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ORIGINAL ARTICLE

Exogenous silicon improves salt tolerance of Fenugreek (*Trigonella foenum-graecum* L.) during seed germination and early seedling stages

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Abstract

In the present study, we investigated the effects of exogenous silicon (Si) treatment on seed germination, embryo viability, seedling growth, antioxidant molecules and osmotic regulation in fenugreek under salt stress. The seeds were germinated in Petri dishes at 25 ± 1 °C for 8 days under 0 mM (control) *versus* 200 mM NaCl (salt stress) with or without 3 mM Si treatment. The obtained results showed that salt stress significantly reduced final germination percentage, germination speed, velocity index, germination energy, peak value, germination value and vitality index, but increased the mean germination time of fenugreek seeds. Salinity also significantly impaired seedling length and fresh weight. However, Si supplementation contracted the negative impacts of salinity and significantly increased all of the studied germination traits except of mean germination time. Additionally, Si alleviated the negative effects of salt on embryo viability and improved its ability to mobilize sugar and protein reserves. Furthermore, 200 mM NaCl increased Na^+ content and induced the oxidative stress reflected by high malonyldialdehyde content, reactive oxygen species accumulation and electrolyte leakage percentage. However, salt-mediated oxidative stress was alleviated by Si treatment through a significant decrease of Na^+ content and an increase of endogenous Si content, which correlated with a significant induction of enzymatic and non-enzymatic molecules with antioxidant function. Altogether, these findings suggested that, the exogenous Si treatment could be a potential method to enhance salt tolerance of fenugreek during seed germination.

Keywords Antioxidant activity · Embryo viability · Fenugreek · Salinity · Seed reserve mobilization · Silicon

Introduction

In Morocco, one among the major fenugreek-producing countries, fenugreek (*Trigonella foenumgraecum* L.) has been used as a spice crop for a long time. It is also widely used in traditional medicine as a remedy against fever and anemia, and for appetite stimulation (Haddad et al. 2003; Benayad et al. 2014). Indeed, it was documented that fenugreek has various medicinal and therapeutic properties, explained by it richness in bioactive molecules such as alkaloids, flavonoids, amino acids, tannins, saponins and some steroidal glycosides (James and Devi 2021). The seeds of this species are used against several diseases like hypercholesterolemia, cancer, constipation and hypertriglyceridemia (Basch et al. 2003; Khorshidian et al. 2016; Hozzein et al. 2020). Also, the extracts of fenugreek seeds contain many molecules with cytotoxicity effect for cancer cells (Alrumaihi et al. 2021). On the other hand, as leguminous species, fenugreek may fix 48% of its total nitrogen (N) during growing season by its symbiotic N-fixation involving soil bacteria called rhizobia (Singh et al. 2008). This process contributes towards soil fertility improvement and reducing the inputs of chemical N fertilizers.

Despite the aforementioned advantages, the productivity and growth stages, including germination and early seedling stages, of this important aromatic and medicinal plant are negatively affected by several abiotic stresses such as salinity stress. In fact, due to climate change, salinization has become a global environmental problem. It was predicted

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that more than 50% of the total arable land will have salinity problems by 2050 due to the annual increase from 1 to 2% rate of salt-affected soils in the world (Bianco and Defez 2009; El Moukhtari et al. 2020), thus as a result, the plants productivity, including aromatic and medicinal plants, will considerably decrease.

Germination is a key stage in the life cycle of plants. Once germinated, seedling establishment is of critical importance to crop productivity, especially under stressed conditions. Salinity grievously affected the seed germination of many aromatic and medicinal plants such as *Trigonella foenumgraecum*, *Thymus daenensis* Celak., *Thymus kotschyanus* Boiss. and *Origanum compactum* Benth. (Khoshsokhan et al. 2012; Laghmouchi et al. 2017; Mahmoudi et al. 2019). The harmful effects of salt stress on seed germination could be explained by inhibition of seed reserve mobilization and activity of some hydrolytic enzymes like α-amylase, *β*-amylase and *α*-glycosidase (Sidari et al. 2008). Salinity has also been reported to cause an ionic toxicity through the accumulation of Na^+ and Cl^- ions, disturbing the ion homeostasis (Farissi et al. 2011). Furthermore, Liu et al. (2019) reported that application of 200 mM NaCl stress significantly decreased gibberellic acid (GA)/abscisic acid (ABA) ratio in *Limonium bicolor* Bag. by down-regulating the expression of *GA20ox* and *GA3ox* (the key genes involved in the biosynthesis of GA), while the expression of *NCED1* and *NCED3* (the key genes involved in the biosynthesis of ABA) was significantly up-regulated in salt stressed seeds. Hence, salt stress might also affect the embryo metabolism by disturbing hormonal balance. On the other hand, Luo et al. (2021) suggested that salinity actives the transcription of NADPH oxidase genes such as *RbohD*, which leads to uncontrolled production and accumulation of reactive oxygen species (ROS), resulting in cell membrane damage and a decrease in seed vigor.

Currently, many strategies are used to alleviate salinity problems on crop production worldwide. One of these strategies focused on the conventional breeding techniques for developing salt tolerant verities, but due to its multigenic traits, this approach has not been successful (İbrahimova et al. 2021). Another approach looks at the use of exogenous substances, as organic or inorganic stimulants, helping plant to tolerate drastic conditions of environment (Hassanvand et al. 2019; Zrig et al. 2019; Robatjazi et al. 2020). However, the mechanism by which these exogenous compounds improved plant tolerance to abiotic stresses is still poorly understood. Likewise, regarding silicon (Si) application, as the second most abundant mineral after oxygen in the earth's crust (Yan et al. 2018), Ivani et al. (2018) reported that nanosized SiO_2 application significantly increased the germination percentage of the fenugreek seeds and enhanced their vigor under salt stress. But, the mechanism by which Si improved salt tolerance of fenugreek during seed germination, are still not fully understood and have not been detailed (Shi et al. 2014; Sun et al. 2021). Therefore, in this study, we investigated the effects of applying exogenous Si on the seed germination of fenugreek under salt stress. Our work focuses on different physiological and biochemical properties associated with fenugreek salt tolerance. The role of Si in salt stress mitigation with link to seed reserve mobilization, embryo viability, enzymatic and non-enzymatic antioxidant system and organic osmolytes were detailed.

Materials and methods

Plant material and treatments

Fenugreek (*Trigonella foenum-graecum* L.) seeds, supplied by the National Institute of Agronomic Research (INRA Morocco), were surface-disinfected for 5 min with sodium hypochlorite solution (6%) and rinsed thoroughly with sterile distilled water (El Moukhtari et al. 2021). After disinfection, every forty seeds were transferred into 9 cm diameter sterile Petri dishes containing two layers of Whatman paper No. 1 and were then moistened with 7 mL of 200 mM NaCl with or without 3 mM Si. Others were soaked with 7 mL of sterile distilled water or 3 mM Si and served as controls. Seeds were germinated in the dark at 25 ± 1 °C and 60%–80% relative humidity for 8 days, with five repetitions per treatment. 200 mM NaCl and 3 mM Si were used according to the results of our preliminary experiments, showing that the germination of fenugreek seeds was not affected by the NaCl concentrations lower than 200 mM, and the 3 mM Si was the effective concentration of Si to alleviate the negative effects of 200 mM NaCl on fenugreek seed germination.

