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Research Article

Assessment of Growth Performance and Histochemical Localisation of Reactive Oxygen Species in Fenugreek under Exogenous Calcium Ion Priming

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Abstract

The objective of present study was to evaluate the response of the fenugreek seeds primed with various elicitors such as calcium chloride (CC) as an exogenous source of calcium ion; a calcium chelator: Ethylene glycol-bis(2-aminoethylether)-N,N,N´,N, tetra acetic acid (EG); and Lanthanum chloride (LC): a calcium channel blocker, under salinity stress. Significant improvement in the growth parameters of fenugreek seedlings was observed comparing with control. The stress tolerance index (STI) and histochemical detection of reactive oxygen species were performed to evaluate the tolerance of the fenugreek against salinity stress. The results exhibited noteworthy inhibitory effect of salinity stress in control set which was significantly mitigated by the exogenous calcium ion application. Furthermore, under the influence of calcium ion antagonists, EG and LC the adverse effect of salinity was more prominent than control set. In conclusion present investigation revealed that exogenous calcium ion is an ideal elicitor for enhancing growth and development of the fenugreek with better salinity stress management.

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Introduction

The environmental factors are known to have significant impact on the morphological, biochemical attributes along with the growth and development of plants. When any of these factors exceed the tolerance level, a stress is imposed on the plant which influences its development and structural, physiological and biochemical processes (Imran et al., 2021). The increase in the salt content above optimum level, which creates salinity stress is considered one among these environmental factors which are responsible for threatening the crop productivity worldwide (Manivannan et al., 2007).

The deleterious effect of salinity which affects the normal growth and development of the plant is attributed to a reduced osmotic potential, specific ion toxicity and nutrient deficiency of the substratum (Nayem et al., 2020). The reduced osmotic potential affects water availability due to the prevention of water uptake by the plants, leading to a condition known as physiological drought (Kim et al., 2009). In addition, salinity is reported to result in the generation of reactive oxygen species which further leads to membrane disruption and metabolic toxicity in plant system (Mittler, 2002).

In plant system, calcium (Ca^{2+}) is considered as a key second messenger as well as signal transducer, which is involved in coupling a wide spectrum of extracellular stimuli to intracellular responses and play vital role in plant growth and development (Arshi et al., 2006). In the cited literature, Ca^{2+} is found to enhance tolerance against various environmental stresses, including salinity by mitigating oxidative stress and regulating membrane stabilization (Larkindale and Knight, 2002; Kader and Lindberg, 2010).

This study aimed to assess the potential calcium ion in improving growth performance and

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alleviating the detrimental effects of salt stress of fenugreek through priming technique.

Materials and method

Elicitation process and germination

The fenugreek seeds were subjected to surface sterilization with 0.1% sodium hypochlorite solution. The sterilized seeds were washed thrice with distilled water and pre-treated with the solutions of 1mM calcium chloride (CC) as an exogenous source of calcium ion; a calcium chelator: 1mM of Ethylene glycol-bis(2-aminoethylether)-N,N,N´,N, tetra acetic acid (EG); and 1mM of Lanthanum chloride (LC): a calcium channel blocker. For control set, seeds were primed with normal water and placed in a rotary shaker along with the treated seeds. After priming for 24h, the seeds were washed thrice with sterile water and kept in the seed germinator for germination. To provide saline conditions the NaCl at the level of (0dS m^{-1}, 4dS m^{-1} and 8dS m^{-1}) was applied to the seeds for 3days.

Measurement of growth parameters

The growth performance of the seedlings was assessed by calculating various morphological parameters. The average length of the roots and shoots of the seedlings of each experimental set up was recorded along with their fresh weight and dry weight.

Relative water content

The relative water content (RWC) of the seedlings was calculated using the following equation (Gonzalez and Gonzalez-Vilar, 2001):

RWC (%) = (Fresh weight - Dry weight) / (Turgid weight - Dry weight) x 100.

Stress tolerance index

The stress tolerance index (STI) of the seedlings was calculated using the following equation (Sen and Mandal, 2018):

STI = (DWs /DWc) × 100

where DWs: Dry weight of seedlings under stress and DWc: Dry weight of control seedlings

Histochemical detection

The histochemical detection of lipid peroxidation was performed according to Pompella et al. 24 (1987). Plasma membrane integrity of the seedlings was detected by the method suggested by Yamamoto et al. (2001). For detection of H_2O_2 localisation in the roots, the fenugreek seedlings were stained for about 40 to 45 min in potassium iodide/starch reagent (4% w/v starch and 0.1M potassium iodide solution) as reported by Olson and Varner, (1993). Superoxide localisation was detected by NBT method (Singh et al., 2009). Stained roots were photographed under a Nikon SLR camera (Model: D3200).

