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Application of sodium nitroprusside and silicon on the enzyme activity of *Solanum lycopersicum* during vegetative growth

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ABSTRACT

One of the most deleterious abiotic stresses is the salinity stress, which causes inhibition of growth and development. Therefore, this investigation was conducted to evaluate the effects of sodium nitroprusside (SNP) and silicic acid (Si) on biochemical response of *Solanum lycopersicum* (cv. Isabella) under different salinity levels during vegetative stage. For this purpose, the seedlings were subjected to different salt stress levels (0, 25, 50, 100, and 150 mM) and supplemented with optimized concentration of silicon (Si) (0, and 2.5 mM of H_4SiO_4) and sodium nitroprusside (SNP) (0, and 100 µM) to assess variations in enzyme activity and biochemical properties of tomato plants during vegetative growth. Salt stress inhibited the chlorophyll and carotenoid contents of tomato plants. The antioxidant enzyme activities such as catalase (CAT) and superoxide dismutase (SOD) as well as the levels of osmolytes (proline, glycine betaine), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) increased in tomato plants due to high salinity. Furthermore, the exogenous use of SNP and Si to alleviate the effect of salinity on the plants increased the antioxidant enzyme activities and osmolyte levels compared to NaCl-treated plants. In addition, in the plants under salt stress, supplemented with SNP and Si, the contents of MDA and H_2O_2 decreased. Therefore, the exogenous use of Si and SNP led to protecting a tomato plant against oxidative damage induced by salt stress by stimulating synthesis of antioxidant enzyme. The findings indicated that, with the improvement in antioxidative defense system, pigment syntheses, and osmolyte accumulation, SNP and Si had the ability to alleviate adverse impact of high salinity on tomato plants.

Keywords: Antioxidant enzyme, chlorophyll content, salinity, tomato.

RESUMO

Aplicação de nitroprussiato de sódio e silício na atividade enzimática de *Solanum lycopersicum* durante o crescimento vegetativo

Um dos estresses abióticos mais deletérios é a salinidade, por inibir o crescimento e desenvolvimento da planta. Portanto, esta pesquisa objetivou avaliar os efeitos do nitroprussiato de sódio (SNP) e do ácido silícico (Si) na resposta bioquímica de *Solanum lycopersicum* (cv. Isabella) sob diferentes níveis de salinidade durante a fase vegetativa. Mudas de tomate foram submetidas a diferentes níveis de estresse salino (0, 25, 50, 100 e 150 mM) e suplementadas com concentração otimizada de silício (Si) (0 e 2,5 mM de H_4SiO_4) e nitroprussiato de sódio (SNP) (0 e 100 µM) para avaliar variações na atividade enzimática e propriedades bioquímicas de plantas durante o crescimento vegetativo. O estresse salino inibiu os teores de clorofila e carotenóides das plantas de tomate. As atividades de enzimas antioxidantes como catalase (CAT) e superóxido dismutase (SOD), bem como os níveis de osmólitos (prolina, glicina betaína), malondialdeído (MDA) e peróxido de hidrogênio (H_2O_2) aumentaram em plantas de tomate devido à alta salinidade. O uso exógeno de SNP e Si para aliviar o efeito da salinidade nas plantas aumentou as atividades das enzimas antioxidantes e os níveis de osmólitos em comparação com as plantas tratadas com NaCl. Nas plantas sob estresse salino, suplementadas com SNP e Si, os teores de MDA e H_2O_2 diminuíram. Portanto, o uso exógeno de Si e SNP levou à proteção de plantas de tomate contra danos oxidativos induzidos pelo estresse salino, estimulando a síntese de enzimas antioxidantes. Os resultados indicaram que, com a melhoria no sistema de defesa antioxidante, síntese de pigmentos e acúmulo de osmólitos, SNP e Si tiveram a capacidade de aliviar o impacto adverso da alta salinidade nas plantas de tomate.

Palavras-chave: Enzima antioxidante, teor de clorofila, salinidade, tomate.

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One of the most deleterious abiotic stresses is defined as salinity stress, which causes the inhibition of development, growth (Roy & Mishra, 2014) as well as a decrease in respiration, protein synthesis, photosynthesis, osmotic stress, production of reactive oxygen species (ROS), and ion toxicity (Mittler, 2002; Wu *et al*., 2010). Therefore, the antioxidant system should be elevated in plant organs to enhance salt stress tolerance and mitigate such stress (Wu *et al*., 2010) or with exogenous application of some compounds which are capable of

alleviating salinity stress.

