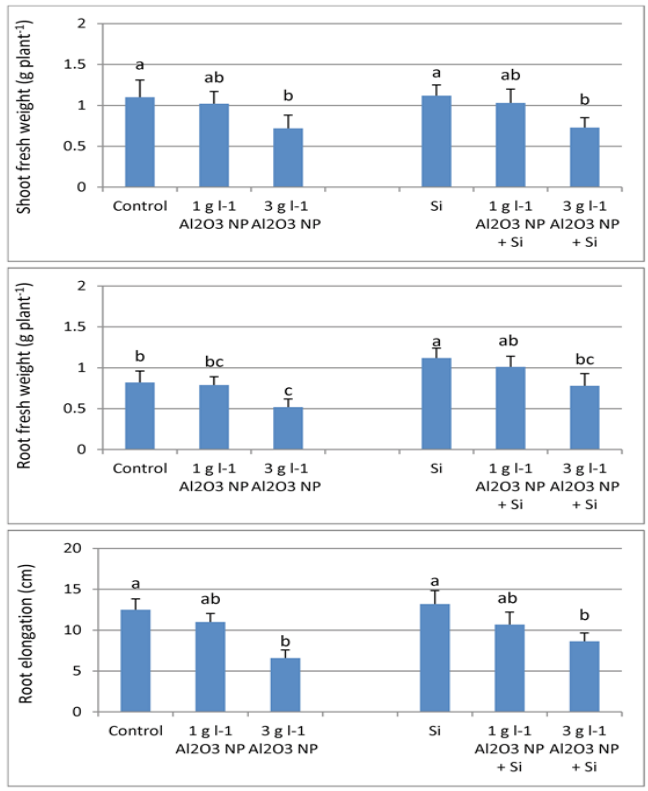

Figure 3: Effects of different concentration of Al_2O_3 NPs on the shoot and root fresh mass, and root elongation of barley seedlings exposed to 2 mM Si for 14 days. Error bars indicate the standard deviation. Bars indicated with the same letter are not significantly different ($p < 0.05$).

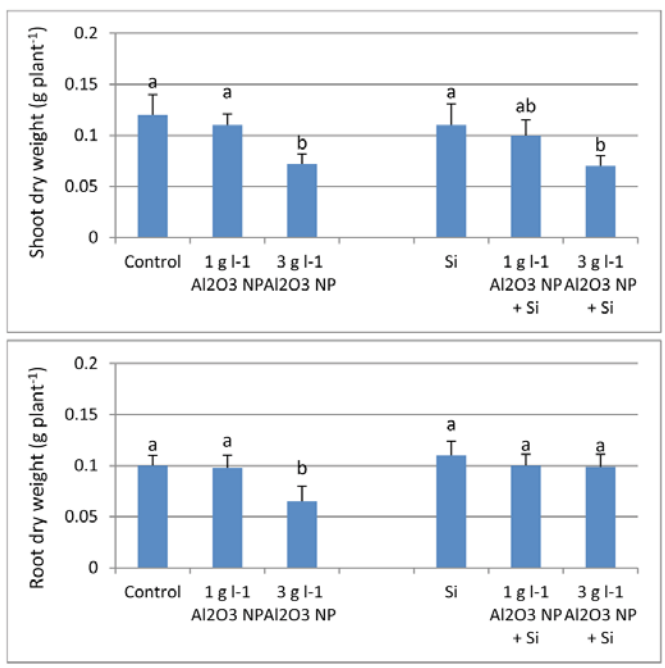

Figure 4: Effects of different concentration of Al_2O_3 NPs on the shoot and root dry mass of barley seedlings exposed to 2 mM Si for 14 days. Error bars indicate the standard deviation. Bars indicated with the same letter are not significantly different ($p < 0.05$).

corn, cucumber, soybean, cabbage, and carrot (Lee et al., 2010). Additionally, our results are in agreement with the findings of Burklew et al. (2012) who reported that root growth and development decreased as the concentration of aluminum oxide nanoparticles increased. Probably, higher concentrations of nano-Al_2O_3 adsorb on the root surface, and interrupt the root functions (Asztemborska et al., 2015). Our results clearly indicated that the diminishing effect of exogenously applied nano-Al_2O_3 on growth parameters of barley plants was dependent on doses of nano-Al_2O_3 used.