Statistical analysis

The statistical tools such as MS Excel 2007 (Microsoft, Redmond, WA, USA), DSAASTAT software (version 1.002; DSAASTAT, Peruglia, Italy), Smith's Statistical Package version 2.5 (prepared by Gary Smith, CA, USA) and Multivariate Statistical Package (MVSP 3.1) were used for statistical analysis of data.

Result and Discussion

Since germination being the initial stage of plant development defines the quality of yield and development of plant; therefore, the plants must be provided with best condition during initial stages for better germination. It has been well known that saline environment constrains the growth and development of plant by virtue of their adverse effect on the various physiological and biochemical processes, including osmolytes accumulation and metabolism along with the antioxidant enzyme system (Li et al., 2014). The basic criteria for a stress tolerant plant is said to be survived under stress are maintenance of biomass production, growth performance, elongation of root and accumulation of biochemical markers such as proline, soluble sugars, polyamines, amino acids and reduced level of lipid peroxidation (Juan et al., 2005).

As a result, it was observed that the growth performance of fenugreek seedlings was extensively affected by the saline condition as it was evident by the reduction growth parameters of the seedlings (Fig. 1). An important parameter, relative water content was calculated and it was observed to be 75.44% and 60.25% for unprimed seedlings, 81.88% and 75.24% for calcium chloride pre-treated, 68.02% and 58.02% for EGTA primed and 66.35% and 62.24% for lanthanum chloride at 4dS m^{-1} and 8dS m^{-1} salinity respectively (**Table 1**). The parameter such as relative water content has been considered as one of the vital factors for the

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assessment of the extent of salinity induced effects and the degree of tolerance in plants towards stress environment. Our findings suggest the positive effect of exogenous calcium priming on the RWC of fenugreek seedlings under salinity stress was in agreement to previous studies on several plants (Khan et al., 2010). The seedlings subjected to calcium chloride priming exhibited minimal reduction in root elongation during saline stress, which is considered to be one of the major physiological parameter for salinity tolerance (Tari et al., 2015). Like calcium ion another signalling molecule, nitric oxide is also reported to mitigate salinity stress in plants (Ahmad et al., 2016). Furthermore, the stress tolerance index was significantly enhanced by the calcium chloride pre-treatment as compared to control, on the other hand the decline in tolerance index under the influence of calcium antagonist suggest potential role of calcium ion in providing tolerance to plant against salinity (**Table 1**).

Fig.1 Fenugreek seedlings at various salinity level under influence of calcium elicitors

Our observation is in agreement with the reports of Joshi et al., (2013) which showed alleviation in salinity stress by calcium chloride priming in *Cucumis sativus*.

Table. 1 Effects of different treatments on the morphological attributes in fenugreek seedlings under saline conditions

Treatment	Salinity	Shoot length (cm)	Root length (cm)	Seedling length (cm)	RWC (%)	STI (%)
Control	4dS m^{-1}	2.03±0.05c	1.49±0.05b	3.52±0.17b	75.44±2.15b	81.84±2.02b
	8dS m^{-1}	1.67±0.09d	0.97±0.06d	2.64±0.19c	60.25±1.02d	61.32±1.05d
CC	4dS m^{-1}	2.42±0.11a	1.86±0.18a	4.28±0.13a	81.88±1.78a	88.13±1.12a
	8dS m^{-1}	2.16±0.09b	1.31±0.07c	3.47±0.15b	75.24±2.22b	78.25±1.02b
EG	4dS m^{-1}	1.55±0.08e	0.94±0.08d	2.49±0.16d	68.02±1.82c	65.25±1.26c
	8dS m^{-1}	1.08±0.04g	0.85±0.05e	1.93±0.11f	58.02±2.82d	51.25±1.14e
LC	4dS m^{-1}	1.33±0.11f	0.72±0.05f	2.05±0.11e	66.35±1.32c	62.42±1.24d
	8dS m^{-1}	0.95±0.06g	0.56±0.07g	1.51±0.05g	62.24±0.76d	54.26±1.76e

The major reactive forms of oxygen also termed as reactive oxygen species such as hydrogen peroxide, and superoxide radicals are known to be the molecules with high toxic potentials to plant tissues (Chen et al., 2022). The histochemical detection was performed for the study of specific localization of free radicals such as H_2O_2 and superoxide anion and their pattern of accumulation in the tissue. A major enhancement in the ROS generation both H_2O_2 (**Fig. 2A**) and superoxide radical (**Fig. 2B**) was observed, wherein the seedlings exposed to saline condition as well as those primed with the antagonists of calcium ion at considerably higher rate of accumulation was noted with respect to those primed with exogenous calcium.