The second most abundant element in soil is silicon (Si), which can alleviate stresses such as drought, heat, chilling, and salt (Wang *et al*., 2010). Some studies have demonstrated that silicon is an essential element in the growth and development of plants (Kim *et al*., 2014). Additionally, several studies reported the beneficial effect of this element to mitigate the salt stress in different plant species such as soybean (Lee *et al*., 2010), sugarcane (Ashraf *et al*., 2010), cherry tomato (Haghighi & Pessarakli, 2013), and squash (Siddiqui *et al*., 2014). Numerous studies suggested that the exogenous application of silicon can ameliorate the production of ROS by regulation of antioxidant enzyme activity (Kim *et al*., 2017; Tripathi *et al*., 2017). Torabi *et al*. (2015) demonstrated that the exogenous application of Si can promote a decrease in CAT and an increase in SOD in borage plants.

Nitric oxide (NO) has a major role as a signaling molecule and also showed various biological functions in plants (Wu *et al*., 2010). NO has a protective impact as a response to biotic and abiotic stresses. In relation to abiotic stresses, application of sodium nitroprusside has been suggested to reduce the adverse impacts of salinity on plants (Fan *et al*., 2012). The NO treatment in plant species resulted in different responses to salinity stress (Begara-Morales *et al*., 2014). Fan *et al*. (2012) found out that the exogenous use of NO ameliorated salinity damage in cucumber seedlings by reducing the adverse impacts of salinity stress and regulating proline metabolism.

Tomato plant is one important greenhouse crop throughout the world. High salinity in the soil heavily influences its growth and it shows different responses to salinity stress depending on cultivar and growth stage (Roy & Mishra, 2014). However, informations on the interaction of salinity, Si and NO applications on tomato seedling's growth and performances are still scarce. Therefore, the objective of the present research was to evaluate de effect of the use of sodium nitroprusside and silicic acid on biochemical response of *Solanum lycopersicum* (cv. Isabella) under different levels of salinity during vegetative stage.

MATERIAL AND METHODS

Plant material and growth conditions

In a greenhouse at Jahrom Azad Agricultural University, Fars, Iran, the experiment was conducted. *Solanum lycopersicum* (cv. Isabella, F_1 hybrid, purity and germination rate 99%, inert 1%) seeds were purchased from Monsanto company. Seeds were planted in plastic pots containing 5 kg sterilized potting mix (sand, clay, and leaf mold, 1:1:2), in a greenhouse where the mean temperature per day/night and relative humidity were 30-20±2°C and 50%, respectively. Some of the physicochemical characteristics of the media are: SO^{-2}_4= 40 meq/L; HCO_3^{-2}= 0 meq/L; CO_3^{-2}= 5.5 meq/L; Ca= 37.5 meq/L; Cl= 7.5 meq/L; Mg= 5.5 meq/L; K= 0.45 meq/L; Na= 12.52 meq/L; EC= 3.84 d/Sm; pH= 7.92; SAR= 2.7.

The seedlings were irrigated with tap water (EC= 0.548 d/Sm) until commencement of salt treatment. When seedlings reached four to six leaf stage, salinity treatments were executed. Sodium chloride treatment solutions containing 0 (distilled water only as control treatment), 25, 50, 100, and 150 mM were applied with irrigation for 40 days duration until flowering stage (from April 3 to May 13, 2018). Before beginning salt treatments, pots were irrigated with tap water to field capacity. Sodium chloride (Merck, Darmstadt, Germany) for the treatments was dissolved in tap water to achieve the desired conductivity. The treatments were applied incrementally, increasing by 25 mM every 1 week, over a 5 week period, to achieve the highest level of salinity in order that an osmotic shock to the plants could be avoided. For each irrigation, the soil moisture content was increased to field capacity level by weighing the pots, according to Gerwal *et al*. (1990). To prevent salt accumulation in the pots, when the EC of pot outlet solution was 1.5 times the EC of the initial solution, the leaching requirement was carried out. At the same time with salinity stress, leaves were sprayed with silicic acid (Si donor) at zero and 2.5 mM and SNP (NO donor) at zero and 100 µM. After 5 weeks the mature leaf samples were harvested for each analysis.

