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RESEARCH ARTICLE

Evaluation of morpho-physiological attributes of Fenugreek (*Trigonella foenum graecum*) genotypes under different water regimes

■ JYOTI CHAUHAN, BAJRANG LAL KAKRALYA AND RAJESH KUMAR SINGHAL

SUMMARY

Drought is one of the major environmental constraints for the agriculture crop worldwide and overcome of yield penalty under drought situations, is the major goal for agriculturist in future. To achieve this goal, screenings of landraces is one of the most important genetic resources for crops improvement especially in dry areas. The present study was carried out during 2015-2016, in order to evaluate drought tolerance in eight fenugreek genotypes, under both control and drought conditions, various parameters were recorded at flowering and pod formation stage. The experiment was laid out in Randomized Block Design and replicated thrice. Physiological and biochemical parameters *viz.*, plant height,relative water content (RWC), chlorophyll a, b and total chlorophyll content, carotenoids content, membrane stability index (MSI) and proline content were used to assess drought tolerance in fenugreek genotypes. Ranking of genotypes based on SY at both flowering and pod formation stage showed that Rmt-1 and Rmt-305 variety has the highest SY among the tested genotypes under control and drought condition. Among the observed parameters all parameters had positive correlations with SY except proline content and recommended for screening of susceptible and tolerant fenugreek genotypes for drought stress.

Key Words : Carotenoids, Drought, MSI, Proline, Relative water content, Seed yield (SY)

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Fenugreek is an annual herb that belongs to the family *Fabaceae* and subfamily *Papilionaceae*, widely grown in Egypt and Middle Eastern countries (Balodi and Rao, 1991 and Petropoulos, 2002). Fenugreek leaves and seeds are widely consumed as a spice in food preparation because of its strong flavor and aroma and also used as an ingredient in traditional medicine (Rajagopalan, 2001). Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses

(Estrogenic, anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, anti-microbial etc.) (Sharma, 1990; Al-Habori and Raman, 2002; Smith, 2003 and Sreeja and Anju, 2010). It is also used as food additive in many countries as stew with rice in Iran, flavor cheese in Switzerland, syrup and bitter rum in Germany, mixed seed powder with flour for making flat bread in Egypt, curries, dyes, young seedlings eaten as a vegetable, roasted grain as coffee-substitute in Africa (Rajagopalan, 2001). The germinated seeds of fenugreek contain Leucine, Lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical and antioxidants like saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and to help lower sugar levels (Smith, 2003 and Khole *et al*., 2014). Nutrient analysis of fenugreek showed that it is a rich source of calcium, iron, alpha-carotene and other vitamins (Meghwal and Goswami, 2012). Fenugreek can be a very useful legume crop for short-termcrop rotation as it fixes nitrogen in the soil.

Drought is one the most important limiting factor for crop production and it is becoming a severe problem in many regions of the world. Drought is one of the major physical parameter of an environment, which determines the success or failure of plants establishment. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by evapotranspiration (Allen *et al*., 1998). Nevertheless, drought tolerance is a complex quantative trait resulting from the contribution of numerous factors (Cattivelli *et al*., 2008). Drought stress has adverse effect on plant growth, cell membrane integrity,photosynthetic activity, pigment content, osmotic adjustment, water relations and imbalance in mineral nutrition lead to yield penalty (Benjamin and Nielsen, 2006; Praba *et al*., 2009 and Farooq *et al*., 2009). A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta (2006). Landraces are the important genetic resources for improvement of crops in dry areas, since they have accumulated adaptation to harsh environment over long period. Collection and characterization of various agronomic and physiological traits of landraces are primary steps in abiotic stress tolerance programme.

With this background, the proposed research studies was planned to understand the physiological and biochemical mechanism that occurs in contrasting fenugreek genotypes under normal and water stress conditions. It is hypothesized that this information will help the plant biologists and plant breeders in developing drought tolerant fenugreek genotype in near future.

MATERIAL AND METHODS

Seeds of fenugreek genotypes obtained from Durgapura Research Station were used for the present study purpose. The experiment was conducted at Research Farm, S.K.N. College of Agriculture, Jobner (26° 05' N and 75° 28' E, 427 m above mean sea level) during *Rabi* season of 2015-16. The soil of the experimental site was loamy sand in texture, slightly alkaline in reaction. Mean annual precipitation and mean annual temperature during crop season were 400 mm and 15.5°C, respectively. Treatments are maintained by withdrawing irrigation at regular interval. Eight fenugreek genotypes were evaluated using Randomized Block Design (RBD) with three replications under irrigation (every two weeks) and drought stress (one irrigation after sowing and rainfall during the season till maturity of 50% of plants in each plot). The crop was sown on 9th November.

Several drought stress tolerance criteria were calculated using the following formula:

$$\text{RWC (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100 \text{ (Turner, 1981)}$$

– Chlorophyll a = 12.21 OD at 663nm - 2.81 OD at 645nm(Hiscox and Israelstam, 1979) DMSO method

– Chlorophyll b = 20.13 OD at 645nm – 5.03 OD at 663nm

– Total chlorophyll = 22.2 O.D. at 663nm + 8.02 O.D. at 645nm or

– Total chlorophyll =chlorophyll a +chlorophyll b

– Carotenoids = (1000 OD 470nm -3.27 chlorophyll a -104 chlorophyll b) /229

– MSI% = $(1-C_1/C_2) \times 100$(Premachandra *et al*., 1991)

where,

C_1: Electrolyte leakage (Conductivity) at 40° C C_2: Electrolyte leakage (Conductivity) at 100°C

Prolinec ontent (mg $100g^{-1}$FW) were measured by method of Bates *et al.* (1973).

Statistical analysis:

Mean values were taken from each treatment of three independent replications; and statistical package for Social Science (SPSS Version 16.0) was used for the analysis of Random Block Design (RBD). Significant differences among various treatments were determined using Duncan's test.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Plant height (cm):

Plant heights were observed at harvesting stage under both control and drought conditions. In Table 1 the highest plant height observed in genotype was Rmt-365 88.89 cm (control) and Rmt-361 72.54 cm (drought), while lowest in Rmt-1 70.59 cm (control) and Rmt-365 55.54 cm. Under drought condition percent reduction was highest in Rmt-365 60.04 per cent and lowest in Rmt-1 6.57 per cent. SPSS analysis showed significant difference between genotype under control and drought condition.

