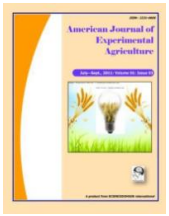

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Mechanism of Nanosilicon-Mediated Alleviation of Salinity Stress in Faba Bean (*Vicia faba* L.) Plants

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aims: In the current study we try to clarify the mechanism that might be involved in the ameliorating effects of Nano-silicon (NSi) and Silicon (Si) on faba bean (*Vicia faba*) plants grown under different levels of salinity stress that we found in a previous study.
Study Design: Factorial completely randomized design Pot experiments were used with NSi and Si applied at 4 concentrations each (0, 1, 2 and 3 mM) and NaCl (0, 50, 100 and 200 mM) were studied.
Place and Duration of Study: Experiments were carried out in the greenhouse of the Experimental Station, Faculty of Science, Princess Nora Bint Abdulrahman University, Kingdom of Saudi Arabia during winter season of 2012/2013.
Methodology: Effects of NSi and Si on membrane characteristics, photosynthetic pigments, sugar content, free proline, antioxidant enzymes and mineral elements were investigated in NaCl stressed and non-stressed faba bean plants. The experiment was arranged in a factorial design with 4 replications at NaCl levels of 0, 50, 100 and 200 mM. The tested NSi and Si concentrations were 0, 1, 2 and 3 mM for each.
Results: NaCl treatments caused an increase in proline content and in some enzyme activities, Chl a, b and carotenoids were decreased. Application of NSi caused a significantly increase in the activity of ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) in plant leaves, but

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caused a decrease in the activity of superoxide dismutase (SOD) as compared to unstressed plants. Oxidative damage, produced by salinity stress, seemed to decrease in accordance with the increase in antioxidant enzymes activity under NSi and Si treatments, thus tolerance against salt stress was observed. The improvement of salt tolerance resulted from NSi and Si treatments was accompanied with improved membrane stability, chloroplast formation and sugar accumulation.
Conclusion: Nano-silicon treatments can reduce the adverse effects of salinity on *V. faba* plants by enhancing the activity of antioxidant enzymes.

Keywords: Nanosilicon; Vicia faba; antioxidant enzymes; salt tolerance.

1. INTRODUCTION

Faba bean (*Vicia faba* L.) is cultivated in the many regions of the world as a source of food for humans and animals. It is also traditionally used as a cover crop to recover nitrogen content and prevent erosion of the soil, and is appreciated for its good agronomic characteristics [1]. The high percentages of protein, carbohydrate, minerals and vitamins in faba bean seeds make it an important source of nutrition in many countries. Being rich in calcium, iron, thiamin, niacin tocopherols, and folic acid faba bean is recommended to strengthen the immune system [2]. In addition, faba bean plants increase the concentration of nitrogenous compounds in the soil and make it more fertile [3]. However, growth and metabolism of faba bean plants are affected by many abiotic stresses such as salinity and water deficit.

Salinity is considered one of the main abiotic stresses which reduce growth and production of many crops [4]. There is a large area of the world adversely affected by high salt concentrations that have a negative impact on the yield of cultivated crops [5]. Therefore, most plants growing in saline conditions have various strategies to allow them to overcome salinity stress. The deleterious effect of salinity on plant growth is attributed to osmotic effect that makes it difficult for a plant to absorb water from saline soils, and/or to ionic effect resulting from accumulation of toxic salt ions to a level at which plants cannot grow well [6]. Both of these components, osmotic and ionic effects, are harmful to growing plants because they can cause changes in cell membrane characteristics, water status, enzyme activities, protein synthesis and gene expression [7]. In addition, reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals, can be produced in saline conditions to a high levels and accumulate in the chloroplasts and other organelles, leading to a disruption in the metabolism within plant cells through lipid peroxidation, protein oxidation and enzyme inhibition [8].

In order to overcome the harmful effect of ROS, produced at salt stress conditions, an efficient antioxidant system, including antioxidant enzymes, should be developed in plant cells. Among these antioxidants are the following enzymes: catalase, peroxidase superoxide dismutase and glutathione reductase [9]. In a previous study by Hernandez et al. [10] they found that salt stress conditions stimulated the stressed plants to activate their antioxidant enzymes in order to endure salt effect. It was found that ROS could harm the photosynthetic apparatus through the effect on chlorophyll structure, plasma membranes and protein formation [11,12]. The accumulation of compatible solutes such as sugars, amino acids, proline and other soluble compounds permits plants to maintain cell turgor pressure necessary for cell expansion during stress conditions; such compounds act usually as osmo-protectants [13]. Proline, in particular, is considered on of the protectants because it has the ablility to scavenge ROS and other free radical compounds, thereby ensuring membrane stabilization and preventing protein denaturation during severe osmotic stress [14]. The protective role of proline in plants under abiotic stress such as salinity or water deficit has been reported in several plant species [15].

The properties of nano-materials and their potential applications are increasing in various fields, especially agricultural biotechnology [16]. However, the investigations on behavior of nanometals such as nanosilica in plants and the mechanism of interaction, its influence, and agricultural application are still under rudimentary stage [17]. Based on current knowledge, Liang et al. [6] concluded that Si is not inert, but acts as a physical or mechanical barrier in plants. It is not only deposited in the cell wall, but is also actively involved in the metabolic and/or physiological

activities, especially in plants subject to environmental stresses [6]. Generally, the ameliorative effect of bulk silicon sources varies genotypically among plant species and particle size [18]. Si (Atom No =14, Atom mass = 28.09, MP 1,410^{o}C, density 2.33 g cm^{-3}) is a metalloid, exists in two forms either crystal plate or non crystal brown powder. Si is a relatively inactive element at room temperature. At higher temperatures, however, silicon becomes much more reactive and it combines with oxygen, nitrogen, sulfur, phosphorus, and other elements.

Nanomaterials, because of their tiny size, may show unique characteristics. For example, they can change physico-chemical properties compared to bulk materials. They have greater surface area than bulk materials, and due to this larger surface area, their solubility and surface reactivity tend to be higher. Si nano-particles were found to increase the growth of different species i.e., maize (16), rice (17) and *V. faba* (19) plants. Therefore, we focus on the effect of nanosilica and its influence on faba bean physiology and metabolic defense compounds. Our previous study [19] revealed improved growth parameters and germination attributes in faba bean plants treated with Si and NSi, but the physiological and biochemical responses to Si and NSi were not well studied. Therefore, the current study was established to examine the effect of nanosilicon (NSi) and silicon particles (Si), as a promising plant development regulatory substance to increase the salt tolerance of faba bean plants grown under salinity stress, and to clarify the mechanism that might be involved in the ameliorating effects of NSi and Si on faba bean plants grown under salt stress conditions.

2. MATERIALS AND METHODS

Pot experiments were carried out in the greenhouse during winter season to investigate the mechanisms that might be involved in the ameliorating effects of NSi and Si on faba bean plants grown under salinity stress conditions. The effect of NSi and Si on on characteristics of cell membrane, chlorophyll a and b, carotenoids, carbohydrates, antioxidant enzymes, free proline and mineral elements were investigated in NaCl stressed and non-stressed faba bean plants. The experiments were arranged in a factorial design with 4 replications at NaCl levels of 0, 50, 100 and 200 mM. The tested Si and NSi concentrations were 0, 1, 2 and 3 mM.