Measurements and observations of leaf samples

Proline content

Using 3% sulfosalicylic acid, 0.2 g of leaf sample was homogenized. Then, acid-ninhydrin was added and the content of proline was measured. A spectrophotometer (UV-120-20, Japan) was utilized to measure the absorbance of the fraction with toluene at 520 nm (Fan *et al*., 2012).

Glycine betaine determination

Using 20 mL deionized water, 0.5 g of leaf sample was homogenized. Then, leaf samples were shaken for 48 hours at 25°C. Using 2N H_2SO_4, the extracts were diluted 1:1. Aliquots were allowed to be cooled down by ice water for one hour. The cold KI-I_2 reagent (0.2 mL) was added and, using a vortex mixer, the reactants were gradually stirred. The tubes were kept at 4°C for 16 h and then centrifuged at 10,000 rpm and 0°C for 15 minutes. The crystals of periodide were dissolved in 9 mL of 1, 2-dichloroethane. After two hours, the absorbance was determined at 365 nm (Torabi *et al*., 2015).

Total phenolic content

The Folin-Ciocalteu reagent was utilized with some modifications to determine the total phenolic content. 200 mg of the sample was extracted with 2 mL of 80% methanol using an orbital shaker at 200 rpm and at room temperature for 2 hours. The mixture was centrifuged at 1000 rpm for 15 minutes and the supernatant liquid was used as extract. 100 µL of the extract and 1.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) were mixed and kept at 22°C for 5 minutes. Then, 1.5 mL of 20% sodium bicarbonate solution was added to the mixture. After 90 minutes, the absorbance was determined at 750 nm

(Abbas *et al*., 2015).

Lipid peroxidation (Malon dialdehyde)

The thiobarbituric acid reaction was utilized to measure the level of lipid peroxidation in the tissue of the leaves. Leaf sample (0.5 g) was homogenized with 10 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 1500 rpm for 15 minutes. 4 mL of 20% TCA that contained 0.5% thiobarbituric acid (TBA) were added to 1 mL supernatant aliquot. The mixture was heated at 95°C for 30 minutes and kept in an ice bath. Then, the mixture was centrifuged at 10,000 rpm for 10 minutes, and the supernatant absorbance was measured at 532 and 600 nm. After subtracting the two wavelengths, the concentration of MDA was determined (Torabi *et al*., 2015).

Peroxide hydrogen assay

The content of hydrogen peroxide (H_2O_2) of the leaves was assayed. The leaves were homogenized with 0.1% (w/v) TCA in an ice bath. The extract was centrifuged at 12,000 rpm for 15 minutes, and the supernatant was added to 0.5 mL 1 M potassium iodide (KI) and 0.5 mL of 10 mM potassium phosphate buffer at pH= 7, and the absorbance was read at 390 nm using Shimadzu UV-1700 spectrophotometer (Jamali *et al*., 2014).

Activity of enzymes

Catalase assay

Half gram leaf tissue was homogenized with 5 mL of 50 mM phosphate buffer that contained 1% insoluble polyvinylpyrrolidone at pH= 7. The homogenate was centrifuged at 15,000 rpm for 10 minutes and the obtained supernatant was utilized as the enzyme extract. The initial rate of disappearance of hydrogen peroxide was measured according to the method employed by Maehly & Chance (1954) to assay the catalase enzyme activity. 1 mL of reaction mixture containing 25 µL of enzyme extract, 15 mM of hydrogen peroxide, and 50 mM of phosphate buffer at pH= 7.0 was used. The reduction in hydrogen peroxide was followed as a decrease in A_{240} with a spectrophotometer (Shimadzu UV-1700) according to Abbas *et al*. (2015).

Superoxide dismutase assay

Fresh sample (0.25 g) was ground to a fine powder in liquid N_2 and then homogenized with 1% PVPP, 2 mM NaEDTA, and 1 mL of 50 mM phosphate potassium buffer at pH= 7.0. The homogenate was centrifuged twice and the supernatants were used. The SOD ability to prevent the photochemical reduction in nitroblue tetrazolium (NBT) at 560 nm spectrophotometrically assay SOD. The enzymatic activity was expressed as U/g FW (Jamali *et al*., 2014).