Relative water content (RWC %) :

RWC content in fenugreek leaves were measured in both control and drought condition at flowering and pod stages. The highest reduction in RWC under drought condition was in genotype Rmt-365 23.7 per cent and 25.14 per cent followed by variety Rmt-143 13.90 per cent and 21.90 per cent, respectively at flowering and pod formation stages. However, minimum RWC content reduction under drought was observed in Rmt-305 and Rmt-303, which was 2 per cent (at flowering stage), 2 per cent (Flowering stage) 1.94 per cent (Pod stage) and 1.43 per cent at pod stage. Highest RWC content under control condition was observed in Rmt-143 80.13 per cent (flowering stage) and 78.68 per cent (pod stage) and lowest in Rmt-361 73.98 per cent (flowering stage) and variety Rmt-351 70.32 per cent (pod stage). Under drought condition highest RWC was observed in Rmt-305 78.89 per cent (flowering stage) and 70.53 per cent (pod stage), while lowest in Rmt-365 63.52 per cent

Table 1 : Representing the plant height (cm) of fenugreek genotypes at harvesting stage. Presented data in table are the mean of three replication and ± represent standard deviation between replication. Within each genotypes different letters indicate significant difference by Duncan`s multiple test at P<0.05

Sr. No	Name of genotype	Control	Drought
Plant height (cm)			
1.	Rmt-1	70.59±0.75[f]	66.23±2.06[b]
2.	Rmt-143	80.32±1.93[c]	63.34±1.84[b,c]
3.	Rmt-303	76.54±1.87[d]	60.20±2.01[c]
4.	Rmt-305	75.34±2.13[d,e]	62.54±2.18[b,c]
5.	Rmt-351	74.32±2.00[d,e]	63.98±1.67[b,c]
6.	Rmt-354	84.39±1.80[b]	72.54±2.36[a]
7.	Rmt-361	72.54±2.08[e,f]	60.38±2.18[c]
8.	Rmt-365	88.89±1.38[a]	55.54±2.73[d]

Table 2 : Representing the seed yield (g plant^{-1}) of fenugreek genotypes at harvesting stage. Presented data in table are the mean of three replication and ± represent standard deviation between replication. Within each genotypes different letters indicate significant difference by Duncan`s multiple test at P<0.05

Sr. No.	Name of genotype	Control	Drought
Seed yield (gplant^{-1})			
1.	Rmt-1	6.90±[a]	6.24±[a]
2.	Rmt-143	6.30±[a]	5.19±[a,b]
3.	Rmt-303	7.02±[a]	5.85±[a,b]
4.	Rmt-305	6.55±[a]	6.08±[a,b]
5.	Rmt-351	6.70±[a]	5.80±[a,b]
6.	Rmt-354	6.82±[a]	5.08±[b]
7.	Rmt-361	6.45±[a]	5.98±[a,b]
8.	Rmt-365	6.98±[a]	5.45±[a,b]

(flowering stage) and 61.32 per cent (at pod stage).SPSS analysis showed significant difference between genotype under control condition but under drought value were non-significant (Fig.1a and b) .

Membrane stability index (MSI %FW):

MSI content (%) in fenugreek leaves were observed in both control and drought condition at flowering and pod stage. Studies on membrane stability index in fenugreek leaves was showed highest MSI % in Rmt-354 73.16 per cent (flowering stage) and 76.54 per cent (pod stage) under control condition, while in drought condition, highest MSI for Rmt-305 63.58 per cent (flowering stage) and 67.54 per cent (pod stage). Under control condition minimum MSI was observed in Rmt-361 64.25 per cent (flowering stage), 63.83 per cent (pod stage). Under drought condition highest reduction was observed in Rmt-354 34.91 per cent and 18.02 per cent at flowering and pod stage, while minimum reduction was in variety Rmt-305. SPSS analysis showed significant difference between genotype under control and drought condition (Fig. 2a and b).

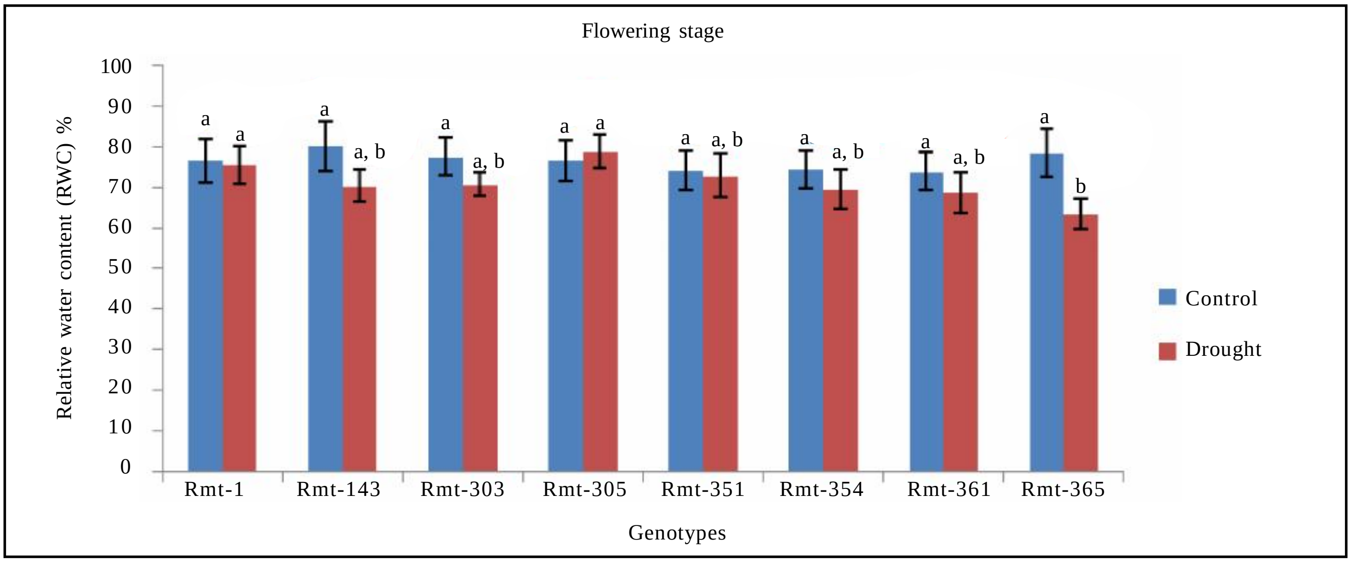

Fig. 1a : Representing the Relative water content (RWC) of fenugreek genotype at flowering stage. Presented data in graph are the mean of three replication and error bar represent standard deviation between replication. Within each genotypes different letters indicate significant difference by Duncan's multiple test at P<0.05

