

## ORIGINAL ARTICLE

# Evaluating Photosynthetic Attributes as Indicator of Salt Tolerance in Fenugreek

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### ABSTRACT

Soil salinity, a major abiotic constraint now becomes a global issue as it is affecting crop yield severely, research has been conducted to acquire crops with improved salinity tolerance. Salt stress impacts numerous characteristic of a plant's physiology, making it difficult to study. Instead, it is more tractable to analyze the plant's response into attributes that are hypothesized to be involved in the overall tolerance of the plant to salinity. Thus, keeping this in mind the current research was carried out in fenugreek, a well known medicinal crop to investigate the effect of salinity on different photosynthetic pigments and correlate it with the tolerance. Five varieties of fenugreek namely RMT-1, RMT-305, Desi, Pusa Early Bunching (PEB) and Kasuri were explored to screen salt tolerance. To create salt stress, sodium chloride at the levels of 0 (as control), 50 mM, 100 mM, 150 mM and 200 mM were used. Results showed significance difference between different photosynthetic parameters with respect to salinity levels and varieties. Low and moderate salinity is not exhibited severe toxic effects. Results exhibited a significant correlation between photosynthetic indices with respect to varietal difference in stress tolerance. The present study concluded that among fenugreek varieties PEB and Kasuri are highly salt sensitive, desi and RMT-1 are moderately salt tolerant and RMT-305 are salt tolerant.

**Key words:** Fenugreek, photosynthetic pigments, salinity, salt tolerance

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### INTRODUCTION

Salt stress is one of the most devastating abiotic factors and becomes a key issue in global agriculture because it adversely causes significant reductions in cultivated land area, agricultural productivity and quality [1, 2]. It was reported that out of 831 million hectares (Mha) of the affected world's land area, 397 Mha (52.2%) and 434 Mha (47.8%) land is affected by salinity and sodicity respectively [3, 4]. In fact, in India, the problem of soil and irrigation water salinity increases every year, which is an ever-present threat to the yield and quality of vegetables [1]. In India about 120.4 Mha (approximately 37%) out of 328.73 Mha land area has been degraded in one way or the other [5]. The major fraction of the predictable 6.74 Mha irrigated land in the country has been inflicted by soil salinity (2.957 Mha) and sodicity (3.79 Mha) [6]. It is estimated that the average soil degradation rate was about 16.4 ton ha<sup>-1</sup>year<sup>-1</sup>, which resulting in an annual entire soil loss of 5.3 billion tonnes throughout the country [7]. The loss in crop production due to salinity in India amounts to 6.2 million tonnes. The projections point out that the country will have 11.7 Mha land area affected by salinity and sodicity by 2025 [8]. In India the foremost salt affected soils were found in Haryana (11.59%), followed by Gujarat (6.77%), Rajasthan (6.24%), Punjab (5.84%), Utrakhand (4.76%) and then by Uttar Pradesh (4.54%) [7].

To overcome salt stress and adaptation of plants to possible high concentrations in the soil profile, researchers and farmers are working from several decades and have been combating the problem by shifting to more salt tolerant crops. Plant species vary in how well they tolerate salt-affected soils. Some plants will tolerate elevated levels of salinity while others can tolerate little or no salinity.

Fenugreek (*Trigonella foenum-graecum* L.) a member of family Fabaceae is one of the most vital medicinal and spice plants in the world [9]. It is mainly a condiment crop and grown for seed, vegetable (green leaves) and fodder purpose and have become a much appreciated healthy food source, rich in mineral nutrients and vitamins. It is an essential ingredient in Indian curries and cultivated in arid and semi-arid areas which have little rainfall [10]. Most commercially grown varieties are developed under non-saline conditions and are not bred to endure salt stress. Very limited information is available about salt stress reactions as well as about the physiological and biochemical parameters that may be useful for the

screening of salt tolerant varieties in fenugreek. Thus, keeping aforesaid views, the objectives were set to evaluate the salt tolerance potential of different fenugreek varieties on the basis of examining photosynthetic pigments analysis.