2.1 Preparing and Sowing of Seeds

Seeds of faba bean (*Vicia faba* L.) were obtained from authorized agriculture company and were sterilized with 10% sodium hypochlorite solution for 10 minutes, washed three times with distilled water, and coated with N-fixing bacteria (*Rhizobium leguminosarum*) using Tween 20 agent as an adhesive and scattering material. Identical seeds were then sown in plastic pots (30 cm inner diameter) filled with 10 kg sandy soil. Physical and chemical properties of the soil used in the study were recorded in Table 1. After sowing, irrigation was applied to supply seedlings with 100% available water, at two-day intervals until the seedlings reached the third leaf stage. Seedlings were then thinned to 3 plants/pot and pots were divided into four main groups for salt-stress treatments, with each group divided into seven subgroups for Si and NSi foliar application. Plants were fertilized with Sangeral complete fertilizer (Sinclair Horticulture LTD, England) in two equal portions; the first during the seedling stage and the second at the start of flowering stage.

At the fourth leaf stage, irrigation solutions containing one of four levels of NaCl (00, 50, 100 or 200 mM) were used and were repeated after two weeks. To avoid osmotic shock due to high concentrations, plants were started with lower salt concentrations, then, salt concentration was increased on a daily basis until each group reached the concentration determined for it. Plants were then irrigated with tap water every three days with the addition of sodium chloride to the irrigation water every two weeks (each pot received 300 ml). Each pot was washed with 500 ml distilled water 2 days before the irrigation with saline solutions to prevent the increase in osmotic potential resulting from the accumulation of salts by the succession of irrigation procedures. Sodium silicate (Na_2SiO_3), as source of Si, and silicon nano-particles (NSi) were purchased from Sigma-Aldrich Company, St. Louis, MO, USA with a purity of 99.5%, through SOMATCO chemical company in Riyadh, Saudi Arabia. Just at the end of each salt treatments, foliar application of Si (35 µm), or NSi (40 nm) in concentrations of 0, 1, 2, 3 mM was performed using a small pressure pump (60 psi) after adding Tween 20 (0.5%) as a wetting agent. The nano particles or Si were suspended directly in distilled water and dispersed by ultrasonic vibration (100W, 40 kHz) for 30 min. Small magnetic bars were placed in the suspension for stirring before use to avoid aggregation of the

particles. Different doses of particles suspensions were prepared as (mM). Each plant received 100 mL of deionized water as well as the solutions of Si and NSi. The experiment consisted of 28 treatments (4 salt treatments with 3 Si treatments, 3 NSi treatments and water as control) and arranged in a factorial completely randomized design with 4 replicates for each treatment to make a sum of 112 pots. Si treatments were represented by Si1, Si2 and Si3; while NSi treatments were represented by NSi1, NSi2 and NSi3.

2.2 Measurements

Three weeks after the second application of Si or NSi (about 100 days after germination), three replicates were taken from each treatment, and the following parameters were measured:

2.2.1 Determination of membrane characteristics

Lipid peroxidation (LP), electrolyte leakage (EL), and membrane stability index (MSI) were determined as followes:

2.2.1.1 Lipid peroxidation

Lipid peroxidation was determined by measuring the amount of MDA according to Unyayar et al. [20]. About 0.5 g of leaf tissues from control and treated groups were cut into small pieces and homogenized by the addition of 5 ml of 5% trichloroacetic acid (TCA) solution (using UP200 Ultra homogenizer; Hielscher Ult. Tech., Germany). The homogenates were then transferred into fresh tubes and centrifuged at 12,000 rpm for 15 min at room temperature. Equal volumes of supernatant and 0.5% thiobarbituric acid (TBA) in 20% TCA solution were added into a new tube and boiled at 96^{o}C for 25 min. The tubes were transferred into ice-bath and then centrifuged at 10,000 rpm for 5 min. The absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm, 0.5% TBA in 20% TCA solution was used as the blank. MDA contents were calculated using the extinction coefficient of 155 M^{-1} cm^{-1}. Values of MDA contents were taken from measurements of three independent samples, and SD of the means were calculated.

2.2.1.2 Electrolyte leakage

Ion leakage was determined as electrical conductivity (EC%) according to Hassanein et al. [2]. Leaf samples were cut into discs of uniform size and placed in 10 ml of double-distilled water at 40°C for 30 min, and its conductivity recorded (C1) using conductivity meter (Jenway 470 portable conductivity meter). Then it was kept in a boiling water bath (100°C) for 15 min and its conductivity also recorded (C2). The percentage of electrolyte leakage was calculated according to this formula: EC (%) = (C1/C2) × 100. Where C1 and C2 are the electrolyte conductivities measured before and after boiling, respectively.

2.2.1.3 Membrane stability index

The membrane stability index (MSI) was determined by placing certain weight (200 mg) of leaf fresh material in 10 ml deionized water in test tubes. Two sets of the test tubes are prepared. One set was heated at 40°C for 30 min in a water bath and, then, the electrical conductivity (C1) was measured using a conductivity meter (ME977-C, Max Electronics, India). The second set of the tubes was boiled at 100°C in a boiling water bath for 10 min and then, the conductivity (C2) was measured again. The MSI value was calculated using the following equation as described by Premchandra et al. [21]:

$$MSI = [1 – (C1/C2)] \times 100$$

2.2.2 Determination of photosynthetic pigments

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined spectrophotometrically according to Metzner et al. [22]. A known fresh weight (500 mg) of leaves was homogenized in 85% aqueous acetone for 5 min. The homogenate was centrifuged and the supernatant was made up to known volume with 85% acetone and measured against a blank of pure 85% aqueous acetone at 3 wavelengths of 452.5, 644 and 663 nm using spectrophotometrically (Shimadzu, RF-5301PC, Japan). Taking into consideration the dilution made, it was possible to determine the concentrations of the pigment fractions (chlorophyll a, chlorophyll b and carotenoids) as *g* /ml using the following equations:

Chlorophyll a = 10.3 E 663 - 0.918 E 644
Chlorophyll b = 19.7 E 644 - 3.87 E 663
Carotenoids = 4.2 E 452.5 - (0.0264 chl a + 0.426 chl b).

Pigments then were calculated on the bases of mg/g fwt.

2.2.3 Determination of soluble sugars

One gram of the dried leaf materials was homogenized with 80% ethanol then placed in a boiling water bath for 15 minutes. After cooling, the extract was filtered and the filtrate was oven dried at 60ºC then dissolved in 50 ml of distilled water to be ready for determination of soluble sugars. Soluble sugars were then determined by the anthrone sulfuric acid method described by Scott and Melvin [23]. Briefly, One ml of the extract was mixed with 9 ml of anthrone sulphuric acid reagent in a test tube and heated for 7 min at 100°C. The absorbance was read spectrophotometrically (Shimadzu, RF-5301PC, Japan) at 620 nm, against a blank containing only distilled water and anthrone reagent. All data were calculated as mg 100 g^{-1} Dwt of leaves.

2.2.4 Determination of free proline

Free proline content was determined colorimetrically in aqueous sulfosalicylic acid as described by Bates et al. [24]. Briefly, lyophilized plant material (0.1 g) was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered through Whatman #2 filter paper. Two ml filtrate was reacted with 2 ml acid-ninhydrin ($C_9H_6O_4$) and 2 ml of glacial acetic acid ($C_2H_4O_2$) in a test tube for 1 h at 100°C, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15–20 s. After 1 h, toluene was added and absorbance at 520 nm was measured by using spectrophotometer (Shimadzu, RF-5301PC, Japan). The standard curve for proline was prepared by dissolving proline in 3% sulfosalicylic acid to cover the concentration range 0.5–10 µg ml^{-1}. The proline concentration of the extract was determined from the standard curve and calculated on a dry weight basis.