3.4 Si PRETREATMENT AMELIORATED TOXIC EFFECTS OF NANO-AL_2O_3 ON ROOT GROWTH

In current study, root growth was reduced by 3 g l^{-1} nano-Al_2O_3, whereas this reduction was alleviated by application of exogenous Si (Figure 4). Exogenous addition of Si can mitigate toxicity symptoms induced by aluminum stress in many plant species (Hammond et al., 1995; Singh et al., 2011; Shen et al., 2014) by enhancing antioxidant protection via modifying the activities of antioxidant enzymes (Shen et al., 2014; Dorneles et al., 2019), and by apoplastic binding of aluminum via formation of aluminosilicate complexes in the cell wall (Wang et al., 2004; Adrees et al., 2015). However, the mechanisms of Si-mediated alleviation of nano-Al_2O_3 stress are still unknown.

3.5 Si PRETREATMENT DID NOT CHANGE THE CONCENTRATION OF PHOTOSYNTHETIC PIGMENTS

Leaf photosynthetic parameters including chlorophyll *b* and carotenoid contents were not significantly influenced under nano-Al_2O_3 stress with or without Si treatment (Figure 5). However, a consistent tendency of chlorophyll *a* to decrease in response to high levels of nano-Al_2O_3 was observed. It has been reported that Si markedly mitigates Al-induced reduction in photosynthetic parameters in peanut plants (Shen et al., 2014). In contrary, our results indicated that the photosynthetic

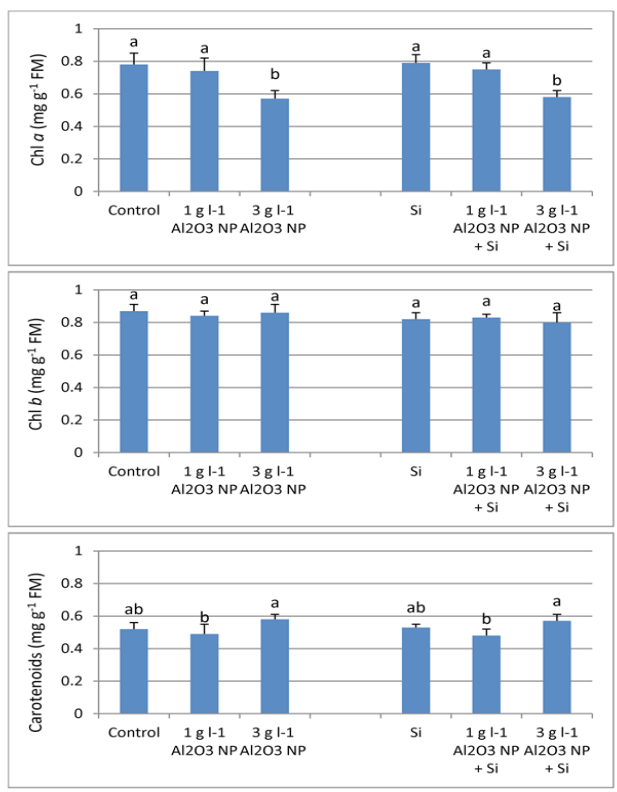

Figure 5: Effects of different concentration of Al_2O_3 NPs on the chlorophyll (Chl) a, b and carotenoid contents in leaves of barley seedlings exposed to 2 mM Si for 14 days. Error bars indicate the standard deviation. Bars indicated with the same letter are not significantly different ($p < 0.05$).

