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Screening of fenugreek genotypes under normal and limited moisture stress condition

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Abstract
A field experiment was conducted with eight fenugreek genotypes comprised of advanced lines and released varieties *viz*. UM-71, UM-68, UM-78, UM-80, UM-90, Rmt-305, Rmt-354 and Rmt-143 to assess the genetic variability under drought condition. Moisture stress was created by withholding irrigation in one set of genotypes at active growth stages while other sets were given normal irrigation. Different physiological, biochemical and antioxidant parameters, yield and yield contributing parameters were recorded under optimal and limited irrigation at flowering and pod filling stages. The result showed that different physiological parameters such as relative water content (RWC), chlorophyll content, carotenoids and membrane stability index decreased while biochemical parameters like proline, glycine betaine content and antioxidant enzymes like superoxide dismutase (SOD) and catalase increased under drought condition.
Most of the physio-biochemical and yield character showed superiority (highest/lowest) in genotype UM-68 followed by Rmt-305and Rmt354. Drought susceptibility index was the lowest, whereas drought tolerance index recorded highest in UM-68 followed by Rmt-305. Drought tolerant genotypes could be selected on the basis of high RWC, MSI, Photosynthetic pigments, proline, GB content, high activity of superoxide dismutase, catalase, yield components and DTI and lower lipid peroxidation and DSI. In the present investigation, UM-68, Rmt-305 and Rmt-354 were found the best suited genotypes under drought stress. These genotypes supposed to carry the drought tolerant genes and may be used for further drought tolerance breeding.

Keywords: Fenugreek genotypes, drought stress, genetic variability, physio-biochemical traits

Introduction

Abiotic stresses such as drought, heat, salinity, etc. are major threats to agriculture and reduce growth and cause severe losses of crop yield due to different physiological, morphological and molecular level changes (Boyer, 1992). Water restriction is expected to results in losses of up to 30% of world crop production by 2025 compared to current yields (Grafton *et al*., 2015). In response to drought stress, there is a wide range of phenotypic diversity and stress damages that allows for a wide range of varieties for drought tolerance not only among the species but also among genotypes related to the same species (Grafton *et al*., 2015; Jaleel *et al*., 2009) [28]. The decline of photosynthesis, metabolic disturbance and finally the death of the plant are the result of high-water stress (Jaleel *et al*., 2009) [28]. In addition, the response of plants to water stress varies significantly depending on the duration and intensity of stress as well as species of plant and the growth stage of plant (Dacosta & Huang, 2007) [14].

Water stress leads to cellular accumulation of reactive oxygen species (ROS), which is the consequence of disequilibrium between electron excitation and utilization through photosynthesis. Oxidative stress as a result of ROS effect can cause detriments to cell membranes, pigments, nucleic acid, proteins, and other essential cellular molecules and processes (Saed-Moucheshi *et al*., 2014) [43]. Once the integrity of the cell membrane has disrupted, a sequence of biochemical and cellular events will be initiated which is measurable by the intercellular concentration of malondialdehyde (MDA) (Hasanuzzaman *et al*., 2013) [23]. During the drought stress, the plant adopts several intrinsic mechanisms to cope with the oxidative damages. The antioxidant defense system, including enzymatic and non-enzymatic antioxidants, is one of the stress-protective strategies to reduce and restrain the destructive effects of oxidative damages (Abid *et al*., 2018) [1]; Hasanuzzaman *et al*.,2013) [23]. For instance, enzymatic antioxidants such as superoxidedismutase (SOD), catalase (CAT), ascorbateperoxidase (APX), glutathione reductase (GR), etc., play critical roles in the ascorbate-glutathione pathway and balance the cellular redox state (Hasanuzzaman *et al*., 2019) [24]; Noctor *et al*., 2016) [35]. Participation of phenolic compounds in the non-enzymatic antioxidant defense system plays a significant protective role by scavenging free radicals (Azizi *et al*., 2019) [7]. Carotenoids act as a non-enzymatic antioxidant by dissipating excess energy and scavenging ROS. Carotenoids ultimately provide photosynthetic membrane constancy and reduce SOD activity (Havaux, 1998) [25]; Pompelli *et al*., 2010) [39]. Another mechanism to maintain plant cellular functions under drought situations is changing water relations by synthesizing and accumulating compatible solutes such as free amino acids and water-soluble carbohydrates (Abid *et al*., 2018) [1]; Mafakheri, 2011) [30]. Noteworthy, after rewatering, recovery of the metabolic activities would be facilitated through osmotic adjustment (Abid *et al*., 2018) [1].