Assessment of germination traits

The number of germinated fenugreek seeds, with extended radicle for at least 2 mm, was recorded every 24 h. After 8 days of germination, seedling fresh weight (FW) and total seedling length were determined and some germination parameters were calculated by using the formulas indicated in Table 1.

Seed reserve mobilization

During seed germination, representative samples of germinated and non-germinated seeds were taken at different germination times and stored at -20 °C. The seed reserve mobilization was evaluated by measuring soluble proteins and sugar contents at 0, 2, 4, 6 and 8 days of germination.

Extraction of soluble proteins was assessed by homogenization of 100 mg fresh samples in 4 mL Tris-HCL buffer

Table 1 All studied germination parameters

Germination parameters	Formula	References
Final germination percentage ***(FGP)***	$\boldsymbol{FGP = (n/N)*100}$ Where *n* is the number of germinated seeds and *N* is the total number of tested seeds	(Farissi et al. 2011)
Germination speed ***(GS)***	$\boldsymbol{GS = \sum (ni/ti)}$ Where *ni* is the number of germinated seeds on day *ti* and *ti* represents the corresponding day of germination	(Hojjat and Kamyab 2017)
Mean germination time ***(MGT)***	$\boldsymbol{MGT = \sum Dn / \sum n}$ Where *n* is the number of seeds newly germinated at time *D* and *D* is the days from the beginning of the germination test	(Moradi Dezfuli et al. 2008)
Velocity index ***(VI)***	$\boldsymbol{VI = \sum (G/t)}$ Where *G* is the germination percentage from 2 days and *t* is the total time of germination	(Farissi et al. 2011; Khan and Ungar 1984)
Germination energy ***(GE)***	$\boldsymbol{GE = \frac{N1}{D1} + \frac{N2-N1}{D2} + \cdots + \frac{Nj-Ni}{Dj}}$ Where *N* is the number of germinated seeds on the counting date and *D* is the number of days	(Calone et al. 2020)
Peak value ***(PV)***	$\boldsymbol{PV = M_{ag} / D}$ Where M_{ag} is the maximum of seeds accumulative germination and *D* is the germination time	(Czabator 1962)
Germination value ***(GV)***	$\boldsymbol{GV = PV*MDG}$ Where *MDG* = number of germinated seeds/ number of days	(Czabator 1962)
Vitality index	$\boldsymbol{Vitality\ index = S*GI}$ Where *S* is the seedling length, and *GI* is the germination index	(Wang et al. 2010)

(0.1 M, pH 7.5). After centrifugation at 14000 rpm for 15 min at 4 °C, 2 mL of Bradford reagent was added to 2 mL of supernatant. The content of soluble proteins was determined after optic density (OD) measurement at 595 nm by referring to standard curve prepared with bovine serum albumin solutions (Bradford 1976).

Assessment of soluble sugars content was realized by grinding 100 mg fresh samples in 4 mL of 80% ethanol. Then, the mixture was centrifuged at 5000 rpm for 15 min at 4 °C and 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid were added to 1 mL of supernatant. After cooling, the OD was measured at 485 nm and the content of soluble sugars was calculated from a standard curve prepared with glucose solutions (Dubois et al. 2002).

Evaluation of embryo viability

Embryo viability of the non-germinated seeds was assessed by a cytochemical method using 2,3,5 triphenyltetrazolium chloride (TTC). TTC assay is based on the fact that viable embryos are red stained due to the reduction of TTC by cell respiratory activity (Verma and Majee 2013). 10 non-germinated seeds from each treatment were taken at different germination times (0 h, 24 h and 48 h), immersed in 10 mg mL^{-1} TTC and incubated at 30 °C in the dark for 24 h. After draining of the TTC solution, seeds were washed three times with distilled water and the embryos were isolated under a binocular magnifying glass with 10 × magnifications and photographed.

Membrane cell stability and oxidative stress markers

Electrolyte leakage percentage (EL) of 8-day-old fenugreek seedlings was determined accordingly to the method used by Ghoulam et al. (2002). Fresh seedling segments were washed and placed in 10 mL of distilled water and then incubated under agitation for 24 h at 25 °C. After incubation, electrical conductivity was measured before (EC_1) and after autoclaving and cooling at 25 °C (EC_2) to calculate the EL by the following formula:

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

Malonyldialdehyde (MDA) content in fenugreek seedlings were estimated using the thiobarbituric acid (TBA) method as described previously by Heath and Packer (1968). Briefly, 100 mg fresh weight were ground in 1 mL of extraction solution, containing 0.5% TBA and 20% trichloroacetic acid (TCA) and incubated at 95 °C for 30 min. After centrifugation at 14000 rpm for 10 min, the OD was determined at 532 nm and 600 nm, and then the content of MDA was calculated using its extinction coefficient of 155 mM^{-1} cm^{-1}.

Hydrogen peroxide (H_2O_2) of 8-day-old fenugreek seedlings was determined following the procedure developed by Brennan and Frenkel (1977) with some modifications. 100 mg fresh samples were homogenized in 2 mL of cold acetone and centrifuged at 5000 rpm for 15 min at 4 °C. Then, 150 µL of titanium (20% titanic tetrachloride in

concentrated hydrochloric acid (HCl), v/v) were added to 1350 µL of supernatant, afterward 300 µL of concentrated ammonia was added to the mixture to precipitate the peroxide-titanium complex. After centrifugation at 10000 rpm for 10 min, the supernatant was discarded and the precipitate was washed five times with cold acetone. The H_2O_2 precipitated was solubilized in 3 mL of 2 N sulfuric acid and its content was determined after OD measurement at 410 nm.

Cytochemical detection of superoxide anion (O_2^-) in the 8-day-old fenugreek seedlings was assessed using the nitro blue tetrazolium chloride (NBT) method (Kumar et al. 2014). For this purpose, 12 seedlings from each treatment were immersed in 0.2% NBT solution, prepared in 50 mM sodium phosphate buffer (pH 7.5), and then they were incubated overnight in the dark. The reaction between O_2^- of seedlings and NBT has given a dark blue stain in the reaction site. After staining, samples were transferred on a paper towel saturated with 60% glycerol until they were photographed using a digital camera.

Determination of antioxidant enzymes activities

Superoxide dismutase (SOD) extraction was assessed by grinding 0.1 g of fresh materials in 1 mL of 50 mM phosphate buffer (pH 7.8), containing 1% of insoluble polyvinylpyrrolidone (PVP) and 0.1 mM ethylenediaminetetraacetic acid. After centrifugation at 12000 × *g* for 20 min at 4 °C, SOD activity was measured using the NBT method (Beyer and Fridovich 1987). One enzymatic unit of SOD was defined as the content of enzyme required to inhibit the reduction of 50% NBT and SOD activity was expressed as an enzymatic unit (U) mg^{-1} proteins.

Polyphenol oxidase (PPO) enzyme was extracted by homogenizing 100 mg of fresh samples in 1 mL of 50 mM phosphate buffer (pH 6), containing 5% of PVP. Afterwards, the PPO activity was assayed according to Hori et al. (1997) by following the oxidation of the 10 mM catechol for 3 min at 410 nm. One unit of PPO activity was defined as the amount of enzyme causing 0.01 absorbance increases.

For both antioxidant enzymes, the content of enzymatic proteins of extracts was determined according to Bradford (1976) method.