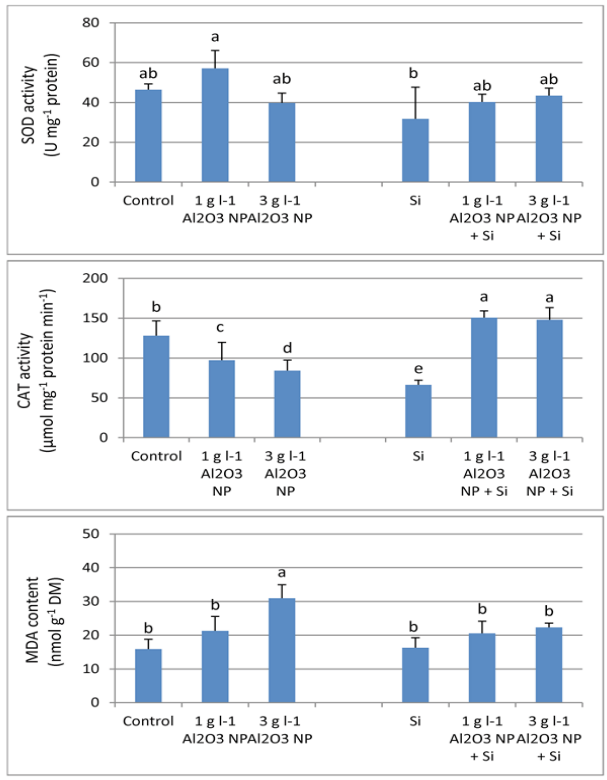

Figure 6: Effects of different concentration of Al_2O_3 NPs on the activity of superoxide dismutase (SOD) and catalase (CAT), and malondialdehyde (MDA) content in leaves of barley seedlings exposed to 2 mM Si for 14 days. Error bars indicate the standard deviation. Bars indicated with the same letter are not significantly different ($p < 0.05$).

pigment concentration was not influenced by Si under nano-Al_2O_3 stress.

3.6 Si PRETREATMENT MITIGATED AL_2O_3 NANOPARTICLE-INDUCED OXIDATIVE STRESS IN BARLEY PLANTS

The activity of SOD was not influenced even under the highest nano-Al_2O_3 levels applied (Figure 6). However, a consistent tendency of CAT activity to decrease in response to nano-Al_2O_3 was observed. We observed that CAT activity was enhanced by Si application in the nano-Al_2O_3-stressed seedlings, which was consistent with the ability of Si to decrease the content of MDA in this plant. We found that the application of 3 g l^{-1} nano-Al_2O_3 was toxic, because it caused the accumulation of MDA, a marker for the ROS-mediated cell membrane damage. However, the MDA content was reduced with Si under nano-Al_2O_3 stress. Similarly, increasing the activity of antioxidant enzymes and mitigating the Al-induced damage to membrane lipids was reported in potato genotypes grown with silicon (Dorneles et al., 2019). Indeed, exogenous addition of Si can mitigate toxicity symptoms induced by aluminum stress via modifying the activities of antioxidant enzymes (Shen et al., 2014; Dorneles et al., 2019; Zargar et al., 2019); however, the mechanisms of Si-mediated inhibition of membrane lipids peroxidation and enhancing the activities of antioxidant enzymes under nano-Al_2O_3 toxicity are still unknown and must be further explored. Furthermore, we showed that Si ameliorated the negative effect of high nano-Al_2O_3 on productivity in barley plants by reducing lipid peroxidation and enhancing antioxidant activity of CAT (Figure 6).

4 CONCLUSION

In summary, we showed that the toxic effect of nano-Al_2O_3 was in a dose-dependent manner. Nano-Al_2O_3 treatment at 3 g l^{-1} decreased the shoot and root mass, and root elongation with regard to controls; however, no changes were observed in 1 g l^{-1}. Si pretreatment ameliorated toxic effects of 3 g l^{-1} nano-Al_2O_3 on root growth. This Si-mediated alleviation of nano-Al_2O_3 toxicity was in parallel with the enhanced antioxidant protection via modifying the activities of antioxidant enzymes and the

restriction of the root-to-shoot translocation of nano-Al_2O_3, as well as lower MDA production.

5 ACKNOWLEDGEMENT

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