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Research Report

Silicon-Mediated Enhancement of Physiological and Biochemical Characteristics of *Zinnia elegans* 'Dreamland Yellow' Grown under Salinity Stress

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Abstract. This study investigated the effects of silicon (Si) nutrition on hydroponically grown *Zinnia elegans* under salinity stress. In this study, six treatments, the control (basal nutrients without NaCl or Si), Si 50 (1.8 mM), Si 100 (3.6 mM), NaCl 50 (50 mM), Si 50 + NaCl 50 (1.8 mM Si; 50 mM NaCl), and Si 100 + NaCl 50 (Si-3.6 mM + NaCl-50 mM), were employed. After 15 days of treatment, growth parameters, biochemical measurements, and antioxidant enzyme activities were examined. Salinity stress significantly reduced plant growth, biomass, photosynthetic parameters, and pigments, and increased the electrolyte leakage potential (ELP), lipid peroxidation, and hydrogen peroxide level. Interestingly, with Si supplementation, *Z. elegans* recovered from salinity stress. Si enhanced growth and photosynthesis, and prevented the decomposition of photosynthetic pigments. Moreover, the addition of Si increased membrane integrity, thereby reducing the ELP and lipid peroxidation levels under salinity stress. Furthermore, Si modulated the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) in scavenging excess reactive oxygen species (ROS). Additionally, Si increased the macronutrient and micronutrient contents. Therefore, augmentation with Si provided salinity resistance and enhanced the growth of *Z. elegans*.

***Additional key words*:** antioxidant enzymes, electrolyte leakage, hydrogen peroxide, lipid peroxidation, reactive oxygen species

Introduction

Salinity affects the majority of agricultural lands worldwide (Perez-Alfocea et al., 1996). Plants are exposed to several environmental stresses, including salinity, which hampers plant growth and productivity. Excess salt impairs water uptake, thereby reducing growth and development. Furthermore, high saline conditions also increase the accumulation of Na^+ and Cl^- ions by plants and counteract the uptake of essential nutrients (Agarie et al., 1998), and also increase the production of harmful reactive oxygen species (ROS), which causes oxidative damage by altering normal cellular metabolism (Zhu et al., 2004). Moreover, in horticultural plants, the association between salinity and mineral nutrition is exceptionally difficult to understand. Hence, an affordable and effective alternative strategy of silicon (Si) supplementation to overcome the problems of salinity in horticultural plants would be valuable.

Si, the second most abundant element in the earth's crust; it is considered to be a 'quasi-essential' element for plants (Ma and Yamaji, 2008). It has been reported to be involved in the enhancement of growth, photosynthetic efficiency, gas exchange, leaf structure, and tolerance to several abiotic and biotic stresses in plants (Liang et al., 2007). However, Si-mediated stress tolerance mechanism and its metabolism and physiological relationship with plants are still under investigation. Si combats the negative effects of salinity stress in several ways. Primarily, it aids in the formation of a thick double-layered cuticle that acts as a physical barrier and balances transpiration loss during salinity stress (Matoh et al.,

1986). In addition, it enhances leaf area and erectness, resulting in maximal light interception for photosynthesis, and maintains the structural integrity of the cell wall (Ma and Yamaji, 2006). Thus, Si improves photosynthetic parameters and gas exchange in stressful environments. The second possible mechanism of stress tolerance is by osmotic adjustment and mitigation of ion toxicity by limiting Na^+ ion transportation (Yin et al., 2013). Si also strengthens plant antioxidant defense by enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) in scavenging the excess reactive oxygen species (ROS) from salinity stress (Zhu et al., 2004).

Apart from the above-mentioned beneficial effects, Si is also reported to enhance nutrient uptake and protein synthesis under normal and stressful conditions (Soundararajan et al., 2014; Zuccarini, 2008). Likewise, the improvement in salinity resistance by silicon has been reported in various plants such as *Oryza sativa*, *Triticum durum*, *Lycopersicum esculentum*, and *Dianthus caryophyllus* (Romero-Aranda et al., 2006; Tuna et al., 2008; Yeo et al., 1999). Although, the promising effects of Si have been extensively studied in several agricultural crops, there is little information on floricultural plants. *Zinnia elegans* (Asteraceae) is one of the most popular summer annuals, planted for its attractive colorful flowers. A previous report by Ranger et al. (2009) showed that Si had positive effects on the biotic stress tolerance of *Z. elegans*, especially upon aphid infection. However, so far no study has documented the influence of Si on salt stress in *Z. elegans*. In order to understand the effects of Si nutrition against salinity stress, the current study extensively investigated the modulations in biochemical and antioxidant enzyme activities occurred during Si-mediated salinity tolerance in *Zinnia elegans* 'Dreamland Yellow'.

Materials and Methods

Plant Material and Treatments

Seeds of *Zinnia elegans* 'Dreamland Yellow' were washed with distilled water and sown on a seed germination tray containing commercial Tosilee medium (Shian Precision Co., Jinju, Korea). After one week, the seedlings were subjected to salinity and Si treatments. For the treatments, seedlings were transplanted to magenta boxes containing 300 mL of nutrient solution formulated according to the method described by Soundararajan et al. (2014). Each magenta box consisted of four plants. Si was administered in the form of potassium silicate (K_2SiO_3). The excess potassium introduced by the K_2SiO_3 was deducted from potassium nitrate, and the nitrate loss was balanced by the addition of nitric acid. Salinity stress was provided by the addition of sodium chloride (NaCl) to the nutrient solution. The pH of the nutrient solution was adjusted to 5.7. Overall, the experiment consisted of six treatments: the control (basal nutrients without NaCl or Si), Si 50 (1.8 mM), Si 100 (3.6 mM), NaCl 50 (50 mM), Si 50 + NaCl 50 (Si-1.8 mM; NaCl-50 mM), and Si 100 + NaCl 50 (Si-3.6 mM + NaCl-50 mM). All the treatments were arranged in a randomized block design with three replicates. The experiment was conducted in a greenhouse at Gyeongsang National University, Jinju, Korea, under normal daylight with night/day set temperatures of 27/19°C and relative humidity (RH) of 60-70%.

