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RESEARCH ARTICLE

Seed priming alleviates salt stress in two fenugreek (*Trigonella foenum-graecum* l.) Cultivars

Sajjad Hussain[1], Hafiza Muzzamil Asif[1], Shakeel Ahmad[2], Muhammad Arif Ali[3], Shaghef Ejaz[1], Tahira Abbas[4], Sakeena Tul-Ain Haider[1], Sezai Ercisli[5], Talaat Ahmed[6], Muhammad Sohail[1], Muhammad Fasih Khalid[6]*

[1]Department of Horticulture, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan, [2]Department of Agronomy, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan, [3]Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan, [4]College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus, Layyah, Pakistan, [5]Department of Horticulture, Faculty of Agriculture Ataturk University 25240 Erzurum, Turkey, [6]Environmental Science Center, Qatar University, 2713, Doha, Qatar

ABSTRACT

Salinity is globally considered a widespread problem of the irrigated soils in arid and semi-arid areas. To minimize the negative effect of salinity seed priming technique is proved as a useful by improve germination and seedling growth. Therefore, the current study was conducted to evaluate the effect of different priming techniques on fenugreek under saline conditions. The priming treatments were potassium nitrate (KNO_3), polyethylene glycol (PEG), gibberellic acid (GA), hydrogen peroxide (H_2O_2), salicylic acid (SA) and distilled water (D/W). Two fenugreek cultivars i.e. Kasuri methi and Local methi were evaluated under two salinity levels *viz*. 0 mM and 100 mM. In Kasuri methi (V1), priming with GA_3 and PEG enhanced germination index (37.52%) and energy of emergence (98.33%) as compared with other treatments. Final emergence percentage (100%) was increased in control plants of both cultivars when treated with SA. Morphological characteristics such as number of leaves (57.50), number of branches (19.16), shoot length (18.03cm), root length (8.98cm), plant fresh (2.34g) and dry biomass (1.21g) was increased in control plants of Kasuri methi (V1) when primed with SA. Leaf chlorophyll "a" (1.06 mg/g) and chlorophyll "b" (1.30 mg/g) was significantly increased in control plants of Local methi (V_2) primed with SA. Antioxidant activity, antioxidant capacity and proline contents in leaves as well as in roots were also increased when treated with SA under salt stress. It is concluded that seeds of Kasuri methi should be treated with SA in order to reduce the effect of salinity and improve the germination, morphological and biochemical characteristics.

Keywords: Defense mechanism; Germination; Growth; Salinity; Seed priming

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is a member of the Leguminosae family Fabaceae. It is an ancient plant having origin in central Asia and dates back to 4000 BC (Altuntas et al., 2005). On a commercial basis, it is widely cultivated in different regions of the world including Pakistan, India, Egypt, Argentina, Turkey, Iran, and Morocco (Flammang et al., 2004; Altuntas et al., 2005). Fenugreek leaves are rich in nutrients such as iron, zinc, and calcium and it is also considered a valuable medicinal herb containing ascorbate and vitamin A in good amounts (Thomas et al., 2011). As it is leguminous and can fix nitrogen it can successfully be grown on marginal lands having salinity issues (Ali et al., 2012; Solorio-Sanchez et al., 2014). The leguminous nature may be used in reducing the effect of salinity and germination issues.

Salinity is considered a widespread problem in the irrigated soils in arid and semi-arid areas (Khalid et al., 2019; Latef et al., 2021a, b, c; Abdelhameed et al., 2021; Dawood et al., 2021). The major causes of the salinity are poor irrigation practices and no drainage consideration, increased evaporation, and saline irrigation water (Munns and Tester, 2008). The success of the crop is greatly affected by the germination ability and seed vigor and these two phases are mostly influenced by the abiotic stresses (Fazlali et al., 2013). Various studies indicated that seed germination percentage decreased and germination time increased under saline conditions (Jamil et al., 2005; Patade et al., 2011; Rouhi et al., 2011; Omid et al., 2012).