Fenugreek (Trigonella foenum-graecum L.) 2n = 16 (Fryer, 1930) popularly known by its vernacular name ‘methi’ is an important condiment crop, largely grown in Northern India during Rabi season. Fenugreek occupies a prime position among various seed spices grown in India. It is an annual herb belonging to sub-family papilionaceae of the family Leguminosae. It is mainly a condiment, but its seeds are also used as carminative and are an ingredient of several ayurvedic medicines, mainly those prescribed for promoting

appetite, correcting disorder and for relieving joints pain particularly in old age life. It is grown both for seed as well as for fodder purpose. It is one of such crops in which every part is consumed in one or other form. In addition to this it serves as a soil renovating crop. The leaves and shoots are quite rich in protein, minerals and vitamin A and C. It is used as a main constituent in curry powder. Seeds are bitter in taste due to presence of an alkaloid "trigonelline". In recent years the importance of fenugreek has further increased due to presence of a steroid called "diosgenin". Diosgenin is used in the synthesis of sex hormone and oral contraceptives. It is well known that drought stress brings about numerous metabolic, biochemical and physiological changes in plants like growth (Ashraf and Iram, 2005; Benjamin and Nielsen, 2006) [5, 11], water status, membrane stability (Bai *et al*., 2006) [8], pigment content and photosynthetic activity (Ekmekc *et al*., 2005) [18]. Drought impacts include growth, yield, membrane integrity, pigment content, osmotic adjustment, water relations, and photosynthetic activity (Benjamin and Nielsen, 2006) [11]; Praba *et al*., 2009). So, the present study was undertaken to evaluate the drought tolerance on the basis of physio-biochemical parameters. The identified genotypes with higher drought tolerance will be use as starting material in the fenugreek drought tolerance breeding program.

Materials and methods

The experiment was carried out at S.K.N. College of Agriculture, Jobner during *rabi* season 2021-22. The cite of experiment situated at latitude of 26005' N, longitude of 75020'E and at the height of 427 m above mean sea level (Shivran *et al*., 2016) In order to achieve the objectives of present investigation the experiment was planned and executed as described below: The seeds of eight fenugreek genotypes comprised of advanced lines and released varieties *viz*. UM-71, UM-68, UM-78, UM-80, UM-90, Rmt-305, Rmt-354 and Rmt-143 were procured from, AICRP on seed spices, Department of Plant Breeding and Genetics, SKN College of Agriculture, Jobner. Seeds of these genotypes were sown in triplicates with three environments namely no water stress, midterm water stress (flowering stage) and terminal water stress (Pod formation stage) at research farm of S.K.N. College of Agriculture, Jobner, Jaipur. Irrigations were given at sowing stages in all three environments. Moisture stress condition was created by withholding irrigation at flowering stage in one set of genotypes (midterm moisture stress) while other two sets were given normal irrigation. Second withdrawal of irrigation was done at pod formation stage (terminal moisture stress) while other two sets were given normal irrigation. The pots were irrigated once every other day pots experiment planted in 3mX 2m plot using the spacing between row to row was kept 30 cm and plant to plant was 10 cm. The recommended dose of fertilizer (20 kgN, 25 kg P2O5 and 40 kg K2O) were applied as per standard agronomic practices. Plant protection measures were also applied as and when needed. The different physio-biochemical parameters were recorded after 8 days of imposing stress. Yield and yield related traits were recorded at the time of harvesting the crop.

Physio-biochemical parameters

Water status and membrane stability: Leaf relative water content (RWC) was estimated according to Barrs and Weatherly (1992) and calculated as:

RWC =)) [(Fresh mass - dry mass)/ (saturated mass - dry mass)] × 100.

For membrane stability index, the shoot portion (100 mg) of control and stressed plants were thoroughly washed and then placed in 10 ml of double distilled water at 40ºC for 30 min (Sairam *et al*. 1997) [44]. Electrical conductivity was measured by conductivity meter (C1). Subsequently, the same samples were placed on boiling water bath (100ºC) for 10 min and their electrical conductivity was recorded again (C2). The membrane stability index (MSI) was calculated as: MSI =)) [1-(C1 /C2)] × 100.