## MATERIAL AND METHODS

### Plant Materials:

The seeds of fenugreek (*Trigonella foenum-graecum* L.) varieties namely RMt-1 and RMt-305 were procured from Jobner Research Centre, Rajasthan. Seeds of Desi variety were collected from local market, whereas, seeds of variety Pusa Early Bunching (PEB) and Kasuri (*Trigonella corniculata* L.) were collected from IARI, New Delhi.

### Sterilization of seeds:

Seeds of uniform size were selected and firstly washed with mild detergent solution of Tween-20 and then surface sterilized in 3% sodium hypochlorite solution for 10 min followed by washings for several times with autoclaved distilled water.

### Experimental design:

The sterilized seeds were germinated on moist filter paper in petri dishes in the dark at a constant temperature of 25°C. The seeds were exposed to different concentrations (50, 100, 150 and 200 mM) of salt (NaCl). Comparison of salt exposed plants was made with untreated (control) plants. After germination, uniform seedlings were selected and inoculated in pots containing soil, sterile sand and vermiculite (2:1:1). Soil was autoclaved at 15 lbs/square per inch for 1 h at alternate days before plantation. The pots were placed into a growth chamber under a mean air temperature of 25±1°C, air relative humidity of 75-80% and photoperiod of 16/8 h. The experiments were conducted in randomized block design (RBD) with three replicates for each treatment. Irrigation was done with water and respective concentration of saline solution for control and treated plants respectively at 10<sup>th</sup> day. Chlorophyll a content, chlorophyll b content, total chlorophyll content, carotenoid content and chlorophyll a/b ratio protein content were scored at 45 DAS.

### Chlorophyll a, chlorophyll b and total chlorophyll content:

Various photosynthetic pigments such as chlorophyll a, chlorophyll b and total chlorophyll content were estimated by Arnon [11]. Fresh leaf material (200 mg) was taken from the second and third nodes of the shoot tip and crushed with mortar and pestle in 10 ml 80% chilled acetone and centrifuge at 2500 rpm for 10 min using High Speed Cooling Microprocessor Centrifuge REMI (C-24BL model, REMI, Mumbai, India). This step was repeated until the pellet became colourless. The supernatant was collected and used for analysis. Different photosynthetic pigments concentrations were measured using spectrophotometer (UV-VIS Double Beam Spectrophotometer (SPUV-26) SCOTECH, Germany) at 453, 645 and 663 nm wavelengths. A solution of 80% acetone was used as a blank. The chlorophyll a, chlorophyll b and total chlorophyll (mg g<sup>-1</sup> FW) concentrations in the leaf tissues were calculated according to the following equations:

$$\text{Chlorophyll a} = [(12.7 \times A_{663}) - (2.63 \times A_{645})] V / (W \times 1000)$$

$$\text{Chlorophyll b} = [(22.9 \times A_{645}) - (4.48 \times A_{663})] V / (W \times 1000)$$

$$\text{Total Chlorophyll} = [(20.2 \times A_{645}) + (8.02 \times A_{663})] V / (W \times 1000)$$

Where,

$A_{663}$  = Absorption at a wavelength of 663 nm.

$A_{645}$  = Absorption at a wavelength of 645 nm.

V = volume of the extract (ml)

W = Weight of leaves taken (g)

### Carotenoid content:

Carotenoid content (mg g<sup>-1</sup> FW) was estimated by MaClachlan and Zalik [12] using following formula:

$$\text{Total carotenoid} = 0.216 \times A_{663} - 1.22 \times A_{645} - 0.354 \times A_{663} + 0.452 \times A_{453}$$

## RESULTS

### Effect of salinity on photosynthetic pigments:

#### Chlorophyll a content:

The chlorophyll a content in the leaves of five fenugreek varieties was determined in non-stressed and salt-stressed conditions. There was very little variation between varieties under non-stressed conditions. Chlorophyll a content was found to decrease significantly in all varieties as compared to their control but varieties RMt-1, RMt-305 and Desi were least affected. The maximum chlorophyll a content (1.8±0.02 mg g<sup>-1</sup> FW) was observed in Desi variety at 50 mM NaCl concentration. Data also showed a gradual increase in chlorophyll a content at 50 mM salt concentration in RMt-305 and Desi variety which was ranging from 1.62 to 1.74 mg g<sup>-1</sup> FW and 1.71 to 1.8 mg g<sup>-1</sup> FW respectively. The maximum increase in chlorophyll a

content was found to be 6.89% in RMT-305 followed by 5% in Desi variety at low salt concentration. Maximum inhibition was observed on basis of % phytotoxicity analysis in PEB followed by Kasuri variety which was 100% and 48.13% respectively at 200 mM concentrations of NaCl, when compared to control plants (Fig. 1A and B).