2.2.5 Determination of antioxidant enzyme activities

2.2.5.1 Enzyme extraction

The samples were prepared as described by Mukherjee and Choudhuri [25]. Fresh leaf samples were submersed for 5 min in liquid nitrogen. The frozen leaves were kept at -80°C for further analyses. Enzymes were extracted by grinding weight of 500 mg fresh leaf materials, using a mortar and pestle, in 5 ml of extraction buffer solution containing 50 mM potassium phosphate buffer (pH 7.6) and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15,000 *g* for 15 min and the supernatant fraction was used to assay for the various enzymes. All steps of enzyme extraction and preparation were performed at 4°C.

2.2.5.2 Superoxide dismutase (SOD)

SOD was assayed using tetrazolium reagent according to Karanlık [26]. The method depends on measuring the absorbance of the nitro-blue tertazolium (NBT) color at 560 nm wavelength. One unit of SOD activity was defined as the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT.

2.2.5.3 Catalase (CAT)

Catalase activity was determined spectrophotometry by monitoring the disappearance of H_2O_2 according to the method of Cakmak and Marschner [27].

2.2.5.4 Ascorbate peroxidase (APX)

The activity of ascorbic acid peroxidase was determined spectrophotometry by measuring the absorbance at 290 nm and estimating the

Table 1. Physical and chemical analyses of the soil used in the experiment

Physical properties		Chemical properties			
Particle size distribution:		**CaCO3 (%)**	**0.41**	**Soluble anions (meq/l)**	
Sand (%)	92.3	OM (%)	0.26	CO32-	0.22
Silt (%)	6.2	ES (dSm^{-1})	0.53	HCO3-	0.86
Clay (%)	1.5	Soluble cations (meq/l)		Cl-	1.83
Soil texture	(Sandy)	Ca2+	2.96	Avail. elements (mg/kg)	
		Mg2+	1.68	N	19.2
		Na+	2.04	P	8.3
pH	8.01	K+	0.21	Fe	2.4

Soil suspension (1 soil: 2.5 water)

consumption of ascorbate substrate. One unit of APX activity was defined as the amount of enzyme required to consume 1 mol ascorbate min^{-1} [27].

2.2.5.5 Glutathione reductase (GR)

GR activity was determined spectrophotometry by measuring the absorbance at wavelength 340 nm. This method depends on the oxidation of NADPH by the enzyme. One unit of GR activity was defined as the amount of enzyme that oxidized 1 mol NADPH min^{-1} [27].

2.2.6 Determination of nutrient elements

Nitrogen (N), phosphorus (P), sodium (Na), potassium (K), and calcium (Ca) analysis, dried shoot samples were ground to pass a 20-mesh sieve and digested with a mixture of H_2SO_4-$HClO_4$ using microwave energy, a modified technique of Lachica et al. [28].

In a mixture of sulfuric and perchloric acid total nitrogen was determined by the micro-Kjeldahl method [29]. For total inorganic phosphorus estimation, 0.5 g of plant material was extracted in 8 ml trichloroacetic acid (6%) and centrifuged for 15 min at 18000 X g. phosphate in supernatant was determined colorimetrically after adding 5 mol sulphuric acid, 2.5% ammonium molybdate and 0.25% 1,2,4-aminonaphtholsulphonic acid solution. After 15 min incubation at 37°C the absorbance was measured at 660 nm [30]. For Measurements of Na+ and K+ concentrations, the leaves were dried in 60°C for 48 h. Then 1 gr of leaves was powdered and burned in 560°C to obtain ash then ashes digested in 10 ml of 1N HCL. The concentration of Na+ and K+ in the digested samples was determined using a flame photometer (Model 420, Sherwood, Cambridge, UK) as described by Yousufinia et al. [31]. Calcium was measured on acid-digested samples by atomic absorption spectrophotometry in a Perkin Elmer Analyst 800 (Perkin Elmer Inc., Wellesley, MA) spectrophotometer equipped with a PE6017 lamp, and measured at 422.7 nm [32].

2.2.7 Statistical analysis

The collected data were analyzed statistically using Factorial Completely Randomized Design (FCRD) and analysis of variance according to Gomez and Gomez [33] with the aid of COSTAT computer program. Treatment means were compared using the least significant difference test (LSD) at 5% level.

3. RESULTS AND DISCUSSION

3.1 Lipid Peroxidation and Electrolyte Leakage

Data registered in (Table 2) indicated clearly that electrolyte leakage (EL), lipid peroxidation (LP) and membrane stability index (MSI) of salt stressed *Vicia faba* leaves showed different patterns of response when treated with NSi and Si. It was clear that the different concentrations of NaCl caused a significant increase in EL and LP, as determined by the amount of Malon dialdehyde (MDA) produced, comparing with the control plants. The results illustrated remarkable increase in Malon dialdehyde content and ion leakage level in response to 100 and 200 mM NaCl. Maximum values of electrolyte leakage and lipid peroxidation were registered at the 200 mM NaCl treatment. However, the application of NSi and Si caused a significant decrease in the EL and LP of the stressed plants as compared with those of the control. In contrast, the MSI of plant leaves was decreased when plants were exposed to NaCl. NSi and Si treatments, on the other side, significantly increased the MSI of salt treated faba bean plants.

Values of electrolyte leakage (EL) and membrane stability index (MSI) can be used to predict indirectly cell membrane damage caused by salt stress [34]. The stimulation effect of saline water on the value of MDA and EL % might be attributed to injury of plasma membrane. That damage caused by ROS which could induce Lipid peroxidation and consequently Electrolyte leakage [35]. Extensive membrane damage and change in membrane integrity, resulting from salinity stress, are shown as increase in lipid peroxidation and electrolyte leakage leading to decrease in MSI values.

Reduction of MDA levels and EL% in response to NSi and Si treatments might be due to induction of antioxidant responses that protect the plants from oxidative damage, increased membrane stability and tolerance of plants which in turn enhanced scavenging of harmful free radicals [36] and elevated ca uptake that protects the plant from the oxidative damage by silicon treatments [37]. On the other side, NSi and Si treatments appeared to have a positive effect on cell membranes and repair the damage mediated by salt stress to plasma membrane. This conclusion was evident from the significant increase in MSI and the significant decrease in EL of NSi and Si treated faba bean plants as

compared with control plants. Similar results were obtained by Hamada [38], who found that brassinolide modifies membrane structure and stability under stress conditions.

In the present study, the decrease in lipid peroxidation, indicated by MDA content, in NSi or Si treated plants may be considered as a mechanism for the improved stability of cell membrane in salinity stressed plants in response to nanosilicon or silicon treatments. Lower lipid peroxidation, lower electrolyte leakage and higher membrane stability have also been reported in salt-tolerant genotypes of rice [39] and sugarcane [40] grown under salt stress conditions.

It is well known that, the deterioration of cell membrane is one of the physiological responses to salt stress. Oxidative stress, generated due to the presence of NaCl stress, promotes lipid peroxidation and causes a simultaneous increase in electron leakage and decrease the MSI. These physiological interferences and membrane disturbances could be mitigated by the addition of silicon and nano-silicon through the activation of antioxidant enzymes that scavenging the reactive oxygen species (ROS), as discussed later.