Pigments and osmolytes: Total chlorophyll content was determined as per method described by Hiscox and Israelstam, 1979) [27]. Finely chopped 50 mg fenugreek leaves were weighed in graduated test tube. Ten ml DMSO was added to each tube and incubated at 65 ºC for 3 hrs. After incubation the tubes were allowed to cool at room temperature and the volume made up to a total of 10 ml by adding DMSO. The optical density (OD) was recorded at 663 and 645 nm by taking DMSO as blank. The amount of chlorophyll present in the sample was calculated using standard formulae:

Total chlorophyll (mg/g) =)) [20.2 ($O.D_{645}$) + 8.02 ($O.D_{663}$)] X

Estimation of Carotenoid content: The same extract of chlorophyll was used for carotenoid content and absorbance was recorded as described by Wellborn, 1994 at 480 nm. Carotenoid content was calculated using the formulae:

Carotenoids (mg g^{-1}fr. wt.) =)) [7.6 ($O.D_{480}$) - 1.49 ($O.D_{510}$)] X

Where,

V = Volume of filtrate

W = Fresh weight of leaf

d = Diameter of cuvette

OD= OD is the absorbance at specific wavelength

Proline

For proline estimation, samples were homogenized in 5 ml of 3% aqueous sulphosalicylic acid and centrifuged at 5000xg for 5 min (Bates *et al*. 1973). An equal volume of glacial acetic acid and ninhydrin solution were added to the extract. The samples were heated to 100ºC for 1 hour and 5 ml toluene was added. The absorbance of the toluene layer was measured at 528 nm. The quantity of proline was calculated using standard curve.

Determination of Malondialdehyde

Malondialdehyde (MDA) concentration was determined by the method described by Heath and Packer, (1968) [26]. Two hundred mg fresh leaf samples were extracted in 5.0 ml of 6% trichloroacetic acid (TCA) solution, centrifuged at 8000 rpm for 10 minutes. Two ml of Thio-Barbituric Acid (TBA) reagent was added in 1 ml of supernatant, mixed well and incubated for half an hour in a boiling water bath. The tubes were than cooled to room temperature. The assay mixture was then centrifuged at 5000 rpm for 10 minutes. Supernatant bearing yellow to light orange colour was read on spectrophotometer at two wavelengths *viz*. 532 nm (major for MDA) and 600 (minor for interfering substance), millimolar concentration of MDA was calculated as follows: MDA (mM) = (O.D.532 -O.D. 600) x 155 (extinction coefficient).

Estimation of Glycine Betaine: (Total Quaternary Ammonium Compounds, QAC)

Glycine betaine concentrations were determined as described by Grieve and Gratten, 1983) [21]. Leaf sample (250 mg - 1.0 g) was finely grind in 20 ml distilled water and shaken mechanically for 24 hrs at 25 ºC. The samples were then filtered. The extract was diluted 1:1 with 2N H_2SO_4. Aliquots (0.5 ml) were measured into 2 ml Eppendorf tubes and cooled in ice water for 1 hr. Cold 0.2 ml KI-I_2 reagent was added to each tube and the reactant gently stirred on a vortex mixture. The tubes were stored at 4 ºC for 16 hrs and then centrifuged at 10,000 rpm for 15 minutes at 0 ºC. The supernatant was carefully aspirated. Per-iodide crystals were dissolved in 9 ml of 1, 2 dichloromethane, and vigorous vortex mixing was frequently required to effect complete solubilization in the developing solvent. After 2-2.5 hrs. the absorbance was measured at 365 nm on a UV-spectrophotometer. Reference standard of Glycine Betain (50-200 micro gm/ ml) were prepared in 1N H_2SO_4. Standard curve was prepared and the GB content of sample was calculated using the formula:

Glycine Betaine = sample O.D. x graph factor x dilution factor.

SOD (Superoxide dismutase)

Superoxide dismutase assay was performed as per the protocol of Dhindsa *et al.* (1981) [16]. 200 mg of leaves were homogenized in a pre-chilled mortar and pestle under ice cold conditions using 3.0 ml of extraction buffer, containing 50 mM sodium phosphate buffer (pH-7.4), 1ml EDTA and 1 per cent (w/v) polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 10,000 rpm for 20 minutes and the supernatants were used for enzymatic measurement.

