Effect of Calcium Silicate Supplementation on the Growth of *Trigonella Foenum-Graecum* L. Variety Hisar Sonali Under Saline Conditions

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Abstract. Salinity is a major abiotic stress which decreases crop productivity. Salt stress also causes osmotic, water, ionic and oxidative stresses. Plants exposure to salt can check their growth by reduction in water and nutrient uptake, osmotic imbalance and cytotoxicity incited by sodium and chloride ions. Present study deals with the impact of salt on germination, growth and physiological components of fenugreek and its alleviation by the application of calcium silicate. The salt stress reduced fenugreek germination and growth but supplementation of calcium silicate to salt stressed seedlings mitigated deleterious impacts of salinity. Various parameters like germination, seedling length, biomass, pigment and protein contents of fenugreek seedlings were significantly improved with calcium silicate under salt stress. Maximum reduction 53.3% in protein amount was recorded in fenugreek seedlings treated with NaCl (10 mM) over control. Significant increase in total antioxidant content in fenugreek seedlings was observed with calcium silicate as it showed the following order: Ca_2SiO_4 > NaCl + Ca_2SiO_4 > NaCl > Control. Hence, application of calcium silicate can be useful for the fenugreek plants growing under saline conditions.

Keywords: Calcium silicate · Growth · Salt stress · *Trigonella foenum-graecum*

1 Introduction

Salinity of soil is major threat which adversely affects arable land and hampers crop productivity [1, 2]. At the global level more than one thousand million hectares of cultivable fields have been adversely affected by salt stress till date [3]. Around thirty three percent of agricultural fields are affected by salt stress which can increase up to fifty percent by 2050 [4]. Exposure of plants to salt can check their growth by reduction in water uptake, osmotic imbalance, nutritional inconsistency and cytotoxicity induced by sodium and chloride ions [5, 6]. High concentration of salt shows imbalance of ions and osmotic strain which leads to adverse impacts on plants morphology and physiological phenomenon [7]. Salt stress enhances ROS level in plants which results in oxidative stress [8]. Silicon is regarded as a beneficial quasi-essential nutrient for development of plants specifically under unfavourable environmental conditions [9]. Silicon is also known as stress reliever as it enhances resistance of plants against abiotic and biotic

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stresses [10]. Calcium silicate acts as an agricultural liming substance applied to control physico-chemical and biological properties of the soil. Elisa et al. [11] reported positive impacts of calcium silicate on growth of plants under environmental pressure. Bokhtiar et al. [12] reported significant role of calcium silicate on biochemical processes and development of sugarcane plants.

Trigonella foenum-graecum L. (family Fabaceae; sub family: Faboideae) also called as Fenugreek or Methi. It is multipurpose crop which can be used as spice, vegetable and medicinal plant. Due to the presence of various phytochemicals, leaves and seeds of fenugreek are widely applied to treat diabetes, heart diseases, gastric problems and cancer etc. [13]. To the best of my information, no investigation has been done earlier to study the effect of calcium silicate on fenugreek plant under saline conditions. Therefore, present investigation was conducted to compare growth and biochemical components of fenugreek seeds grown under salt stress and effect of calcium silicate in alleviation of unfavourable impacts of salinity on fenugreek.

2 Material and Methods

The present experiments were conducted in Plant Physiology Laboratory, Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida. Uniform fenugreek seeds (*Trigonella foenum-graecum* L. variety Hisar Sonali) were obtained from seed agency of Ghaziabad. Fenugreek seeds were kept in sterilized plastic containers to avoid impurities.

2.1 Preparation of Salt Solution and Calcium Silicate

The NaCl solution (10 mM) and calcium silicate (5 mM) [Formula: Ca_2SiO_4 (molecular weight: 172.24 g/mol) procured from LOBA Chemie private limited, Mumbai) were prepared with autoclaved water. Effect of salt solution (10 mM) and calcium silicate (5 mM) alone and their combined effect (10 + 5 mM) was analyzed on growth and biochemical components of fenugreek seeds.