3.2 Photosynthetic Pigments

The present results showed that chl *a*, chl *b*, carotenoids (Table 3), total chlorophyll content (Fig. 1) and chl *a*: chl *b* ratio (Fig. 2) were all significantly decreased in faba bean leaves as salinity stress increased. The decrease in pigment concentrations of salt treated plants was more observed in the absence of NSi and Si treatments. Data in the table indicate clearly that the 50 mM salt treatment caused a slight increase in chl a, and chl b and carotenoid content as compared with salt untreated control (Table 3). This observation was true either with or without Si and NSi treatments. At this salinity level, the most observed increase in total chlorophyll (Chl a + b) content was recorded under NSi2 treatment (Fig. 1). In this regard, Chookhampaeng [41] found that chlorophyll content increased in pepper plants under low levels of salinity. One reason of that was the thicker leaves produced under salt stress. Increases in leaf thickness tended to compensate slightly for the negative effects of salinity on leaf chlorophyll [41].

Increasing salinity levels from 50 to 100 and 200 mM significantly decreased chlorophyll a, chlorophyll b and carotene contents. At 200 mM of NaCl, Chla, Chl b and carotene decreased by about 50%, 50% and 44%, respectively, as compared with control treatment. The application of Si and NSi as foliar spray alleviate the deleterious effect of salinity on the chlorophyll content. Application of 2 mM of Si or NSi produced the highest values of chlorophyll a, b and carotene as compared to the other treatments or control (Table 3). At no salt treatments, Si2 and NSi2 treatments caused an increase in Chl a by about 34% and 50%, respectively, Chl b by about 20% and 30%, respectively and carotene by about 20% and 50%, respectively, as compared with silicon untreated plants. It seems that, at any salt treatment Si and NSi treatments decrease the harmful effect of salinity stress on chlorophyll content. The reduction in leaf chlorophyll content under NaCl stress has been reported in early studies on *V. faba* [12], Zea maize [42] and green bean [43]. In this regard, Yuvakkumar et al. [42] found that total chlorophyll content of maize was increased by (13–17%) when treated with nanosilicate.

The decrease in chlorophyll content under salinity conditions is reported by Yasar et al. [43] and might have been due to salt-induced increase in the activity of the chlorophyll degrading enzyme, chlorophylase [44]. In this concern, a decrease in cholorophyll content (chl. a, b and total chl) of fennel plants under salt stress was observed [5]. The results obtained in this study are in agreement with those of Azooz et al. [12] for *V. faba*. The decrease in chlorophyll content of salt-stressed faba bean plants that associated with the decrease in enzyme activities (Table 4) and the increase in proline content (Table 5) is reliable with the suggestion that nitrogen might be redirected to the synthesis of proline instead of chlorophyll. In addition, Djanaguiraman and Ramadass [45] ascribed the decrease in pigment content of salt-stressed rice plants to the increase in the activity of chlorophyllase or to the disruption of the fine structure of the chloroplast, as well as to the instability of the chloroplast membrane and pigment-protein complex. In this regard, Sevengor et al. [46] attributed the reduction in leaf chlorophyll content under NaCl stress to the destruction of chlorophyll pigments and to the instability of the pigment-protein complex.

Table 2. Effects of nano-silicon (NSi) and silicon (Si) on electrode leakage (EL), lipid peroxidation (LP) and membrane stability index (MSI) of faba bean plants grown under different levels of salinity stress

Silica treatments	Salinity levels (mM)											
	00	50	100	200	00	50	100	200	00	50	100	200
	Electrolete leakage (%) (EL %)				Lipid peroxidation (MDA µg/g fwt)				Memberane stability index (MSI %)			
Cont	13.5	15.4	19.8	20.5	6.41	7.92	9.52	12.2	86.5	84.6	80.2	79.5
NSi1	11.6	14.2	17.3	18.6	5.23	6.23	8.53	10.2	88.4	85.8	82.7	81.4
NSi2	10.4	11.3	15.2	17.8	4.82	5.42	7.31	7.63	89.6	88.7	84.8	82.2
NSi3	13.2	14.7	14.6	18.7	5.14	5.55	7.13	8.24	86.8	85.3	85.4	81.3
Si1	11.2	13.8	16.6	18.2	5.12	6.06	8.26	9.85	88.8	86.2	83.4	81.8
Si2	10.4	11.5	14.4	17.3	4.24	5.21	7.08	7.43	89.6	88.5	85.6	82.7
Si3	10.0	11.1	13.7	16.8	4.32	5.04	6.55	7.22	90.0	88.9	86.3	83.2
LSD (5%)	1.41	2.33	2.16	1.45	0.55	0.62	1.24	1.45	2.56	2.14	2.22	1.57

Table 3. Effects of nano-silicon (NSi) and silicon (Si) on chlorophyll a (Chl a), chlorophyll a (Chl a) and carotenoids (Carot.) of faba bean plants grown under different levels of salinity stress

Silica treatments	Salinity levels (mM)											
	00	50	100	200	00	50	100	200	00	50	100	200
	Chl a (mg/g fwt)				Chl b (mg/g fwt)				Carot. (mg/g fwt)			
Cont	1.41	1.46	1.02	0.66	1.05	1.08	0.87	0.52	0.62	0.77	0.72	0.35
NSi1	1.53	1.66	1.28	1.06	1.28	1.31	1.18	1.05	0.78	0.81	0.66	0.45
NSi2	2.12	2.18	1.87	1.25	1.78	1.79	1.36	1.16	0.94	0.98	0.78	0.48
NSi3	1.66	1.72	1.46	1.01	1.25	1.28	1.05	0.88	0.55	0.58	0.52	0.38
Si1	1.45	1.48	1.15	1.04	1.12	1.14	1.02	0.77	0.71	0.79	0.73	0.41
Si2	1.89	1.92	1.53	1.11	1.25	1.28	1.07	0.82	0.75	0.82	0.75	0.45
Si3	1.52	1.61	1.36	1.09	1.16	1.12	0.97	0.78	0.65	0.72	0.68	0.37
LSD (5%)	0.22	0.18	0.17	0.11	0.12	0.14	0.16	0.14	0.11	0.15	0.10	0.11

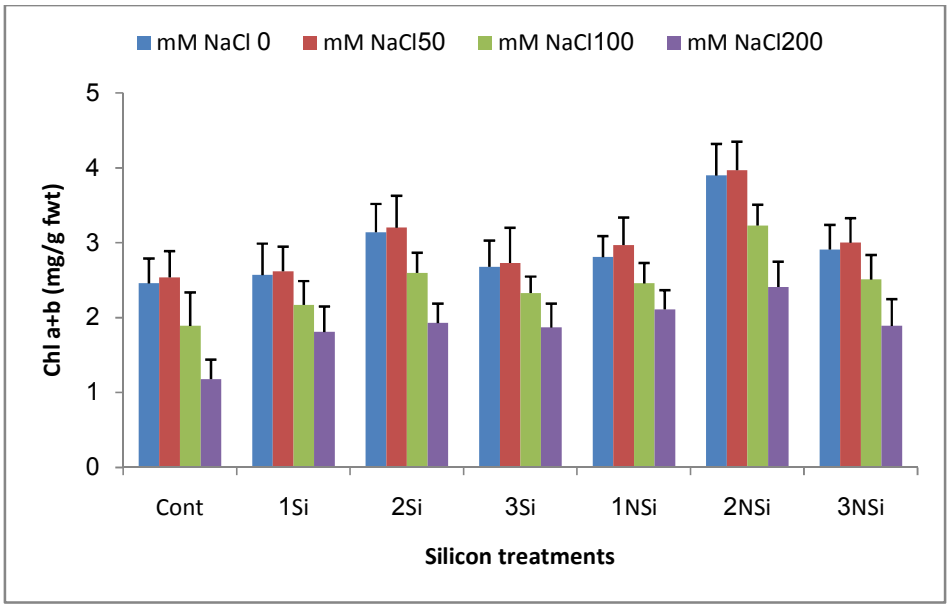

Fig. 1. Effects of nano-silicon (NSi) and silicon (Si) on total chlorophyll (Chl a +b) content of faba bean plants grown under different levels of salinity stress