To increase seed germination different researchers have analyzed different mechanisms among which seed priming is the most applicable technique. Priming enables the seeds to quickly obtain

***Corresponding author:**
Muhammad Fasih Khalid, Environmental Science Center, Qatar University, 2713, Doha, Qatar. **E-mail:** fasihkh65@gmail.com

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sufficient moisture and constant oxygen to facilitate germination by overcoming germination barriers, especially under adverse conditions such as salinity, drought, etc (Khalid et al., 2019a; Sadeghi et al., 2011). The seed germination can be improved by different mechanisms and the most applicable phenomenon is the seed priming. Seed priming increased the seed germination and germination speed by overcoming germination barriers that emerge during germination. (Farooq et al., 2006, 2009). The achievable results of priming are more significant under unfavorable than favorable environments (Ashraf and Foolad, 2005; Chen, 2011). The significant results of seed priming under a saline environment have been reported by various scientists such as chili pepper (*Capsicum annuum*) (Khan et al., 2009), tomato (*Solanum lycopersicum* L.) (Pradhan et al., 2014), okra (*Abelmoschus esculentus* L.) (Dkhil et al., 2014) and lettuce (*Lactuca sativa* L.) (Nasri et al., 2011). Priming enhanced the metabolic activity of different germination affecting enzymes and it also affected the mobilization of organic compounds in the embryo. (Ahmed et al., 2021; Nasri et al., 2011) reported that acid phosphatase and phytase activities increased under salt stress when primed with KNO_3.

Seed priming stimulates the functions which are related to germination and enhances germination rate, vigor, growth, development, and many other attributes in plants. Furthermore, priming increases the plant biochemical process by induction and hydrolyses synthesis (e.g., α-amylase) and sugar levels through germination while nitrate reductase activity and nitrogen content in the growing seedlings under the normal condition to concerning untreated seeds (Anaytullah and Bose, 2007).

Seed priming with growth regulators increased the antioxidant enzyme activities and made the seed tolerant to the saline conditions (Hela et al., 2012; Younesi and Moradi, 2015). Gibberellic acid reduced abscisic acid (ABA) activity and enhanced seed germination. Salicylic acid (SA) is another stress releasing chemical and priming with SA exhibited higher seed germination under saline conditions as compared with control. Salicylic acid improves the production of antioxidant enzymes such as peroxidase, catalase, and ascorbate reductase involved in stress tolerance (Azooz, 2009). As discussed earlier the fenugreek production is declining due to salinity and to conquer that cheapest way is the seed priming technique. Therefore, the current study was conducted to evaluate the role of different seed priming agents on the physical and biochemical characteristics of fenugreek cultivars under salt stress conditions.

MATERIALS AND METHODS

The present study was conducted to assess the salt tolerance of fenugreek cultivars primed with different compounds. The experiment was performed in the nursery area, the Department of Horticulture, Bahauddin Zakariya University, Multan. There were six priming treatments were selected after the preliminary study, *viz*. T_1= Potassium nitrate (KNO_3) (20%), T_2= Polyethylene glycol (PEG) (20%), T_3= Gibberellic acid (GA_3) (20%), T_4= Hydrogen peroxide (H_2O_2) (30%), T_5= Salicylic acid (SA) (30%) and T_6= distilled water. There were two fenugreek cultivars i.e. Kasuri methi and Local methi and two salinity levels *viz*. 0 mM and 100 mM. Seeds were purchased from the local seed market. Seeds were dipped in respective priming solutions for 8 hours and then dried for 24 hours (Singh et al., 2015). After drying, seeds were sown in plastic containers filled with silt as a growing medium. Line sowing was done with R×R= 5 cm. On every irrigation, normal irrigation water was applied to the 0 mM treatment whereas NaCl solution was applied to the 100 mM treatment. The experiment was arranged according to a Completely Randomized Design.

Data collection

Data for the following parameters were collected

Germination characteristics

Data for different germination characteristics were recorded after one week of germination.

The emergence index and energy of emergence were calculated according to the method developed by the Association of Official Seed Analysts (1983). Final Emergence Percentage (FEP) was determined by using the following formula.

$$\text{Final emergence percetnatge} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

Growth attributes

At the end of the experiment, tagged plants were uprooted and the following growth parameters were determined. Leaves and branches were counted. The length of the shoot and root was measured with a measuring scale. Plant fresh biomass was calculated by weighing the fresh weight of all plant parts using an electronic weighing balance and then drying in an oven at 65 °C for 48 hours. The dry weight of the respective plant parts was taken and dry biomass was calculated.