Measurement of Growth, Biomass, Photosynthesis, and Pigments

After 15 days of treatment, the growth parameters such as stem length, stem diameter, root length, number of roots, and fresh and dry weight were measured. The net photosynthesis rate (*Pn*), stomatal conductance (*Gs*), and transpiration rate (*Tr*) were measured using an LI-6400 portable photosynthetic measurement system (Li-COR, Inc, Lincoln, NE, USA) according to Muneer et al. (2014). Photosynthetic pigments such as chlorophyll and carotenoid contents were measured by following the method of Arnon (1949). Briefly, 0.1 g of leaf tissue was extracted with 80% (v/v) acetone and the absorbance was measured at 645, 663, and 470 nm using a UV-spectrophotometer (Uvikon 992, Kotron Instrumentals, Milano, Italy).

Estimation of Tissue Silicon Content

Si uptake was estimated by the wet autoclaved digestion method (Elliott and George, 1991) with 0.1 g dried samples. The sample preparation and estimation of Si were performed according to the method described by Soundararajan et al. (2014).

Determination of Electrolyte Leakage Potential (ELP) and Malondialdehyde (MDA) Content

For ELP measurements, the leaf discs (0.5 cm) were washed with distilled water, immersed in 10 mL distilled water for 22 h, and autoclaved for 120 min at 90°C. The electrical conductivity (EC) was measured before and after autoclaving to determine the electrolyte leakage. The ELP % was calculated according to Campos et al. (2003).

The lipid peroxidation level in the leaves was estimated based on the MDA content according to Zhu et al. (2004). Briefly, 0.1 g leaf samples were homogenized in trichloroacetic acid (TCA) solution (0.1%, 5 mL) and centrifuged at 18,000 × *g* for 15 min. The supernatant (0.5 mL) was added to 5 mL of 0.5% thiobarbituric acid (TBA) solution prepared in 20% TCA. The reaction mixture was incubated in a hot water bath (95°C) for 30 min and the reaction was terminated by placing the mixture in ice. Then, the samples were centrifuged

for 5 min at 10,000 × *g*. The absorbance was determined at 532 nm by subtracting the non-specific values at 600 nm and the MDA content was calculated according to Sivanesan et al. (2011).

Estimation of Hydrogen Peroxide Content and Antioxidant Enzymes Activity

The spectrophotometric determination of H_2O_2 was carried out according to Christou et al. (2014). Briefly, 0.1 g leaf sample was homogenized in 1 mL of 0.1% trichloroaceticacid (TCA) and centrifuged at 10,000 × *g* for 15 min. Then, 0.5 mL of supernatant was mixed with 10 mM phosphate buffer (0.5 mL, pH 7.0) and 1 mL potassium iodide (1M). The mixture was incubated at room temperature for 30 min and the absorbance was measured at 390 nm. The H_2O_2 content was determined from the standard calibration curve.

For antioxidant enzyme analysis, samples were prepared according to our previous report (Manivannan et al., 2015). The activity of SOD was assayed by following the protocol described by Giannopolitis and Ries (1977) for the nitro blue tetrazolium (NBT) inhibition method. GPX activity was measured based on the amount of enzyme required for the formation of tetraguaiacol per minute according to the method described by Shah et al. (2001). The CAT enzyme activity was determined according to the method described by Cakmak and Marschner (1992). APX activity was estimated by following the protocol described by Nakano and Asada (1981). The total protein content of the samples was estimated according to Bradford's method (Bradford, 1976) using the BSA standard curve.

Macronutrient and Micronutrient Analysis

The elemental analysis was carried out according to Yin et al. (2013). Briefly, the samples were dried and powdered using a stainless mill (Cytclotec, Model 1093, Tector, Hoganas, Sweden). For elemental analysis, the samples were ashed for 4 h at 525°C in a Naberthern muffle furnace (Model LV 5/11/B180, Lilienthal, Breman, Germany). Digestion of the samples was carried out according to the procedure outlined by Sivanesan et al. (2014). Both the macronutrients and micronutrients were determined using an inductively coupled plasma (ICP) spectrometer (Optima 4300DV/5300DV, Perkin Elmer Inc., Waltham, MA, USA).

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test at $p \leq 0.05$, and an F-test was carried out to find statistically significant differences among the treatments using the SAS (Statistical Analysis System, V. 6.12, Cary, USA) program. All sets of data were the means of three replicates.

Results and Discussion

Effects of Si on Growth Parameters of *Z. elegans*

Si augmentation in the hydroponic medium significantly increased the growth of *Z. elegans* and alleviated salinity stress (Fig. 1). The growth characteristics observed after 15 days of salinity and Si treatments are shown in Table 1. The results suggested that Si fortification greatly enhances the growth of *Z. elegans*. Si 100 treatment had the longest shoots (50.7% higher than the control), followed by Si 50 treatment (15.21% higher than the control). Salt stress drastically decreased the shoot length by 18.4% compared with the control. However, Si alleviated NaCl stress and improved the stem length by 28.8 and 33.9% in Si 50 + NaCl 50 and Si 100 + NaCl 50 treatments, respectively. Similarly, Si treatments also enhanced the stem diameter. Under normal conditions, Si 50 and Si 100 increased the stem diameter by 7.5 and 14.4%, respectively, compared with the control. On the other hand, salinity stress reduced stem diameter by 47.2% compared with the control. Interestingly, Si 50 + NaCl 50 and Si 100 + NaCl 50 treatments had significantly recovered the stem diameter by 71.4% and 93.5% than NaCl alone treatment. Previous studies have suggested that Si has a plant hormone-like property that might play a vital role in stem elongation (Soundararajan et al., 2014). In addition, Si supplementation along with nitrogen has increased the levels of gibberellic acid, a primary plant growth regulator linked with stem elongation (Hwang et al.,

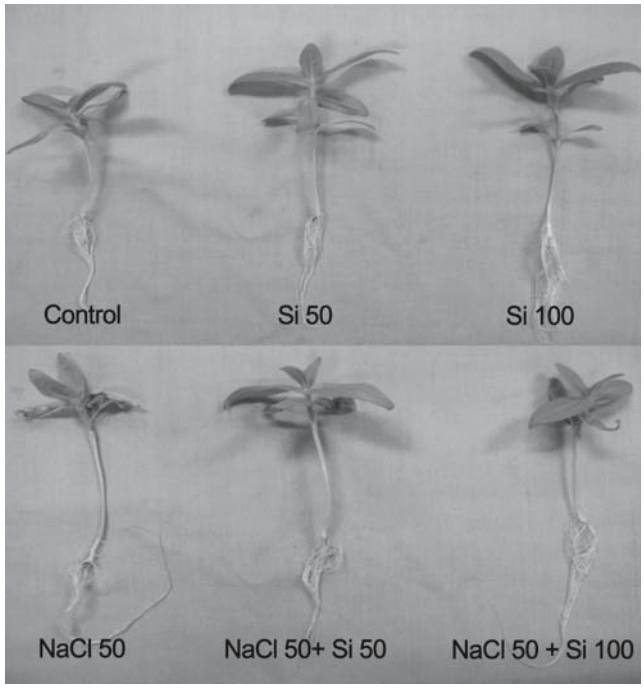

Fig. 1. Effects of Si nutrition and salinity stress on the growth of *Zinnia elegans* after 15 days of treatment.