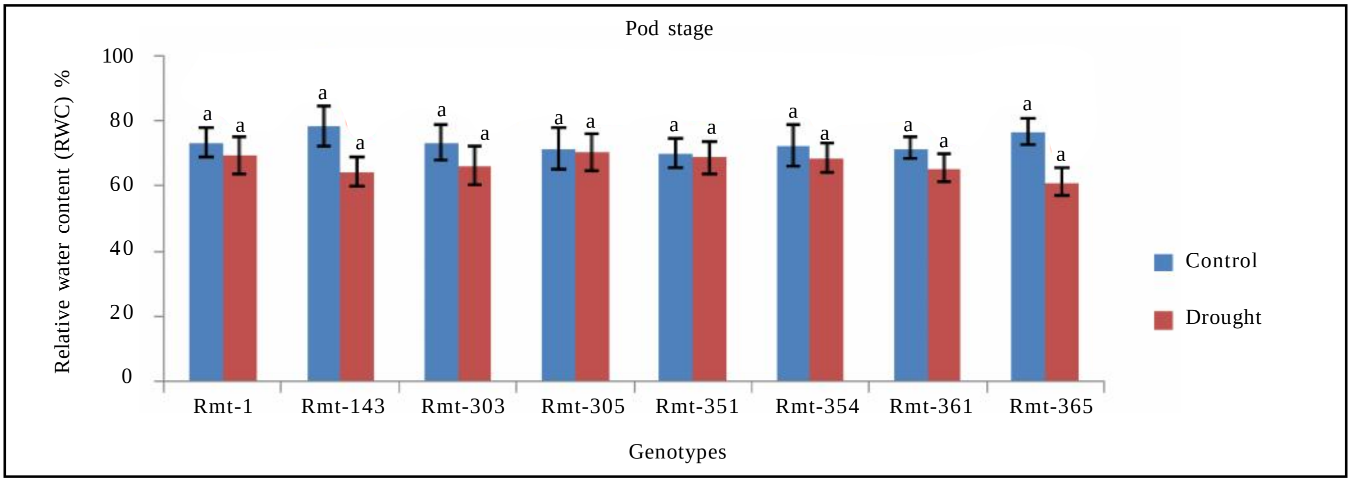

Fig. 1b : Representing the Relative water content (RWC) of fenugreek genotype at pod stage. Presented data in graph are the mean of three replication and error bar represent standard deviation between replication. Within each genotype same letters indicate non-significant difference by Duncan's multiple test at P<0.05

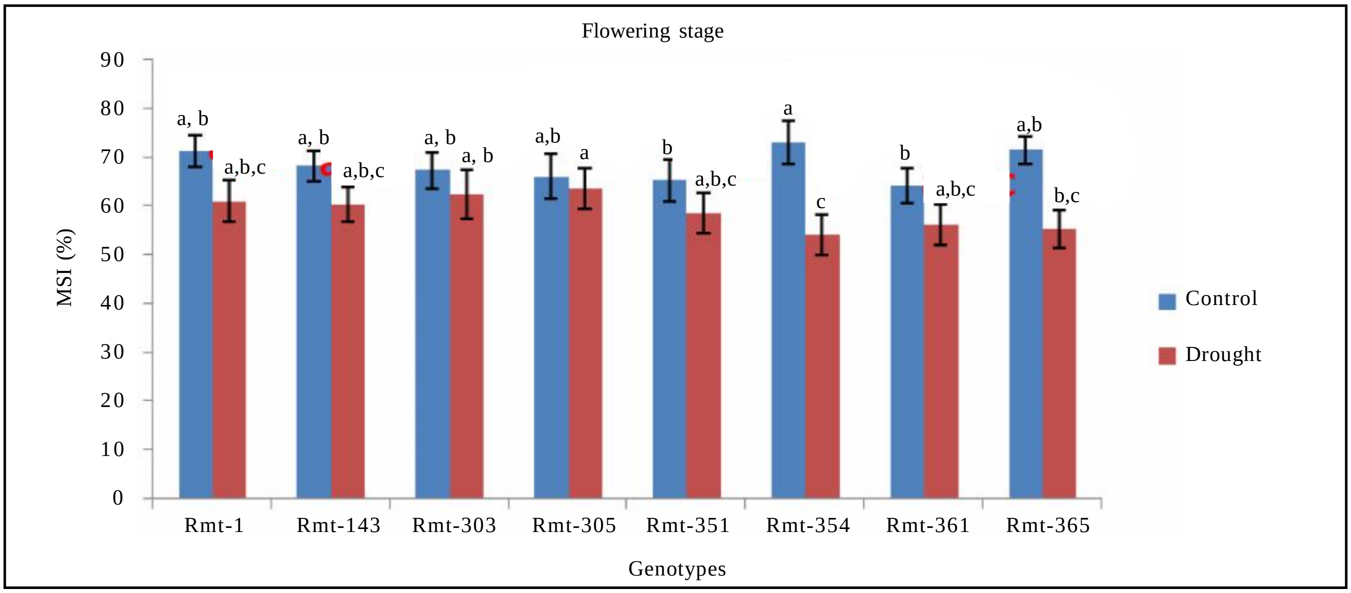

Fig. 2a : Representing the membrane stability index (MSI % FW) of fenugreek genotypes at flowering stages. Presented data in graph are the mean of three replication and error bar represent standard deviation between replication. Within each genotypes different letters indicate significant difference by Duncan's multiple test at P<0.05