Biochemical parameters

Fresh samples of leaves and roots were used for biochemical analysis. Leaf chlorophyll 'a' and 'b' content was determined using the modified method of Harborne (1973) using following equations:

$$\text{Chlorophyll 'a' (mg/g)} = (A663 \times 0.0127) - (A645 \times 0.00269) / 0.5 \times 100$$

$$\text{Chlorophyll 'b' (mg/g)} = (A645 \times 0.0229) - (A6633 \times 0.00468) / 0.5 \times 100$$

Antioxidants and total phenols

Antioxidants and total phenolic contents were determined using the modified method of Ozgen et al. (2010) and absorbance was read at 515 nm at (UV-1900) spectrophotometer. Antioxidant activity was calculated using the following equation

$$\text{Antioxidant activity \%} = \frac{A\ \text{control} - A\ \text{sample}}{A\ \text{sample}} x\ 100$$

Antioxidant capacity was estimated by comparing the sample absorbance with a standard graph of Trolox. Total phenolic contents were determined by applying the methodology of singleton and Rossi (1965) using Folin-Ciocalteu's phenol reagent at 750 nm using (UV-1900) spectrophotometer and total phenols were calculated by comparing the absorbance values with the standard curve of gallic acid and results were computed in GAE/g FW.

Proline determination

Proline estimation in leaf and roots was carried out in freshly harvested samples. Proline in the sample was determined in ug proline/ml through the standard curve of proline while on fresh weight basis proline was calculated using the given equation (Bates et al., 1973)

$$\text{Proline } (\mu\text{moles } g^{-1}\ \text{FW}) = \frac{\mu g\ \text{proline per ml} \times 4 / 115.5\ \mu g\text{mole}^{-1}}{\textit{grame sample} / 5}$$

Statistical analysis

Data were analyzed using the statistical tool Statistics 8.1 (Tallahassee Florida, USA). LSD test was applied at a 5% probability to compare the treatment means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The data regarding different germination, physical as well as chemical parameters were statistically analyzed. The interactive effect of salinity, seed priming, and fenugreek cultivars was found statistically significant and the results are described as follows.

Germination characteristics

Various germination attributes were significantly affected by the salt stress and also among the priming treatments in fenugreek cultivars. The maximum emergence index (37.52 %) was recorded in control plants of Kasuri methi (V_1) primed with GA_3 (Fig.1). The energy of emergence was found to be maximum (98.33%) in control plants of Local methi (V_2) primed with GA_3 (Fig. 1). A statistically similar result was observed in PEG-treated plants. Regarding final emergence percentage, the maximum value (100%) was recorded in control plants of both cultivars when treated with SA (Fig. 1). Our results revealed that germination ability in fenugreek seeds was reduced in saline conditions. As the upper soil layer contained higher salt particles as compared