Total SOD (1.15.1.1) activity was measured spectrophotometrically based on inhibitions in the photochemical reductions of nitroblue tetrazolium (NBT). The 3 ml reaction mixture contained 50 mM Sodium phosphate buffer (pH-7.8), 13 mM methionine, 75 uM NBT, 2 uM riboflavin, 0.1 mM EDTA and 0.1 ml enzyme extract, riboflavin was added last, (Van Rossun *et al.,* 1997). After addition of all these components and mixing, test tubes was placed on stand 30 cm below a light source consisting of four 15-W fluorescent lamps. The photochemical inhibitions was allowed to happen for 10 minutes and stopped by switching off the light source. The photo reduction in NBT was measured as increase in absorbance at 560 nm. Blanks and controls were run in the same way but without illuminations and enzyme respectively. One unit of SOD was defined as the quantity of enzyme required to inhibit the reductions of NBT by 50 per cent in comparison to control. One unit of sod was calculated according to formula given below:-

$$\text{SOD unit} = \frac{\text{O.D. control (without enzyme) - O.D sample (with enzyme)}}{\text{O.D. (control)/2}}$$

$$\text{SOD U/mg .f. wt} = \frac{\text{SOD unit}}{\text{mg .f. wt}}$$

Catalase

The catalase activity was assayed as per the protocol of Chance and Maehly (1955) [13]. Sample were prepared by grinding 0.5 g fresh leaves in ice cold 50 mM potassium phosphate buffer (P ^{H} 7.0) containing 0.1 mM EDTA and 1 per cent polyvinylpyrrolidone (PVP). The homogenate was filtered through four layers of cheese cloth and then centrifuged at 4 ^{0}C for 20 min. at 15000 rpm. The supernatant was diluted and an appropriate aliquot dilution of the crude extract was taken for enzyme assay. CAT activity was measured by following the decomposition of H_2O_2 at 240 nm (Ɛ = 39.4 mm $^{-1}$cm $^{-1}$) in a reaction mixture containing 50 mM phosphate buffer (P ^{H}-7.0) and 15 mM H_2O_2 decomposed mg $^{-1}$ f.wt. min $^{-1}$.

ΔO.D.

$$\text{mmol/min/ mg .f. wt} = \frac{\Delta\text{O.D.}}{\text{Enzyme conc. (g)} \times \text{mg f. wt.} \times €}$$

Drought susceptibility Index was calculated for yield over limited moisture (Stress) and normal (non-stress) environment as per formula given by Fisher and Maurer (1978) [19].

DSI= (1-YD/YP)/D

Where YD= Mean of the genotype in limited moisture environment

YP= mean of the genotype in limited moisture environment

D= Stress intensity= 1-(Mean Y_D of all genotypes/Mean Y_P of all genotypes)

Drought Tolerance Index (DTI)=)) [(YP x (YD)/(YP)2]

YP= Yield of genotypes under normal condition

YD= Yield of genotypes under normal condition

Five competitive plants were selected at random for recording the observations on number of pods per plant, Number of seed per pod, 1000-seed weight (g) and Seed yield per plants (g).

Statistical analysis: All the observation was taken in each genotype, replications and sets. The data was statistically analyzed as per Panse and Sukhatme, (1985) [36]

Results and discussion

Fenugreek crop responds to drought in the form of changes in various physiological, biochemical and molecular level has gained momentum in many laboratories around the world. In the present study, eight genotypes varying in performance in response to moisture stress, in terms of physiological attributes (relative water content, chlorophyll, carotenoids, membrane stability index, proline) and antioxidant like SOD, MDA, catalase, Glycine Betain monitored at midterm and terminal moisture stress stages. Besides this seed yield and contributing traits were also measured at maturity. All the parameters helped in assessing tolerant versus susceptible genotypes at physiological and biochemical levels to limited moisture at two developmental stages of crops.