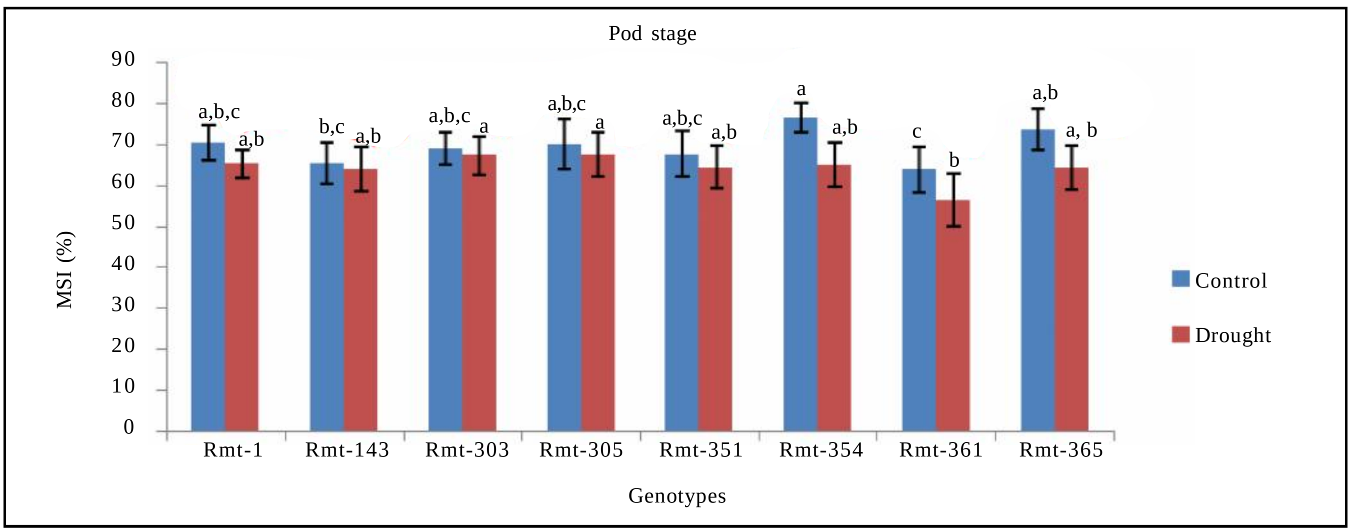

Fig. 2b : Representing the membrane stability index (MSI % FW) of fenugreek genotypes at pod stage. Presented data in graph are the mean of three replication and error bar represent standard deviation between replication. Within each genotypes different letters indicate significant difference by Duncan's multiple test at P<0.05

Proline content (mg100^{-1} FW) :

Studies on proline content in fenugreek leaves were observed in both control and drought condition at flowering and pod stage. Highest proline content under control was observed in Rmt-305 64.81 (flowering stage) and 254.45 (pod stage), while lowest in Rmt-143 51.79 (flowering stage) and 149.54 (pod stage). Under drought condition highest proline content were measured in Rmt-143 102.17 (flowering stage) and 388.31 (pod stage), while minimum in Rmt-365 57.93% (flowering stage) and 220.80 (pod stage). SPSS analysis showed significant difference between genotype under control and drought condition (Fig. 3a and b).

Chlorophyll (a,b), total chlorophyll and carotenoid content (mgg^{-1} FW):

Chlorophyll (Chl. a, b), total chlorophyll and carotenoids contents were measured in fenugreek

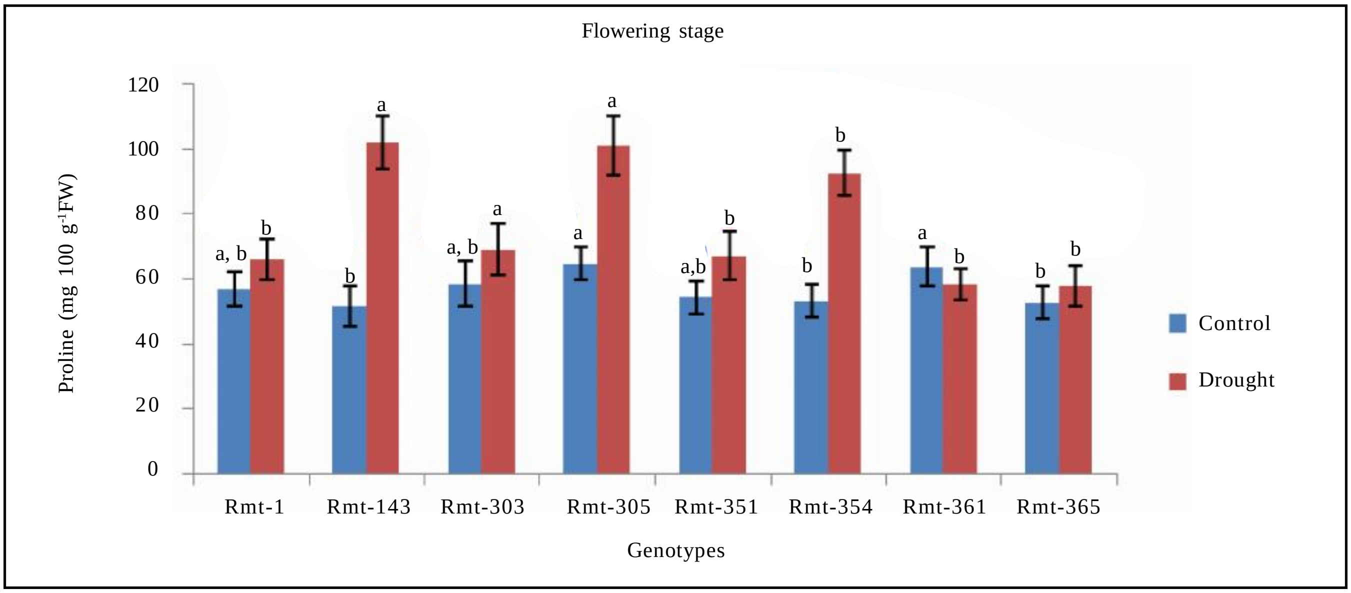

Fig. 3a : Representing the proline content of fenugreek genotypes at flowering stage. Presented data in graph are the mean of three replication and error bar represent standard deviation between replication. Within each genotypes different letters indicate significant difference by Duncan's multiple test at P<0.05.

