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ORIGINAL PAPER

Beneficial role of exogenous silicon on yield, antioxidant systems, osmoregulation and oxidative stress in fenugreek (*Trigonella foenum-graecum* L.) under salinity stress

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Abstract

Purpose In the Earth's crust, silicon (Si) is the most abundant element after oxygen, while, under salt stress, its role in the tolerance of aromatic and medicinal plants (AMPs) is not yet detailed. For this reason, in this study we evaluated the effect of exogenous Si on some tolerance-related parameters in salt-stressed fenugreek, as an important AMP.

Methods 3 mM of exogenous Si was applied to assess its impact on plant biomass and on some tolerance-related parameters in fenugreek (*Trigonella foenum-graecum* L.) grown under 150 mM NaCl stress.

Results Results showed that salinity reduced growth parameters, relative water content, photosystem II efficiency, stomatal conductance and K^+ and Ca^{2+} contents, while it increased the Na^+ content, which could explain the obtained reduction in fenugreek growth and yield. However, Si supply reversed the depressive effects of salinity and improved fenugreek growth and yield. Adding exogenous Si also caused a significant reduction in Na^+ content and increased K^+ and Ca^{2+} concentrations. The content of malonyldialdehyd and hydrogen peroxide and the level of electrolyte leakage were significantly increased in salt-stressed fenugreek, while were significantly decreased after Si supplementation. The reduction in oxidative stress markers in Si-treated plants was correlated with a significant increase in both enzymatic and non-enzymatic antioxidant systems and an important accumulation of compatible solutes.

Conclusion Therefore, exogenous Si was directly involved in the central defensive mechanisms to enhance salt tolerance of fenugreek, thus its application could be a promoting strategy to alleviate the damages of salinity on fenugreek growth and yield.

Keywords Fenugreek · Oxidative Stress · Salinity · Silicon · Tolerance · Yield

1 Introduction

In recent years, climate change has given rise to several abiotic stresses. Soil salinization is one of the most of these environmental challenges, because approximately 7% of the world land area, 50% of the irrigated land and 20% of cultivated land are affected by high accumulation of salt ions, causing a considerable decrease in agricultural systems, in terms of production and yield [1]. In fact, salinity stress induces oxidative stress and consequently destructs most of the vital plant processes, including seed germination, photosynthesis, ions uptake, membrane permeability and cell homeostasis [2–4]. Salt stress significantly reduced the content of photosynthetic pigments and the efficiency of both photosystems, thus it greatly affected photosynthetic process activity, as a vital mechanism in

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the plant life cycle [5]. Membrane stability also drastically affected under salt stress due to high accumulation of malondialdehyde (MDA) and reactive oxygen species (ROS), like hydrogen peroxide and superoxide [6, 7]. In this context, Luo et al. [8] found that salinity stress actives transcription of NADPH oxidase genes like *RbohD*, leading to uncontrolled production and accumulation of ROS, and in turn disturbs cell membrane permeability. In plant rhizosphere, accumulation of salt ions like sodium (Na^+), chloride and sulfate, causes an osmotic stress and eventually reduces water and nutrients accessibility to plant root [9]. In plant, excess of Na^+ ions negatively affect nutritional balance by disrupting plant nutrient uptake, explaining by reduction in the content of essential elements, like potassium, in plant tissues during exposure to salt stress [10]. Following their above-mentioned injurious effects, salt stress can destruct and inhibit the growth process of various plants, including aromatic and medicinal species.

Fenugreek (*Trigonella foenum-graecum* L.), as an old medicinal plant, it has long been cultivated as a spice crop in the Mediterranean area, where it has been used by people as one of the ingredients in daily diet [11, 12]. Regarding the medicinal and therapeutical properties, it has been reported that fenugreek seeds are used for two main pharmacological properties; hypocholesterolaemic and antidiabetic activities [13, 14]. More than that, in a recent study, which aimed to evaluate the anticancer potential of methanolic fenugreek seed extract, Alrumaihi et al. [15] documented that fenugreek seed extracts have many substances with significant cytotoxicity effect for cancer cells. On the other hand, fenugreek, as other legume plants, is known for its atmospheric nitrogen fixation ability by its symbiosis with rhizobia. In this context, Singh et al. [16] estimated that fenugreek can fix 48% of its total nitrogen (N) during growing season. Thus, in addition to their medicinal properties, fenugreek can be used as a good soil renovator and a best green manure [17]. However, salt stress and other environment-stressed factors drastically affected growth and yield of fenugreek. Indeed, germination parameters i.e., seedling biomasses, embryo viability and seed reserve mobilization of fenugreek seeds are negatively affected under 200 mM salinity stress [7]. Nasseri et al. [18] found that plant biomasses, chlorophyll content and membrane integrity in fenugreek were significantly reduced with addition of NaCl to the growth medium. Also, Zaghdoudi et al. [19] demonstrated that 150 mM NaCl salt stress decreased the activities of both photosystem I and II, explaining the significant decrease in photosynthetic activity, as well as fenugreek growth. The large above-cited medicinal and agricultural advantages of this medicinal plant encouraged future researchers to develop new and ecofriendly strategies, such as treatment with exogenous nutrients like silicon (Si), to enhance the growth and production of fenugreek under stressed conditions.

In terms of abundance, Si is the most abundant element after oxygen in earth [20]. Various plant species are known for its ability to absorb and accumulate Si in their cell tissues [20, 21]. Many studies reported that Si is a beneficial element that enhance plant growth and improve plant tolerance to several abiotic stresses like heavy metal [22], drought [23], phosphorus deficiency [24] and salt stress [6]. However, in case of AMPs, the effect of exogenous Si has not yet received more attention. Nasseri et al. [18] evaluated only the effect of Si treatment on plant growth, relative water content, electrolyte leakage and chlorophyll content in salt-stressed fenugreek plants but, their effect on antioxidant system, osmoregulation and photosystem efficiency is not yet assessed and detailed. For this reason, in the present work, the effect of exogenous Si application on photosynthetic parameters, oxidative stress markers, antioxidant systems and osmoregulation were assessed in fenugreek, as an AMP, to understand the mechanism by which Si improved plants tolerance and yield under salt-stressed conditions.

2 Materials and Methods

2.1 Plant Material and Growth Conditions

Fenugreek (*Trigonella foenum-graecum* L.) seeds were supplied by the National Institute of Agronomic Research (INRA Morocco) and used as plant material. Eight fenugreek seeds, disinfected with 5% of sodium hypochlorite solution for 5 min, were sown in plastic pot, containing 160 g of sterilized sand-peat mixture (1:4 v:v), in a growth chamber at 25 ± 1 °C, 60%—80% relative humidity and 16 h photoperiod. One week after germination, 4 fenugreek seedlings were kept and irrigated with Hoagland nutrient solution [25], containing KH_2PO_4 (250 µM L^{-1}), KNO_3 (600 µM L^{-1}), K_2SO_4 (0.75 mM L^{-1}), $MgSO_4$ (1 mM L^{-1}), $CaCl_2$ (1.65 mM L^{-1}), Fe-EDTA (16 µmol L^{-1}), H_3BO_3 (4 µM L^{-1}), $MnSO_4$ (6 µM L^{-1}), $ZnSO_4$ (1 µM L^{-1}), $CuSO_4$ (1 µM L^{-1}) and Na_2MoO_4 (0.1 µM L^{-1}). Two weeks after sowing, plants were divided into two plots: plants treated with 0 mM NaCl and plants treated with 150 mM NaCl supplied to the nutrient solution. Each plot was divided into two subplots: plants treated with 0 mM Si and plants treated with 3 mM Si supplied to the nutrient solution in $CaSiO_3$. For each treatment, 12 pots containing 4 plants each were considered. Stress was applied for one month, and then some growth attributes, photosynthetic characteristics and other biochemical parameters associated with salt tolerance, like level of oxidative stress markers and antioxidant molecules, were evaluated.