Table 1. Growth of *Zinnia elegans* affected by salinity stress and Si supplementation after 15 days of treatment

Si ($mg \cdot L^{-1}$)	NaCl (mM)	Shoot length (cm)	Shoot diameter (mm)	Root length (cm)	No. of roots	Fresh wt. (g)	Dry wt. (mg)
0	0	7.2 ± 0. 68d[z]	1.5 ± 0.04c	9.7 ± 0.10c	25.6 ± 0.03c	3.6 ± 0.15c	7.4 ± 0.13c
	50	5.9 ± 0.00e	0.7 ± 0.12e	11.3 ± 0.04b	30.3 ± 0.05c	1.8 ± 0.06d	3.3 ± 0.02d
50	0	8.3 ± 0.16b	1.6 ± 0.07b	11.1 ± 0.08b	37.6 ± 0.08b	4.4 ± 0.11b	14.3 ± 0.05ab
	50	7.6 ± 0.10cd	1.3 ± 0.08d	11.6 ± 0.16b	38.6 ± 0.15ab	3.6 ± 0.08c	11.0 ± 0.21bc
100	0	10.9 ± 0.15a	1.7 ± 0.15a	13.8 ± 0.03a	39.6 ± 1.00ab	5.2 ± 0.22a	19.3 ± 0.04a
	50	7.9 ± 0.04bc	1.5 ± 0.08c	13.0 ± 0.12a	44.7 ± 0.31a	4.0 ± 0.11bc	17.0 ± 0.12a

[z]Mean separation within columns by Duncan's multiple range test at $p \leq 0.05$.
Data are represented as mean ± SE from three replicates.

2007). In agreement with our results, Si-mediated enhancement of stem thickness has been observed in sunflowers by Kamenidou et al. (2008).

The longest roots were in Si 100 (14.4% higher than control) and Si 100 + NaCl 50 (15.0% higher than NaCl 50) treatments. However, no significant differences in root length were observed among Si 50, NaCl 50, and Si 50 + NaCl 50 treatments. Interestingly, the maximum number of roots was produced by Si 100 + NaCl 50 treatment (74.2% greater than the control) followed by Si 100 (54.7% greater than the control) and Si 50 + NaCl 50 treatments (50.7% greater than the control). There was no significant difference between the control and NaCl 50 treatments in the number of roots. In rice, Si augmentation enhanced root parameters such as root length and the number of roots (Agarie et al., 1998). The improvement in root parameters upon Si addition could be due to the auxin-like nature of Si that directs the hydraulic conductivity of the roots to maintain the osmotic balance and uptake of nutrients (Liu et al., 2014). Recently, Si-mediated enhancement of roots for alleviating drought and aluminum stress has been reported in *Triticum aestivum* (Ahmed et al., 2014) and *Arachis hypogea* (Shen et al., 2014).

Effects of Si on Biomass

As expected, Si treatments under normal and salt-stressed conditions greatly increased the biomass of *Z. elegans*. Si supplementation increased the fresh weight by 46.2% and 22.8% in Si 100 and Si 50 treatments, respectively, compared with the control. On the other hand, salinity stress drastically reduced the fresh weight by 48.5% compared with the control. However, the addition of Si mitigated the stress and remarkably increased the fresh weight by 114% and 94.6% in Si 100 + NaCl 50 and Si 50 + NaCl 50 treatments, respectively, compared with NaCl 50 treatment. Likewise, the dry weight of *Z. elegans* was also significantly improved with Si addition. Specifically, Si 100 treatment enriched the dry weight by 160.0% and Si 50 treatment by 93.24% compared to the control. However, a notable reduction in dry weight was evident from the salt stress in NaCl 50 treatment (55.4% less than the control). However, Si treatments alleviated the effect of NaCl and immensely enhanced the dry weight by 415% and 233% in Si 100 + NaCl 50 and Si 50 + NaCl 50 treatments, respectively, compared with NaCl 50 treatment. Recently, the biomass enhancement by Si has been well-documented in tomatoes, especially under salinity stress (Muneer et al., 2014). Salinity could impair growth due to osmotic reduction or the excessive accumulation of Na^+ and Cl^- ions (Gunes et al., 2007). In general, Si increases biomass by hardening tissues to maintain optimal water status (Agarie et al., 1998).

Effects of Si on Photosynthesis

In the current study, salinity negatively influenced the photosynthetic parameters in *Z. elegans* (Fig. 2). However, both concentrations of Si (Si 50 and Si 100) improved the photosynthetic traits by mitigating NaCl stress. Interestingly, Si treatments alone or under stressed conditions significantly enhanced the net photosynthesis rate (Fig. 2A) by 158.3% in Si 50 and 251.3% in Si 100 treatments, respectively, compared with the control. However, NaCl 50 treatment drastically reduced the net photosynthesis rate by 76.0%. Si augmentation greatly increased photosynthesis by 396.1% (Si 50 + NaCl 50) and 667.4% (Si 100 + NaCl 50) compared with NaCl 50 treatment. Stomatal conductance and the transpiration rate were similarly decreased by salinity stress, as shown in Fig. 2B, C. Si-mediated improvement in photosynthesis could be attributed by the formation of a double-layered cuticle in the leaves that both provides mechanical strength and maintains photosynthesis in plants (Ma and Yamaji, 2006). Our results are consistent with several studies on the enhancement of photosynthesis by Si under both normal and salt stress conditions (Murillo-Amador et al., 2007; Yin et al., 2013).