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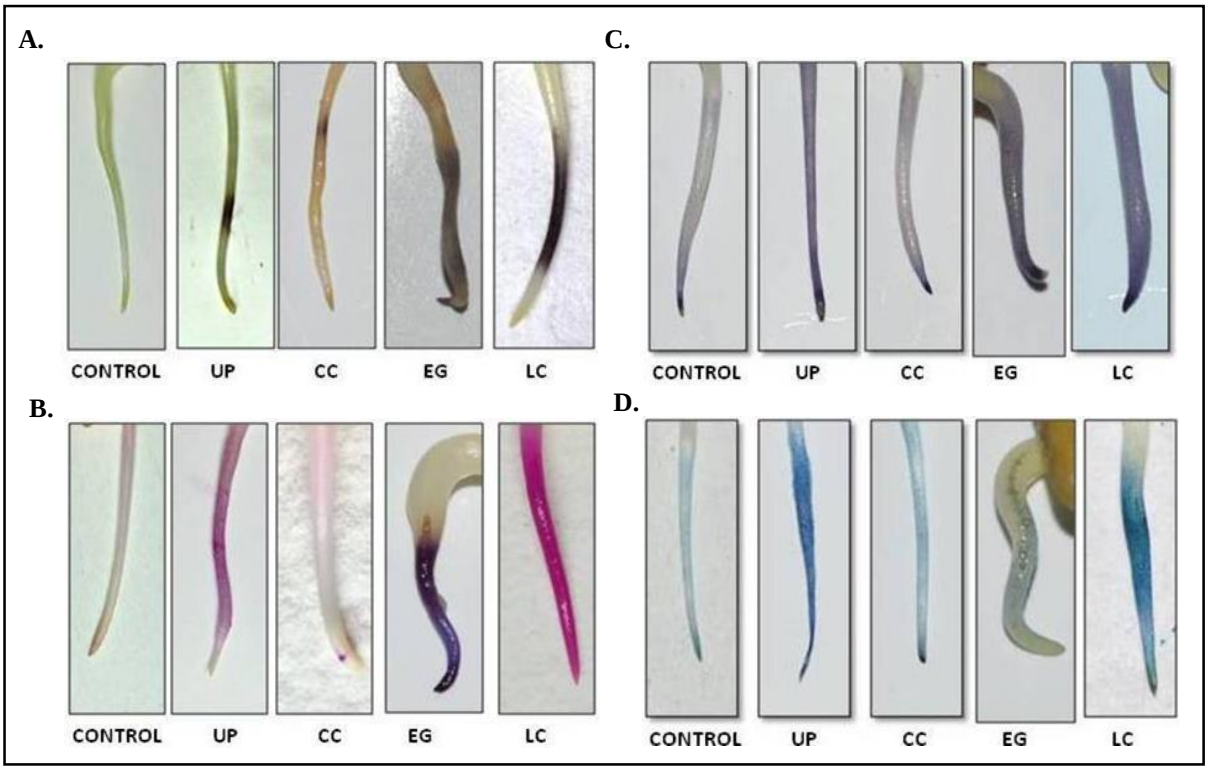

Fig. 2 Histochemical detection of ROS localisation in fenugreek seedlings under salinity stress. (A) Hydrogen peroxide, (B) Superoxide radical, (C) Lipid peroxidation and (D) Plasma Membrane Integrity.

The peroxidation of lipid bilayer of the cell membrane is one of the prominent adverse effects of salinity stress in biological system (Ahmad et al., 2014). Such damages and injuries of biological membranes due to lipid peroxidation are measured in terms of malondialdehyde content (Hogg and Kalyanaraman, 1999) which indicates the degree of stress experienced by the tissue. Consequently, the membrane fluidity is disrupted leading to ion leakage from the tissue. In agreement to aforesaid statement, it was observed that the degree of lipid peroxidation was found to increase considerably in response to salinity and further elevation was observed in the seedlings under the influence of antagonists as shown in Fig 2C. Similarly, the plasma membrane integrity was found to be affected by salinity and the antagonists (Fig. 2D). The seedlings pre-treated with calcium chloride were found to be resistant and exhibited high degree of membrane integrity in concomitant with low amount of ROS localization. Furthermore, in agreement with our result, the protective role of calcium ion is also found to be reported in other plant system under salinity stress condition (Bhattacharjee, 2009; Tian et al., 2015).

Conclusion

In conclusion, our results suggest that priming of fenugreek seeds with exogenous source of calcium enhanced the morphological and biochemical attributes under saline condition, which was further substantiated by the occurrence of adverse effects of salinity on the seeds which were unprimed and also those primed with the antagonists of these signalling molecules.

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