Determination of total polyphenols and flavonoids

Total polyphenols and flavonoids were extracted by grinding 100 mg of fresh samples in 1 mL of methanol (80%). After centrifugation at 12000 × *g* for 20 min at 4 °C, the supernatant was stored at -20 °C until total polyphenols and flavonoids determination. The content of total polyphenols was determined through the Folin-Ciocalteu method and their concentration was described as mg gallic acid equivalents g^{-1} FW (Singleton and Rossi 1965). However, the content of flavonoids was assessed by using the method described by Chang et al. (2002), where the flavonoids content was calculated referring to a standard range of different concentrations of quercetin and expressed as mg quercetin g^{-1} FW.

Determination of organic osmolytes

Method of Bates et al. (1973) was used for proline content determination. 100 mg of fresh matter were homogenized in 1 mL of aqueous sulfosalicylic acide (3%) and centrifuged at 14000 rpm for 10 min at 4 °C. To 400 µL of resulted supernatant, 400 µL of ninhydrine reagent and 400 µL of concentrated acetic acid were added and the mixture was incubated at 95 °C for 1 h. After cooling down, 800 µL of toluene was added and the OD of the pink phase was read at 520 nm. Afterward, the proline content was calculated from a standard curve prepared with proline.

Regarding glycine betaine measurement, the method descripted by Grieve and Grattan. (1983) was used with some modifications. Indeed, 500 mg of dried materials were mechanically shaken with 15 mL of distilled water for 48 h at 25 °C. After filtration, the filtrate was diluted 1:1 with 2 N sulfuric acid, and then the mixture was incubated under agitation and cooled in ice water for 1 h. Then, 0.4 mL of cooled potassium iodide-iodine ($KI-I_2$) reagent was added to 1 mL of mixture and incubated at 4 °C for 16 h. After centrifugation at 10000 rpm for 15 min at 0 °C, the supernatant was carefully aspirated and the precipitate was dissolved in 6 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.

Determination of Si, potassium (K^+), sodium (Na^+) and calcium (Ca^{2+}) contents

The mineral analysis of 8-day-old fenugreek seedlings was conducted according to Liu et al. (2013) with some modifications. Briefly, 100 mg of dry weights were incinerated at 600 °C for 6 h in a Protherm Furnaces (PLF 120/12). Afterward, the obtained ashes were acid digested by using a mixture of 3 mL of concentrated nitric acid and 6 mL of concentrated HCl, then the mixture was heated for 1 h at 200 °C. The content of Si, K^+, Na^+ and Ca^{2+} was determined using inductively coupled plasma optical emission spectroscopy (Optima 8000 ICP-OES).

Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA II) using SPSS version 22. Tukey's test was used to compare the means of control and treatments and the difference at $P < 0.05$ was considered as significant. XLSTAT

version 2014.5.03 was used to perform the Pearson's correlation matrix at $P < 0.05$.

Results

Effect of Si and salt stress on germination traits

The effects of 200 mM NaCl stress and 3 mM Si treatment on germination traits, including FGP, GS, VI, GE, PV, GV, MGT and vitality index (For key to abbreviations see Table 1), are shown in Table 2. The obtained results indicated that exposure of fenugreek seeds to salt stress significantly ($P < 0.001$) reduced FGP, GS, VI, GE, PV, GV and vitality index by 32%, 56%, 44%, 58%, 46%, 63% and 77%, respectively. However, the MGT was significantly ($P < 0.001$) increased by 87.5% compared to control. However, Si supply alleviated the negative impacts of salt stress on seed germination. In fact, FGP, GS, VI, GE, PV, GV and vitality index were 1.29, 1.2, 1.28, 1.24, 1.23, 1.60 and 2.11-fold higher in Si-treated salt-stressed seeds as compared to Si-untreated ones. Results also showed that Si supplementation to salt- stressed seeds reduced MGT by 20% as compared to salt-stressed seeds. In addition, under non-stress conditions, no significant difference was observed between Si-treated and untreated seeds ($P > 0.05$) for all studied germination parameters.

The seedling fresh weight and length was significantly ($P < 0.001$) reduced from 0.15 g and 7.7 cm to 0.06 g and 4 cm, respectively under salt stress in comparison with control (Fig. 1). However, Si supplementation to rooting medium alleviated significantly ($P < 0.05$) the salt stress-induced reduction in fenugreek seedling fresh weight and length. Under unstressed conditions, Si supplementation has no significant effect ($P > 0.05$) on seedling growth as compared to the untreated control.

Table 2 Effect of silicon (Si) and salt stress on germination parameters of fenugreek seeds. The represented data are the means of three replicates ± standard error (SE). Between treatments, the means followed by the same letters at same parameters are not significantly different at $P < 0.05$

Germination parameters	Treatments		
		- Si	+ Si
FGP	- NaCl	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	+ NaCl	67.50 ± 0.96^{c}	87.50 ± 0.96^{b}
GS	- NaCl	55.90 ± 0.90^{a}	55.06 ± 0.71^{a}
	+ NaCl	24.51 ± 0.27^{c}	29.93 ± 0.85^{b}
VI	- NaCl	38.02 ± 0.64^{a}	36.90 ± 0.36^{a}
	+ NaCl	21.04 ± 0.28^{c}	27.00 ± 0.56^{b}
MGT	- NaCl	0.16 ± 0.00^{c}	0.16 ± 0.00^{c}
	+ NaCl	0.30 ± 0.00^{a}	0.24 ± 0.00^{b}
GE	- NaCl	18.67 ± 0.17^{a}	18.83 ± 0.34^{a}
	+ NaCl	7.69 ± 0.06^{c}	9.57 ± 0.20^{b}
PV	- NaCl	70.62 ± 1.20^{a}	68.43 ± 0.36^{a}
	+ NaCl	37.81 ± 0.60^{c}	46.77 ± 1.00^{b}
GV	- NaCl	353.12 ± 6.01^{a}	342.18 ± 1.80^{a}
	+ NaCl	127.73 ± 3.81^{c}	204.66 ± 5.16^{b}
Vitality index	- NaCl	434.18 ± 10.85^{a}	401.88 ± 3.29^{a}
	+ NaCl	97.98 ± 1.50^{c}	206.86 ± 9.44^{b}

-NaCl: 0 mM NaCl; + NaCl: 200 mM NaCl; -Si: 0 mM Si; + Si: 3 mM Si; FGP: Final germination percentage; GS: Germination speed; VI: Velocity index; MGT: Mean germination time; GE: Germination energy; PV: Peak value; GV: Germination value

Effect of Si and salt stress on seed reserve mobilization

The content of soluble proteins and soluble sugars were followed in fenugreek seeds during 8 days of germination to evaluate the effect of salt and Si treatment on seed reserve mobilization. From the beginning to the end of germination period, the protein content was decreased by 26% in salt-stressed seedlings, whereas this reserve was more decreased by 65% and 30%, respectively in Si-treated and untreated seeds germinated under non-stressed conditions (Fig. 2a). Therefore, salt stress hinders the mobilization of protein reserves by the embryo during the germination process. However, the Si treatment enhanced seed protein mobilization in salt-stressed seeds. In fact, the content of soluble proteins was decreased from 2.48 µg mg^{-1} FW, during the germination initiation, to 1.75 µg mg^{-1} FW at the end of the germination period in Si-treated salt-stressed seeds.