2.2 Seed Germination Test

Seeds of fenugreek were cleaned with tap water for 5 min, after that treated with 10:1 distilled water/bleach (commercial sodium hypochlorite solution) for five minutes, finally with autoclaved water. Ten seeds were kept in test tubes with 10 ml of NaCl (10 mM) and calcium silicate (5 mM) solution respectively for five hours. Control seeds were kept in distilled water. Three different sets of petriplates were arranged and ten fenugreek seeds of each treatment i.e. salt solution (10 mM), calcium silicate (5 mM) and distilled water were kept in petridishes respectively, and finally in growth chamber at 25 ± 2 °C with 16/8 h photoperiod. Seed germination was calculated by observing germinated seeds after 24 h interval up to ten days.

2.3 Growth Variables

The growth parameters of fenugreek seeds like percentage of germination, germination rate and index, length of seedlings, biomass and vigour index were calculated by procedure of Li [14].

(1). **Germination (%)** = Total number of seedsgerminated/total number of seeds taken for germination × 100.
(2). **Relative germinatio rate** = germination(%in treatment/germination (%) in control.
(3). **Germination index** = ΣGt/Dt.
Gt = seeds germinated in t days; Dt = number of corresponding germination days.
(4). **Seedling Length**
Length of radicle and plumule of fenugreek seeds were measured with ruler and expressed in cms [15].
(5). **Vigour Index**
Vigour index was estimated by Abdul - Baki and Anderson [16]. Vigour index = Length of seedling × germination (%).
(6). **Biomass Analysis**
Biomass of fenugreek seedlings was measured after ten days of seedling growth, then seedlings were kept in oven at 65 °C for three days for estimation of dry weight.

2.4 Relative Water Content

After estimation of fresh weight of fenugreek seedlings, these were kept in sterilized water at 25 °C under dark conditions. Turgid weight was calculated after 12 hours, and seedlings were kept for two days at 80 °C for measurement of dry weight. Bars and Weatherly method was used for calculation of relative water content [17].

$$\text{RWC} = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100$$

2.5 Estimation of Biochemical Components

2.5.1 Pigment Content

Chlorophyll content was measured in fenugreek seedlings by following Lichtenthaler [18]. Leaves of control and treatment were grounded with 80% acetone and centrifuged at 3000 rpm for ten minutes. The optical density of supernatant was calculated at 645 and 663 nm and chlorophyll contents were analyzed as given below:

$$\textbf{Total Chlorophyll}\ (\text{mg/g}) = 20.2 \times \text{OD}_{645} + 8.02 \times \text{OD}_{663} \times \text{V}/100 \times \text{W}$$

$$\textbf{Chlorophyll a}\ (\text{mg/g}) = 12.7 \times \text{OD}_{663} - 2.69 \times \text{OD}_{645} \times \text{V}/100 \times \text{W}$$

$$\textbf{Chlorophyll b}\ (\text{mg/g}) = 22.9 \times \text{OD645} - 4.68 \times \text{OD}_{663} \times \text{V}/100 \times \text{W}$$

V = supernatant volume (ml), W = leaves fresh weight (g), OD = absorbance.

Chlorophyll stability index was calculated by Sairam et al. [19]. CSI = Total chlorophyll in treatment/Total chlorophyll in control × 100.

2.5.2 Estimation of Sugar

Hedge and Hofreiter [20] procedure was followed for analyses of sugars. Fenugreek seedlings were macerated in 95% ethanol and centrifuged at 4000 rpm for 15 minutes and mixed with autoclaved water and anthrone reagent. Test tubes were placed at boiling water bath for 15 minutes and absorbance was taken at 620 nm, sugar amount was measured by standard curve of glucose.

2.5.3 Measurement of Proline and Protein Contents

Proline amount was analyzed by procedure of Bates et al. [21]. Fenugreek seedlings were extracted with 3% sulphosalicylic acid, acid-ninhydrin, acetic acid and kept at water bath for one hour at 100 °C and extracted with toluene. Chromophore absorbance was measured at 520 nm and amount of proline was analyzed with standard curve and denoted as μmol g^{-1}FW.