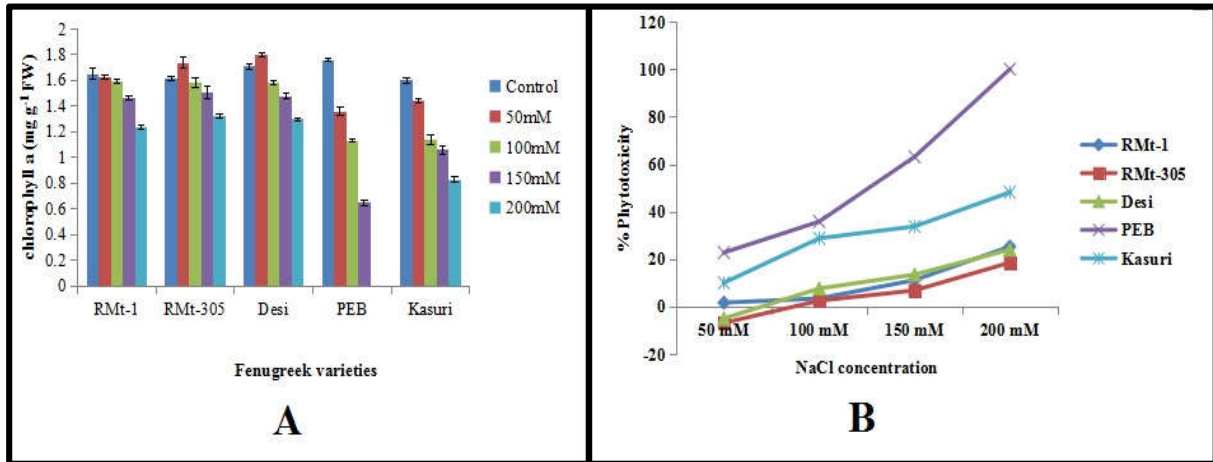


Figure 1: Effect of salinity on (A) chlorophyll a content and (B) chlorophyll a % phytotoxicity of five fenugreek varieties. Data represent mean of three replicates±SD.

**Chlorophyll b content**

The data revealed more detrimental effect of salinity stress on chlorophyll b content as compared to chlorophyll a in all fenugreek varieties. Chlorophyll b content was found to decrease significantly in all varieties under stress as compared to their control. In control chlorophyll b content was recorded in all fenugreek varieties which were ranged from 0.22±0.018 mg g<sup>-1</sup> FW (minimum) in Kasuri to 1.46±0.019 mg g<sup>-1</sup> FW (maximum) in PEB. The minimum inhibition of chlorophyll b was observed in RMT-305 followed by RMT-1, then by Desi, then Kasuri and highest reduction was in PEB with salt stress. At 200 mM salt concentration minimum decrease of 25% was observed in RMT-305 at 45 DAS. Severity of salinity was observed at higher salt concentration in PEB, Kasuri and Desi which was 100%, 76.44% and 46.15% respectively (Fig. 2A and B).