Likewise, salt stress affected the mobilization process of soluble sugars as compared to control. Indeed, the soluble sugars were more mobilized under non stressed conditions, from 205 µg mg^{-1} FW, during the germination initiation, to 9 µg mg^{-1} FW at the end of the experiment, whereas, it was from 205 µg mg^{-1} FW to 13 µg mg^{-1} FW in stressed seeds (Fig. 2b). Conversely, Si treatment enhanced the use of sugar reserves by the embryo under salt stress, which was explained by a reducing in the content of soluble sugars by 96% from the beginning to the end of the germination test.

Effect of Si and salt stress on embryo viability

The effect of salt and exogenous Si application on embryo viability are presented in Fig. 3. Results showed that, dependent on the treatment and time of exposition to the applied treatments, the embryo showed different staining intensity. At the beginning of seed germination, embryos from different treatments were bright red. However, the embryo from the seeds treated with 200 mM NaCl has become yellow after 24 h of germination. Therefore, the embryo could become unviable after 48 h of salt stress.

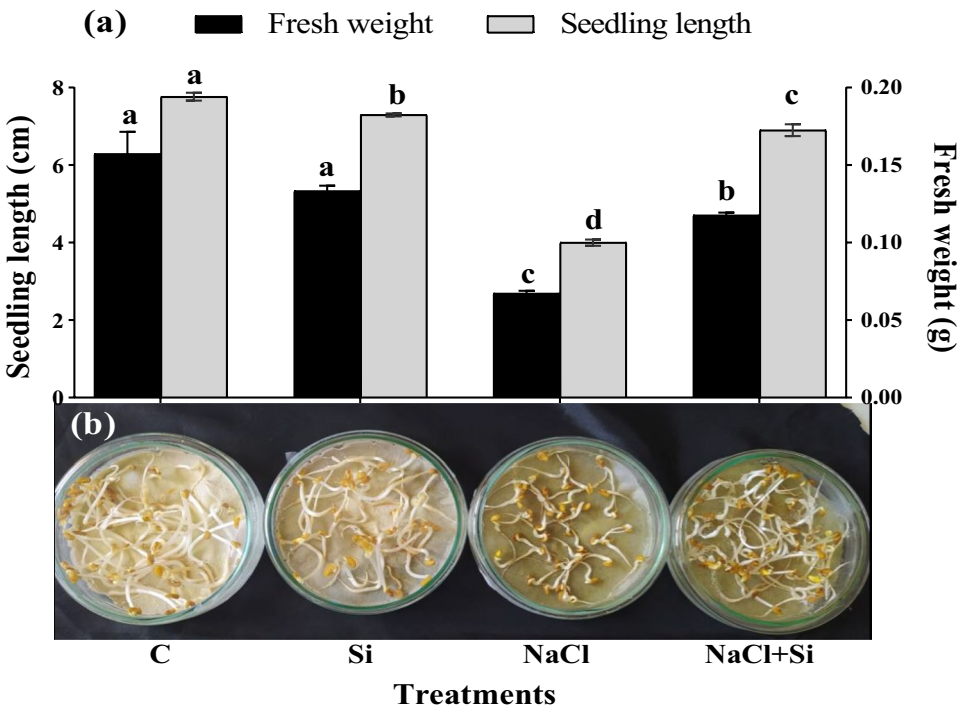

Fig. 1 Effect of silicon (Si) and salt stress on fresh weight (a), length (a) and phenotype (b) of 8-day-old fenugreek seedlings. Bars represent standard errors of three replicates and the values followed by different small letters are significantly different at $P < 0.05$. C: Control; Si: 3 mM Si; NaCl: 200 mM NaCl

However, this effect was alleviated by exogenous Si supplementation. Indeed, when Si was applied to 200 mM NaCl stressed-seeds, the embryo retained a red color during the whole experiment. The same was observed under control or Si-treatment alone, where the embryos were bright red during the whole experiment.

Effect of Si and salt stress on cell membrane integrity and oxidative stress markers

Salt stress induced significant effects on cell membrane integrity and oxidative stress markers. As compared to control, EL, MDA and H_2O_2 contents were significantly ($P < 0.05$) increased by 270%, 53% and 400% under salt stress. However, the presence of Si in rooting medium remarkably reduced all of the EL (22%), MDA (23%) and H_2O_2 (48%) relative to salt-stressed seedlings. Under non-stressed conditions, there was no significant difference ($P > 0.05$) between control and Si-treatment alone for EL and MDA content (Fig. 4a and b). However, for H_2O_2 content (Fig. 4c), Si supplementation obviously reduced H_2O_2 content by 60% relative to Si-untreated seedlings.

Results of histochemical staining indicated a significant accumulation of O_2^- in the fenugreek seedlings, which was represented by high intensity of the dark blue stain when seedlings are exposed to salt stress (Fig. 4d). However, the exogenous supplementation of Si remarkably reduced the intensity of the dark blue stain of formazan in salt-stressed seedlings. Furthermore, under unstressed conditions, no difference was noted between Si-treated and untreated fenugreek seedlings.

Effect of Si and salt stress on enzymatic and non-enzymatic antioxidant activity

Salinity and exogenous Si affect significantly ($P < 0.001$) the antioxidant activity of 8-day-old fenugreek seedlings as depicted in Table 3. The results indicated that salt stress significantly ($P < 0.001$) decreased the activity of both SOD and PPO by 75% and 73%, respectively as compared to control. However, Si treatment markedly ($P < 0.001$) enhanced the activity of SOD and PPO by 85% and 158% under salt stress, respectively in comparison to Si-untreated salt-stressed seedlings. However, when compared to control, the treatment with Si alone has no significant effect ($P > 0.05$) on SOD and PPO activities.

Regarding non-enzymatic antioxidant molecules, Si treatment significantly ($P < 0.001$) increased total polyphenol content. The increase was more furthered when Si was applied simultaneously with NaCl. Indeed, upon Si treatment, total polyphenol content was increased by 76% in Si-treated seedlings alone and 194% in Si - treated salt-stressed seedlings as compared to control. Under salt stress, total polyphenol content was decreased by more than 20% ($P < 0.001$). However, for flavonoid content, although Si and salt treatment alone had no significant effect, the simultaneous application of Si and NaCl significantly ($P \leq 0.001$) enhanced the content of flavonoid by more than 243% and

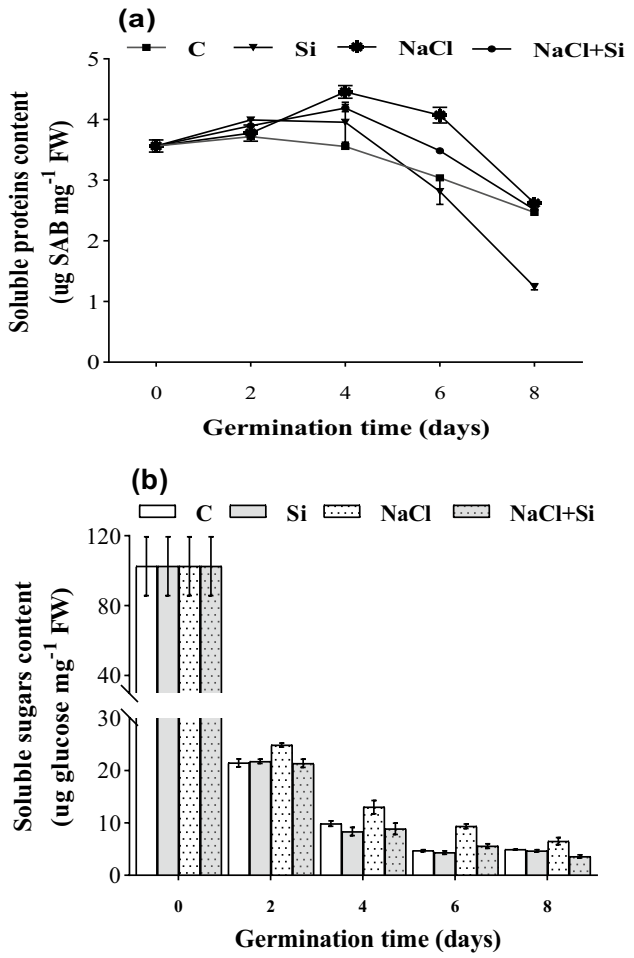

Fig. 2 Effect of silicon (Si) and salt stress on use of soluble proteins (a) and soluble sugars (b) by embryo of fenugreek seeds during seed germination. Bars represent standard errors of three replicates. C: Control; Si: 3 mM Si; NaCl: 200 mM NaCl