Effects of Si on Photosynthetic Pigments

Addition of Si significantly enhanced the photosynthetic

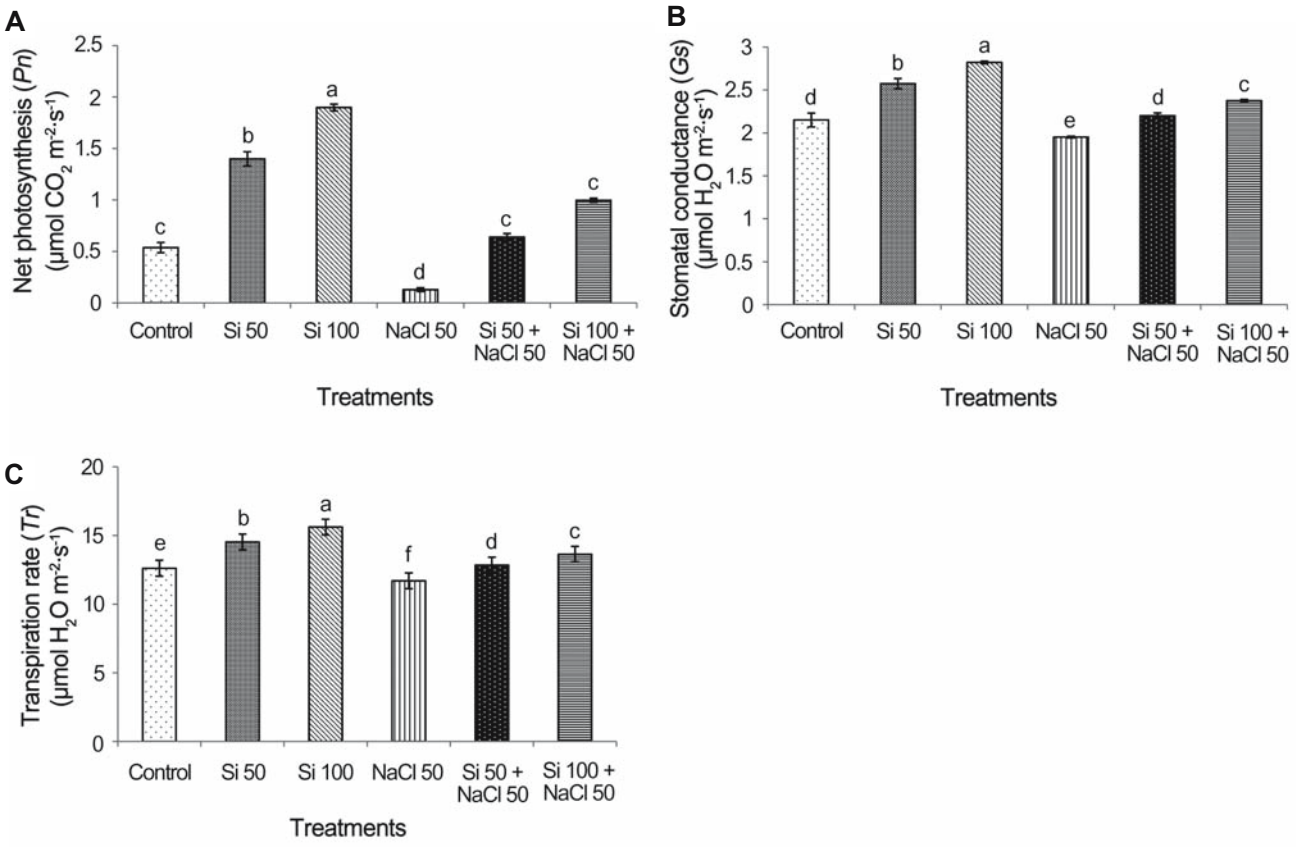

Fig. 2. Effects of Si nutrition and salinity stress on photosynthetic parameters of *Zinnia elegans* after 15 days of treatment. A, net photosynthetic rate; B, stomatal conductance; C, transpiration rate. Data are represented as mean ± SE from three replicates. Different letters within each panel indicate statistically significant differences at $p \leq 0.05$ using Duncan's multiple range test.

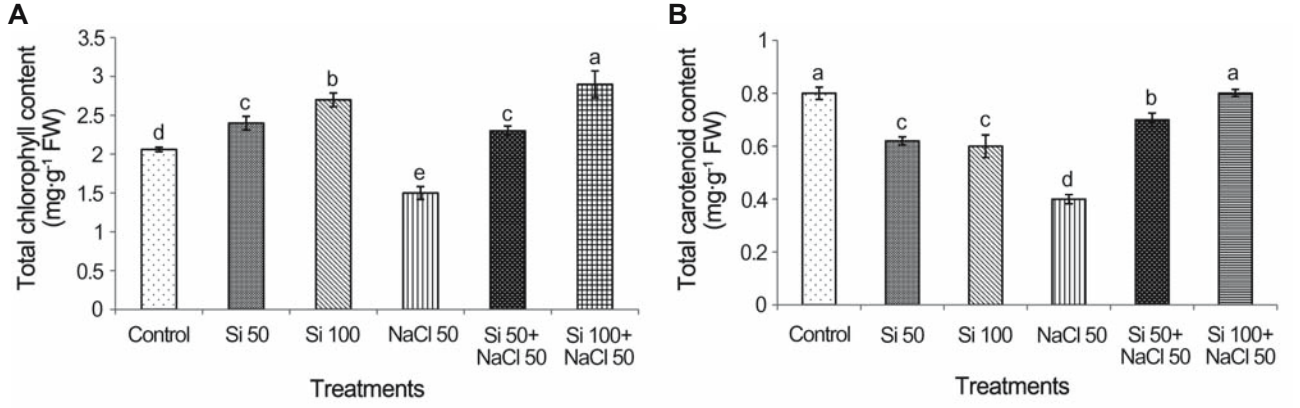

Fig. 3. Effects of Si nutrition and salinity stress on photosynthetic pigments of *Zinnia elegans* after 15 days of treatment. A, total chlorophyll content; B, carotenoid content. Data are represented as mean ± SE from three replicates. Different letters within each graph indicate statistically significant differences at $p \leq 0.05$ using Duncan's multiple range test.