Chlorophyll and carotenoid

The content of chlorophyll was spectrophotometrically measured at 480 nm for carotenoid and at 645 and 663 nm for chlorophyll (Lee *et al*., 2010).

Chl *a* = $0.0127\ A_{663}$-$0.00269\ A_{645}$

Chl *b* = $0.00229A_{645} - 0.00468\ A_{663}$

Carotenoid = ($1000A_{470}$ - 1.8 chl *a* - 85.2 chl *b*)/198

Statistical analysis

The experiment was carried out based on a completely randomized factorial design with five replications (one seedling in each replication). The SAS software (Version 9.4) was used to analyze the data and LSD was utilized to compare significant differences between the mean data at $P \leq 0.05$.

RESULTS AND DISCUSSION

The data analysis of variance (results not displayed) indicated that there was significant interaction between the factors. On this basis, the mean comparison of the interaction between factors has been explained.

Proline

The results (Figure 1) showed that, as salinity increased, proline content significantly increased without application of Si and SNP. Under higher levels of salinity, proline content increased with application of 2.5 mM Si.

MDA concentration, glycine betaine, and H_2O_2

The addition of Si dramatically enhanced MDA content of the leaf under salinity stress (Figure 2A). The addition of NaCl in the presence of SNP and Si slightly increased a MDA content of tomato plant leaves stressed with 150 mM NaCl, in which lowest MDA content was observed in Si + SNP in 150 mM NaCl. As salinity increased, glycine betaine content increased without application of Si and SNP. Glycine betaine reached highest level in 150 mM NaCl with application of SNP + Si. The minimum content of glycine betaine was observed in control (no salt stress) (Figure 2B). In the absence of Si and SNP, salt stress at 150 mM enhanced the H_2O_2 content, however, added Si and SNP reduced the content of H_2O_2 under the highest level of salt stress (150 mM NaCl) (Figure 2C).

Total phenolic content

Total phenolic content significantly increased as salinity increased, however, with application of Si and SNP, phenol content decreased (Figure 3). The maximum and minimum content of phenol was observed in application of Si and SNP under 150 mM NaCl, respectively (Figure 3).

Enzyme activities

As the result indicated in Figure 4A, CAT activity increased in the application of 100 µM SNP under salinity of 150 mM NaCl and decreased with Si in plants treated with 25 mM NaCl, whilst, the activity of SOD enhanced with foliar application of Si in 150 mM NaCl. SOD decreased with application of SNP in plants stressed with 25 mM NaCl (Figure 4B).

Chlorophyll and carotenoid content

Amounts of chlorophyll *a*, chlorophyll *b* and carotenoid dramatically decreased under conditions of salinity stress. However, the addition of Si and SNP raised amounts of chlorophyll *a*, chlorophyll *b* and carotenoid under conditions of salinity stress. Amounts of chlorophyll *a*, chlorophyll *b* and carotenoid decreased in the highest level of salinity, but Si at 2.5 mM improved amounts of chlorophyll *a* and chlorophyll *b* at 150 mM NaCl. SNP at 100 µM enhanced

carotenoid content in plants treated at 150 mM NaCl (Figure 5A, B, C).

Salinity stress showed to dramatically change the biochemical responses of Isabella tomato cultivar. Exogenous application of silicic acid and sodium nitroprusside decreased the effect of salinity stress, thereby improving biochemical responses of the plant. The findings indicated that SNP and Si improved proline content under salinity condition (Figure 1). Similarly, the impacts of SNP and Si have been documented in relation to tomato plants (Manai *et al*., 2014) and cucumber seedlings (Siddiqui *et al*., 2014), respectively. Plants produce several different compatible solutes such as total phenolic content, glycine betaine, and proline under stress conditions. The results of our research demonstrated that Si and SNP increased the proline and glycine betaine content (Figures 1, 2B). The current result of this study is in line with earlier studies (Fan *et al*., 2012; Abbas *et al*., 2015), where an increase in proline and glycine betaine was indicative of an osmotic adjustment capacity of plants under conditions of salt stress. In another study on pepper cultivar, improving potential for an osmotic adjustment in terms of increasing compatible solutes by Si resulted in high photosynthetic activities and considerable growth under conditions of salt stress (Fan *et al*., 2012). Generally, it can be presumed that Si can alleviate the damaging impacts of salinity stress by an increase in the accumulation of organic osmolytes that reduced an osmotic shock induced by NaCl stress owing to ion toxicity.