2.2 Growth Attributes

After one month of salt stress and Si treatment, some growth attributes, such as shoot and root dry weight, plant length and leaf area were assessed. Just before the harvest, plant height was determined in three random plants from each treatment using a ruler graduated to centimeters and millimeters. Plants were then harvested, and shoots were separated from the roots, oven dried at 80 °C for 48 h and their dry weight was determined.

Leaf area was determined in three random leaves from three random plants according to El Moukhtari et al. [6]. Briefly, leaves were cut and laid out on a white sheet containing a scale and scanned using a digital scanner. Leaf area was measured using Mesurum software version 3.4.4.0.

After 3 months, fenugreek plants were hand-harvested and the number of immature seeds per pod (NSP) was recorded, with three replicates for each treatment.

2.3 Relative Water Content

Relative water content (RWC) was determined as described in [26]. The third fully expanded youngest leaf from top was excised from three random plants from each treatment and their fresh weight (FW) was recorded immediately. Samples were then cut and transferred to distilled water for 8 h and their turgid weight (TW) was determined. Samples were then kept in an oven at 70 °C for 24 h and their dry weights (DW) was measured. RWC was calculated following the formula below:

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100$$

2.4 Photosynthetic Pigment Content

Photosynthetic pigments were determined following Arnon's [27] method. Fresh leaf material (0.1 g) was homogenized at 4 °C in 2 mL of acetone (80%) using mortar and pestle. Homogenate was then centrifuged at 10 000 rpm for 10 min at 4 °C, and the supernatant was used to read the optical density (OD) at 645 nm, 663 nm and 480 nm. Chlorophyll (Chl) a, Chl b, total Chl and carotenoid contents were calculated according to D'souza and Devaraj [28], with three replicates for each treatment.

2.5 Quantum Efficiency of the Photochemistry of PS II (F_v/F_m) and Stomatal Conductance

Regarding photosystem II efficiency, the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) was determined in 9 leaves from each treatment after 20 min of darkness adaptation using a portable fluorescence meter (Handy PEA, Hansatech, England) according to Mouradi et al. [29]. For stomatal conductance (gs), a leaf porometer (SC1 Model, Decagon Devices, version 2012) was used. Five replicates for each treatment were considered. Measurement was taken between 9 and 12 p.m. and before each measurement, the instrument was calibrated to ensure an accurate reading [30].

2.6 Malonyldialdehyde (MDA) and Hydrogen Peroxide (H_2O_2) Contents and Electrolyte Leakage Percentage (EL)

MDA content was estimated in three replicates using the thiobarbituric acid (TBA) method [31]. 100 mg of fresh leaf material were homogenized in 1 mL of 0.5% TBA prepared in 20% trichloroacetic acid (TCA) and the resulted homogenate was heated at 95 °C for 30 min. After cooling down, samples were centrifuged at 14 000 rpm for 10 min, and the absorbance of supernatant was determined at 532 nm and 600 nm. MDA content was determined using its extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as µmol MDA g^{-1} FW, with three replicates for each treatment.

H_2O_2 content in fenugreek leaves was determined following the method of Brennan and Frenkel [32]. 100 mg of fresh leaf materials were ground in 2 mL of cold acetone and centrifuged at 5000 rpm for 15 min at 4 °C. Afterward, to 1350 µL of supernatant, 150 µL of 20% titanium, prepared in concentrated hydrochloric acid (HCl), (v/v), and 300 µL of concentrated ammonia were added and the mixture was centrifuged at 10 000 rpm for 10 min. Supernatant was then discarded and the precipitate was washed five times with cold acetone and recovered in 3 mL of 2 N sulfuric acid to determine H_2O_2 content after absorbance measurement at 410 nm. H_2O_2 was calculated using a standard curve prepared with known concentration of H_2O_2 and expressed as mmol H_2O_2 g^{-1} FW, with three replicates for each treatment were considered.

According to Ghoulam et al. [26], the EL was determined in three replicates per treatment. Three leaves from each treatment were cut and washed thoroughly with deionized water, to remove all surface electrolytes, and immersed in 10 mL of distilled water. After 24 h of agitation at 25 °C, the initial electrical conductivity (EC_1) was measured using a conductivity meter (DDS-12DW, Benchtop Conductivity Meter). Samples were then autoclaved at 120 °C for 20 min and the finale electrical conductivity (EC_2) was measured. EL was calculated by the following formula:

$$EL(\%) = (EC_1/EC_2) * 100$$

2.7 Enzymatic Antioxidant Activity

Polyphenol oxidase (PPO) was extracted by grinding 100 mg of fresh leaf material in 1 mL of 50 mM phosphate buffer (pH 6), containing 5% of polyvinylpyrrolidone (PVP). The PPO activity was determined according to Hori et al. [33], following the oxidation of catechol for 3 min at 410 nm. One unit of PPO activity was defined as the amount of enzyme causing 0.01 absorbance increases. PPO activity was expressed as enzymatic unit (EU) min^{-1} mg^{-1} protein, with three replicates for each treatment.

For superoxide dismutase (SOD), 0.1 g of fresh material was ground in 1 mL of 50 mM phosphate buffer (pH 7.8), containing 1% of PVP and 0.1 mM ethylenediaminetetraacetic acid. The mixture was centrifuged at 12 000 × *g* for 20 min at 4 °C and the resulted supernatant was used for SOD activity as reported previously [34]. One enzymatic unit of SOD was defined as the amount of enzyme required to inhibit the reduction of 50% NBT. SOD activity was expressed as EU min^{-1} mg^{-1} protein, with three replicates for each treatment.

For both antioxidant enzymes, Bradford [35] method was followed to determine the content of enzymatic proteins of the extracts.

2.8 Non-Enzymatic Antioxidant Content

Co-extraction of total polyphenols and flavonoids was realized as reported previously by Lamsaadi et al. [7]. 100 mg of fresh plant materials were homogenized in 1 mL of methanol (80%) at 4 °C using mortar and pestle. After 20 min of centrifugation at 12 000 × *g* at 4 °C, the supernatant was recovered and stored at -20 °C until evaluation of the total polyphenols and flavonoids contents.

For total polyphenols, Folin-Ciocalteu (FC) method was adopted [36]. 50 µL of the resulted supernatant was mixed with 250 µL of FC reagent and the volume was adjusted to 5 mL with distilled water. After incubation for 3 min at room temperature, the volume was adjusted to 6.5 mL with Na_2CO_3 (20%) and the resulted mixture was incubated at the dark for 1 h at room temperature. The OD was then read at 725 nm and the content of total polyphenols was determined and expressed as mg gallic acid equivalents g^{-1} FW, with three replicates for each treatment.

Flavonoids content was assessed following the method of Chang et al. [37]. Briefly, 300 µL of methanol (95%), 20 µL of 10% aluminum chloride ($AlCl_3$), 20 µL of potassium acetate (1 M) and 560 µL of distilled water were added to 100 µL of supernatant. After incubation for 30 min at room temperature, the absorbance of the resulted mixture was read at 415 nm and the flavonoids content was calculated referring to a standard curve prepared from different concentrations of quercetin. Flavonoid content was expressed as mg quercetin g^{-1} FW, with three replicates for each treatment.