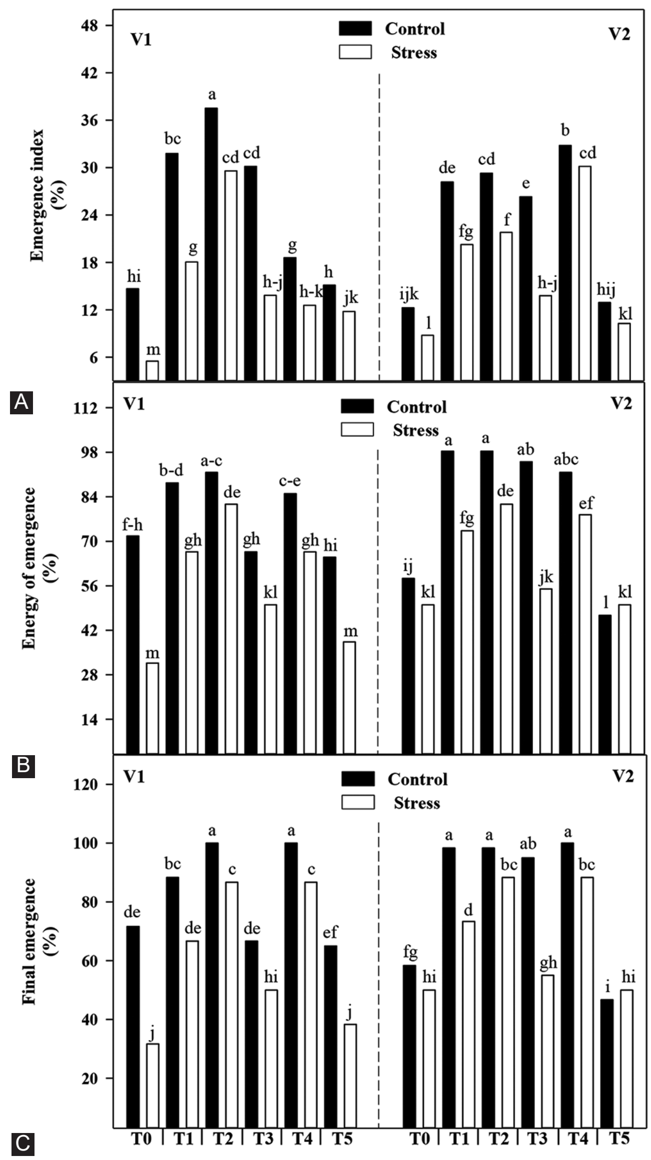

Fig 1. (A) Emergence index; (B) Energy of emergence; (C) Final emergence in two fenugreek cultivars treated by different priming solutions under salt stress. Values are mean ± S.E. at $p < 0.05$ (n = 3).

with the lower portion and seed is planted in the upper 10 cm layer (in most crops) (Esechie, 1995). Seed germination is strictly affected by abiotic stresses i.e., delayed germination (Khalid et al., 2019a). Germination of fenugreek decreased with salinity increment in the soil surface and germination was inhibited in higher salt concentrations (Khan and Weber, 2008). GA_3 plays a vital role in conservation and induction of seed dormancy and increases seed germination (Rao et al., 2019). Seeds treated with GA_3 significantly improved the seed germination percentage and germination index (Tahir et al., 2019). A similar positive role of GA_3 in saline conditions had been reported by (Sedghi et al., 2010) and (Hela et al., 2012) in sweet fennel and lettuce seeds. Final emergence percentage was found to improve when seeds were treated with SA. (Azooz, 2009) reported that SA enhanced the enzymatic activity of SA treated seeds, and resultantly final seed emergence was increased.

Physical attributes

Seed priming greatly affected the various physical characteristics of fenugreek cultivars under salt stress. The maximum number of leaves (57.50) was counted in control plants of Kasuri methi (V_1) primed with SA (Fig. 2). The number of branches (19.16) was maximum in control plants of Kasuri methi (V_1) primed with SA (Fig. 2). The maximum root length (8.98cm) and shoot length (18.03cm) were recorded in control plants of Kasuri methi (V_1) treated with SA (Fig. 2). Similarly, Kasuri methi (V_1) produced maximum plant fresh biomass (2.34 g) and plant dry biomass (1.21 g) in control plants primed with SA (Fig. 3). The maximum SPAD value (45.62) was recorded in control plants of Kasuri methi (V_1) primed with SA (Fig. 3). According to our results, SA induced a stimulatory effect in the control plants and also mitigated the inhibitory effect of salt stress in both genotypes. SA acts as a signaling molecule in the plant and altered plants' response biotic and abiotic factors. It has been reported that SA reduced the concentration of cytokines and other molecules, which results in the better division of cells in roots apical meristem and increased plant growth and development (Shakirova et al., 2003). SA also plays a vital role in the biosynthesis of organic foods, enhancing the uptake of water and increasing the availability of nutrients to the plants under salinity (Kalaivani et al., 2016). Similar findings have been reported by (Azooz, 2009) in two faba