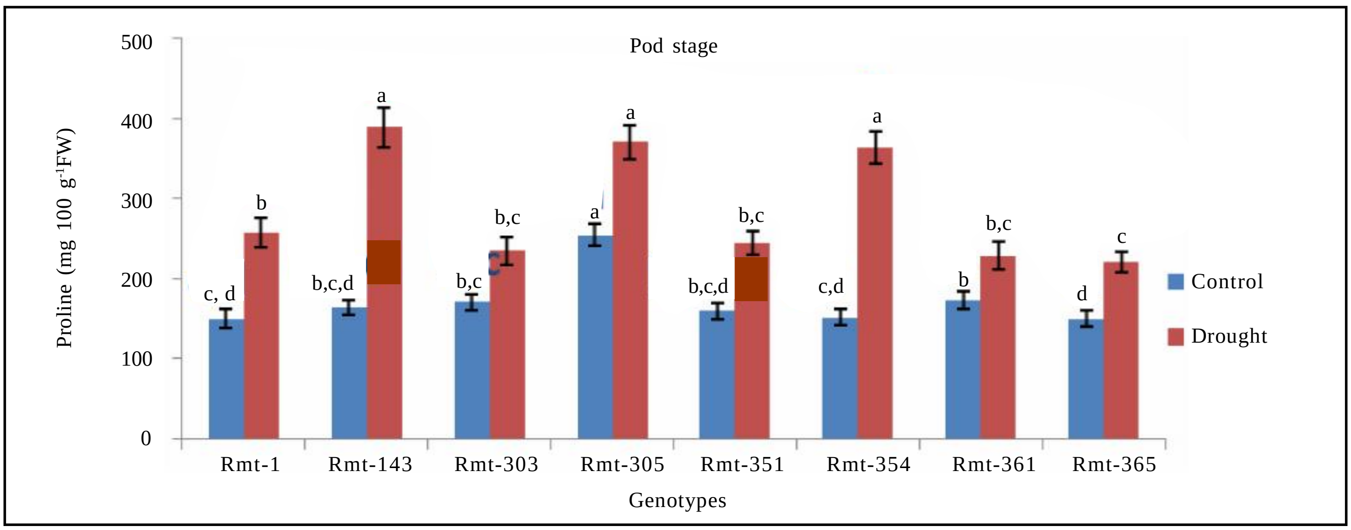

Fig. 3b : Representing the Proline content of fenugreek genotypes at pod stage. Presented data in graph are the mean of three replication and error bar represent standard deviation between replication. Within each genotypes different letters indicate significant difference by Duncan's multiple test at P<0.05

leavesunder both control and drought condition at flowering and pod stages. Result showed that chlorophyll (Chl. a, b), total chlorophyll and carotenoids contents were highest in the variety Rmt-365 followed by Rmt-361 and Rmt-351 under control condition at flowering and pod stage.Under drought condition chlorophyll (Chl. a, b), total chlorophyll and carotenoids contents were highest in the Rmt1 at flowering and pod stage. Under drought condition highest reduction in chlorophyll (Chl. a, b), total chlorophyll and carotenoids contents were observed 90.44%, 93.75%, 90.76%, 45.45% (flowering stage) and 84.09%, 86.30, 80.56%, 60.71% (pod stage) in Rmt-365. SPSS analysis showed significant difference between genotype under control and drought condition (Fig 4a - h).

Seed yield (g plant^{-1}) :

Seed yield per plant observed in all genotype under both control and drought conditions and it was found that in control condition highest seed yield from genotype Rmt-