2.9 Compatible Solutes Accumulation

The proline content was determined by homogenizing 100 mg of fresh materials in 1 mL of aqueous sulfosalicylic acid (3%) according to Bates et al. [38], with three replicates for each treatment. The homogenate was centrifuged at 14 000 rpm for 10 min at 4 °C and to 400 µL of the resulted supernatant, an equal volume of ninhydrin reagent and concentrated acetic acid were added. After 1 h of incubation at 95 °C, the reaction was stopped using an ice bath. Afterward, 800 µL of toluene was added and the absorbance of the pink phase was read at 520 nm. The content of proline was determined using a standard curve prepared with known concentrations of proline and expressed as mmol proline g^{-1} FW.

Glycine betaine content was measured according to Grieve and Grattan [39]. 250 mg of dried plant materials were mechanically shaken with 7.5 mL of distilled water for 48 h at 25 °C, and the resulted filtrate was diluted 1:1 with 2 N sulfuric acid. After incubation in an ice bath under agitation for 1 h, 0.2 mL of cooled potassium iodide-iodine ($KI\text{-}I_2$) reagent was added to 0.5 mL of mixture and incubated at 4 °C for 16 h. Then, after centrifugation at 10 000 rpm for 15 min at 0 °C, the supernatant was carefully recovered and the precipitate was dissolved in 3 mL of 1.2 dichloroethane. The OD of the dichloroethanic phase was measured at 365 nm and the content of glycine betaine was determined from a standard curve and expressed as mmol glycine betaine g^{-1} DW, with three replicates for each treatment were considered.

As described by Dubois et al. [40], the content of soluble sugars was determined by homogenizing 100 mg of fresh leaf samples in 4 mL of 80% ethanol (v/v), with three replicates for each treatment. After 15 min of centrifugation at 5000 rpm at 4 °C, 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid were added to 1 mL of supernatant. The mixture was left to cool down, and then the absorbance was measured at 485 nm. The content of soluble sugars was calculated from a standard curve prepared with glucose solutions and expressed as mg glucose g^{-1} FW.

2.10 Sodium (Na^+), Potassium (K^+) and Calcium (Ca^{2+}) Determination

Na^+, K^+ and Ca^{2+} contents in fenugreek plants were determined according to Oukaltouma et al. [41]. 0.5 g of dry fenugreek plants were incinerated for 6 h at 600 °C in a Protherm Furnaces (PLF 120/12). The resulted ashes were recovered in 3 mL of 10 N HCl and the volume was adjusted to 50 mL using deionized water. The amount of Na^+, K^+

and Ca^{2+} was determined using a flame emission photometer (AFP100 Model, Biotech Management Engineering Co. Ltd., UK).

2.11 Statistical Analysis

Data were analyzed using two-way analysis of variance (ANOVA II), where Si and salinity were the independent variables. Means were compared using Tukey's test at 95% confidence level. Pearson's correlation matrix was realized by using XLSTAT statistical software, version 2014.5.03 at $p < 0.05$.

3 Results

3.1 Growth Attributes

Results indicated that salt stress significantly ($p < 0.05$) reduced shoot dry weight (SDW), root dry weight (RDW), plant height (PH) and leaf area (LA) by 60%, 66%, 145% and 89%, respectively, as compared to control (Table 1; Fig. 1a-b). However, 3 mM of Si supplementation to the growth medium of salt-stressed fenugreek plants alleviated the negative impact of salt and increased SDW, RDW, PH and LA by 100%, 100%, 60% and 40%, respectively, relative to Si-untreated salt-stressed plants. Under normal conditions, Si supply increased SDW by 20%. Regarding water status of fenugreek plants, relative water content (RWC) was significantly ($p < 0.05$) decreased from 64 to 42% under salt stress. Si supplementation improved leaf RWC of fenugreek plants under either stressed or unstressed conditions. Indeed, RWC of stressed and unstressed plants was 1.6 and 1.2-fold higher under Si treatment relative to their respective Si-untreated control. (Table 1).

Under salinity conditions, number of seed per pod (NSP) was 1.72-fold higher in Si-treated fenugreek plants as compared to plants without Si (Table 1). Moreover, under unstressed conditions, Si improved NSP by 14% relative to Si-untreated control.

3.2 Photosynthetic Pigments

Results presented in Fig. (2a-d) showed that photosynthetic pigments were significantly ($p < 0.05$) reduced upon salt stress. Indeed, chlorophyll (Chl) a, Chl b, total Chl and carotenoids were 2.59, 1.79, 2.32 and 2.20-times lower under 150 mM NaCl treatment as compared to the salt-untreated control. However, Si supply to the growth medium of salt-stressed fenugreek plants significantly improved Chl a, Chl b, total Chl, and carotenoids, respectively, by 40%, 33%, 38% and 37% as compared to Si-untreated salt-stressed plants. Under normal conditions, Si treatment has no significant ($p > 0.05$) effect on Chl a, Chl b and total Chl.

3.3 Stomatal Conductance and Photosystem II Efficiency

Stomatal conductance (Fig. 3a) and the photosystem II efficiency (F_v/F_m; Fig. 3b) were decreased, respectively from 66.8 to 24.4 mmol H_2O m^{-2} s^{-1} and from 0.89 to 0.64 in response to 150 mM NaCl. However, the treatment of salt-stressed plants with 3 mM Si significantly ($p < 0.05$) improved the stomatal conductance and the photosystem II efficiency by 43% and 19% relative to Si-untreated salt-stressed plants. In non-stressed plants, there was no significant ($p > 0.05$) difference between Si-treated and untreated fenugreek plants (Fig. 3a-b).

3.4 Oxidative Stress Markers and Membrane Cell Integrity

Exposure of fenugreek plants to 150 mM NaCl significantly ($p < 0.001$) increased the oxidative stress markers such as malonyldialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents and electrolyte leakage (EL) as compared to the control (Fig. 4a-c). In fact, when compared to the unstressed control, MDA and H_2O_2 contents and EL (%) were 18.1, 1.87 and 3.4-fold higher in fenugreek plants exposed to salinity stress. However, when salt-stressed fenugreek plants were supplied with 3 mM of exogenous Si, MDA and

Table 1 Effect of exogenous silicon (3 mM Si) treatment on SDW, RDW, PH, LA, NSP and RWC of fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. Data are the mean of three replicates ± standard error, and the different letters show a significant difference at $p < 0.05$

Treatments	SDW (mg plant^{-1})	RDW (mg plant^{-1})	PH (cm plant^{-1})	LA (cm^2)	RWC (%)	NSP
C	153.33 ± 5.13b	27.33 ± 0.64a	35.66 ± 0.20a	4.89 ± 0.29a	63.88 ± 0.82b	14.00 ± 0.76b
Si	180.00 ± 7.69a	26.35 ± 0.51a	36.80 ± 0.15a	4.76 ± 0.22a	73.45 ± 3.01a	16.00 ± 0.38a
NaCl	60.00 ± 3.84d	10.00 ± 0.48c	14.56 ± 0.29c	2.59 ± 0.09c	42.40 ± 1.97c	6.00 ± 0.38d
NaCl + Si	123.33 ± 6.41c	19.66 ± 0.25b	23.46 ± 0.24b	3.62 ± 0.17b	68.51 ± 2.71ab	10.33 ± 0.64c