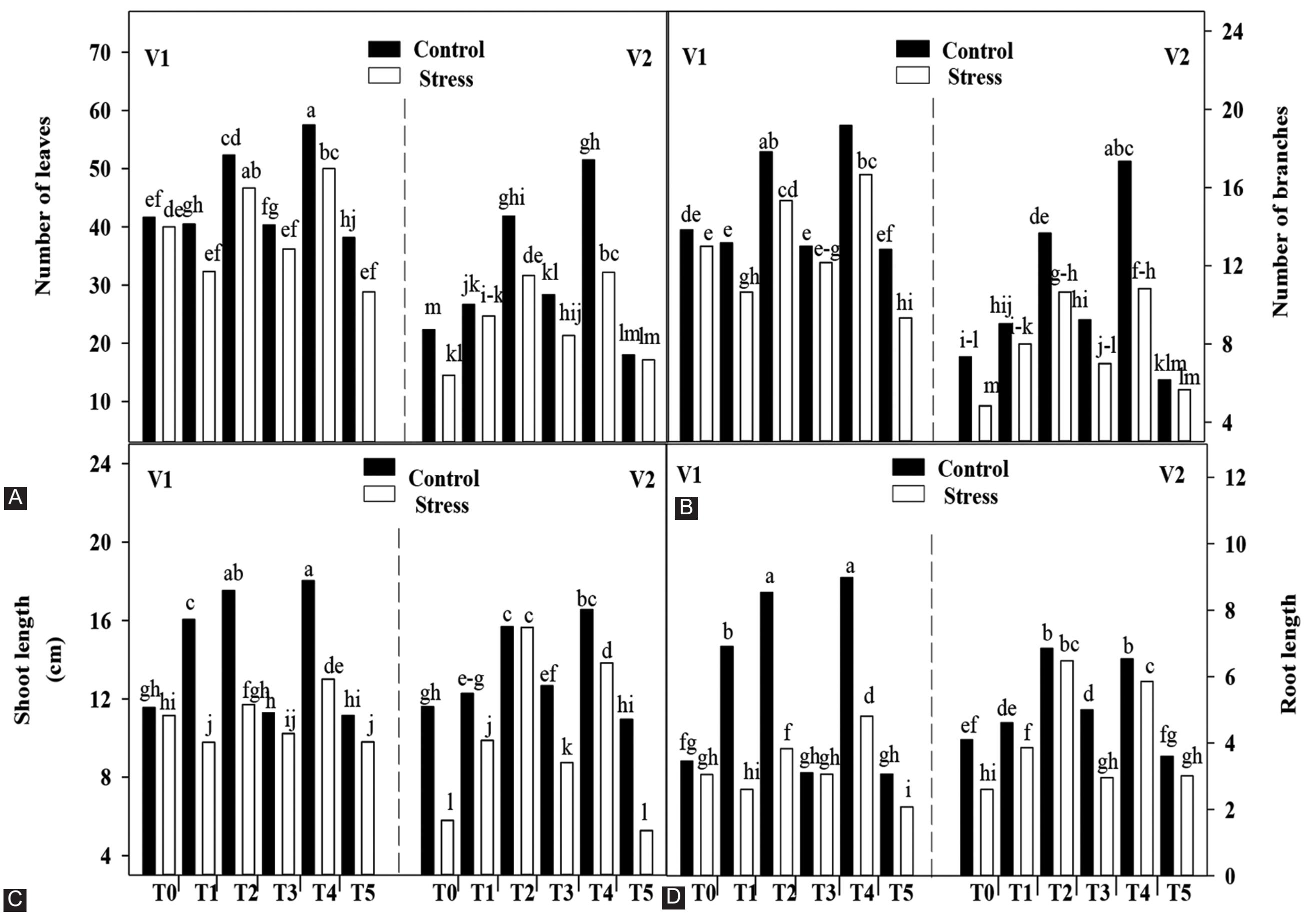

Fig 2. (A) Number of leaves; (B) Number of branches; (C) Shoot length; (D) Root length in two fenugreek cultivars treated by different priming solutions under salt stress. Values are mean ± S.E. at $p < 0.05$ (n = 3).