Lowry et al. [22] method was applied for protein assessment. The fenugreek seedlings were crushed with 1 N sodium hydroxide for five minutes at 100 °C. The alkaline copper reagent was mixed at room temperature for 10 min. Folin - Ciocalteu reagent was mixed and absorbance was calculated at 650 nm after half an hour. Protein amount was measured with standard curve of lysozyme.

2.5.4 Antioxidant Content

Antioxidant content in fenugreek seedlings was assessed by Prieto et al. [23]. Total antioxidant content was measured in fenugreek seedlings (150 mg) after mixing with ethanol, reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4 and 4 mM $(NH_4)_2MoO_4$). Optical density was calculated at 695 nm.

2.6 Statistical Analysis

Treatment was arranged in randomized block design with 3 replications. Results were statistically analyzed by ANOVA. Treatment mean was assessed by DMRT at $p < 0.05$.

3 Results and Discussion

Salt stress effect was studied on the growth and physiological constituents of fenugreek seeds and function of calcium silicate in plant growth and mitigation of salt stress was also analyzed.

3.1 Seed Germination and Growth Variables

The growth variables like seed germination, relative germination rate, length of seedlings, vigour index and fenugreek seedlings biomass exhibited differences under different treatment. In control, 93% germination was observed in fenugreek seeds. Enhancement in germination and growth of fenugreek was reported with supplementation of calcium silicate. Maximum seed germination 95% was observed with calcium silicate and it

Table 1. Impact of salt and calcium silicate on seed germination of *Trigonella foenum-graecum* L. variety Hisar Sonali.

Treatment	Germination (%)	Relative germination rate (RGR)	Germination Index (GI)
Control	$93^{a} \pm 0.69$	–	$9.3^{a} \pm 0.07$
NaCl (10 mM)	$21^{c} \pm 0.11$	$0.23^{c} \pm 0.05$	$2.1^{c} \pm 0.04$
Ca_2SiO_4 (5 mM)	$95^{a} \pm 0.87$	$1.02^{a} \pm 0.42$	$9.5^{a} \pm 0.21$
NaCl + Ca_2SiO_4 (10 + 5 mM)	$88^{b} \pm 0.52$	$0.95^{a} \pm 0.17$	$8.8^{a} \pm 0.06$

Mean ± SD values followed by different letters in each group show significant differences at $P < 0.05$ (ANOVA and DMRT).

Table 2. Impact of salt and calcium silicate on the growth variables of *Trigonella foenum-graecum* L. variety Hisar Sonali.

Treatment	Length of radicle (cms)	Length of plumule (cms)	Vigour index
Control	$3.1^{b} \pm 0.09$	$7.7^{b} \pm 0.18$	10044
NaCl (10 mM)	$1.9^{c} \pm 0.02$	$3.2^{c} \pm 0.24$	1092
Ca_2SiO_4 (5 mM)	$4.3^{a} \pm 0.13$	$8.5^{a} \pm 0.72$	12160
NaCl + Ca_2SiO_4 (10 + 5 mM)	$3.8^{a} \pm 0.21$	$7.3^{b} \pm 0.35$	9768

Mean ± SD values followed by different letters in each group indicates variation at $P < 0.05$ (ANOVA and DMRT).

was 2% more than control. The Ca_2SiO_4 treatment showed high germination index as compared to control (Table 1).

Length of seedlings, fresh and dry weight and vigour index were reported in control and treatment. Maximum radicle and plumule length, vigour index were observed in fenugreek seedlings treated with calcium silicate (Table 2).

Fresh and dry weight of fenugreek seedlings were reduced with NaCl treatment but enhanced with calcium silicate (Table 3). Maximum 10.21 g fresh and 3.18 g dry weight of fenugreek seedlings were observed with calcium silicate. Higher relative water content 95.39% was observed in fenugreek seedlings with calcium silicate treatment whereas 8.77% was reported in control. The enhancement in germination and growth parameters have shown the following order: Ca_2SiO_4 > Control > NaCl + Ca_2SiO_4 > NaCl.

3.2 Biochemical Contents

Total chlorophyll content 2.45 mg/g was reported in fenugreek seedlings with calcium silicate treatment in comparison to 1.82 mg/g in control. The highest chlorophyll stability index was observed with Ca_2SiO_4 treatment. The rise in total chlorophyll content in

Table 3. Impact of salt and calcium silicate on biomass of *Trigonella foenum-graecum* L. variety Hisar Sonali.