C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; SDW, shoot dry weight; RDW, root dry weight; PH, plant height; LA, leaf area; NSP, number of seeds per pod; RWC, relative water content

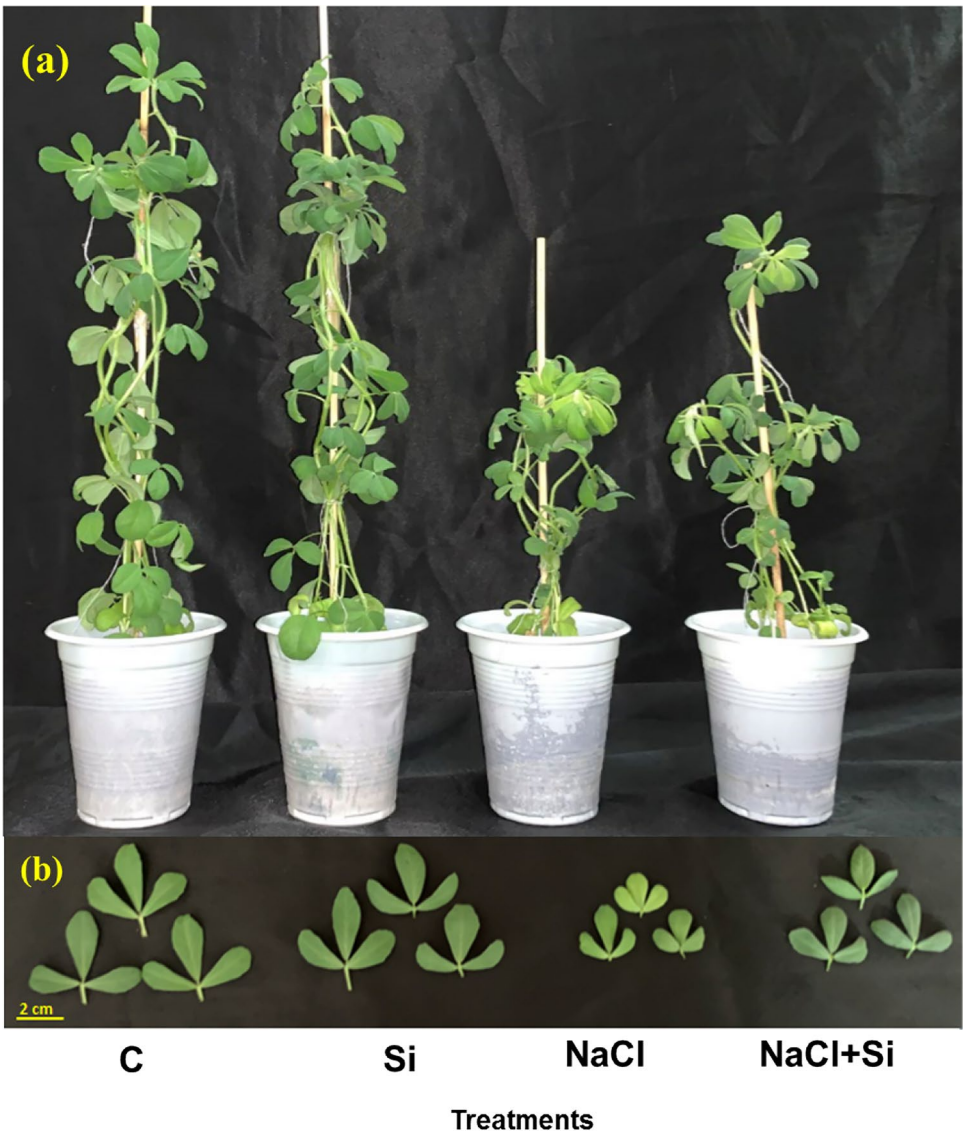

Fig. 1 Effect of exogenous silicon (3 mM Si) treatment on plant phenotype (**a**) and leaf area (**b**) of fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl + Si, combination of NaCl and Si

H_2O_2 contents and EL were reduced by 40%, 18% and 40%, respectively relative to Si-untreated salt-stressed plants. No significant difference was observed between Si-treated and untreated fenugreek plants under normal conditions for all investigated oxidative stress markers (Fig. 4a-c).

3.5 Enzymatic and Non-Enzymatic Antioxidant Activity

Results illustrated in Table 2 revealed that the contents of total polyphenols and flavonoids were drastically reduced upon salt stress. Indeed, 150 mM NaCl stress significantly ($p \leq 0.001$) decreased total polyphenols from 9.72 to 4.32 mg gallic acid g^{-1} FW and flavonoids from 20.50 to 6.36 mg quercetin g^{-1} FW reflected 56% and 69% of reduction rates, respectively, as compared to the control. However, Si supplementation markedly alleviated the negative impacts of salt stress and significantly ($p < 0.001$) improved total polyphenols and flavonoids contents.

Regarding enzymatic antioxidant activity (Table 2), the activity of superoxide dismutase (SOD) and polyphenol oxidase (PPO) was significantly ($p < 0.05$) increased by 166% and 72%, respectively under salt stress as compared to the control. Additionally, the activity of both SOD and PPO was further increased in salt-stressed fenugreek plants when supplied with exogenous Si. In fact, under combined treatment of 150 mM NaCl and 3 mM Si, the activity of SOD and PPO was increased by 124% and 14%, respectively as compared to salt stress alone. When applied to the unstressed plants, Si has no significant effect ($p > 0.05$) on SOD and PPO activities.