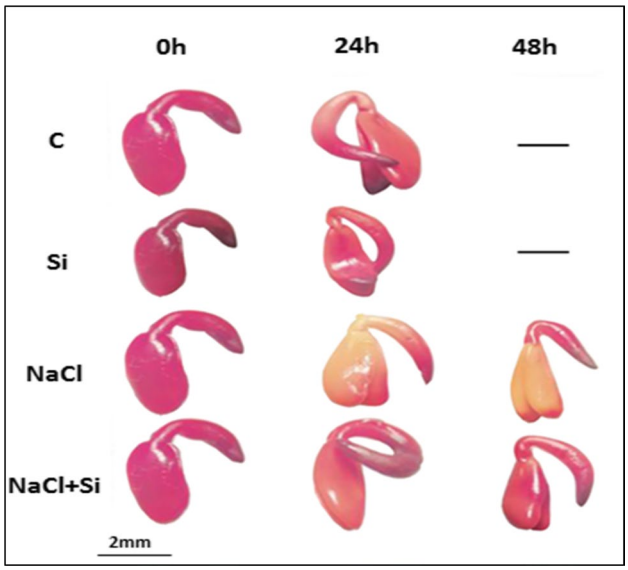

Fig. 3 Effect of silicon (Si) and salt stress on embryo viability of fenugreek seeds at different germination times (0 h, 24 h and 48 h after the beginning of seed germination). The embryo viability was evaluated by staining pattern and red color intensity of embryo after their immersion in 2, 3, 5 triphenyltetrazolium chloride (TTC) solution. Living embryo tissues are red stained, while dead embryo tissues are unstained with red. At 48 h of germination time, hyphens mean that all tested seeds have been germinated. C: Control; Si: 3 mM Si; NaCl: 200 mM NaCl

171%, respectively as compared to control and NaCl-treated seedlings (Table 3).

Effect of Si and salt on compatible solutes

Glycine betaine and proline contents in 8-day-old fenugreek seedlings are presented in Fig. 5. The obtained data showed that glycine betaine content was significantly ($P < 0.001$) reduced by 79% under salt stress. However, 3 mM Si application significantly ($P < 0.001$) alleviated this effect and increased the glycine betaine content in salt-stressed seedlings by up to 122% as compared to salt-stressed and Si-untreated seedlings. In contrast, the content of this compatible solute was decreased after Si supplementation under non-stressed conditions.

For proline content, relative to control, a significant ($P < 0.001$) increase of 95% was observed upon salt stress and interestingly this increase was further important when Si was combined with NaCl (147%). However, no significant ($P > 0.05$) difference was found between Si-treated and untreated seedlings under non-stressed conditions (Fig. 5).

Effect of Si and salt stress on Si, Ca^{2+}, Na^{+} and K^{+} contents

The effects of NaCl stress and Si treatment on nutrient contents in 8-day-old fenugreek seedlings are presented in Table 4. As compared to control, Si content was significantly increased in Si-treated and stressed seedlings ($P \leq 0.001$) or non-stressed ones ($P < 0.001$). In addition, our results showed that Si application has no significant ($P > 0.05$) effect on Ca^{2+}, K^{+} and Na^{+} contents as compared to Si-untreated seedlings under unstressed conditions. However, in comparison to the control, the content of Ca^{2+}, K^{+} and Na^{+} was respectively 2.48, 2.65 and 10.72-fold higher in the presence of NaCl in rooting medium. The application of Si to stressed seedlings decreased all of the Ca^{2+}, K^{+} and Na^{+} contents by 20%, 68% and 70%, respectively relative to salt stress.

Pearson's correlation matrix analysis

Analysis of Pearson's correlation matrix (Fig. 6) presented a significant negative correlation between Na^{+} content and

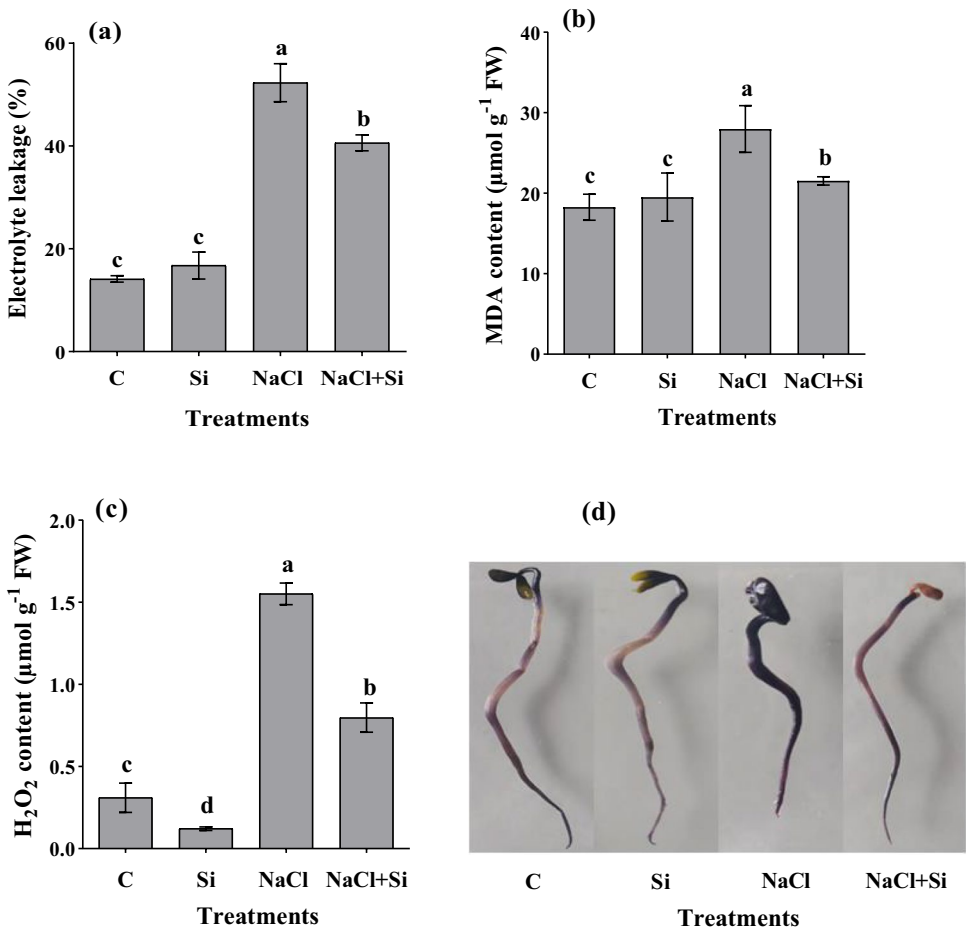

Fig. 4 Effect of silicon (Si) and salt stress on electrolyte leakage percentage (a), malonyldialdehyde (MDA) (b) and hydrogen peroxide (H_2O_2) (c) contents and superoxide anion (O_2^-) accumulation (d) in 8-day-old fenugreek seedlings. Histochemical detection of O_2^- was assessed by staining with nitro blue tetrazolium chloride (NBT). Bars represent standard errors of three replicates and the values followed by different small letters are significantly different at $P < 0.05$. C: Control; Si: 3 mM Si; NaCl: 200 mM NaCl