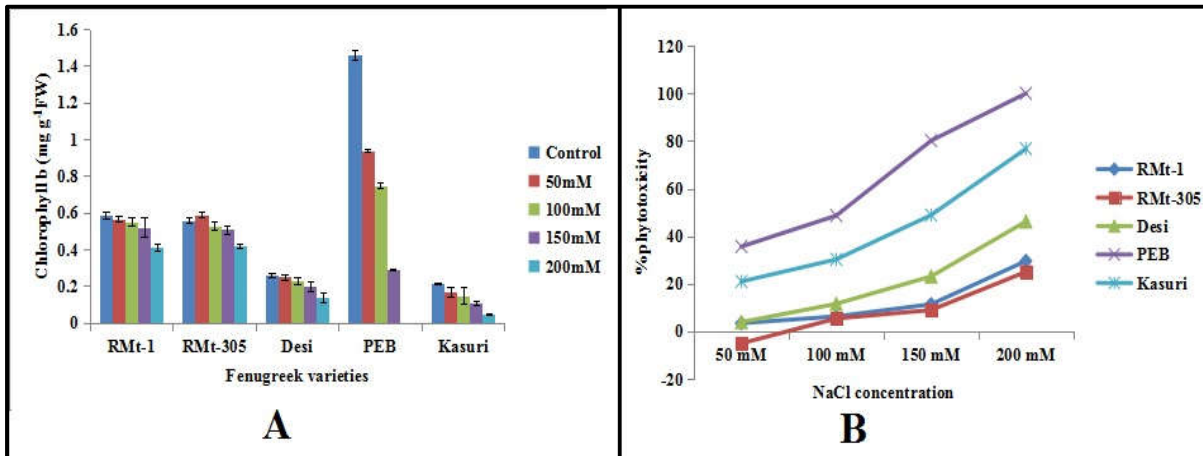


Figure 2: Effect of salinity on (A) chlorophyll b content and (B) chlorophyll b % phytotoxicity of five fenugreek varieties. Data represent mean of three replicates±SD.

**Total Chlorophyll content:**

Decrease in total chlorophyll content was found highly significant in saline soil grown fenugreek plants with respect to normal soil grown plants. It was observed that total chlorophyll content in RMT-305 and Desi was slightly increased with low salinity level from 2.18 to 2.33 mg g<sup>-1</sup> FW and 1.97 to 2.05 mg g<sup>-1</sup> FW respectively. In salt treatment maximum percent decrease in total chlorophyll was observed in PEB which was 100%, followed by Kasuri with 51.52%, Desi with 26.9%, RMT-1 with 26.34% and then by RMT-305 with 20.18% reduction (Fig. 3A and B).

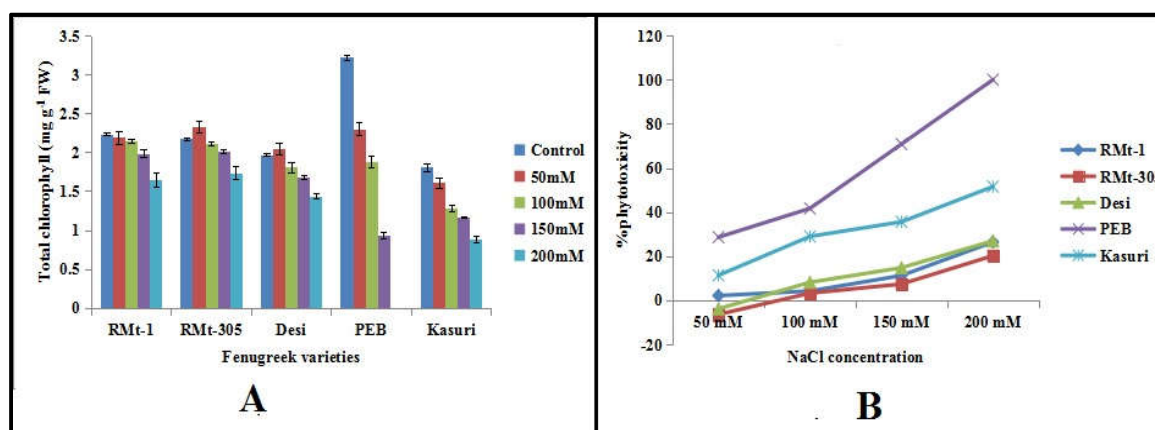


Figure 3: Effect of salinity on (A) total chlorophyll content and (B) total chlorophyll % phytotoxicity of five fenugreek varieties. Data represent mean of three replicates $\pm$ SD.