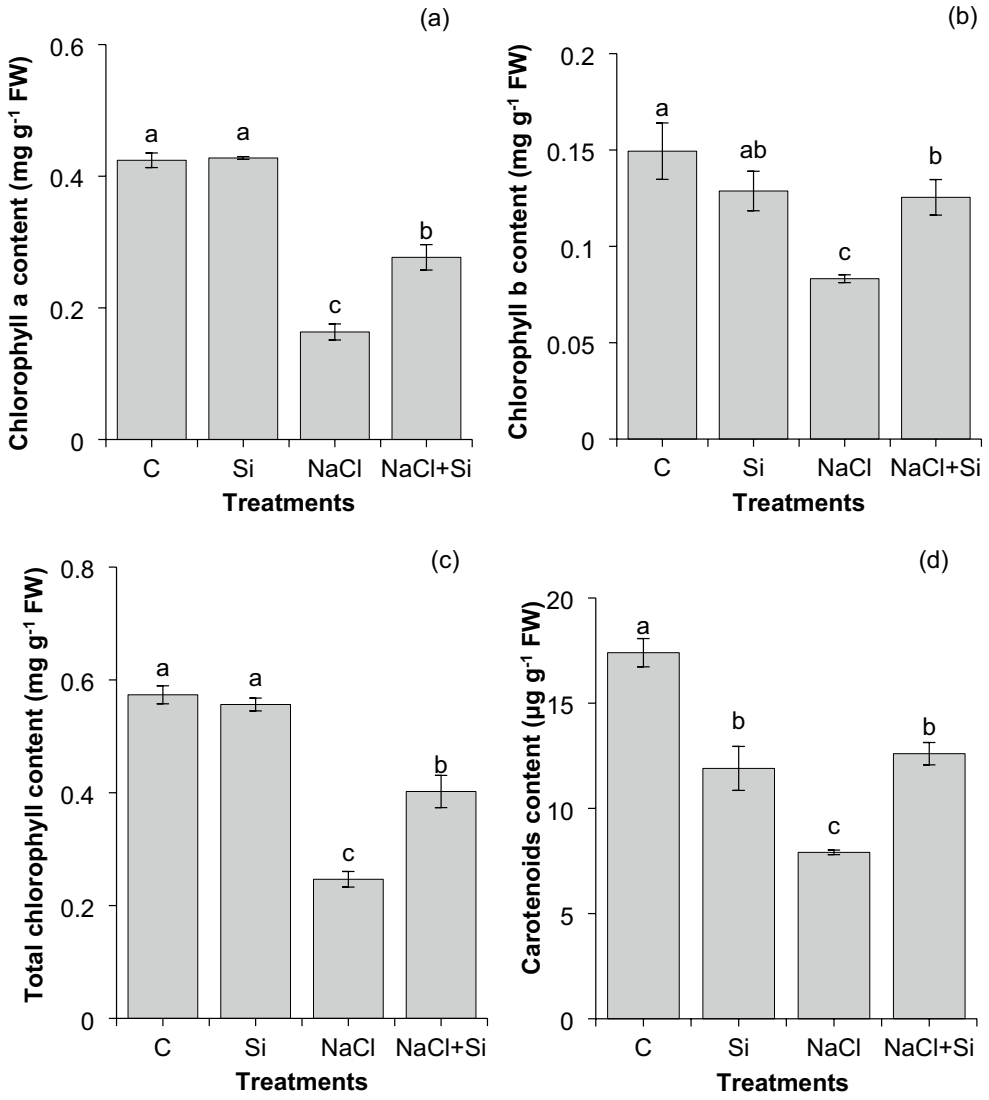

Fig. 2 Effect of exogenous silicon (3 mM Si) treatment on chlorophyll a (**a**), chlorophyll b (**b**), total chlorophyll (**c**) and carotenoids (**d**) contents of fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. Bars represent standard errors of three replicates and the values followed by different letters show a significant difference at $p < 0.05$. C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl + Si, combination of NaCl and Si

3.6 Compatible Solutes Accumulation

Salt stress caused a significant increase in the content of compatible solutes, in terms of soluble sugars, proline and glycine betaine, and this increase was more furthered ($p < 0.01$) when salt stressed fenugreek plants were treated exogenously with 3 mM Si (Fig. 5). Indeed, in Si-treated salt-stressed plants, soluble sugars, proline and glycine betaine contents were significantly increased by 42%, 56% and 14%, respectively, relative to plants treated with NaCl alone (Fig. 5). Si treatment had no significant ($p > 0.05$) effect on compatible solutes under unstressed conditions.

3.7 Mineral nutrition

Results in Table 3 showed that salt stress imposition elevated the content of sodium (Na^+) by 217%, while it significantly ($p < 0.05$) reduced potassium (K^+) and calcium (Ca^{2+}) by 53% and 29%, respectively relative to the untreated control. In addition, the increase in Na^+ and the decrease in K^+ reduced K^+/Na^+ ratio from 0.61 to 0.12 reflected 80% of reduction rate (Table 3). However, treatment with Si significantly ($p < 0.05$) increased K^+, while it significantly decreased Na^+ content leading to a higher K^+/Na^+ ratio (0.26) compared to Si-untreated salt-stressed fenugreek

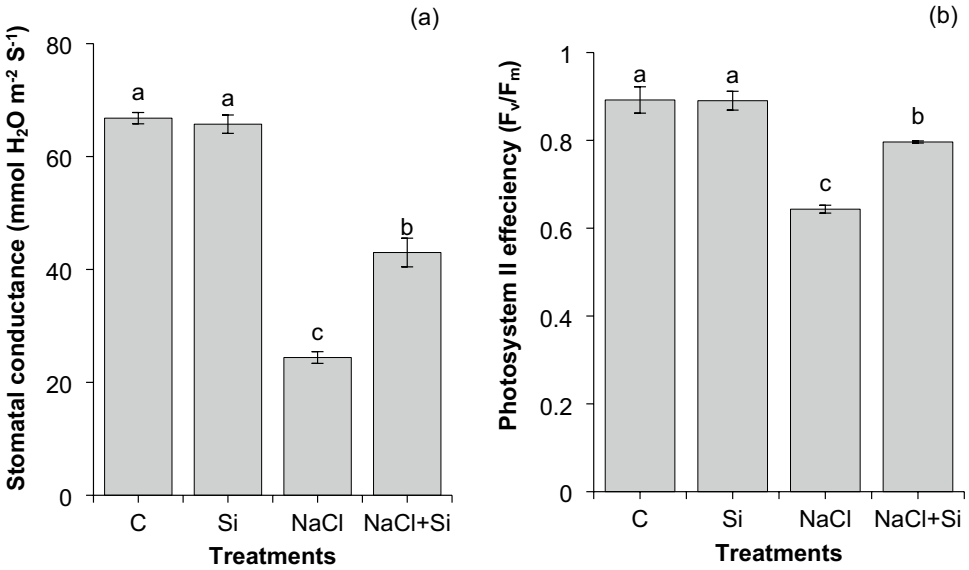

Fig. 3 Effect of exogenous silicon (3 mM Si) treatment on stomatal conductance (**a**) and photosystem II efficiency (**b**) of fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. Bars represent standard errors of three replicates and the values followed by different letters show a significant difference at $p < 0.05$. C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl + Si, combination of NaCl and Si