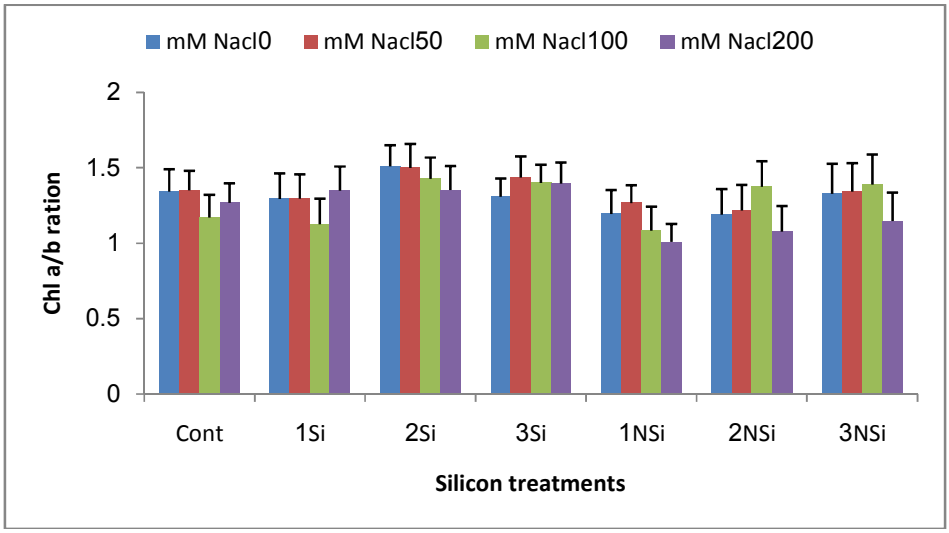

Fig. 2. Effects of nano-silicon (NSi) and silicon (Si) on Chl a/Chl b ration in faba bean plants grown under different levels of salinity stress

In the present study NSi or Si reduced the damage effects of salt stress on photosynthetic pigments through decreasing the electrolyte leakage and increasing the membrane stability index MSI (Table 2) compared with those of the control. Moreover, the chlorophyll content may be protected probably because of the high antioxidant enzyme activities that increased with NSi and Si and prevented degradation of leaf chlorophyll [46;47]. The results showed that nano Si could stabilize the integrality of chloroplast membrane and protect the chloroplasts from salt stress. Therefore with nano Si treatment, content of chlorophyll (a + b), was higher than the control (Fig. 1). In fact, NSi can improve structure of chlorophyll and can facilitate manufacture of pigments and protect chloroplasts from ageing [48]. However, bulk Si effects on pigments was not as significant as nano Si, as the grain size of nano Si is much smaller than that of bulk Si, which entered Faba bean cells more easily.

It was found that ROS produced by salt stress affect cellular membranes and cause a destruction of chroroplasts [12], while scavenging capacity during salt stress was increased with NSi and Si, exhibited as increased of antioxidant enzymes activity. Ameliorative effects on salinity stress may be attributed to silicon supplements included reductions in tissue Na content, maintenance of chloroplast cell membrane integrity, reductions in lipid peroxidation and lignification, and increases in ROS scavenging capacity. These findings are in accordance with those reported by Lei et al. [49] and Gao et al. [50] in a study on the effects of different concentrations of nano particles on the spinach traits, concluded that, chlorophyll amounts in treatment with nano particles showed significant increase, and it was 17 times of control amount. In another study, Yang and Hong [51] found that nano TiO2 increased protein and chlorophyll content of leaves more than bulk particles.

3.3 Soluble Sugar Contents

Effects of salt treatments and NSi or Si on soluble sugar contents of *V. faba* plants are shown in (Fig. 3). It was clear that total soluble sugars decreased with increasing NaCl concentration, to produce the lowest value of soluble sugars at the highest level of salt concentration, as compared with those of salt unstressed plants. In this regard the 200 mM NaCl resulted in a decrease of about 36% in the soluble sugar content as compared to unstressed plants. NSi and Si applications caused significant increases in the content of soluble sugars in faba bean plants. The maximum content of soluble sugars was estimated at moderate level of NaCl (100 mM) particularly with NSi2 and Si2 treatments compared with that determined in control plants. In this concern, NSi2 and Si2 treatments resulted in an increase of about 86% and 89%, respectively, in plants treated with 100 mM of NaCl.

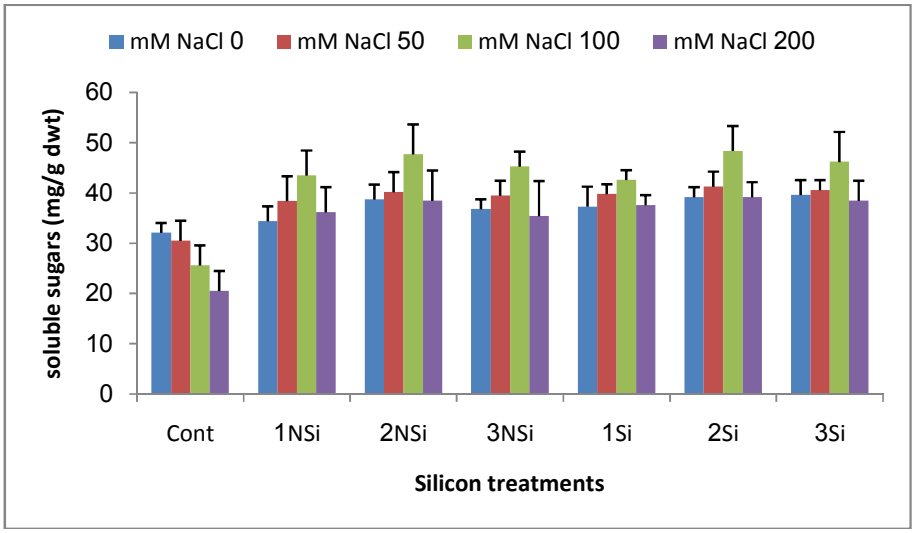

Fig. 3. Effects of nano-silicon (NSi) and silicon (Si) on total soluble sugars in faba bean plants grown under different levels of salinity stress

It was clear that soluble sugar content of plant leaves decreased significantly in salt-stressed *V. faba* plants compared to the unstressed plants. Similar results showing inhibitions in sugar formation under salinity stress were recorded in earlier studies [52,53]. The decrease in soluble sugar content and the reduction in photosynthetic pigment concentration under salt stress conditions were directly proportional to the applied concentration of NaCl in the growth media. These findings may lead to the conclusion that NaCl inhibits photosynthetic activity or increase partial utilization of carbohydrates in other metabolic pathways. Generally, NSi and Si enhanced the accumulation of sugars in salt-treated *V. faba* plants and alleviate the inhibitory effects of salt stress on sugar formation [47].