Treatment	Fresh weight (gm)	Dry weight (gm)	Relative water content (%)
Control	$8.34^{b} \pm 0.53$	$2.92^{b} \pm 0.18$	$87.7^{a} \pm 0.82$
NaCl (10 mM)	$4.65^{c} \pm 0.23$	$1.21^{c} \pm 0.05$	$70.64^{c} \pm 0.18$
Ca_2SiO_4 (5 mM)	$10.21^{a} \pm 0.86$	$3.18^{a} \pm 0.82$	$95.39^{a} \pm 0.94$
NaCl + Ca_2SiO_4 (10 + 5 mM)	$9.13^{a} \pm 0.72$	$2.47^{b} \pm 0.47$	$81.62^{b} \pm 0.79$

Mean ± SD values followed by different letters in each group reflect variations at $P < 0.05$ (ANOVA and DMRT).

Table 4. Impact of salt and calcium silicate on biochemical constituents of *Trigonella foenum-graecum* L. variety Hisar Sonali.

Treatment	Total chlorophyll content (mg/g)	Chlorophyll stability index (%)	Sugar (mg/g FW)	Proline (μmol/g FW)	Protein (mg/g FW)
Control	$1.82^{b} \pm 0.84$	–	$64.19^{b} \pm 0.62$	$0.49^{b} \pm 0.27$	$53.82^{a} \pm 0.45$
NaCl (10 mM)	$1.01^{c} \pm 0.09$	55.49	$21.71^{d} \pm 0.21$	$1.05^{a} \pm 0.93$	$25.12^{c} \pm 0.31$
Ca_2SiO_4 (5 mM)	$2.45^{a} \pm 0.92$	134.62	$89.42^{a} \pm 0.85$	$0.38^{c} \pm 0.05$	$59.26^{a} \pm 0.54$
NaCl + Ca_2SiO_4 (10 + 5 mM)	$1.49^{b} \pm 0.17$	81.87	$52.64^{c} \pm 0.41$	$0.96^{a} \pm 0.71$	$46.32^{b} \pm 0.32$

Mean ± SD values followed by various letters in each group indicate variations at $P < 0.05$ (ANOVA and DMRT).

fenugreek seedlings followed the order: Ca_2SiO_4 > Control > NaCl + Ca_2SiO_4 > NaCl (Table 4).

The sugar content 89.42 mg/g was recorded in fenugreek seedlings with calcium silicate in comparison to 64.19 mg/g in control. Significant enhancement 39.31% in sugar amount was observed in fenugreek with calcium silicate treatment over control (Table 4). The significant reduction in sugar content 66.18% was observed with NaCl treatment over control. Fenugreek seedlings showed that salt treatment significantly enhanced proline content. The increase in proline content showed the following trend: NaCl > NaCl + Ca_2SiO_4 > Control > Ca_2SiO_4 (Table 4).

Significant increase in total protein content in fenugreek seedlings has been reported with calcium silicate. The total protein content 59.26 mg/g was recorded in fenugreek seedlings with calcium silicate in comparison to 53.82 mg/g in control. Highest reduction 53.3% in protein amount was reported in seedlings of fenugreek with 10 mM salt treatment over control (Table 4).

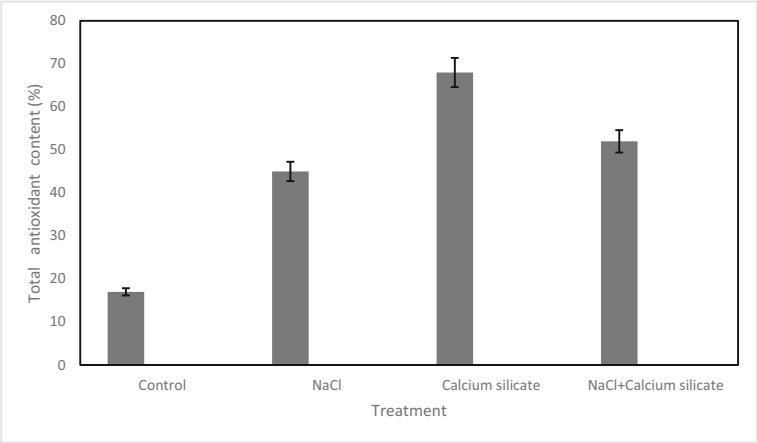

Fig. 1. Total antioxidant content in fenugreek seedlings under different treatment.