Nevertheless, some studies described that Si and SNP enhanced total phenolic content (Jamali *et al*., 2014; Abbas *et al*., 2015). Our research demonstrated that the SNP and Si treatments had lower total phenolic content than non-SNP and Si treated plants (Figure 3). It seemed that reduction of this osmolyte by application of Si and SNP on tomato

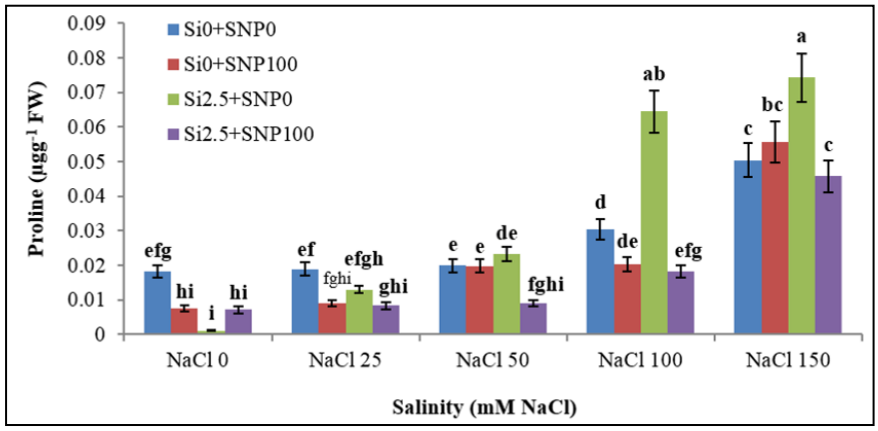

Figure 1. Impact of Si and SNP on proline content under different salinity levels. The columns having same letters are not different according to LSD test ($P \leq 0.05$). Jahrom, Iran, Jahrom Azad Agricultural University, 2021.