It is well known that organic solutes play a major role in the mitigation of salt stress. Accumulation of these compatible solutes reduces osmotic potential in the cytoplasm and contributes to maintaining water homeostasis among several cellular compartments. Among all organic solutes, soluble sugars and proline represent the most osmotically active organic solutes. A strong correlation between soluble sugar accumulation and salt tolerance has been widely reported [12,47]. In this regard, the enhancement effect of silicon on carbohydrate biosynthesis, especially soluble sugars, was found to be the main organic osmotica in a number of glycophytes when exposed to salt stress conditions [2]. This effect highlights another possible mechanism by which silicon plays a positive role in alleviation of the harmful effects of salt stress. Subjecting water stressed faba bean plants to silicon synergistically increased the amounts of soluble sugars than in untreated stressed ones which indicated that accumulation of these compounds by silicon plays a key role in retaining the water capacity of stressed cells which thereby can tolerate severe drought and salinity stress [36,54,55].

3.4 Antioxidant Enzyme Activities

The effect of NSi and Si treatments on the activity of antioxidant enzymes in faba bean plants, grown at different concentrations of NaCl, is presented in Tables 4 and 5. The recorded date showed a progressive increase in the activity of enzymes SOD, CAT, POD and APX with increasing salinity level in the growth media, whereas the activity of GR significantly decreased with increasing NaCl concentration as compared with those recorded for control plants. Adding Si or NSi had significant effects on the activity of all enzymes under salt stress Table 4 and 5. With increasing Si treatments a gradual increase in the activity of SOD, CAT, POD and APX was observed regardless of salinity treatment. Whereas, with NSi the highest increase in enzyme activity was observed under NSi2 treatment above which activities of the enzymes tended to decrease. The addition of Si or NSi markedly decreased the GR activity as compared with control treatment.

Table 4. Effects of nano-silicon (NSi) and silicon (Si) on SOD, CAT and POD antioxidant enzyme activities of faba bean plants grown under different levels of salinity stress

Silica treatments	Salinity levels (mM)											
	00	50	100	200	00	50	100	200	00	50	100	200
	SOD (unit g^{-1} FW)				CAT (µM H2O2 oxidized g^{-1} FW)				POD (O.D g^{-1} FW min^{-1})			
Cont	29.1	35.1	64.1	52.5	4.41	4.02	3.56	3.24	2.25	4.33	4.46	3.16
NSi1	34.3	36.6	66.5	60.6	5.12	6.82	7.11	5.64	2.88	4.75	5.75	3.65
NSi2	40.2	42.5	71.2	62.5	5.76	7.45	8.45	6.55	3.05	5.12	5.96	4.32
NSi3	28.6	30.7	60.4	48.5	4.14	5.16	7.06	5.14	2.62	4.23	4.67	3.12
Si1	30.4	36.5	67.3	53.3	4.56	6.12	7.68	5.12	2.45	4.65	5.11	3.57
Si2	34.7	38.2	69.2	58.2	4.64	6.86	7.94	5.34	2.87	4.87	5.32	3.84
Si3	36.3	40.5	69.8	55.2	4.86	7.11	8.12	6.45	3.11	4.46	5.65	4.11
LSD (5%)	2.88	2.12	2.45	3.62	NS	1.05	2.12	1.44	0.66	0.45	0.32	0.44

Table 5. Effects of nano-silicon (NSi) and silicon (Si) on APX andGR antioxidant enzyme activities and proline content of faba bean plants grown under different levels of salinity stress

Silica treatments	Salinity levels (mM)											
	00	50	100	200	00	50	100	200	00	50	100	200
	APX (mM ascorbate g^{-1} FW min^{-1})				GR (µg g^{-1} FW)				Proline (mg per 100 g DW)			
Cont	0.22	0.30	0.48	0.37	0.52	0.44	0.35	0.27	3.22	5.34	7.75	6.14
NSi1	0.37	0.41	0.49	0.38	0.47	0.39	0.33	0.25	3.11	4.01	5.66	6.01
NSi2	0.45	0.48	0.62	0.48	0.43	0.35	0.29	0.26	2.82	4.12	5.98	6.06
NSi3	0.26	0.35	0.50	0.40	0.52	0.45	0.33	0.30	3.27	4.50	6.14	6.15
Si1	0.28	0.34	0.52	0.39	0.50	0.41	0.30	0.31	3.20	3.35	5.11	6.34
Si2	0.32	0.37	0.56	0.42	0.45	0.40	0.26	0.28	2.24	3.88	5.29	6.84
Si3	0.36	0.43	0.58	0.46	0.40	0.38	0.24	0.24	2.11	3.96	5.88	6.98
LSD (5%)	0.12	0.24	0.16	0.04	0.05	0.03	0.05	0.03	0.03	0.46	0.34	0.02

It is well known that salinity stress causes generation of excessive reactive oxygen species (ROS), which leads to cell toxicity, membrane dysfunction and cell death [41]. Plants have developed enzymatic and nonenzymatic mechanism to scavenge ROS [2]. Among the active oxygen species superoxide is converted by SOD enzyme to H2O2, which is further scavenged by CAT and APX. Over expression of the APX gene in plants has showed improvement in protection against oxidative stress [43]. In the present study we found that the activities of antioxidant enzymes, SOD, POD and APX, in *V. Faba* plant leaves were enhanced when plants subjected to salinity stress condition. These results are in agreement with those of Hassanein *et al.* [53], who observed that salt stress increased the activities of antioxidant enzymes in leaves of *Zea mays* plants. In addition, Farag [56] reported that in pea (*Pisum sativum*), high concentrations of NaCl (110–130 mM) enhanced the activities of cytosolic and chloroplastic SOD. Increased activity of these antioxidant enzymes is considered to be a salt-tolerance mechanism in most plants [57].

The present results showed that salt stress caused a decrease in GR activity. GR deactivation by salt stress may be a result of prevention of new enzyme synthesis [6]. GR activates the glutathione-ascorbate cycle and converts GSSG to reduced glutathione (GSH) [58]. In addition, GR regulates GSH/GSSG ratio and supplies GSH for GPX and DHAR, which convert H_2O_2 to H_2O and reduce oxidized ascorbate, respectively. The changes in GR activity (Table 5) and lipid peroxidation (LP), as pointed out by the increase in MDA concentration (Table 2), in plants exposed to different levels of salinity stress and treated with NSi and Si were recorded. The GR activity gradually decreased with increasing NaCl concentration, whereas lipid peroxidation showed a gradual increase with increasing salt concentration as compared with control plants. In this regard, the maximum reduction in GR activity that reached 43% of that of the controls plants, and the maximum increase in MDA (LP) content which reached 77.7% of that of the control plants, was detected in faba bean plants treated with 200 mM NaCl (Table 2).