#### Chlorophyll a/b ratio:

Chlorophyll a/b ratio of fenugreek varieties were showed significant variation under stress conditions in all the varieties. Under low and moderate saline condition the variation was non-significant in RMT-1 and RMT-305. Interaction between varieties and stress was found significant at higher salt concentrations. The maximum Chlorophyll a/b ratio was observed in Kasuri variety under both control and salt stressed plants which was 7.44 and 16.6 at 200 mM respectively. The minimum chlorophyll a/b ratio was observed in PEB which was 1.21 and 0 at 0 and 200 mM NaCl concentration respectively (Fig. 4).

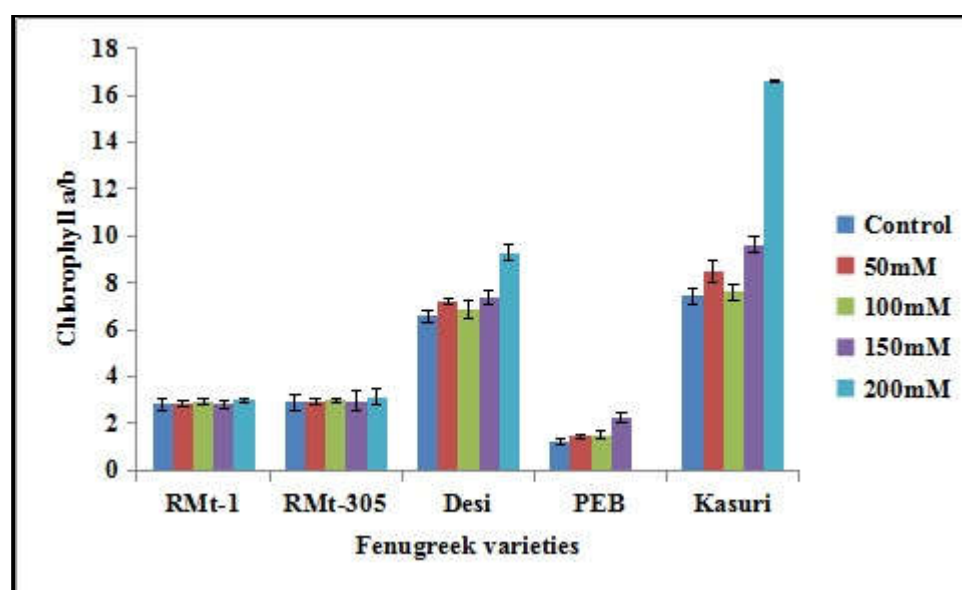


Figure 4: Effect of salinity on chlorophyll a/b ratio of five fenugreek varieties. Data represent mean of three replicates $\pm$ SD.

#### Carotenoids content

Carotenoids content among five fenugreek varieties was also decreased significantly under salt stress conditions. The percent decrease in carotenoids content was increased with increasing salt concentration. Under control condition maximum carotenoids content was observed in RMT-305 followed by RMT-1, then by PEB, Desi and then by Kasuri variety which was 0.38, 0.37, 0.32, 0.21 and 0.16 mg g<sup>-1</sup> FW respectively. At 200 mM concentration the highest and lowest carotenoids content was 0.28 mg g<sup>-1</sup> FW in RMT-305 and 0.02 mg g<sup>-1</sup> FW in Kasuri respectively. PEB showed maximum decrease of 100% followed by Kasuri that exhibited 87.5% decreases with 200 mM salt concentration (Fig. 5 A and B).