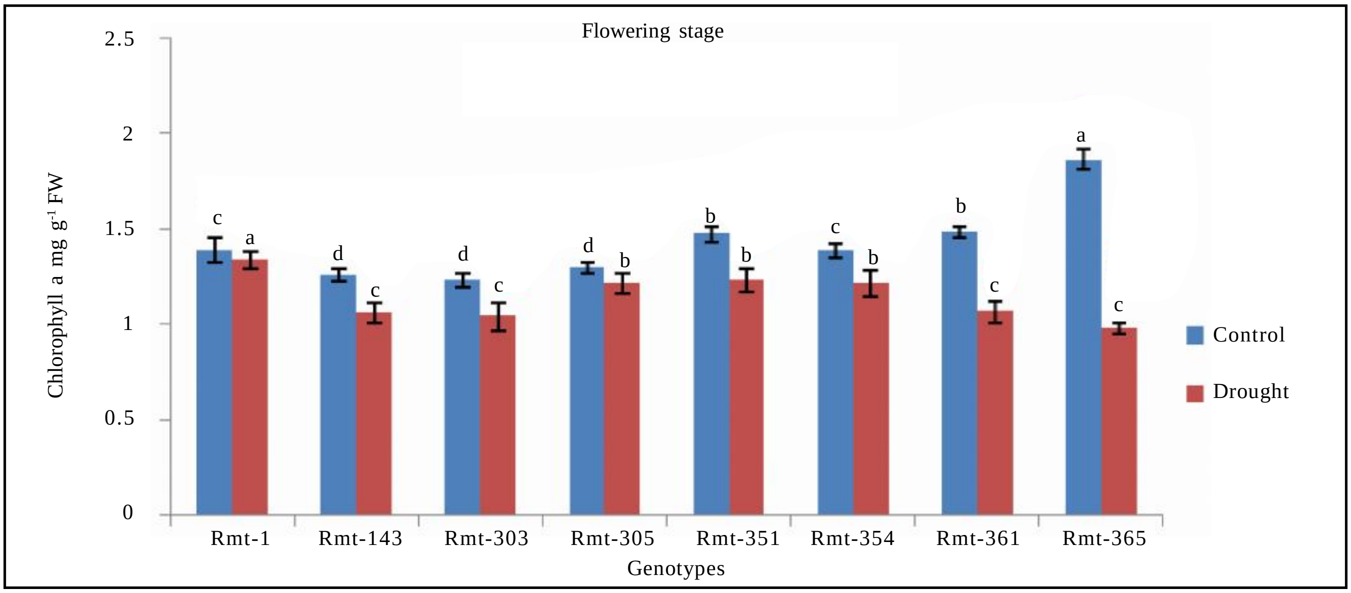

Fig., 4 a

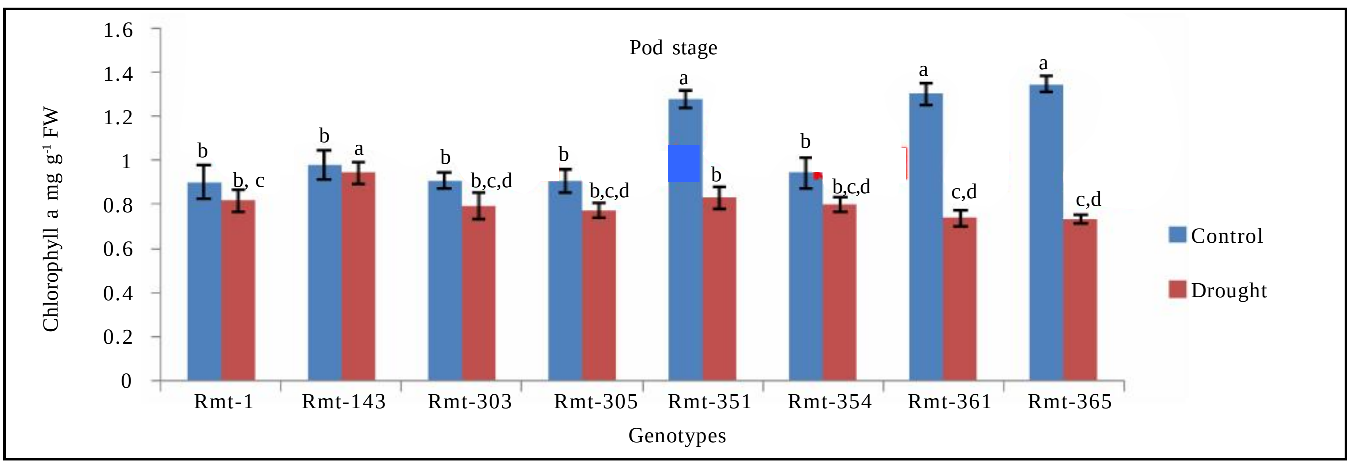

Fig., 4 b

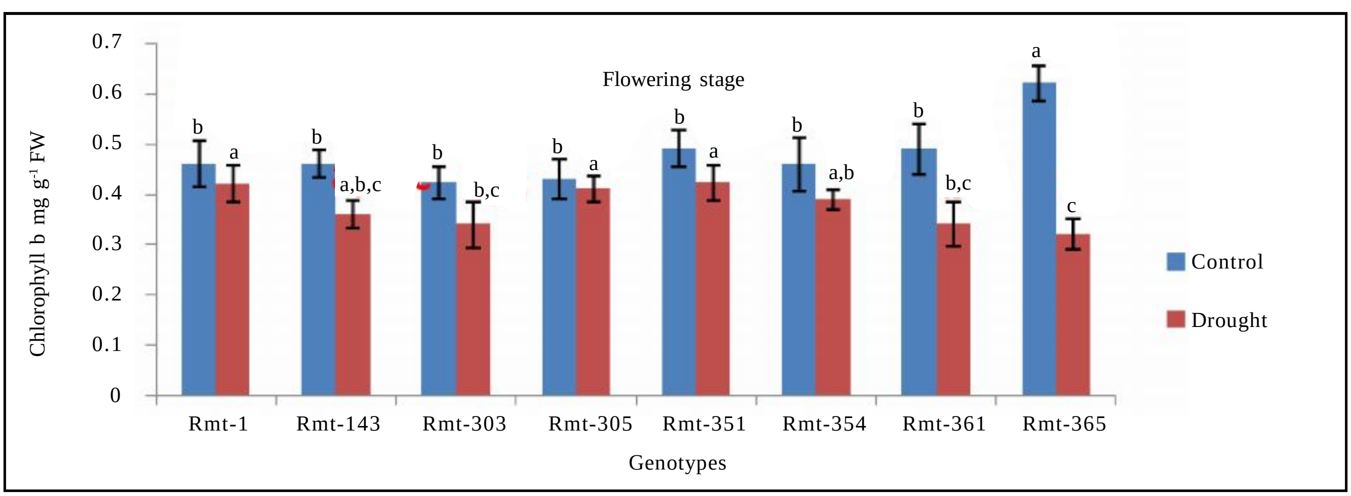

Fig., 4c

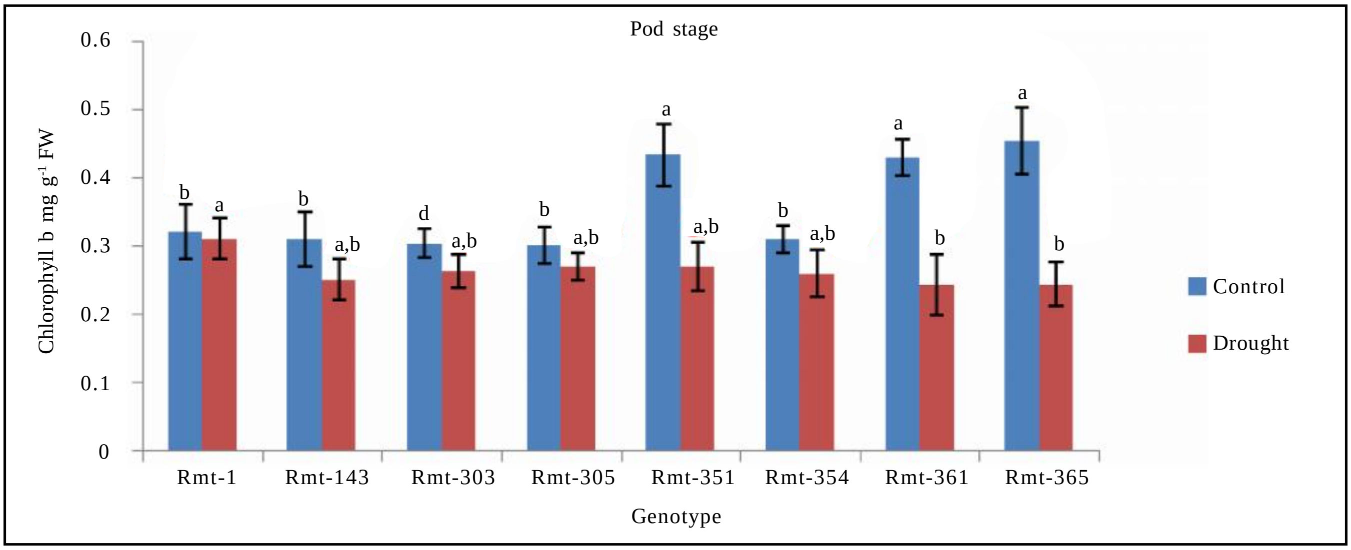

Fig. 4 d

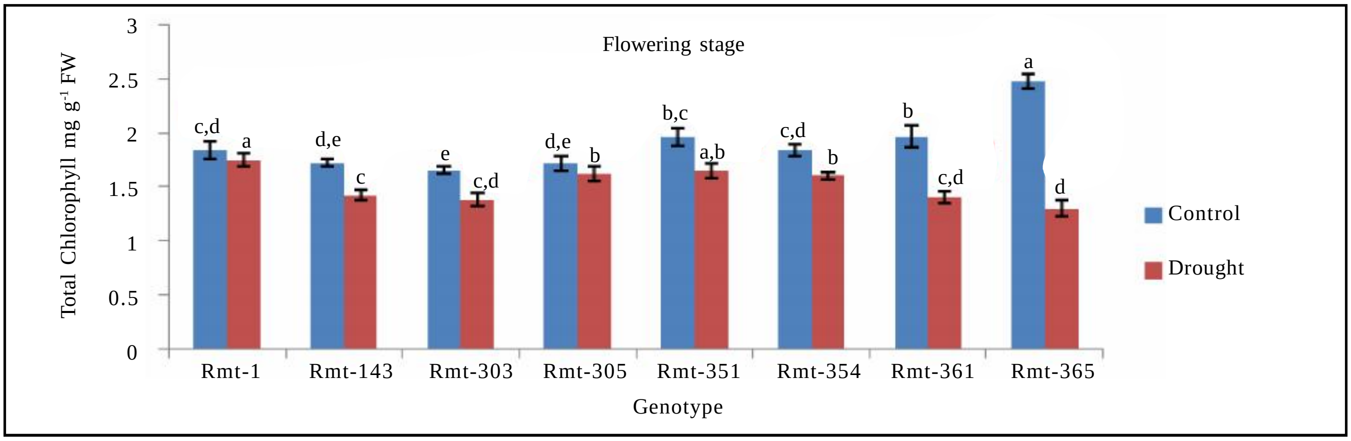

Fig. 4 e

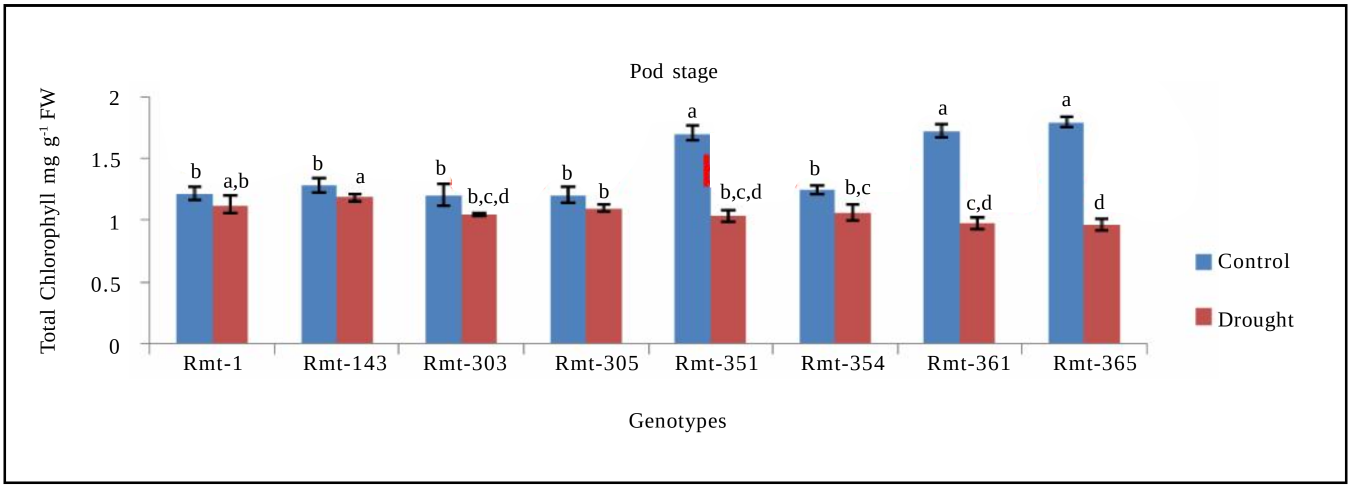

Fig. 4 f

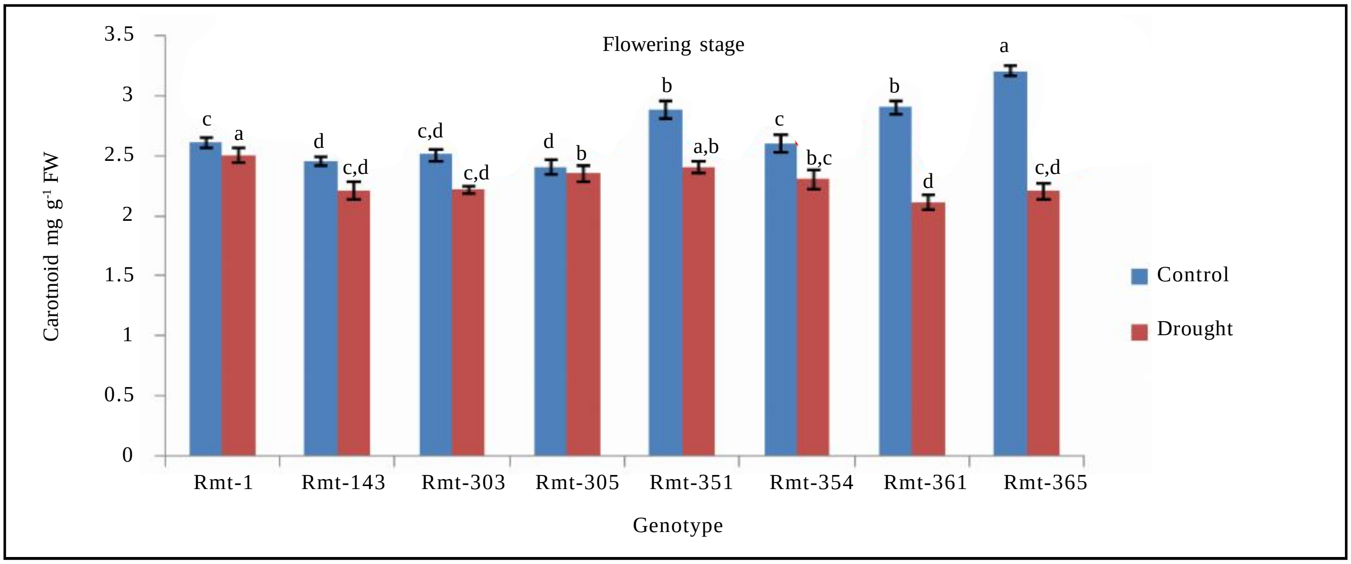

Fig. 4 g

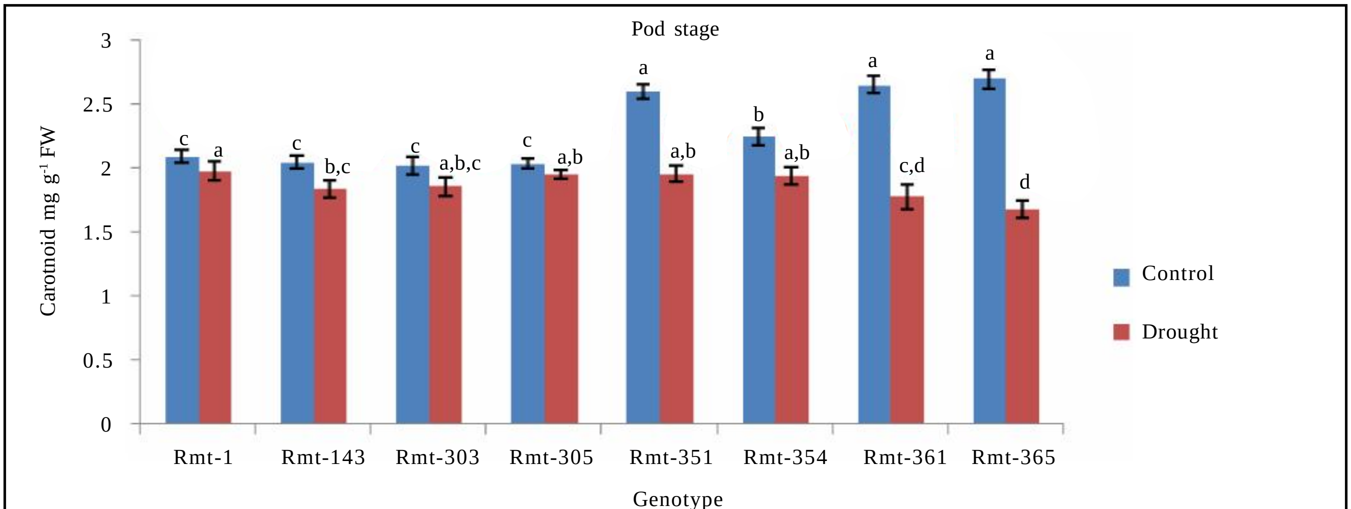

Fig. 4h

Fig. 4a-h are representing the Chlorophyll (Chl. a, b), total chlorophyll and carotenoids contents (mgg^{-1}FW) content of fenugreek genotypes at flowering and pod stage respectively. Presented data in graph are the mean of three replication and error bar represent standard deviation between replication. Within each genotypes different letters indicate significant difference by Duncan's multiple test at P<0.05

303 7.02 followed by Rmt-365 6.98, while lowest in Rmt-143 6.30 followed by Rmt-361 6.45. Under drought highest seed yield was observed in genotype Rmt-1 6.24 followed by Rmt-305, while lowest in Rmt-354 5.08 followed by Rmt-143 5.19 g plant^{-1}. SPSS analysis showed significant difference between genotype under drought condition but under control data were non-significant. Observations on seed yield plant^{-1} under control and drought conditions are represented in Table 2.