pigment content such as total chlorophyll and carotenoid contents (Fig. 3). Although salt stress (NaCl 50 treatment) greatly reduced the photosynthetic pigment content (total chlorophyll 27.2% and carotenoid 50% less than the control), Si supplementation in both Si 50 + NaCl 50 (total chlorophyll 53.3% and carotenoid 75% greater than NaCl 50 treatment) and Si 100 + NaCl 50 treatments (total chlorophyll 93.3% and carotenoid 50% greater than NaCl 50 treatment) removed the detrimental effects of salinity on pigments. Similarly, Liang (1999) observed a protective effect of Si on chlorophyll content in salt-stressed barley leaves. Moreover, Si-mediated protection of chlorophyll might be due to the deposition of Si on the cell walls which resulted in the leaf erection leading to higher light interception for photosynthesis (Ma and Yamaji,

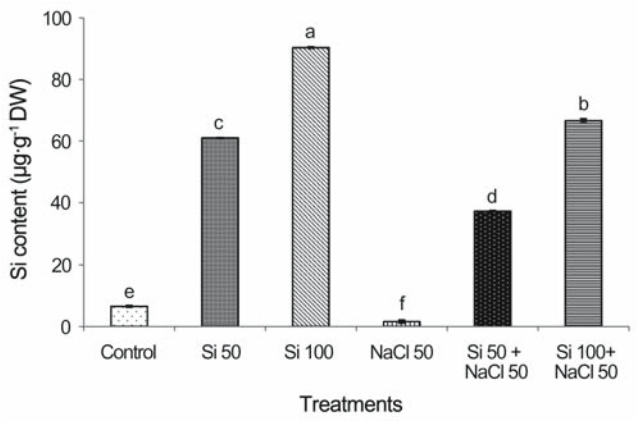

Fig. 4. Accumulation of Si in *Zinnia elegans* after 15 days of treatment. Data are represented as mean ± SE from three replicates. Different letters indicate statistically significant differences at $p \leq 0.05$ using Duncan's multiple range test.

2006). Similar findings have been reported in different crops, such as tomatoes, cucumbers, and corn (Al-aghabary et al., 2005; Feng et al., 2010; Gottardi et al., 2012).

Estimation of Si Uptake

In *Z. elegans*, Si uptake was concentration dependent. Among the treatments, the highest Si content was in Si 100 (90.4 $\mu g \cdot g^{-1}$ DW) treatment, followed by Si 100 + NaCl 50 (66.6 $\mu g \cdot g^{-1}$ DW) treatment (Fig. 4). However, under salinity stress, Si uptake was reduced by 38.9% and 26.3% in Si 50 + NaCl 50 and Si 100 + NaCl 50 treatments, respectively, compared with Si 50 and Si 100 treatments. The decrease in Si content under salinity treatment could be due to the competitive ion uptake and transportation process in plants. Moreover, the deposited Si acted as the salt-binding site, which hinders the translocation of salt in sugarcane (Ashraf et al., 2010). In addition, the small amount of Si deposition in the control and NaCl treatment could be acquired by the plants during the germination process because the seedlings were irrigated with normal tap water during seed germination and grown in Tosilee medium, a substrate with a negligible amount of silicon. *Z. elegans* is considered the best accumulator of Si among the dicots (Frantz et al., 2008), so maximizing Si accumulation may help the plant to combat salinity stress.

Effects of Si on Electrolyte Leakage and Lipid Peroxidation

The electrolyte leakage potential (ELP) was the highest in NaCl 50 treatment, illustrating the salinity-induced cell membrane damage (Fig. 5A). However, the addition of Si mitigated the leakage potential by 13.6 and 19.7% in Si 50 + NaCl 50 and Si 100 + NaCl 50 treatments, respectively, compared with NaCl 50 treatment. Interestingly, Si supplementation decreased the membrane leakage potential by 27.1 and 25.0% in Si 50 and Si 100 treatments, respectively, compared with the control. These results suggest that the addition of Si increased the membrane stability under both normal and salt-stress conditions. In general, membrane permeability can be ascertained by the electrolyte leakage potential. The dysfunction of the cellular membrane caused by salinity stress increases its permeability to ions and electrolytes (Demidchik et al., 2014), but the added Si alleviated the detrimental effect of NaCl treatment. Similarly, He et al. (2010) also observed a reduction in ELP by Si with chilling injury in *Paspalum vaginatum*.

As a result of electrolyte leakage, the greatest lipid peroxidation level in terms of MDA content was observed in NaCl treatment (Fig. 5B). NaCl treatment increased the MDA content by 92.1% compared with the control. However, the attenuation of lipid peroxidation was apparent in Si 50 + NaCl 50 and Si 100 + NaCl 50 treatments, in which the MDA levels were reduced to 64.6 and 25.9%, respectively, of that in NaCl 50 treatment. However, there was no significant difference in the MDA level between the control and Si 50 treatment. Among the treatments, the lowest lipid peroxidation was observed in Si 100 treatment, which was 54.8% less than the control. In several plants, salinity increased the peroxidation of lipids, implying that functional disturbances occurred in the cellular membranes as the result of salinity-mediated oxidative damage (Gong et al., 2005). However, Si application significantly reduced the lipid peroxidation level. Our results are in agreement with Agarie et al. (1998), who suggested that Si-mediated enhancement of lipids present in the cell membrane in rice prevent the structure and function of the cell membrane during environmental stress. Likewise, Si augmentation decreased the plasma membrane permeability of barley leaves and reduced lipid peroxidation (Liang, 1999). Thus, our results suggest that Si mitigated salinity stress by increasing the membrane integrity and reducing the peroxidation of lipids in salt-stressed *Z. elegans*.