It seems that nanosilicon could alter the activity of antioxidative enzymes in plant organs to improve the salt tolerance. Results in Tables 4 and 5 showed that application of Si or NSi caused an increase in the activity of SOD, POD,

APX and CAT in *V. Faba* plants and this could ameliorate the effect of salinity. High activity of CAT in Si and NSi treated plants under salt stress suggests that the treated plants possess a better scavenging ability. The present study was consistent with the results reported by Mazorra et al. [58]. The effect of Si on the antioxidant enzymes activity under salt stress has been reported by Liang et al. [59] who described an increase in SOD activity in salt-stressed barley leaves and increases in SOD, GPX, CAT and GR activity in salt-stressed barley roots. In the present study, applying Si to Faba bean under NaCl stress significantly increased SOD, POD, APX and CAT activity. In another study, it was found that applying Si and nSi to plants under salt stress could increase antioxidative enzymes activity which played great role to counterbalance salinity damages [47] and [60]. In this regard Helaly et al. [61] reported that SOD, CAT and POX activities were increased significantly in banana plants when the nanoparticles doses increased. Moreover, Bao-shan et al. [62] tested nanoparticle silicon dioxide (NSi) on growth of Changbai larch (*Larix olgensis*) and soybean plants and observed that NSi showed the highest activity of nitrate reductase, superoxide dismutase, catalase and peroxidase. In contrast to our results, Rubinowska et al. [36] reported that at interaction of both salinity and NSi the rate of GR reduction was high. It was indicated that NSi might have toxic effects or it can stimulate salinity effects. Although there was a report on toxicity of $NSiO_2$ on *Arabidopsis thaliana*, but these toxicity was not stronger as other nanoparticles such as N-ZnO and N-Fe_3O_4 [63].

3.5 Proline Content

Analysis of the proline content of *V. Faba* showed that NaCl application significantly increased free proline contents while under Si treatment, the free proline contents of plant leaves markedly decreased but NSi treatment enhanced the accumulation of proline (Fig. 4). Proline content increased with increasing salinity level up to 100 mM of NaCl then, tended to decrease at 200 mM of NaCl (Fig. 4). At 100 mM of NaCl proline content increased in silicon untreated plants by about 140% as compared to 0 mM NaCl control treatment (Table 5). Si and NSi treatment decreased proline accumulation particularly when plants were not under salt stress. While at salt stress Si and NSi seemed to enhance proline accumulation.

Our results showed that the accumulation of proline in *V. faba* plants was linked with the increase in salinity stress level. These findings were in agreement with those obtained by Rahimi et al. [5] who reported that proline accumulation, in response to environmental stress, such as salinity stress and water stress, protected plant cells via osmotic adjustment effect by balancing the osmotic pressure of the cytosol with that of the vacuole and the external environment [5]. In this regard, proline accumulation could be a protective response, because of the osmoregulation role of proline that prevents water deficit stress under high salinity, and also because of its capability to scavenge the active radicals, produced at salt stress conditions, and stabilize the protein [15]. In addition, proline accumulation was reported to serve as a nitrogen storage compound and thus protects the cellular structure [5].

The amount of free proline in sole Si treated plants were however similar to that of control treatments. The free proline contents significantly increased under NaCl stress, while Si caused a reduction in free proline contents. Various earlier studies reported that proline contents significantly increased in many species such as common bean [64] and soybean [65] under salt stress condition. Current results also suggest that proline contents decreased with Si application, which show the favorable role of Si in mitigating the adverse effects of salt stress on plants. It was thus concluded that addition of Si and nSi is beneficial in hydroponically grown plants as it significantly improves growth attributes and effectively mitigate the adverse effects of NaCl induced salt stress. However, further studies are needed for understanding the mechanism of physiological or biochemical roles of Si in higher plants. Crusciol et al. [54] found that silicon increased proline (a key solute in osmotic adjustment) content in stressed plant tissue. In this regartd, Helaly et al. [61] found that total proline was increased significantly in banana plants when the nanoparticles doses increased.

3.6 Nutrient Elements Content

Data in Table 6 indicated clearly that the low values of N, P and Ca content of plant shoots were recorded by plants growing under salinity when compared with control or plants grown under low saline soil. A gradual reduction in N and Ca concentrations were recorded with increasing salinity level. The reduction in element concentration was more pronounced at the highest salinity level. In this regard, the 200 ppm

of NaCl reduced the N% and Ca% by about 25% and 19%, respectively, as compared with control plants. On the other side, P% and K% in plant shoots increased with increasing salinity level up to 100 ppm then decreased at 200 ppm of NaCl, while Na% increased progressively with increasing salt level in the growth medium to reach its maximum at 200 ppm of NaCl. Therefore, K/Na ration decreased at high salt concentration as compared to non salinized plants. The negative effect of salinity stress on nutrient elements of bean plants was reported by Matijević et al. [66].

It is obvious from the data in Table 6 that all Si treatments caused an observed reduction in N% and Na% in plant shoots. While P, Ca, K, and Na concentrations increased with Si application. On the other side, low and medium levels of NSi (NSi1 and NSi2) caused a significant increase in N, P and K contents. The increase in K/Na ratio with the application of NSi may be attributed to the enhancing effect of nanosilica on the uptake of K or to the reduction in the absorbtion of Na. These results were agreed with those reported by Hanafy Ahmed et al. [67] on wheat. Phosphorus (P) tended to accumulate in the faba bean plants grown under low and moderate saline conditions but was reduced at strongly saline compared to control values. It might be due to an adaptation mechanism developed by the plants to overcome osmotic stress caused by salinity while further decrease in P might be related to the antagonistic relation between salt ions and P [68]. Potassium content was lower in plant shoots grown under high salinity levels than those grown in non saline conditions, while K accumulated at low levels of salinity.

As for the interaction effect of salinity stress and silicon treatments on nutrient composition of faba bean shoots, it is clear from the data recorded in Table 7 that Si treatments did not cause significant changes on N% of shoots under salt treatments, while NSi treatments showed an observed increase in N% of shoots under salinity stress particularly at 50 ppm of NaCl. Moreover, P and Ca concentrations in shoots of salt treated faba bean plants were also enhanced by the application of Si and NSi treatments. The most effective treatments in this regard were Si2 and NSi2, which produced the highest percent increase in P and Ca of salt stressed shoots (Table 7).

In addition, K concentration in salt stressed shoots was increased significantly by the application of Si and NSi as compared with salt stressed shoots without Si or NSi treatments (Table 8). Again, the most pronounced effect was recorded at Si2 and NSi2 treatments. At which K% in the 50 ppm NaCl treated shoots was increased by about 10% and 11% at Si2 and NSi2 treatment, respectively, as compared with the values obtained at 50 ppm NaCl in the absence of Si or NSi. The analogous increase in K content at 100 ppm NaCl were about 37% and 47%, respectively. While the corresponding increases at 200 ppm NaCl were about 6% and 28%, respectively. Contrary, Na concentration in salt stressed shoots was significantly decresed by Si and NSi applications. A gradual decrease in Na, of salt stressed shoots, with increasing Si or NSi concentrations was observed. The most reduction in Na was recorded at Si3 and NSi3 treatments, at which the Na% was decreased by about 37% and 32%, respectively at 50 ppm NaCl treatment, and by about 37% and 34%, respectively, at 100 ppm treatment and by about 36% and 48%, respectively, at 200 ppm treatment as compared with salt treated plants without Si or NSi application. In this regard, Liang et al., (2007) [59] reported a significant increase in K uptake and decrease in Na uptake under salt stress when Si was included because of increased activity of plasma membrane H-ATPase. K/Na ratio was significantly lower under salinity stress when Si was not applied. Silicon application enhanced K/Na selectivity ratio in Faba bean shoots thus enhancing pod and shoot yield. Application of Si in the salinity soil increased the K/Na. The exclusion of Na+ ions and a higher K/Na ratio in bean plants grown under saline conditions have been confirmed as important selection criteria for salt tolerance [69].