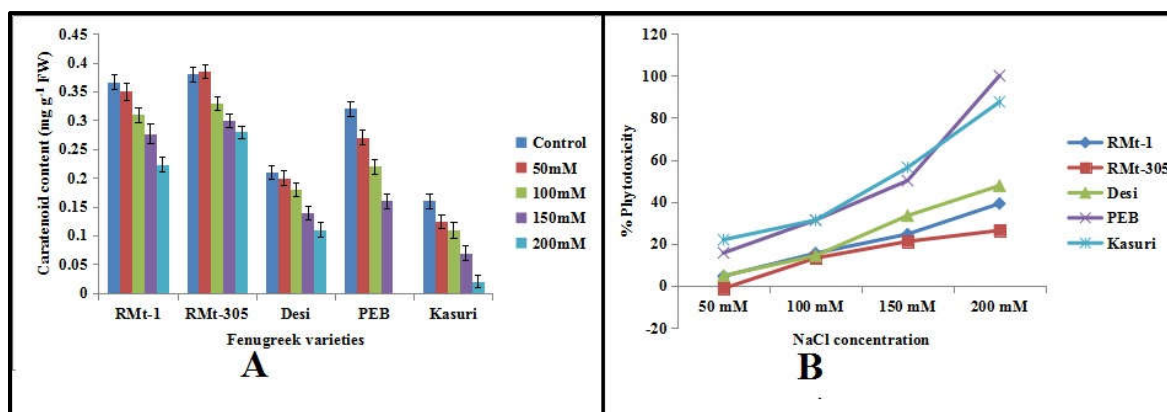


Figure 5: Effect of salinity on (A) carotenoid content and (B) % carotenoid phytotoxicity of five fenugreek varieties. Data represent mean of three replicates  $\pm$ SD.

## DISCUSSION

Fenugreek is considered as most important medicinal crop in the world and is grown all over the world under different climatic conditions. It is one of the major cash crops of India but its productivity is still very low to fulfil international demand because of several constraints including soil salinity. Thus, evaluation of the potential of different fenugreek varieties is necessary before releasing the best salinity tolerant fenugreek varieties, by keeping these necessities under consideration the current investigation was undertaken on five fenugreek varieties namely RMt-1, RMt-305, Desi, PEB and Kasuri. The effects of salinity on different photosynthetic pigments were analysed and results obtained on these aspects have been discussed as follows:

### Effect of salinity on photosynthetic pigment status:

Chlorophyll is present in all photosynthetic plants and marked as the principle pigment. Chlorophyll content of leaf is one of the essential physiological parameters, which influences photosynthesis and thus, indirectly associated with growth and yield of crop plants [13]. The Chlorophyll degradation under saline condition is well-known phenomenon and had been reported in several plants, such as mung bean [14], faba bean [15] etc. Chlorophyll content can be considered as one of the indices to screen genotypes for salt tolerance since salinity induce chlorophyll reduction and consequently the reduction in photosynthesis [16, 17]. Al-Saady *et al.* [13] observed a highly significant decrease in chlorophyll concentration with increasing salinity in salt-sensitive genotypes, while a much lesser effect was recorded in salt-tolerant genotypes.

In the present study, significant percent decrease in chlorophyll a, chlorophyll band total chlorophyll content were observed in all screened fenugreek varieties with varying degrees of NaCl treatment. From this data, it is clear that the chloroplast integrity has been damaged by salinity stress in different varieties of fenugreek. These results corroborate with previous work of Talukdar [18], Agarwal *et al.* [19] and Sadak *et al.* [15]. Loss of photosynthetic pigments might be due to different reasons including chloroplastid membrane deterioration, inhibition of *de novo* synthesis of proteins which are the structural component of chlorophyll, inhibition of several photosynthesis responsible enzymes, decrease production efficiency of photosynthetic pigments such as protochlorophyll, chlorophyll and carotenoid, photosynthetic electron transport, stomatal functioning, gaseous exchange attributes, carbon metabolism, photophosphorylation in photosynthesis, destruction of chlorophyll pigments by salt induced generation of ROS, increased chlorophyllase activity (a chlorophyll degrading enzyme) and the instability of the pigment protein complex [13, 20].

Variation in content of chlorophyll a was noticed among the varieties. Variety RMt-305 and Desi shows increase in chlorophyll content at 50 mM salt concentration only. Less reduction in chlorophyll content as observed in RMt-305 compared to other four varieties with increasing saline conditions suggested that RMt-305 are capable of maintaining high pigment composition in response to salinity stress which might contribute for high photosynthetic activity and thus making them to salt tolerant.