Effects of Si on Endogenous Hydrogen Peroxide Levels and Antioxidant Enzyme Activity

The H_2O_2 content was significantly increased by salt stress (Fig. 5C). On the other hand, the addition of Si significantly decreased the H_2O_2 induced by salinity stress. The higher accumulation of H_2O_2 by NaCl 50 treatment (66.6% higher than the control) was decreased by 36.3 and 32.7% in Si 50 + NaCl 50 and Si 100 + NaCl 50 treatments, respectively. However, there was no significant difference in the H_2O_2 levels among the control, Si 50, Si 100, and Si 50 + NaCl 50 treatments. Similarly, supplemental Si reduced the H_2O_2 content in salt-stressed cucumber leaves (Zhu et al., 2004). The elevated accumulation of endogenous H_2O_2 represents the oxidative damage level and the complex signaling mech-

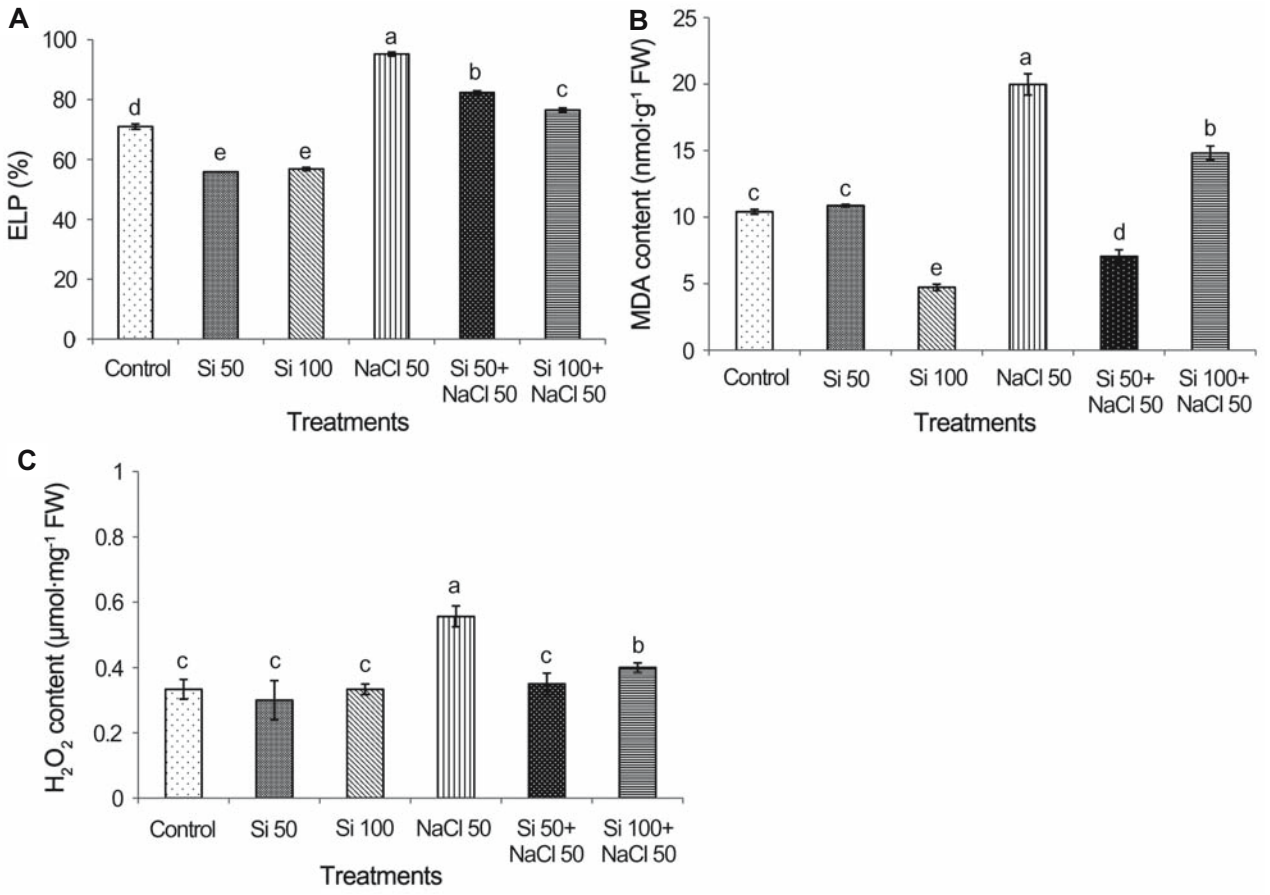

Fig. 5. Effects of Si supplementation and salinity stress on biochemical stress markers in *Zinnia elegans* after 15 days of treatment. A, electrolyte leakage potential (ELP); B, malondialdehyde content (MDA); C, hydrogen peroxide content (H_2O_2). Data are the mean ± SE from three replicates. Different letters within each graph indicate statistically significant differences at $p \leq 0.05$ using Duncan's multiple range test.

anism involved during abiotic stresses (Gunes et al., 2007). Moreover, the intracellular H_2O_2 concentration also determines the activity of antioxidant enzymes (Mittler, 2002).

Si augmentation significantly increased the SOD enzyme activity in both the normal and salt-stressed treatments (Fig. 6A). Si 50 and Si 100 treatments augmented SOD activity by 164% and 141%, respectively, compared with the control. However, NaCl 50 treatment enhanced the SOD activity by 72% compared with the control. Under salt stress, the added Si increased the activity of SOD in Si 50 + NaCl 50 (37.9%) and Si 100 + NaCl 50 (48.6%) treatments compared with NaCl 50 treatment. Generally, the antioxidant enzymes prevent cell damage from harmful reactive oxygen species (ROS) by catalyzing a cascade of reactions. As the first line of defense, the enzyme SOD converts the unstable superoxide (O_2^{-1}) radical into stable H_2O_2 and molecular oxygen (Halliwell and Gutteridge, 2007). Subsequently, the H_2O_2 molecules are scavenged by several other enzymes such as GPX, CAT, and APX (Zhu et al., 2004).

In the present study, Si treatments greatly enhanced the activities of GPX, CAT, and APX. Si significantly increased the activity of GPX (Fig. 6B) by 126 and 226% in Si 50 and Si 100 treatments, respectively, compared with the control. When Si was added under the salt-stress conditions in Si 50 + NaCl 50 and Si 100 + NaCl 50 treatments, the GPX activity increased by 140% and 170%, respectively, compared with NaCl 50 treatment. In plants, the GPX enzyme acts as an active scavenger of H_2O_2 and catalyzes the metabolism of polyphenols that enrich the antioxidant activity of plants (Shi et al., 2005). Similarly, CAT was increased by 52.9 and 70.9% in Si 50 and Si 100 treatments compared with the control (Fig. 6C). However, there was no significant difference in the CAT activity between the control and NaCl 50 treatments. However, the addition of Si increased the CAT activity in Si 50 + NaCl 50 (32.7%) and Si 100 + NaCl 50 (43.6%) treatments compared with NaCl 50 treatment. CAT is a universal oxido-reductase responsible for the decomposition of H_2O_2 into water and molecular oxygen and maintains the fine regulation of H_2O_2 for signaling processes (Gill and Tuteja, 2010). Moreover, the activity of APX also followed a similar trend to GPX and CAT. The APX activity was significantly increased in Si treatments under both non-stressed