germination parameters including FGP (r = -0.98), GS (r = -0.86), VI (r = -0.92), GE (r = -0.86), PV (r = -0.90), GV (r = -0.92) and vitality index (r = -0.92). Additionally, glycine betaine content (r = -0.84) and antioxidant activity, in terms of SOD (r = -0.92) and PPO (r = -0.94), were also negatively correlated with Na^+ content. However, between Na^+ content and MGT (r = 0.94), proline content (r = 0.55) and oxidative stress markers, such as MDA (r = 0.81), H_2O_2 (r = 0.97) and EL (r = 0.88), there were significant positive correlations, explaining the harmful effects of 200 mM NaCl stress on seedling length (r = -0.97) and fresh weight (r = -0.87). In addition, a significant negative correlation (r = -0.86) was obtained between Na^+ and Si content. In contrast, our results showed a significant positive correlation between Si content and FGP (r = 0.82), GS (r = 0.61), VI (r = 0.69), GE (r = 0.62), PV (r = 0.65), GV (r = 0.69), vitality index (r = 0.67), SOD (r = 0.69), PPO (r = 0.67) and total polyphenol (r = 0.66). Whereas, there were significant negative correlations between MGT (r = -0.72), oxidative stress indicators (MDA (r = -0.71), H_2O_2 (r = -0.85) and EL (r = -0.64)) and Si content, indicating that Si treatment interestingly improved seedling length (r = 0.83) and fresh weight (r = 0.63) as well as seed germination of fenugreek.

Discussion

Seed germination, as a critical stage in the plant life cycle, is the most sensitive stage to abiotic stresses including salinity, which causes in the same time osmotic stress and ionic toxicity (Farissi et al. 2011; Peng et al. 2016). For this reason, salinity inhibited seed germination and seedling growth of various aromatic and medicinal plants such as *Thymus daenensis, Thymus kotschyanus* and *Origanum compactum* (Khoshsokhan et al. 2012; Laghmouchi et al. 2017). According to Calone et al. (2020), determination of germination parameters is among the most important and suitable criteria to assessing salt stress tolerance in plants. In this context, Gou et al. (2020) found that germination percentage, germination index and seedling vigor was significantly reduced in cucumber under 200 mM NaCl. In addition, Hosein and Keshavarzi (2012) showed that germination traits including plumule length, radicle length, seed vigor and fresh and dry seedling weight of *Artemisia annua* L. were drastically decreased upon salt stress. Similar to the above studies, our findings showed that salinity stress, induced by 200 mM NaCl, significantly ($P < 0.001$) reduced germination traits of fenugreek. Inhibition of seed germination of fenugreek

Table 3 Effect of silicon (Si) and salt stress on enzymatic and non-enzymatic antioxidant activity of 8-day-old fenugreek seedlings. The represented data are the means of three replicates ± standard error (SE). Between treatments, the means followed by the same letters are not significantly different at $P < 0.05$

Treatments		Non-enzymatic antioxidant activity				Enzymatic antioxidant activity			
		Total polyphenol (mg gallic acid g^{-1} FW)		Flavonoid (mg quercetin g^{-1} FW)		SOD (U mg^{-1} proteins)		PPO (U min^{-1} mg^{-1} proteins)	
		- Si	+ Si	- Si	+ Si	- Si	+ Si	- Si	+ Si
- NaCl		3.06 ± 0.10^{c}	5.40 ± 0.25^{b}	1.05 ± 0.06^{b}	1.33 ± 0.40^{b}	96.22 ± 0.37^{a}	93.81 ± 0.80^{a}	1.19 ± 0.01^{a}	0.90 ± 0.00^{b}
+ NaCl		2.44 ± 0.01^{d}	9.02 ± 0.14^{a}	1.33 ± 0.00^{b}	3.61 ± 0.26^{a}	23.83 ± 0.00^{c}	44.11 ± 2.32^{b}	0.31 ± 0.00^{d}	0.80 ± 0.01^{c}

-NaCl: 0 mM NaCl; + NaCl: 200 mM NaCl; -Si: 0 mM Si; + Si: 3 mM Si; SOD: Superoxide dismutase; PPO: Polyphenol oxidase

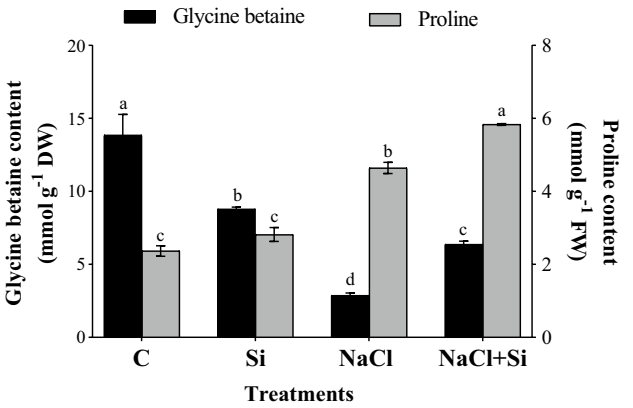

Fig. 5 Effect of silicon (Si) and salt stress on glycine betaine and proline contents in 8 day-old fenugreek seedlings. Bars represent standard errors of three replicates and the values followed by different small letters are significantly different at $P < 0.05$. C: Control; Si: 3 mM Si; NaCl: 200 mM NaCl

due to salinity was also reported by Mahmoudi et al. (2019). However, in this study, the treatment with exogenous Si markedly improved all tested germination parameters ($P < 0.05$) by reducing significantly MGT ($P < 0.001$). In the same way, Gou et al. (2020) observed that 0.3 mM Si could raise seed germination percentage and germination index of cucumber under NaCl stress. Similarly, the germination rate, germination index and velocity index of salt stressed *Momordica charantia* L. seeds were significantly improved after treatment with exogenous Si (Wang et al. 2010). Also, Zhang et al. (2017) showed that 1 mM Si addition ameliorated germination rate, germination index and seedling vitality index of *Glycyrrhiza uralensis* Fisch. seeds under NaCl stress combined with drought stress. Furthermore, in this work, results recorded for fresh weight and seedling length, suggested that Si treatment markedly enhanced growth and phenotype of fenugreek seedlings under salt stressed conditions. The same results were reported in seedlings of *Glycyrrhiza uralensis* (Zhang et al. 2015), *Lycopersicum esculentum* Mill. (Haghighi et al. 2012) and *Cucumis melo* L. (Zhang et al. 2020). In the same line, Almutairi (2016) showed that tomato seedling growth, in terms of root length and fresh weight, were significantly improved after treatment with nano- Si under salt stress.