3.3 Total Antioxidant Content

Antioxidants are regarded as defense system of plants against oxidative pressure. Rise in content of antioxidants in seedlings of fenugreek observed with calcium silicate treatment and it showed the following order: Ca_2SiO_4 > NaCl + Ca_2SiO_4 > NaCl > Control (Fig. 1).

Reduction in plant growth under saline conditions because of decrease in cell division and elongation [24]. Earlier reports reveal that plant exposure to NaCl can retard growth and developmental processes via impaired photosynthesis, imbalance in nutrients and phytohormones, cytotoxicity incited by excessive intake of sodium and chloride ions, changes in structures of chloroplast and mitochondria [6, 25, 26]. Plant cells generate osmolytes such as proline and sugars for its protection against salt stress. Osmolytes give osmotic adjustment and promotes salt tolerance ability of plants [7]. The adverse impact of salt stress on growth, biochemical processes and productivity have been observed in various plants like *Phaseolus vulgaris* [27], *Vicia faba* [28] and *Triticum aestivum* [29]. Salt treatment (10 mM) exhibited significant reduction in seed germination and biomass of fenugreek seedlings (Table 1 and 3). Decreased relative water content in fenugreek seedlings showed water deficiency because of inhibition in water uptake under saline condition [30]. The reduced radicle and plumule length of fenugreek seedlings may be adoption by plants to regulate water requirement in the presence of salt stress [31]. Positive correlation was reported between calcium silicate and various growth variables of fenugreek in this investigation (Table 1, 2 and 3). It might be because of nutritional characteristics of calcium silicate which enhances cell division and growth which is needed for development of seedlings. In combined treatment, under the influence of calcium silicate significant increase in growth of fenugreek seedlings was due to rise in CO_2-fixing capacity with increase in germination with biomass production.

Enhancement in photosynthetic rate promotes plant growth. The total chlorophyll content was declined with NaCl but significantly increased with calcium silicate in fenugreek seedlings suggested that calcium silicate alleviated adverse impacts of salt

on chlorophyll content. The rise in biochemical components like pigment and protein contents with calcium silicate treatment might cause promotion in germination and growth of fenugreek. Donega [32] observed that silicon improved architecture of plants and increased photosynthesis. Silicon accumulation in the cell wall maintains leaves rigidity with enhanced surface area of leaves to absorb light for synthesis of sugars via photosynthesis. Silicon supplementation improved nitrogen use efficiency, yield and nutritional value of crops as observed in rapeseed [33] and maize [34].

Proline acts as potent antioxidant which checks programmed cell death [35]. Results reflected that proline contents reduced with calcium silicate application, which show favourable role of calcium silicate in alleviating harmful impacts of salt stress on fenugreek. The increase in protein contents with foliar use of calcium silicate may be because of its role in synthesis of protein and DNA [36] and functioning of mRNA [37]. Due to accretion of proline and sugars, cell osmotic potential become less which promotes water absorption and maintains turgor pressure.

Silicon plays a protective role by removal of free radicals in plants [38]. Supplementation of silicon reduces adverse impacts of abiotic stresses on the plants by decreasing ROS generation and promoting antioxidant system of plants [39]. The enhanced amount of total antioxidants provide protection to the plant cells against oxidative pressure by lowering peroxidation of lipids in fenugreek seedlings (Fig. 1). Qutab et al. [40] stated that plants utilize antioxidant enzymes to combat oxidative strain.

In present study, calcium silicate acts as protective agent and increases seed germination, growth and physiological components of fenugreek under salt stress. Further in-depth investigations are required for identification of its optimum doses, exposure time and mechanism of action for development of the crop plants grown under saline conditions.

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