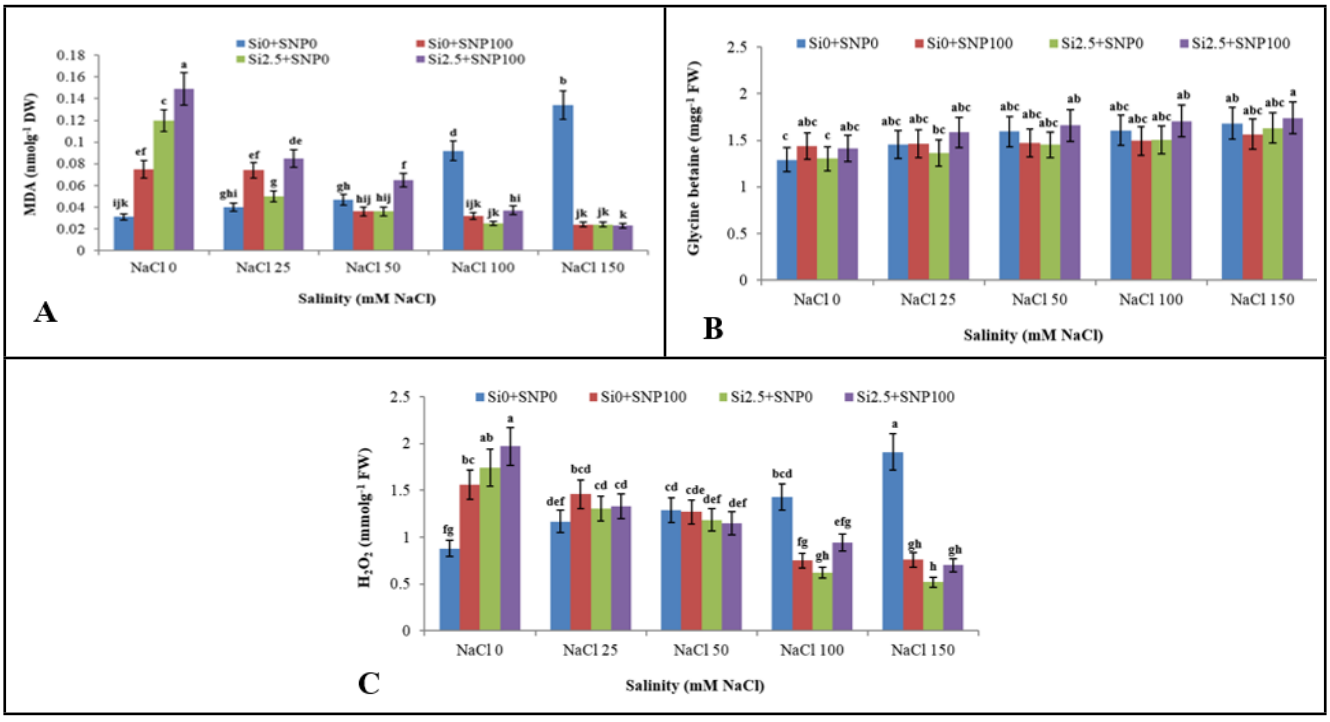

Figure 2. Impact of Si and SNP on MDA (A), glycine betaine (B) and H_2O_2 (C) content under different salinity levels. The columns having same letters are not different according to LSD test ($P \leq 0.05$). Jahrom, Iran, Jahrom Azad Agricultural University, 2021.

leaves probably depended on genotype, cultivar and severity of salinity stress. Therefore, more detailed study is needed to investigate such difference in tomato.

The present study suggested that Si and SNP treatments reached highest CAT and SOD activity during salinity condition (Figure 3A, B). The findings supported the results of previous studies done on distinct plant species, in which CAT and SOD activities increased by the use of SNP and Si under conditions of salt stress (Kim *et al*., 2014; Jamali *et al*., 2014; Siddiqui *et al*., 2014). Due to high ROS production under conditions of salt stress, plants provide an antioxidant mechanism to prevent damage to cellular components and increase tolerance to salinity by the ROS scavenging system (Alscher *et al*., 2002). Additionally, foliar applied-Si intensified the ROS scavenging under saline condition. Tripathi *et al*. (2013) and Farooq *et al*. (2013) found that Si is a possible element to regulate various activities of antioxidant enzymes. The simultaneous use of NaCl and sodium nitroprusside raised the CAT and SOD activities in a tomato plant (Figure 3A, B). This finding is in line with the results of other studies done on mustard (Khan *et al*., 2012), tomato (Manai *et al*., 2014) and chick pea (Ashraf *et al*., 2010) plants. Nitric oxide (NO) can play an important role in ROS scavenging and antioxidant system stimulation to increase the expression of the genes that encode antioxidant enzymes (Grob *et al*., 2013). Furthermore, the exogenous NO use might induce the synthesis of endogenous NO to act as above-mentioned roles (Zhao *et al*., 2009; Fan *et al*., 2012). Consequently, it is possible that SNP and Si eliminated ROS by elevating the antioxidant activities and thus mitigated toxicity induced by NaCl in a tomato plant depending on severity of salinity.

In our study, Si considerably ameliorated the drastic impact of lipid peroxidation on the membrane under salinity condition by reducing the content of the MDA in tomato plants (Figure 2A). Therefore, Si lead the cell membrane integrity and sustained their permeability under abiotic stress conditions.

The leaves of tomato plants under salinity stress accumulated a high level of H_2O_2 content (Figure 2C), which cause oxidative damage. The exogenous use of SNP and Si decreased H_2O_2 in NaCl-treated tomato plants, which is in harmony with the observation of Sim *et al.* (2009) and Khan *et al.* (2012). Consequently, using SNP and Si might lead to protecting plants against oxidative damage induced by salinity stress. Some studies also suggested that SNP and Si possess the ability to defend cell membranes by alleviating damage to the cell membrane system and lowering the permeability of membranes.