Benmahioul et al. (2009) reported that 256.6 mM NaCl might reduce the germinated embryo survival rates from 100% to 62.9% referred to control in *Pistacia vera* L. The same was found in this study after TTC staining, suggesting that the embryonic cells of salt-stressed seeds was became unviable after 48 h of exposure to salt stress. Conversely, the embryo in Si treated seeds stayed completely viable at different times of experimentation under salinity. These results explained the observed reductions in the germination parameters of salt-stressed seeds, also suggested that the

Table 4 Effect of silicon (Si) and salt stress on Si, calcium (Ca^{2+}), sodium (Na^{+}) and potassium (K^{+}) contents in 8-day-old fenugreek seedlings. The represented data followed by the same letters at same parameters are not significantly different at $P < 0.05$

Treatments	Ions content							
	Si (mg g^{-1} DW)		Ca^{2+} (mg g^{-1} DW)		Na^{+} (mg g^{-1} DW)		K^{+} (mg g^{-1} DW)	
	- Si	+ Si	- Si	+ Si	- Si	+ Si	- Si	+ Si
- NaCl	70.62c	96.10a	14.41c	14.83c	46.17c	46.20c	102.89b	104.34b
+ NaCl	37.81d	83.43b	35.83a	28.65b	495.28a	147.35b	272.90a	86.15c

-NaCl: 0 mM NaCl; + NaCl: 200 mM NaCl; -Si: 0 mM Si; + Si: 3 mM Si

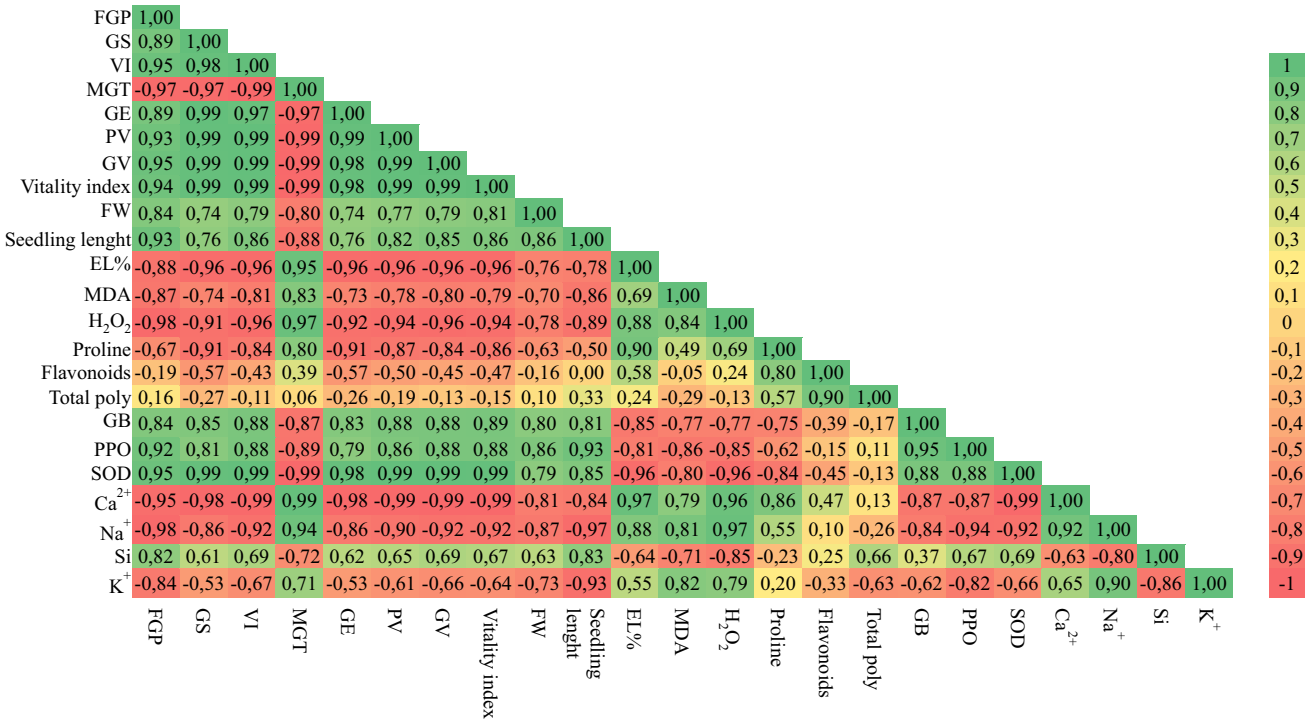

Fig. 6 Pearson's correlation matrix between germination parameters, oxidative stress markers, compatible osmolyte, ions content and enzymatic and non-enzymatic antioxidant systems of 8 day-old fenugreek seedlings treated with 0 or 200 mM NaCl with or without 3 mM Si. In addition to correlation coefficient (r), the positive correlations are displayed in green while red color revealed the negative correlations. FGP: Final germination percentage; GS: Germination speed; VI: Velocity index; MGT: Mean germination time; GE: Germination energy; PV: Peak value; GV: Germination value; GB: Glycine betaine; MDA: Malonyldialdehyde; H_2O_2: Hydrogen peroxide; EL: Electrolyte leakage; SOD: Superoxide dismutase; PPO: Polyphenol oxidase; Si: Silicon; Ca^{2+}: Calcium; Na^{+}: Sodium; K^{+}: Potassium; FW: Fresh weight of seedlings

exogenous Si kept embryo viability as well as seed germination process under salt stress.

The negative effects of salt stress on seed germination could be the results of an osmotic stress, as the water content of the seed was reported to diminish (Salahuddin et al. 2017). It was also reported that this abiotic stress unregulated genes expression of gibberellic acid and abscisic acid and consequently disturbing hormonal balance in seed during germination stage (Sebei et al. 2007; Liu et al. 2019). On the other hand, several studies were correlated the deleterious effects of salt stress on seed germination with ions toxicity, which induced by higher accumulation of Na^{+} and Cl^{-} in embryotic cells during the exposure of seeds to salt stress (Farissi et al. 2011; Li et al. 2019). In the same way, Bonilla et al. (2004) suggested that most toxic effects of NaCl can be attributed to Na^{+} toxicity. Similar results were found in the present work, where salt stress caused a significant ($P < 0.001$) increase of Na^{+} content comparatively to control. Also, this increase of Na^{+} content was negatively correlated with all studied germination parameters exempt MGT, explaining the negative effects of salt stress on fenugreek seedlings growth. In contrast, the exogenous Si treatment reduced Na^{+} accumulation by 70%, but enhanced endogenous Si content under even stressed or unstressed

conditions. More than that, the result of statistical analysis showed significant positive correlations between Si content and all germination traits exempt MGT, which was negatively correlated with endogenous Si content. Therefore, in this work, the observed salt alleviating effects through Si on seed germination might be due to higher Si uptake and accumulation in Si-treated seedlings under salinity stress. Similarly, Biju et al. (2017) explained the positive effects of Si on seed germination of lentil under drought stress by deposition of Si in cell walls of lentil. In the same line, Al-Saeedi et al. (2017) reported that addition of 300 mg L^{-1} of Nanosilica significantly reduced Na^+ ion accumulation in *Phaseolus vulgaris* L. seedlings under Na^+ stress.