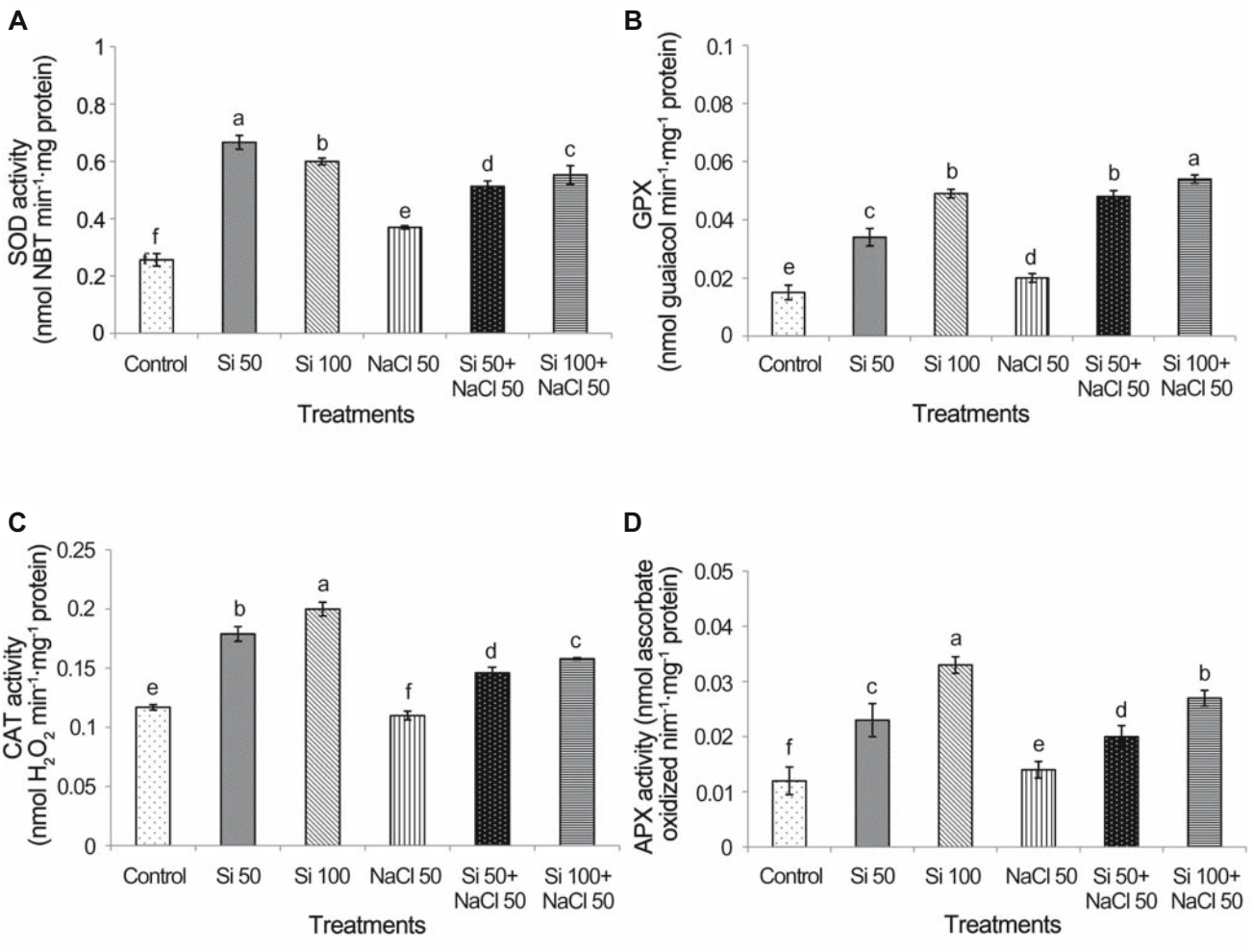

Fig. 6. Effects of Si supplementation and salinity stress on the antioxidant enzyme activity of *Zinnia elegans* after 15 days of treatment. A, superoxide dismutase (SOD); B, guaiacol peroxidase (GPX); C, catalase (CAT); D, ascorbate peroxidase (APX). Data are represented as mean ± SE from three replicates. Different letters within each graph indicate statistically significant differences at $p \leq 0.05$ using Duncan's multiple range test.

and stressed conditions (Fig. 6D). More specifically, Si 50 and Si 100 treatments enhanced the APX activity by 91.6 and 175%, respectively, compared with the control. Furthermore, Si 50 + NaCl 50 and Si 100 + NaCl 50 treatments increased the activity of APX by 42.8 and 92.8% compared with NaCl 50 treatment. APX is an important antioxidant enzyme in the ascorbate-glutathione cycle involved in the reduction of H_2O_2 by using ascorbate as the substrate electron donor (Mittler, 2002; Rennenberg, 1980).

Overall, the activities of SOD, GPX, CAT, and APX (H_2O_2 scavengers) were enhanced with Si supplementation in both normal and salinity-stress conditions. Our findings are concordant with previous reports suggesting Si-mediated modulation of antioxidant enzymes renders abiotic stress tolerance (Liang, 1999; Zhu et al., 2004).

Effects of Si on Macronutrients

The concentrations of macronutrients such as K, Na, Ca, P, S, and Mg were significantly modulated by salinity stress (Table 2). In particular, salt stress highly reduced the K content in *Z. elegans* by 21.6% compared with the control. On the other hand, Si supplementation significantly increased the K concentration by 22.8 and 31% in Si 50 + NaCl 50 and Si 100 + NaCl 50 treatments, respectively, compared with NaCl 50 treatment. Si also enhanced the concentration of K in normal plants without NaCl treatment. One salinity mitigation mechanism attributed to Si is the reduction of Na uptake by increasing the counter-uptake of K (Liang, 1999; Tuna et al., 2008). Moreover, the enhancement of K content by Si could help maintain membrane integrity during salinity stress.