The reduction in the amounts of chlorophyll *a* and chlorophyll *b* in the leaves of tomato plants observed under stress condition (Figure 5A, B) might be recognized as the destruction of chlorophyll pigments that resulted in reducing chlorophyll syntheses (Rasool *et al*., 2013). The synthesis of carotenoids lead to decreasing them in

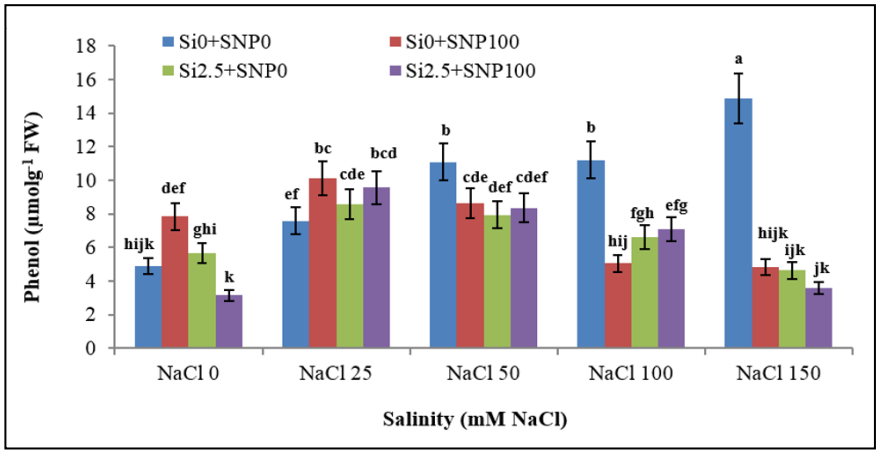

Figure 3. Impact of Si and SNP on total phenol content under different salinity levels. The columns having same letters are not different according to LSD test ($P \leq 0.05$). Jahrom, Iran, Jahrom Azad Agricultural University, 2021.

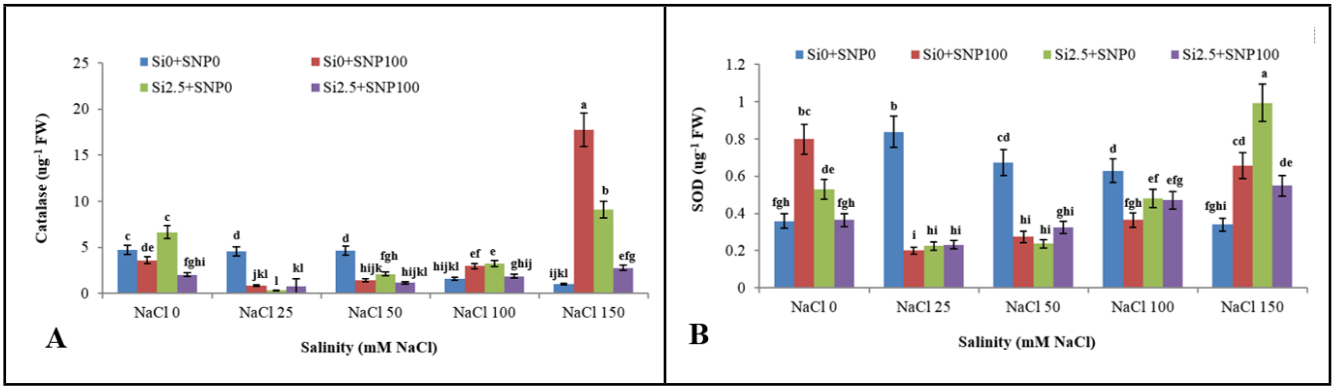

Figure 4. Impact of Si and SNP on catalase (A) and SOD (B) enzymes activity under different salinity levels. The columns having same letters are not different according to LSD test ($P \leq 0.05$). Jahrom, Iran, Jahrom Azad Agricultural University, 2021.