Under salt stress, seed reserve mobilization is inhibited by immobilization of nutrient reserve such as amino acids and sugars stored at albumen (Sebei et al. 2007; Aghaei and Komatsu 2013). Similarly, the present research proved that salt stress decreased the use of soluble proteins and sugars by embryo during seed germination. Indeed, it was reported that many important hydrolytic enzymes like amylase and glycosidase are drastically affected during seed germination under salt stress. In this context, Liu et al. (2019) reported that the activity of amylase was significantly inhibited under 200 mM NaCl in *Limonium bicolor*. Also, Hua-long et al. (2014) found that the activities of α-amylase, β-amylase and the total amylase were significantly reduced during seed germination of rice under salt stress. In our study, we noted that Si addition enhanced seed reserve mobilization under salt stress, which could explained by the decrease in soluble protein and soluble sugar contents during seed germination of Si-treated seeds under salinity stress. To understand the seed reserve mobilization enhancement by exogenous Si, Zhang et al. (2020) demonstrated that Si treatment up-regulated amylases genes expressions, which in return improved significantly its activity in melon seed under auto-toxicity stress. Also, Biju et al. (2017) found that Si supplied under drought stress significantly enhanced the activity of α and β-amylase and α-glycosidase in *Lens culinaris* L. The same was reported by Gou et al. (2020) of α-amylase activity, where the added Si significantly improved α-amylase activity in cucumber seeds under salt stress. Therefore, these findings exhibited that Si might have a crucial effect on reserve mobilization to enhance seed germination of fenugreek under salt stress.

It was reported that abiotic stresses, including salinity stress, affected seed germination by triggering the over production and accumulation of ROS such as O_2^- and H_2O_2, resulting in membrane peroxidation and even cell death (Luo et al. 2021). This is in agreement with our results, where O_2^- and H_2O_2 accumulations were markedly raised in fenugreek seedlings under salt stress, inducing in turn a significant increase in MDA content and EL ($P < 0.05$). In addition, exposure of fenugreek seeds to 200 mM NaCl caused a significant ($P < 0.001$) decrease in the activities of SOD and PPO antioxidant enzymes and the content of total polyphenol and flavonoid. In the same line, our result found a significant positive correlation between oxidative stress markers, in terms of MDA ($r = 0.81$), H_2O_2 ($r = 0.97$) and EL ($r = 0.88$), and the content of Na^+, which was negatively correlated with SOD ($r = -0.92$) and PPO ($r = -0.94$). According to these results, reduction in both enzymatic and non-enzymatic antioxidant activities could decreased the ability of fenugreek seedlings to scavenge O_2^- and H_2O_2 radicals, inducing in turn an accumulation of ROS in seed under salt stress, which might cause the membrane oxidative damages, reflected by high MDA content and EL. The decline in the SOD activity, during seed germination, was reported also by Sekmen et al. (2012), where the activity of SOD and CAT enzymes and the intensity of their isoenzymes were significantly decreased in *Gypsophila ablanceolat* Bark. under 50 mM NaCl stress as compared to control. Also, at plant stage, Ben Taârit et al. (2012) found that, the total polyphenol and antioxidant activity of *Salvia sclarea* L. were reduced with an increase in salt stress from 50 to 75 mM NaCl. In the same way, Jaleel et al. (2007) demonstrated that 80 mM NaCl treatment significantly decreased overall growth of *Catharanthus roseus* L. and reduced the proteins content and the activities of some antioxidant enzymes like POX, SOD and PPO. Other studies confirmed these findings regarding decline in the antioxidant activity under salt stress (Abdul Jaleel et al. 2008; Perveen et al. 2011; Amraee et al. 2020). However, in this work, Si treatment significantly ($P < 0.001$) reduced O_2^- and H_2O_2 accumulations under salt stress, suggesting that Si has a crucial role on reduction of ROS production to alleviate oxidative damage induced by 200 mM NaCl stress. In addition, an important decline in MDA content and EL was also obtained after Si treatment. Thus, Si could protect membrane from salt-induced lipid peroxidation. The decrease in oxidative stress markers through exogenous Si supplementation could be explained by the improvement of antioxidant activity of fenugreek seedlings under salt stress. In fact, the present study showed that Si treatment significantly ($P \leq 0.001$) enhanced the content of total polyphenol and flavonoid and the activities of SOD and PPO, thus it improved both enzymatic and non-enzymatic antioxidant activities of fenugreek under salt stressed conditions. These finding were confirmed by the statistical analysis, which showed a significant positive correlation between Si content and SOD ($r = 0.69$), PPO ($r = 0.67$) and total polyphenol ($r = 0.66$), while there was a significant negative correlation between endogenous Si content and all of Na^+ content ($r = -0.86$), MDA ($r = -0.71$), H_2O_2 ($r = -0.85$) and EL ($r = -0.64$). Similar results were suggested by Wang et al. (2010) in *Momordica charantia*. In the same way, Zhang et al. (2015) suggested that supplemented Si enhanced seed germination of *Glycyrrhiza uralensis* by reducing MDA content and improving antioxidant enzyme

activity under salt stress. Also, Shi et al. (2014) reported that Si application significantly reduced oxidative stress by enhancing the activities of antioxidant enzymes like SOD and CAT in *Solanum lycopersicum* L. under water stress.

On the other hand, several studies demonstrated that during germination phase, the seeds accumulate a variety of organic and inorganic solutes to induce an osmotic adjustment, and in order to maintain their germination under salt stress (Thakur and Sharma 2005; Farissi et al. 2011). In fact, osmotic regulation is one of the important mechanisms to increase the concentration of cell fluid to maintain the ability of cell absorption or water retention. In this context, it was reported that glycine betaine and proline are important compatible solutes (İbrahimova et al. 2021). Indeed, when the cell is dehydrated, proline acts as a chaperone and prevents the structure of macromolecules from destruction (Tang et al. 2015). Also, glycine betaine, as an organic compound, is maintains the structure of macromolecules by scavenging ROS under salt and drought stresses (Cha-Um and Kirdmanee 2010). Importantly, our results found that application of exogenous Si interestingly improved the content of proline and glycine betaine, as key compatible solutes, in salt stressed fenugreek seedlings, alleviating in turn osmotic stress induced by 200 mM NaCl stress. Similarly, Zhang et al. (2017) reported that Si addition significantly increased proline content in *Glycyrrhiza uralensis* seedlings to regulate osmotic stress induced by salinity and drought stresses. Altogether, to enhance seed germination of fenugreek under salt stressed conditions, supplemented Si reduced oxidative stress by improving antioxidant activity and accumulation of osmolyte compounds. In the same line, Wang et al. (2010) reported that exogenous Si may increase GR, GI and VI through their contribution to reducing oxidative stress and increasing antioxidant activity in *Momordica charantia* under NaCl stress.

Conclusion

Taken together, the treatment with exogenous Si significantly increased germination parameters, fresh weight and seedling length of fenugreek under salt stress. Furthermore, the Si treatment improved seed reserve mobilization and embryo viability under this abiotic stress. The enhancement of salt tolerance through Si during seed germination might explained by a significant increase in the content of endogenous Si, which was correlated with a significant decrease in lipid peroxidation and ROS production. In addition to these beneficial effects, exogenous Si also significantly improved the antioxidant activity and the accumulation of osmolyte compounds like proline and glycine betaine in fenugreek seedlings under salt stress. Hence, according to above results, application of Si could alleviate the harm effects of salt stress on seed germination of fenugreek and enhance its salt tolerance by improving seed reserve mobilization, antioxidant activity and osmolytes accumulation, thus it might be a potential approach to improve seed germination of fenugreek in salt-affected soils.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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