Application of Si significantly increased the contents of P and K, and the K/Na ratio and decreased Na ion contents of salt-affected plants. Therefore, the results shown in Table 6 agree with experimentations of Faba bean by Abdelhamid et al. [69] which indicate that salt tolerance is associated with an enhanced K/Na discrimination trait. The ability of plant to limit Na transport into the shoot is critically importance for the maintenance of high growth rates and protection of the metabolic processes in elongation cells from the toxic effects of Na. Sodium content was higher in plants grown under saline soil condition; however Si application significantly reduced Na content in shoot of Faba bean plants. Increased K content and reduced Na in shoot may be one of the possible mechanisms of increased salinity tolerance by Si application in Faba bean plants

[70]. In this regard, X-ray analysis revealed silicon deposits in unique silica filled cells of leaf margins located mainly near hydathodes and around trichome bases and along the leaf margins [71]. This leaf silica deposition may function in leaf strengthen, reduction in transpiration, and increasing biotic and abiotic resistance [72].

Table 6. Effects of salt stress and silicon treatments on mineral contents of faba bean plants

	PPM	N%	P%	Ca%		PPM	K%	Na%	K/Na
Salt treatments	00	2.39	0.26	1.37	Salt treatments	00	2.95	2.61	1.14
	50	2.17	0.28	1.27		50	3.23	2.83	1.16
	100	2.03	0.31	1.11		100	3.52	2.92	1.24
	200	1.79	0.22	0.86		200	2.46	2.97	0.83
LSD5%		0.11	0.04	0.16	LSD5%		0.46	0.26	0.28
		N%	P%	Ca%			K%	Na%	K/Na
Silicon treatments	Con	2.16	0.22	1.19	Silicon treatments	Con	2.72	3.67	0.76
	NSi1	2.21	0.29	1.19		NSi1	3.27	2.82	1.26
	NSi2	2.22	0.32	1.17		NSi2	3.38	2.69	1.25
	NSi3	2.05	0.25	1.02		NSi3	2.80	2.44	1.14
	Si1	2.03	0.25	1.21		Si1	3.09	2.85	1.08
	Si2	1.98	0.29	1.23		Si2	3.17	2.76	1.15
	Si3	2.04	0.28	1.08		Si3	2.69	2.43	1.20
LSD5%		0.05	0.05	0.06	LSD5%		0.15	0.23	0.14

Table 7. Effects of silicon (Si) and nano-silicon (NSi) on N, P and Ca percentages in faba bean plants under different levels of salinity stress

	N (%)				P (%)				Ca (%)			
	00	50	100	200	00	50	100	200	00	50	100	200
Con	2.45	2.22	2.11	1.85	0.22	0.23	0.24	0.18	1.52	1.27	1.11	0.85
NSi1	2.43	2.32	2.15	1.94	0.26	0.29	0.33	0.25	1.46	1.28	1.12	0.90
NSi2	2.46	2.38	2.10	1.88	0.30	0.32	0.38	0.28	1.36	1.30	1.17	0.86
NSi3	2.30	2.26	2.11	1.52	0.25	0.28	0.30	0.19	1.20	1.15	1.04	0.65
Si1	2.42	2.00	1.91	1.80	0.24	0.26	0.28	0.20	1.44	1.40	1.12	0.91
Si2	2.32	1.99	1.85	1.79	0.27	0.32	0.34	0.22	1.41	1.36	1.22	0.95
Si3	2.38	2.03	1.96	1.80	0.28	0.30	0.32	0.23	1.26	1.15	1.02	0.87
LSD5%	0.02	0.07	0.06	0.04	0.03	0.05	0.03	0.03	0.06	0.04	0.05	0.04

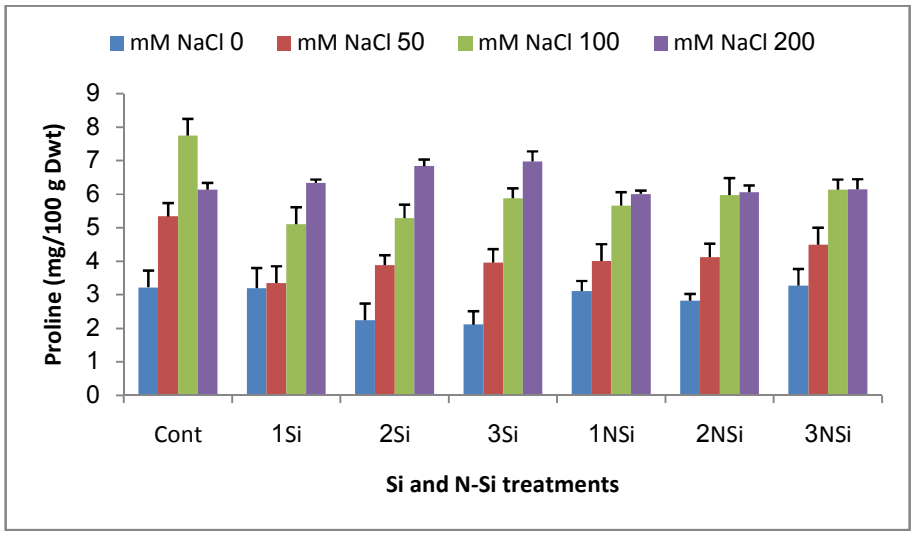

Fig. 4. Effects of nano-silicon (NSi) and silicon (Si) on free proline content of faba bean plants grown under different levels of salinity stress

Table 8. Effects of silicon (Si) and nano-silicon (NSi) on K, Na and K/Na ration in Faba bean plants under different levels of salinity stress

	K (%)				Na (%)				K/Na			
	00	50	100	200	00	50	100	200	00	50	100	200
Con	2.86	3.11	2.66	2.24	2.88	3.74	3.95	4.11	0.99	0.83	0.67	0.55
NSi1	2.88	3.30	3.88	3.02	2.75	2.88	2.89	2.77	1.05	1.14	1.34	1.09
NSi2	3.32	3.45	3.90	2.87	2.60	2.72	2.88	2.58	1.28	1.27	1.36	1.11
NSi3	2.67	3.15	3.26	2.12	2.45	2.55	2.62	2.14	1.09	1.24	1.24	0.99
Si1	2.95	3.21	3.89	2.30	2.76	2.82	2.86	2.95	1.07	1.14	1.36	0.78
Si2	3.21	3.42	3.65	2.38	2.60	2.77	2.80	2.90	1.23	1.23	1.30	0.82
Si3	2.82	3.02	3.44	2.28	2.24	2.37	2.45	2.65	1.26	1.27	1.42	0.86
LSD5%	0.44	0.12	0.25	0.11	0.15	0.25	0.18	0.26	0.16	0.11	0.14	0.23

4. CONCLUSION

The present study revealed that when faba bean plants were subjected to salt stress conditions the plants might not be able to overcome the oxidative damage. Meanwhile, nanosilicon and silicon treatments enhanced the activity of antioxidant enzymes, thus reduced the oxidative damage and decreased the absorption of Na ions in NaCl-salt stress conditions. The increase in the degree of salt tolerance induced by NSi and Si was also reflected by the improvement in the contents of photosynthetic pigments and carbohydrates in the presence of salinity. Therefore, data of this study provide an additional evidence for the stimulatory effects of nanosilicon to induce salt tolerance in faba bean plants. The results also highlight the role of NSi in regulating salinity responses, and indicate that nanosilicon could protect faba bean plants against the hazardous effect of salinity. There were no meaningful differences between the silicon (Si) and nanosilicon (NSi) applications; both forms of silicon were effective at lower levels in most measured characteristics.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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