Salt stress also significantly reduced Ca by 29.6% compared with the control. However, Ca was increased by Si augmentation in Si 50 + NaCl 50 and Si 100 + NaCl 50 treatments. According to Liang (1999), Si-mediated enhancement of Ca could also alleviate the Na-induced Ca deficiency

Table 2. Macronutrient contents of *Zinnia elegans* as affected by salinity stress and Si supplementation after 15 days of treatment

Si ($mg \cdot L^{-1}$)	NaCl (mM)	K ($mg \cdot g^{-1}$ DW)	Ca ($mg \cdot g^{-1}$ DW)	Mg ($mg \cdot g^{-1}$ DW)	Na ($mg \cdot g^{-1}$ DW)	S ($mg \cdot g^{-1}$ DW)	P ($mg \cdot g^{-1}$ DW)
0	0	100.5±0.22c[z]	37.8±0.07b	12.13±0.45b	3.67±0.03d	5.90±0.24c	78.8±0.18c
	50	78.8±0.04f	26.9±0.61e	13.23±0.59a	57.1 ±0.27a	7.38±0.09a	31.1±0.07f
50	0	107.8±0.19c	37.1±0.12b	12.31±0.05b	2.5 ±0.33d	5.89±0.13c	80.5±0.60b
	50	102.0±0.26d	35.7±0.36c	13.08±0.16a	31.4 ±0.50b	4.76±0.55d	62.7±0.31e
100	0	132.9±0.52a	28.8±0.75d	11.17±0.81c	2.40±0.41d	4.87±0.69d	82.3±0.02a
	50	114.1±0.83b	40.0±0.17a	12.32±0.25b	26.40±0.13c	6.27±0.19b	71.7±0.38d

[z]Mean separation within columns by Duncan's multiple range test at $p \leq 0.05$.
Data are represented as mean ± SE from three replicates.

Table 3. Micronutrient contents of *Zinnia elegans* affected by salinity stress and Si supplementation after 15 days of treatment

Si ($mg \cdot L^{-1}$)	NaCl (mM)	Mo ($mg \cdot g^{-1}$ DW)	Cu ($mg \cdot g^{-1}$ DW)	Zn ($mg \cdot g^{-1}$ DW)	Mn ($mg \cdot g^{-1}$ DW)	Fe ($mg \cdot g^{-1}$ DW)	B ($mg \cdot g^{-1}$ DW)
0	0	0.027±0.58b[z]	0.097±0.26b	0.427±0.22a	1.5±0.71a	1.00±0.19b	0.220±0.06ba
	50	0.042±0.11a	0.093±0.07b	0.308±0.50c	0.8±0.22c	0.84±0.09c	0.187±0.33c
50	0	0.027±0.87b	0.099±0.19b	0.394±0.61b	1.1±0.55b	0.83±0.32c	0.199±0.25bc
	50	0.017±0.24c	0.085±0.08c	0.381±0.05b	1.5±0.16a	0.91±0.08c	0.267±0.84a
100	0	0.016±0.31c	0.079±0.66d	0.259±0.09e	0.7±0.51d	1.06±0.22b	0.200±0.05a
	50	0.029±0.06b	0.113±0.71a	0.288±0.45d	1.1±0.35b	1.25±0.90a	0.220±0.37b

[z]Mean separation within columns by Duncan's multiple range test at $p \leq 0.05$.
Data are represented as mean ± SE from three replicates.

from salt stress. In addition, Murillo-Amador et al. (2007) reported that Ca ions can compete with Na for the membrane binding site. However, the Mg and S contents were significantly increased in NaCl 50 treatment. In the present study, the addition of Si significantly reduced the Na content by 45% in Si 50 + NaCl 50 treatment and by 53.8% in Si 100 + NaCl 50 treatment. In accordance with our results, several studies reported that Si lowered Na content (Ahmad et al., 1992; Liang, 1999; Soundararajan et al., 2014), suggesting that Si-mediated salt tolerance could be associated, at least partly, with Na exclusion in tissues. The internal concentration of P has been reported to play a vital role in several metabolisms and to act as a basic structural element in nucleic acids and amino acids. Hence, the improvement of P content in Si-fed plants could help protect against the negative effects of salinity in cell organelles (Ma and Takashi, 1991).

Effects of Si on Micronutrients

Salinity-induced reduction of micronutrients such as Zn, Mn, Fe, and B was significantly alleviated with Si addition (Table 3). NaCl 50 treatment reduced the Zn concentration by 27.8% compared with the control, but Si 50 + NaCl 50 treatment enhanced the Zn level by 21.0%. However, the higher Si concentration in Si 100 + NaCl 50 treatment decreased the Zn content, possibly due to the formation of a Zn-silicate complex in cell wall (Neumann and zur Nieden, 2001). Among the micronutrients, the Mn content was highly decreased by 43.8% in NaCl treatment, while the addition of Si improved the Mn concentration by 43.8% (Si 50 + NaCl 50) and 22.2% (Si 100 + NaCl 50). According to Cramer et al. (1991) salinity negatively affected Mn transportation in barley. In addition, Wang and Han (2007) reported the enhancement of Mn by Si in barley. In *Z. elegans*, the tissue Fe concentration was reduced by NaCl 50 treatment (25.0% less than the control), but was increased by 33.9% in Si 100 + NaCl 50 treatment. Hence, the added Si ameliorated the Na-mediated Fe deficiency and enriched the Fe content.

Salinity also affected the B content and reduced its concentration by 21.7%, but Si addition, especially in Si 50 + NaCl 50 treatment, increased the B content by 30.3 and 15.4% in Si 100 + NaCl 50 treatment compared with NaCl 50 treatment. Unlike the results reported by Gunes et al. (2007), which suggested that Si prevents B uptake, none of the negative effects were observed in the present study. Further, the elemental content of Mo was significantly increased under salinity stress. According to previous reports, NaCl stress generally increased the Cu level in several plants (Wang and Han (2007)), but in this study the level of Cu was not significantly affected by salt treatment. In brief, the enhancement of macronutrients and micronutrients by Si supplementation improved the mechanical strength and resistance against

salinity stress in *Z. elegans.*

In conclusion, Si augmentation during hydroponic cultivation of *Z. elegans* enhanced growth, photosynthesis, pigmentation, activity of antioxidant enzymes, membrane integrity, and nutrient contents. Si supplementation also imparted salinity stress tolerance to *Z. elegans* by providing mechanical strength, maintaining osmotic balance, and modulating the activity of antioxidant enzymes. Overall, the results of the current study strongly suggest that Si can effectively mitigate salinity stress in hydroponically grown *Z. elegans*. In the future, this study could be extended to understand the molecular rationale behind Si-mediated stress alleviation.

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