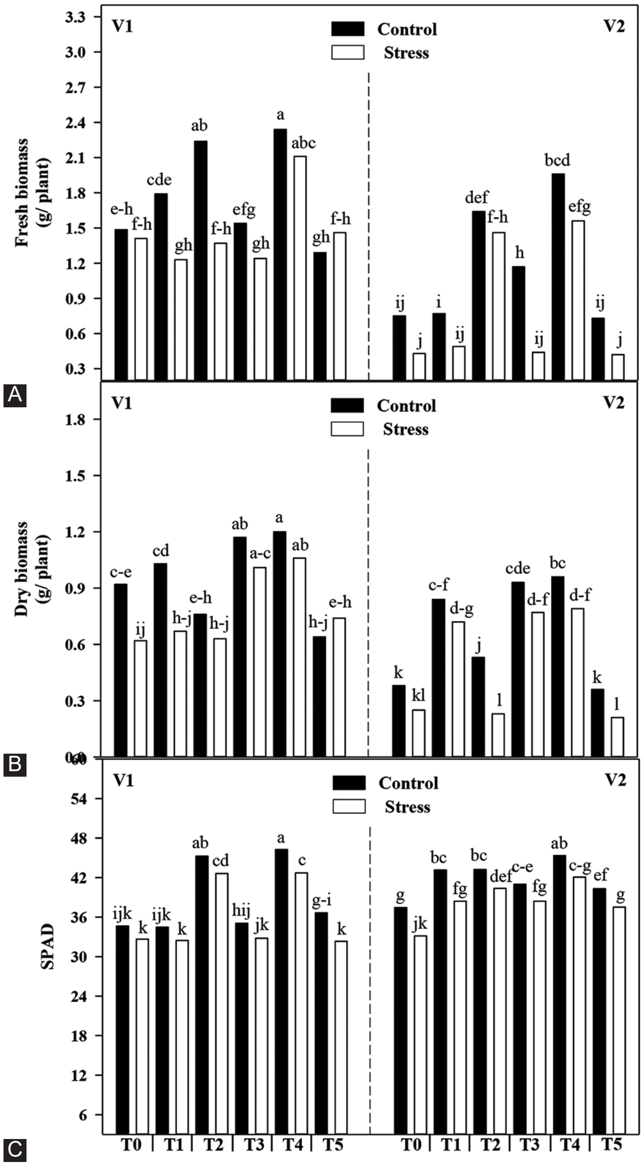

Fig 3. (A) Fresh biomass; (B) Dry biomass; (C) SPAD in two fenugreek cultivars treated by different priming solutions under salt stress. Values are mean ± S.E. at $p < 0.05$ (n = 3).

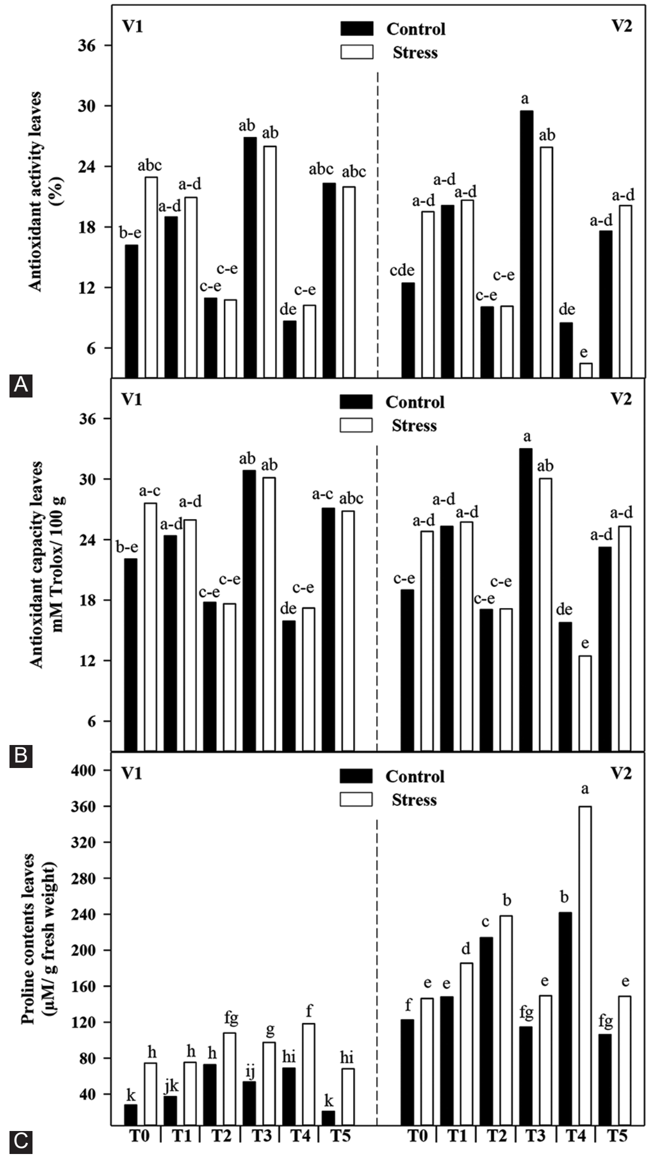

Fig 4. (A) Antioxidant activity leaves; (B) Antioxidant capacity leaves; (C) Proline content leaves in two fenugreek cultivars treated by different priming solutions under salt stress. Values are mean ± S.E. at $p < 0.05$ (n = 3).

bean and (Kalaivani et al., 2016) in rice genotypes when treated with SA under saline conditions.