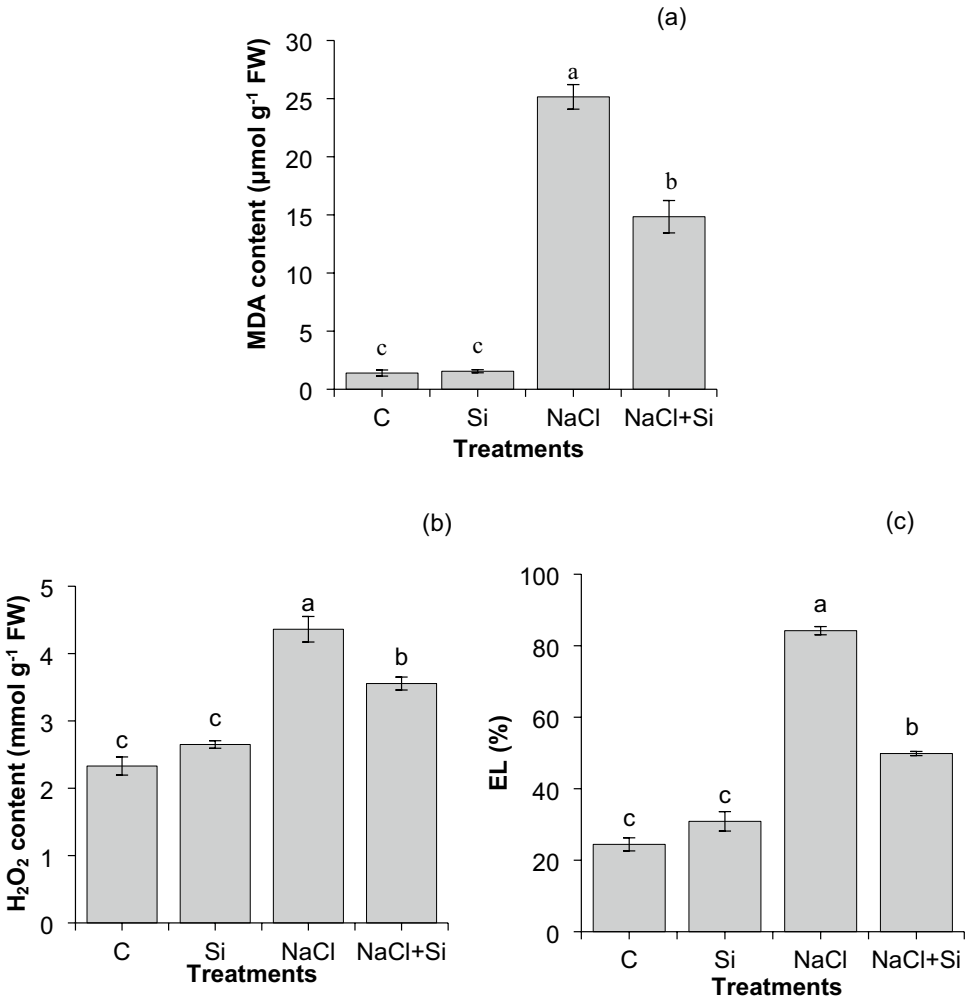

Fig. 4 Effect of exogenous silicon (3 mM Si) treatment on malondialdehyde (MDA) (**a**) and hydrogen peroxide (H_2O_2) (**b**) contents and electrolyte leakage (EL) (**c**) in fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. Bars represent standard errors of three replicates and the values followed by different letters show a significant difference at $p < 0.05$. C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl + Si, combination of NaCl and Si

Table 2 Effect of exogenous silicon (3 mM Si) treatment on total polyphenols and flavonoids contents and SOD and PPO activities in fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. The represented data are the mean of three replicates ± standard error, and the different letters show a significant difference at $p < 0.05$

Treatments	Non-enzymatic antioxidant content		Enzymatic antioxidant activity	
	Total polyphenols (mg gallic acid g^{-1} FW)	Flavonoids (mg quercetin g^{-1} FW)	SOD activity (EU min^{-1} mg^{-1} protein)	PPO activity (EU min^{-1} mg^{-1} protein)
C	9.72 ± 0.24b	20.50 ± 0.66a	33.32 ± 3.13c	0.40 ± 0.03c
Si	16.38 ± 1.14a	19.46 ± 0.04a	31.81 ± 7.11c	0.38 ± 0.05c
NaCl	4.32 ± 0.66d	6.36 ± 0.40c	88.84 ± 12.82b	0.69 ± 0.01b
NaCl + Si	7.64 ± 0.31c	14.51 ± 0.40b	199.03 ± 5.97a	0.79 ± 0.01a

C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl + Si, combination of NaCl and Si; SOD, superoxide dismutase; PPO, polyphenol oxidase; EU, enzymatic unit; FW, fresh weight

plants (0.12). Moreover, Si treatment alleviated the negative effect of salinity and significantly ($p < 0.05$) improved Ca^{2+} content. Under normal conditions, Si application caused a significant increase in the content of Na^+, K^+ and Ca^{2+}, but it remarkably decreased K^+/Na^+ ratio.

4 Discussion

Reduction in plant growth is a common response to salt stress, which is might be due to a reduction in cell division and cell elongation [42]. Similarly, our findings revealed that under salt stress conditions, fenugreek plants growth was severely reduced as reflected by a significant decrease in shoot and root dry weight, plant height and leaf area. Salinity also remarkably reduced the number of seeds per pod by 57% relative to the unstressed control. Therefore, exposure to 150 mM NaCl interestingly reduced both fenugreek growth and yield. However, exogenous Si supply to the growth medium significantly alleviated the harmful effects of 150 mM NaCl stress on all the above studied growth and yield parameters. These findings are in agreement with those of El Moukhtari et al. [6], who reported that 3 mM Si significantly improved plant biomass, plant height, leaf number and leaf area in *Medicago sativa* L. under 120 mM NaCl stress. Likewise, Ali et al. [10] found that Si was able to improve plant biomass and RWC in exposed maize to salinity. According to Bayat et al. [43], when applied under salt stress, Si caused a significant increase in calendula growth traits including shoot and root dry weight, plant height and leaf area. Similar findings were reported in purslane [44], basil [45] and honeysuckle [46]. Several studies demonstrated that, under stressed conditions, the plants biomass reduction could be the results of photosynthesis capacity reduction. Indeed, the decrease in plants growth parameters is significantly correlated with a decrease in chlorophyll synthesis and in other photosynthetic parameters in various plants species like lavender [9], *Vigna angularis* [47] and cucumber [48]. In the present study, salt-stressed fenugreek plants had reduced photosynthetic pigments (Chl a, Chl b, total Chl and carotenoids) as compared to controls. This effect is often attributed to the toxic effect of Na^+ and Cl^- on chlorophyll synthesis machinery, where there is a close negative correlation between shoot Na^+ content and photosynthetic pigments, like total chl ($r = -0.89$; $p \leq 0.05$; Fig. 6). Another explanation of the decreased Chl content under salt stress is the increase in the activities of Chl degrading enzymes such as chlorophyllase, Chl-degrading peroxidase and pheophytinase [49]. Yang et al. [50] reported that photosynthesis inhibition is one, among others, factors that will minimize growth under salt stress. This was clearly observed in our study as indicated by the highly significant correlation (Fig. 6) observed between root dry weight and Chl a ($r = 95$; $p \leq 0.05$), Chl b ($r = 69$; $p \leq 0.05$), total Chl ($r = 93$; $p \leq 0.05$) and carotenoids ($r = 71$; $p \leq 0.05$). However, supply of Si in salt-stressed fenugreek plants led to significant increases of Chl a, Chl b, total Chl and carotenoids. Another consequence of salt stress on photosynthesis is the decrease of Chl fluorescence, especially the photosystem II parameter; F_v/F_m ratio [51]. This might be a consequence of Chl reduction in response to salt. Our results revealed that F_v/F_m ratio was remarkably reduced upon salt stress and this negative effect was reversed by Si treatment. Interestingly, F_v/F_m ratio was positively correlated with Chl content ($r = 0.89$, $p \leq 0.05$ for total chl), confirming the finding of Ganieva et al. [52]. Previous studies indicated that Si supplementation significantly improved photosynthetic pigments due to its ability to increase the activities of some Chl synthesis enzymes, including δ-aminolevulinic acid dehydratase and porphobilinogen deaminase, under salt stress [49].