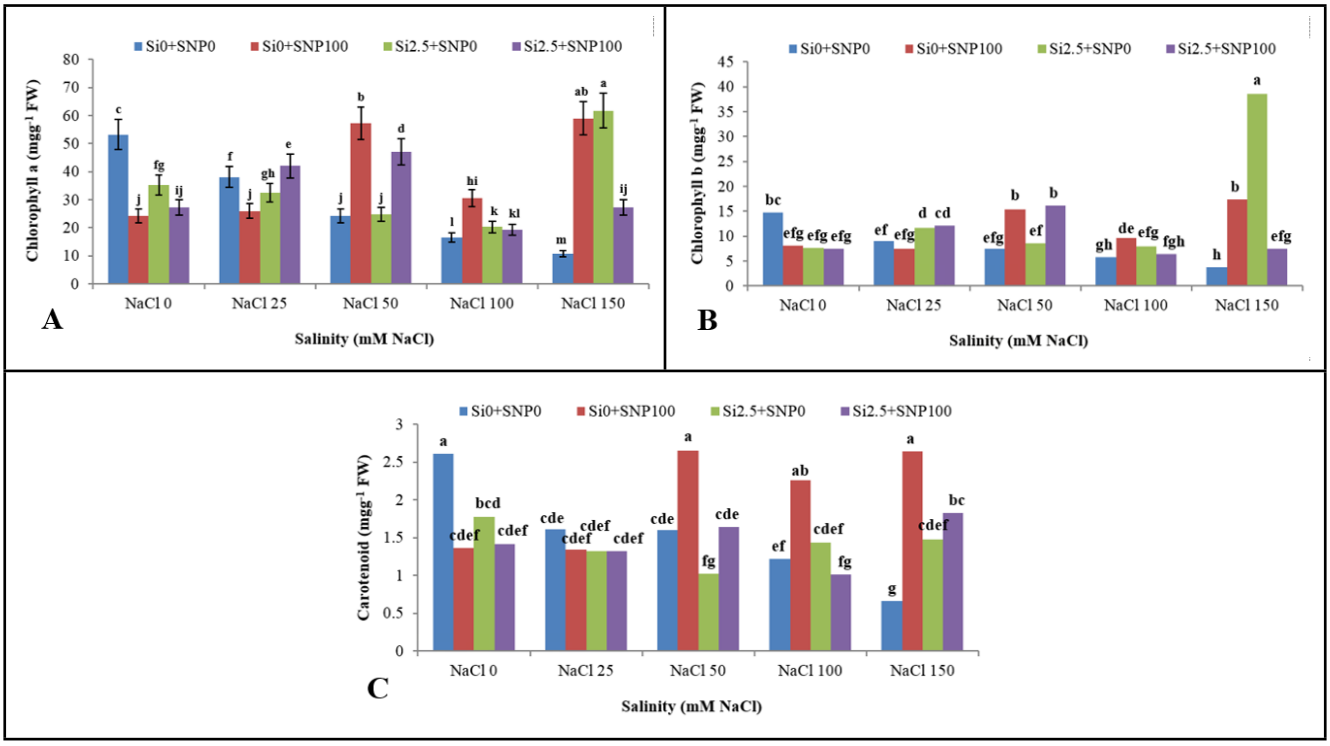

Figure 5. Impact of Si and SNP on chlorophyll *a* (A), chlorophyll *b* (B), and carotenoid (C) content under different salinity levels. The columns having same letters are not different according to LSD test ($P \leq 0.05$). Jahrom, Iran, Jahrom Azad Agricultural University, 2021.

the leaves of tomato plants under NaCl stress. However, SNP and Si (Figure 5C) have been shown to stimulate improvement in the photosynthetic pigments in tomato plants, because these compounds have a major role in mitigating oxidative damage caused by NaCl stress. This study is in accordance with previous researches on tomato (Wu *et al.*, 2010) and rice (Habib *et al.*, 2013) plants.

Stress induced by NaCl led to a decrease in malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and green pigments, and thereby resulting in decrease in catalase (CAT) and superoxide dismutase (SOD) activities and accumulation of osmolytes (glycine betaine and proline). Improvement in growth owing to spraying with sodium nitroprusside and silicon is caused by an increase in the antioxidant activities and of certain osmolyte levels. These increases resulted in rising carotenoids and total chlorophyll content under NaCl stress. Consequently, it is recommended that sodium nitroprusside (SNP) and Si are effective agents for mitigating stress in order that tomato plants can be protected against toxicity induced by NaCl. Therefore, the exogenous use of silicic acid (Si) and SNP led to protecting a tomato plant against oxidative damage induced by salt stress by stimulating synthesis of antioxidant enzyme. Finally, under saline stress, plant growth improved. The findings indicated that with the improvement in antioxidative defense system, pigment syntheses, and osmolyte accumulation, SNP and Si had the ability to alleviate adverse impact of high salinity on tomato plant.

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