Biochemical characteristics

The different biochemical characteristics were significantly affected by the salinity and priming in fenugreek cultivars. Results for leaves and roots' chemical attributes are described as follows. The maximum antioxidant activity (29.48 %) and antioxidant capacity (33 mM Trolox/100g) in leaves were recorded in control plants of Local methi (V_2) primed with H_2O_2 (Fig. 4). Leaf total phenolic contents in leaves were found maximum in salt-stressed plants of Local methi (V_2) primed with H_2O_2 (Fig. 5). Leaf proline contents were found maximum (359.55 µmoles/g fresh weight material) in salt-stressed plants of Local methi (V_2) primed with SA (Fig. 4). Leaf chlorophyll contents were also affected by the salinity and priming treatments. The maximum chlorophyll "a" (1.06 mg/g) and chlorophyll "b" (1.30 mg/g) was recorded in control plants of Local methi (V_2) primed with SA (Fig. 5). Root antioxidant activity (12.68 %) and antioxidant capacity (19.21 mM Trolox/100g) were maximum in salt-stressed plants of Kasuri methi (V_1) primed with SA (Fig. 6). Root proline content (583.99 µmoles/g fresh weight material) was maximum in salt-stressed plants of Local methi (V_2) (Fig. 6). Priming of fenugreek seeds markedly increased the biochemical properties of fenugreek leaves as well as roots. SA had a profound positive role in increasing the antioxidant constituents of fenugreek leaves and roots. Proline contents increased significantly in leaves

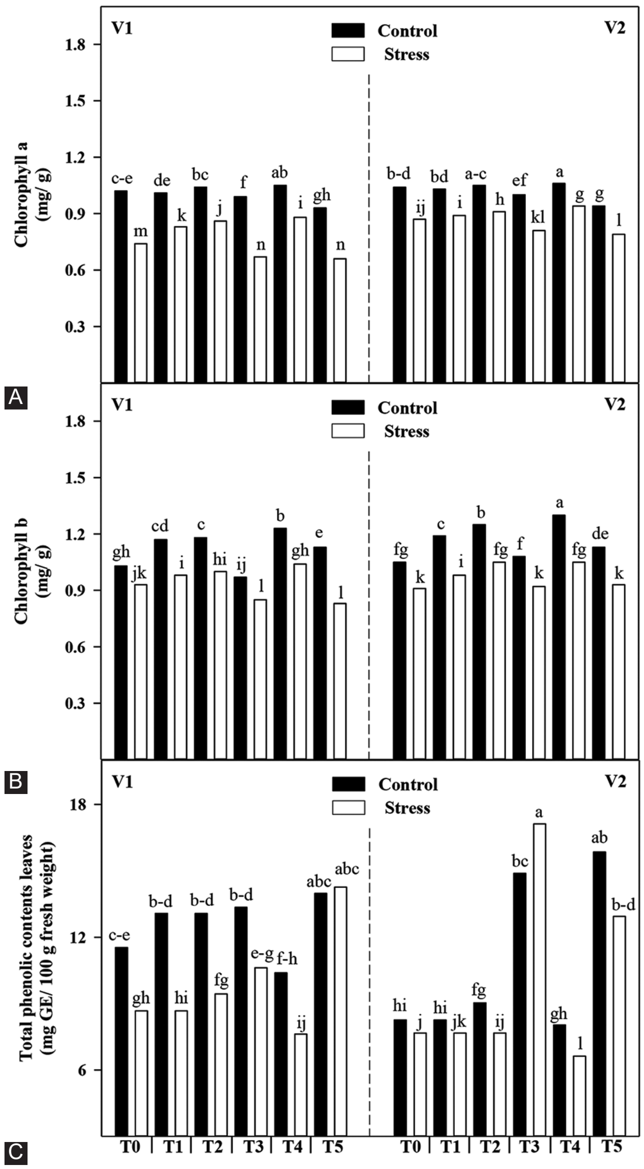

Fig 5. (A) Chlorophyll a; (B) Chlorophyll b; (C) Total phenolic contents leaves in two fenugreek cultivars treated by different priming solutions under salt stress. Values are mean ± S.E. at $p < 0.05$ (n = 3).