Added exogenous Si also contracted the inimical effects of 150 mM NaCl constraint on nutrition balance by enhancing fenugreek nutrition, in terms of high content of K^+ and Ca^{2+} and a significant decrease of Na^+ accumulation, which

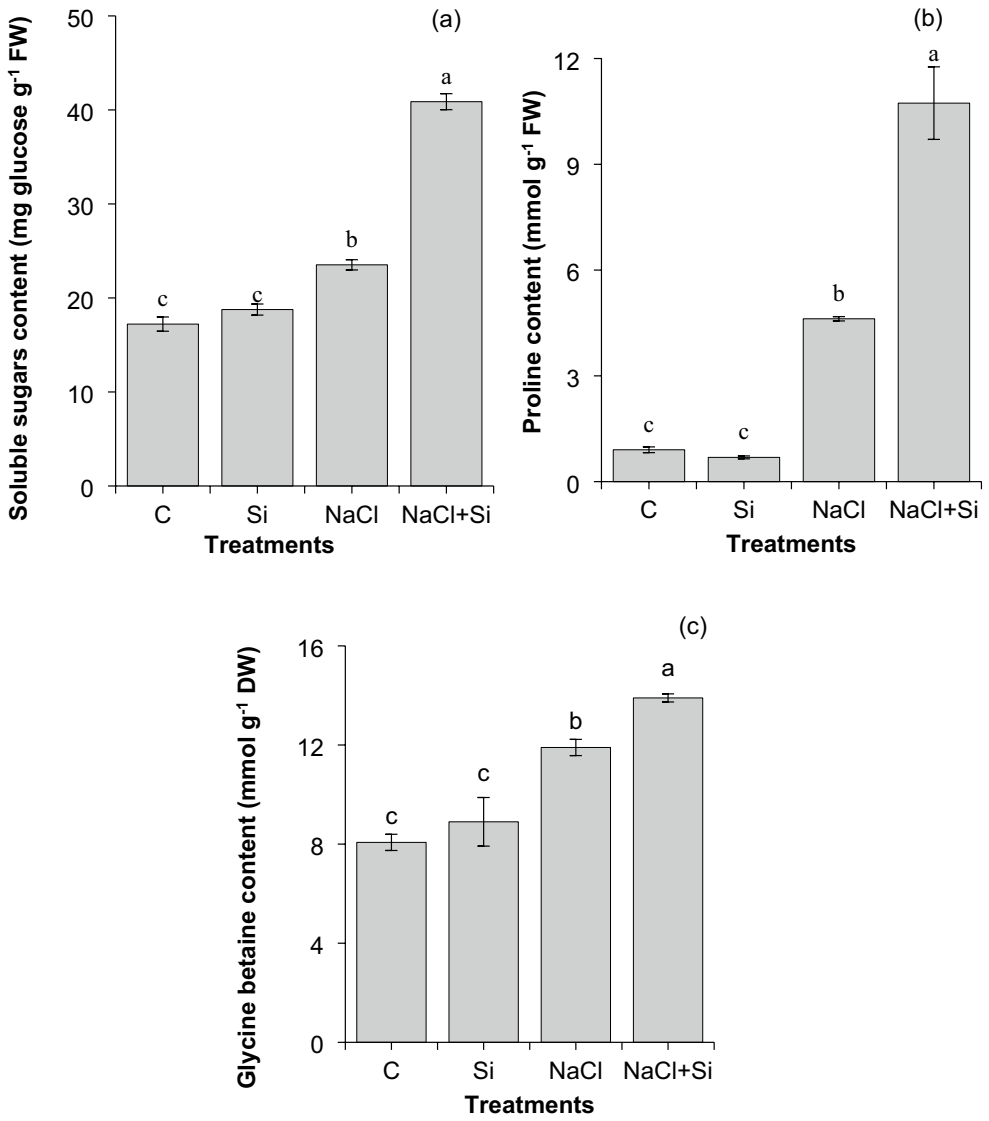

Fig. 5 Effect of exogenous silicon (3 mM Si) treatment on the content of soluble sugars (**a**), proline (**b**) and glycine betaine (**c**) in fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. Bars represent standard errors of three replicates and the values followed by different letters show a significant difference at $p < 0.05$. C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl + Si, combination of NaCl and Si

Table 3 Effect of exogenous silicon (3 mM Si) treatment on Na^+, K^+ and Ca^{2+} contents and K^+/Na^+ ratio in fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. The different letters show a significant difference at $p < 0.05$

Treatments	Na^+ (mg g^{-1} DW)	K^+ (mg g^{-1} DW)	Ca^{2+} (mg g^{-1} DW)	K^+/Na^+
C	35.33d	48.35c	44.69c	0.61a
Si	70.43c	60.48a	52.08b	0.42b
NaCl	112.04a	31.55d	31.50d	0.12d
NaCl + Si	107.70b	59.80b	73.05a	0.26c

Na^+, sodium; K^+, potassium; Ca^{2+}, calcium; C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl + Si, combination of NaCl and Si

might be the reason of improved biomasses and phenotype of fenugreek plants under salt stress. In agreement to the above findings, Shekari et al. [53] found that application of Si decreased Na^+ concentration and increased K^+ concentration in roots and shoots of *Anethum graveolens* L. plants, correlating with a significant amelioration in chlorophyll content and plant biomasses under saline condition. Therefore, the positive effect of Si on photosynthetic pigment can also be attributed to the involvement of Si in reducing Na^+ uptake by salt-stressed plants. Shen et al. [54] demonstrated that Si supplementation resulted in reduced Na^+ content and improved K^+ content, photosynthetic pigments content and gas exchange parameters, which in return

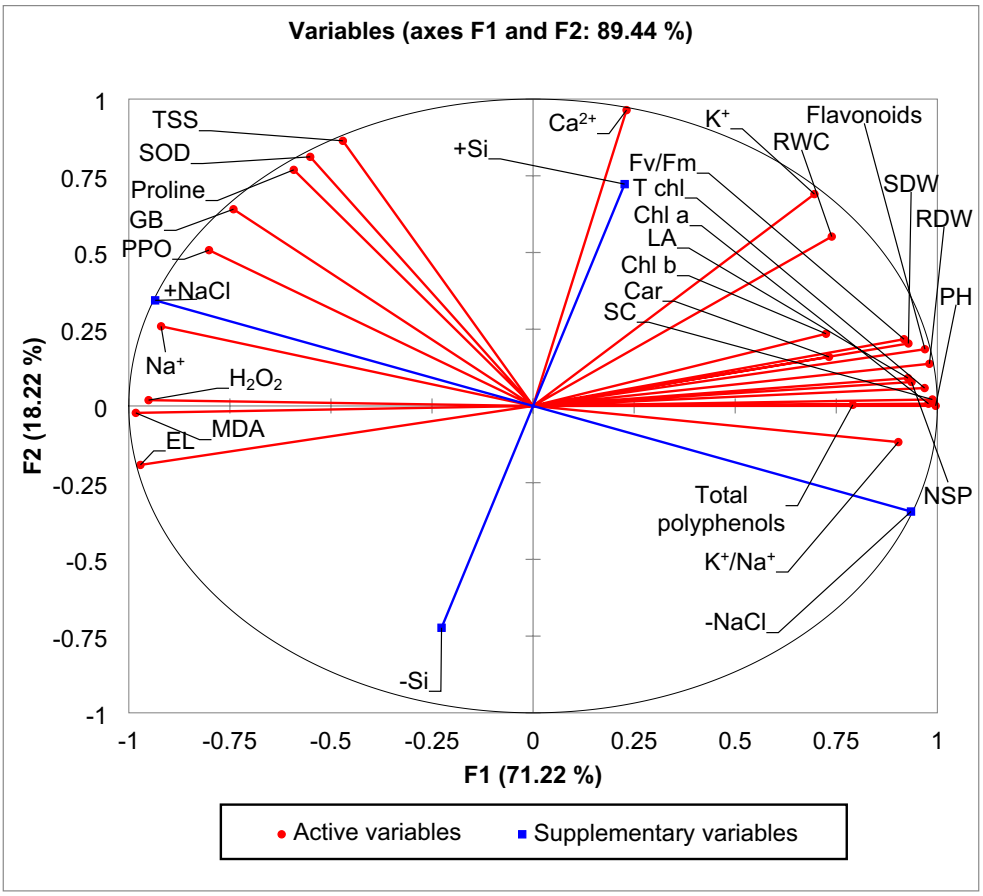

Fig. 6 Principal component analysis (PCA) of all studied parameters related to response of fenugreek to salt stress and Si supplementation in the growth medium. The most variables (arrows), Si treatment and 150 mM NaCl treatment are projected onto the F1-F2 principal factorial plane that explains 89.44% of the variation. SDW: Shoot dry weight; RDW: Root dry weight; PH: Plant height; LA: Leaf area; NSP: Number of seed per pod; T Chl: Total chlorophyll; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Car: Carotenoids; F_v/F_m: Photosystem II efficiency; SC: Stomatal conductance; RWC: Relative water content; MDA: Malonyldialdehyde; H_2O_2: Hydrogen peroxide; EL: Electrolyte leakage; SOD: Superoxide dismutase; PPO: Polyphenol oxidase; TSS: total soluble sugars; GB: Glycine betaine; Si: Silicon; Ca^{2+}: Calcium; Na^+: Sodium; K^+: Potassium