In present study the percent decrease in chlorophyll b content was found to be more as compared to percent decrease in chlorophyll a content in all varieties of fenugreek which was in corroboration to previous work of Farahmandfar *et al.* [21]. Decrease in chlorophyll b can be explained by conversion of this into chlorophyll a that resulted in an increased chlorophyll a/b ratio. It was observed that the ratio of chlorophyll a/b was least affected for the fenugreek varieties RMt-1 and RMt-305 as compared to other lines showing least increase in ratio.

Further, it was found that total chlorophyll content was also exhibited varietal difference in terms of salinity exposure. Severe inhibition was observed in variety PEB and Kasuri revealing that these varieties are highly salt sensitive as compared to other three varieties. It was observed that variety RMT-305 not only exhibited least inhibition but also showed increased total chlorophyll content at low salt concentration further, revealed that this variety is salt tolerant. Plant synthesized sugar during vegetative stage, which is broken down during respiration by plants. Increase in different chlorophyll content at 45 DAS as observed in present study cope with increased carbohydrate content requirement of plants. Reduction in chlorophyll content occurs due to transportation of sugars carbohydrate towards sink (immature pod) and increased activity of chlorophyllase enzyme resulted in degradation of chlorophyll molecules [19]. PEB, Kasuri and Desi variety also showed necrosis at higher salt concentration further revealed inhibition of all photosynthetic pigment. Visualization of necrosis in PEB, Kasuri and Desi fenugreek variety only indicates superiority of other screened varieties of fenugreek. According to Parida and Das [22] plant growth in terms of biomass productions is a measure of net photosynthesis thus, saline environment affecting growth also affects photosynthesis.

Carotenoids, a cardinal pigments are known for protecting the photosynthetic apparatus from photo-inhibition, can quench triplet excited chlorophyll molecules and reactive singlet oxygen molecules. Thus, play an important role in salt stress tolerance [18]. In present study, it was found that variety RMT-305 is salt tolerant as it exhibited increase in carotenoids content at low salt concentration as well as less decrease at higher salt concentration as compared to other varieties. Radi *et al.*[23] had also been reported that salt tolerant genotypes have a higher chlorophyll and carotenoid content than salt sensitive ones. A detrimental effect of salinity on carotenoids content was observed at higher concentration which was also reported by Kapoor and Pande [24]. Kumawat *et al.* [25] also reported detrimental effects of salinity on various parameters of fenugreek. It was also observed that percent decrease in carotenoids content was higher in Desi, PEB and Kasuri varieties which further correlated with previous finding and confirmed that carotenoids plays an important role in maintaining salt tolerance in fenugreek.

## CONCLUSION

Fenugreek (*Trigonella foenum-graecum* L.), a member of family Fabaceae is mostly cultivated in arid and semi-arid areas of the world because of its medicinal and nutritional importance. Globally, there is growing interest in the medicinal properties of fenugreek which requires its commercial cultivation. Although, India is the leading producer of fenugreek, the average productivity of this crop is still low because of several constraints including salinity. Most clinical researchers exploring the effects of fenugreek do not consider the genetic and environmental response variability of the crop. The improvement works done in this crop is limited. The low success in fenugreek salt tolerance breeding is, at least partially, due to the lack of effective evaluation methods for salt tolerances among genotypes, low selection efficiency using overall agronomic characters and a complex phenomenon involving morphological, physiological and biochemical parameters among varieties. Thus, keeping this in mind the research was carried out and the results revealed that as the level of salt in the soil increases, the concentrations of chlorophyll a, chlorophyll b, total chlorophyll and carotenoides content declined gradually. Result findings suggested that fenugreek variety RMT-305 has maintained the homeostasis of chloroplast due to which there was an increase in all chlorophyll content at 50 mM and lesser reduction at higher salt concentration in comparison to other varieties. The chlorophyll a/b ratio showed differences between control and stressed plants at all levels of salinity. This indicates that chlorophyll b was more affected by NaCl then chlorophyll a as well as chlorophyll b plays a significant protective role in fenugreek against salt stress by maintaining chlorophyll a content. Finally the research findings conclude that among five fenugreek varieties RMT-305 is salt tolerant whereas, PEB and Kasuri variety are salt sensitive.

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