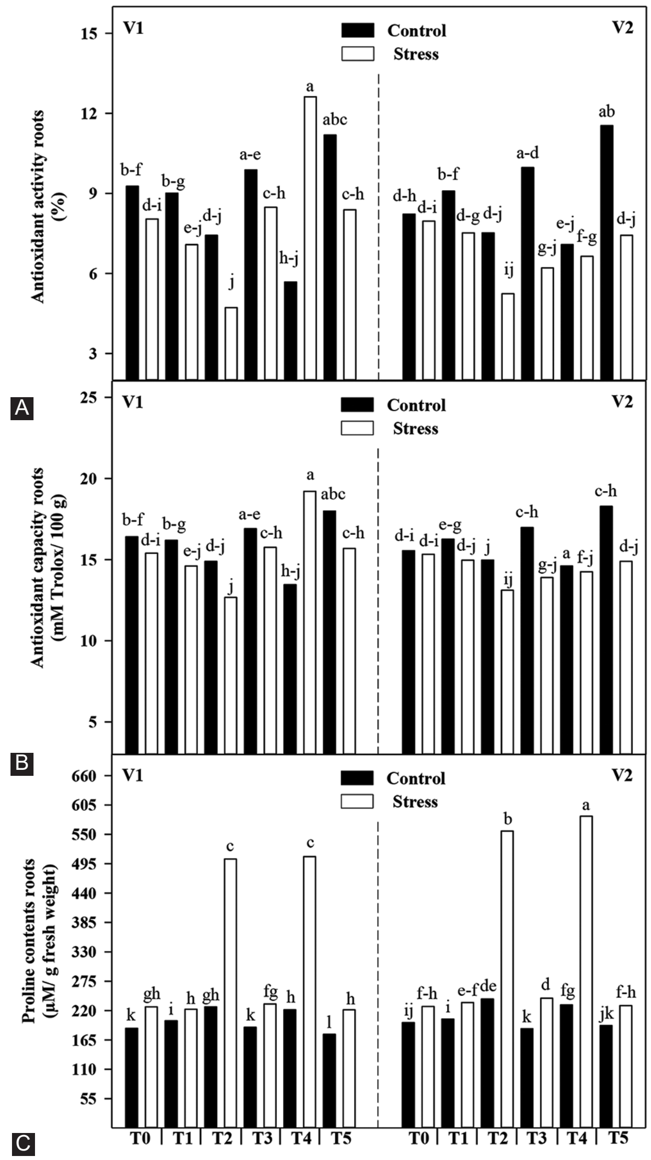

Fig 6. (A) Antioxidant activity roots; (B) Antioxidant capacity roots; (C) Proline content roots in two fenugreek cultivars treated by different priming solutions under salt stress. Values are mean ± S.E. at $p < 0.05$ (n = 3).

as well as in roots in salt-stressed plants when treated with SA. Proline is an important amino acid which mitigates the effect of different stresses in plants and is produced by the plants in stressed conditions. Priming with SA increased its concentration in plant parts making fenugreek plants more tolerant to saline conditions. SA takes a part in important in plant defense mechanisms i.e., the antioxidant defense system to protect plants against abiotic stresses (Sheteiwy et al., 2019). SA enhanced the production of antioxidant enzymes such as catalase, superoxidase, and ascorbate reductase physiologically involved in the salt tolerance mechanism (Azooz, 2009). Tolerant genotypes showed more antioxidant capacity and phenolic contents under salinity as compared to sensitive (Khalid et al., 2020; Sheteiwy et al., 2019).

CONCLUSIONS

It is concluded from the above results that priming had a significant impact on the salinity tolerance of the fenugreek plant. Seed treatment with GA_3 and PEG enhanced seed emergence index and energy of emergence. The final emergence percentage was increased when primed with SA as compared with other priming agents. SA also improved the physical attributes of fenugreek in control conditions as well as in saline conditions. Antioxidant compounds and proline contents were also increased when treated with SA under salt stress. Regarding the fenugreek cultivar, Kasuri methi (V1) comparatively performed better under saline conditions when treated with different priming agents. Therefore, seeds of Kasuri methi should be treated with SA to mitigate the salinity stress and improve the morphological and biochemical properties.

Author contributions

Conceptualization was done by Sajjad Hussain. **Formal analyses** were performed by Hafiza Muzamail Asif and Muhammad Sohail. **The investigation** was done by Tahia Abbas and Sakeena Tul-Ain Haider. **Writing—original draft preparation** was done by Muhammad Fasih Khalid. **Writing—review, and editing** were done by Muhammad Arif Ali, Shaghef Ejaz, and Shakeel Ahmad. **Writing and revision of the draft** were done by Sezai Erscili and Talaat Ahmed.

Conflicts of interest

The authors declare no conflict of interest.

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