enhanced *Glycyrrhiza uralensis* and *G. inflata* growth under salinity stress. Previously, the significant improvement of plant growth, photosynthetic activity and nutrition balance in response to adding exogenous Si under salt stress has been reported, also, in several plant species such as wheat [55], basil [45] and *Crocus sativus* L. [56]. In salt-stressed okra plant, Abbas et al. [57] reported that foliar spray of Si enhanced stomatal conductance, photosynthetic rate, transpiration rate and number and size of stomata. More than that, Gou et al. [58] showed that added Si could significantly decrease chlorophyll degradation and tomato plant senescence under salt stress. Based on above cited positive effects, Si-mediated increase in growth, yield and photosynthetic activity of fenugreek plants might be partly attributed to different mechanisms, including decrease in salt ions uptake, like Na^+, increase in mineral nutrition (K^+ and Ca^{2+}), modification in gas exchange and photosystems performance under salt stress.

Closing stomatal pores is a common response of plants to overcome water loss by transpiration especially under osmotic stress conditions [59]. However, this led to reduction of CO_2 assimilation and to perturbation of photosynthetic activities. In the present study, salt-stressed fenugreek plants showed a lower RWC as compared to control, indicating an osmotic stress. The decrease in RWC in salinity conditions was positively and significantly correlated to a decrease in stomatal conductance ($r = 0.78$, $p \leq 0.05$) (Fig. 6). However, as previously reported by Siddiqui et al. [60] and Avestan et al. [61], Si supply along with NaCl significantly improved both RWC and stomatal conductance. Si improved RWC under salt stress has been reported in several plant species including maize [62], wheat [55], cucumber [48] and turfgrass [63]. On the one hand, it was reported that, after the uptake, Si accumulates on the epidermis of various plant tissues mainly as a polymer of hydrated amorphous silica, and consequently raised the wax content of the plant epidermis

to regulate the water use efficiency and water evaporation [64–66], which could explain the enhanced RWC in Si-treated salt-stressed plants.

Compatible osmolytes are small molecules that can act as osmoprotectant, alleviating salt stress by regulating cellular osmotic pressure [67, 68]. Furthermore, the ability of stressed plant to accumulate compatible osmolytes may define their tolerance capability [69]. In the present study, salt-stressed fenugreek plants have accumulated numerous compatible solutes, including organic (proline, glycine betaine and soluble sugars) and inorganic (K^+) compounds. More interestingly, the increased compatible solutes in salt-stressed plants were further enhanced when they were treated with 3 mM Si, which could explain the RWC amelioration under salt-mediated osmotic stress and in return enhanced morphological aspect and growth of stressed plants. Previous research indicated that Si increased salt tolerance of plants by regulating osmolytes accumulation, allowing osmotic potential adjustment. For example, in a study conducted on salt-stressed Cucumber by Mousavi et al. [70], Si incorporation in cultured media resulted in a significant increase in the content of proline and soluble sugars. The same has been reported in other plant species such as wheat [55] and okra [57]. According to Zhu et al. [71], exogenous Si was involved directly in proline biosynthesis by inhibiting the activity of proline dehydrogenase and enhancing that of pyrroline5-carboxylase synthase, which resulted in an increase in plant proline content.

The effect of salt stress in plants can also be seen in the form of oxidative stress. Elevated Na^+ content particularly in the aerial parts led to a dramatic accumulation of H_2O_2 ($r = 0.90$, $p < 0.05$) in the leaves. If not metabolized, H_2O_2 could induce membrane damages [72]. In our study, the increase in H_2O_2 content was significantly correlated with MDA content ($r = 0.94$, $p < 0.05$) and electrolyte leakage percentage ($r = 0.90$, $p < 0.05$), indicating an oxidative damage. Similar findings were obtained by Ahanger et al. [47], who found that, under salinity, the accumulation of ROS was positively correlated with an increase in lipid peroxidation. However, in the current study, incorporation of Si to the growth medium significantly mitigated the adverse effects of salt stress on membrane integrity by decreasing H_2O_2 and MDA contents and electrolyte leakage value. Similarly, several studies reported that ROS generation and membrane cell instability were significantly declined in response to exogenous Si application under salt stressed conditions [45, 61, 73]. To overcome salinity-mediated oxidative stress, tolerant plants adopt some tolerant strategies. This includes the induction of the enzymatic and non-enzymatic antioxidant pathways [74]. Importantly, in the current study, supply of Si to salt-stressed plants furthered the increase in the activity of SOD and PPO, together with an increase in the content of non-enzymatic antioxidant compounds (total polyphenols and flavonoids). Thus, Si treatment induced ROS detoxification by promoting the activities of antioxidant enzymes, and enhancing the content of non-enzymatic antioxidant compounds, such as total polyphenol and flavonoids. Likewise, in salt-stressed tomato, Al-aghabary et al. [73] reported that Si treatment decreased lipid peroxidation and H_2O_2 content, while it increased SOD and catalase activities. Si-mediated reduction in oxidative stress under salinity stress was also reported in rice [75], okra [55], *Anethum graveolens* L. [53] and *Glycyrrhiza uralensis* [76] during response to salinity constraint. Thus, in addition to the enhancement of photosynthetic process performance and accumulation of osmoprotectant compounds, incorporation of exogenous Si to the stressed growth medium was also able to alleviate the harmful effect of 150 mM NaCl on fenugreek plants by activating both enzymatic and non-enzymatic antioxidant systems, as well as detoxification of oxidative stress markers.

5 Conclusion

Overall, salt stress significantly reduced fenugreek growth and yield, due to reduction in relative water content, photosystem II efficiency and chlorophyll content. Also, salinity caused a significant induction of oxidative stress, reflecting by high accumulation of MDA and ROS in salt stressed fenugreek plants. However, Si addition alleviated salt-induced reduction in plant growth and yield by enhancing photosynthesis, relative water content and the uptake of indispensable nutrients like K^+ and Ca^{2+}. Exogenous Si also decreased Na^+ accumulation and saved the membrane permeability, due to a decrease in oxidative stress markers. In addition, adding Si induced defense-related mechanisms via the activation of both enzymatic and non-enzymatic antioxidant systems and accumulation of organic compounds, in terms of proline, glycine betaine and soluble sugars. Thus, under salt stressed conditions, Si treatment might be a useful method for improving fenugreek tolerance and yield.

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Data Availability Not applicable.

Declarations

Competing Interests The authors declare no competing interests.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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