Water is one of the major factor which limits the crop production. Fenugreek crop responds to water stress in the form of changes in various physiological, biochemical and molecular processes. In the present study, with eight genotypes varying in field performance in response to water stress, morpho-physiological parameters are evaluated. All these parameters helped in assessing tolerant versus susceptible genotypes at physiological levels between the two critical stages of water stress.

Result of present study showed that the relative water content decreased significantly in all the varieties at both flowering and pod formation stage during drought condition. Exposure of plants to drought stress substantially decreased the leaf water potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature, which negatively affect the seed yield (Siddique *et al*., 2001). In present investigation a significant and positive correlation of relative water content with seed yield was observed at flowering and pod formation stages.

In the present study, Chlorophyll a, b, total chlorophyll and carotenoid content decreased significantly in all the varieties at both the stages due to water stress condition. Low level decrease in chlorophyll in genotypes indicates that their photosynthetic apparatus is able to resist adverse conditions. On the other hand accumulation of higher chlorophyll content at flowering compared to pod formation stages may be due to sugar synthesized in photosynthesis and breaks down during respiration by plants. β-carotene present in the chloroplasts of all green plants is exclusively bound to the core complexes of photosystem I and photosystem II. β-carotene functioning as an accessory pigment, antioxidant through direct quenching of triplet chlorophyll Therefore, for better yields under stress, higher chlorophyll and chlorophyll content contributes to higher plants productivity. The resulted are supported work of Wahid (2007) and Agrawal *et al*. (2013). Exposure of plants to certain environmental stresses quite often leads to the generation of reactive oxygen species (ROS). ROS cause lipid peroxidation and consequently membrane injuries, protein degradation and enzyme inactivation. It is generally accepted that the maintenance of integrity and stability of membranes (MSI) underwater stress is a major component of drought tolerance in plants (Premachandra *et al*., 1991 and Sairam *et al*., 2005).

In the present study, higher levels of proline were observed in all genotypes subjected to water stress as compared to control. Hence, the results of present investigation showing high accumulation of proline at pod formation compared to flowering in the stressed tissues of all genotypes indicates pod formation stage to be a more responsive stage in terms of cellular osmotic adjustment. In present study, the varieties Rmt-301 and Rmt-305 exhibited higher grain yield, thus showing the existence of drought tolerance mechanism.

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