Relative water content decreased significantly in all genotypes in drought condition compared to control at both flowering and pod formation stages. The mean reduction in RWC was 17 per cent at both flowering and pod formation stages. Genotypes like UM-68 and Rmt-305 exhibit the maximum relative water content in comparison to other genotypes under limited moisture condition at midterm and terminal moisture stress stages. A decrease in (RWC) in response to moisture stress has been noted in wide variety of

plants. Drought stress leads to reduction in water status during crop growth (Almeselmani *et al*., 2011) [4]. The values for stability of cellular membrane in the fenugreek genotypes revealed that there was decline in MSI of stressed plants in all genotypes at both stages. The decrease in MSI under limited moisture condition as compared to control was 12.56 and 14.83 per cent respectively at midterm and terminal moisture stress stages. The maximum membrane stability index was recorded in UM-68 followed by Rmt-305 under limited moisture condition at both stages. These genotypes indicate the highly tolerant genotypes under drought condition. The membrane stability index is one of the key indicators of drought tolerance and the rate of injury to cell membrane by drought can be estimated by measurement of electrolyte leakage from the cells. Rahbarian *et al*. (2014) [41] studied the effect of water deficit stress on cell membrane stability of dragonhead (*Dracocephalum moldavica* L.). Cell membrane stability declined rapidly in Kentucky bluegrass exposed to drought and heat stress simultaneously (Wang and Huang, 2004) [48]. Stress induced membrane damage has been biochemically marked by the presence of malondialdehyde content (MDA) as one of the Thio barbituric acid reducing substance (TBARS) that accumulates as a consequence of membrane lipid peroxidation. The MDA values in drought plants were found higher over normal at both stages in all genotypes. The content of MDA was higher at pod terminal than midterm moisture stress stage in all genotypes. The minimum MDA content was reported in genotype UM-68 under drought condition at both flowering (25.33 mM) and pod formation stages(27.99 mM) followed by Rmt-354. These genotypes have better stability to membrane than other genotypes.

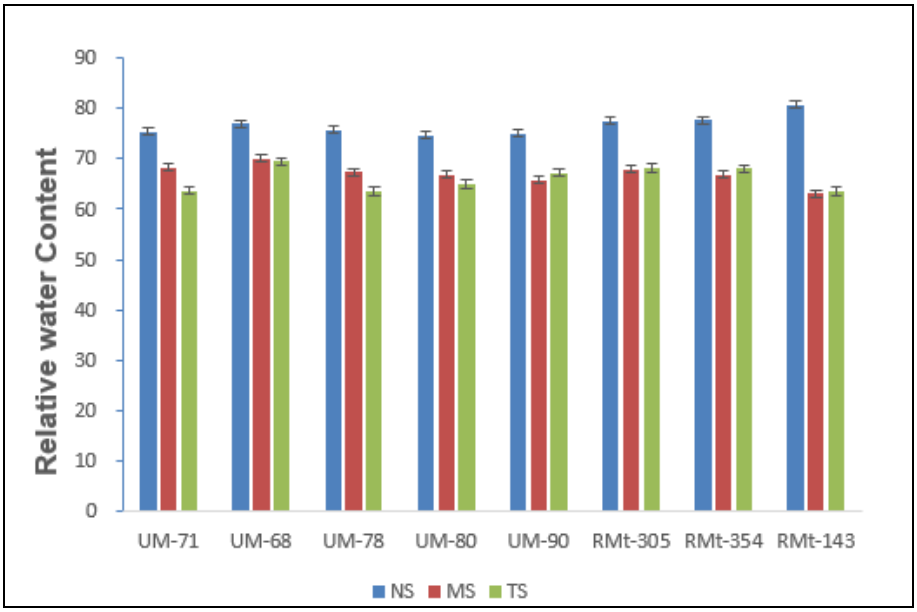

Fig 1: Effect of limited moisture on relative water content of fenugreek genotypes at midterm and terminal moisture stress condition

Table 1: Effect of limited moisture on membrane stability index and malondialdehyde (MDA) content of fenugreek genotypes at midterm and terminal moisture stress condition

Genotypes	Membrane stability index			Malondialdehyde content (mM)		
	NS	MS	TS	NS	MS	TS
UM-71	66.33	55.97	52.35	22.70	28.32	30.96
UM-68	69.23	68.02	58.26	25.28	25.33	27.99
UM-78	66.72	62.56	54.46	18.83	30.89	37.54
UM-80	66.33	54.27	53.36	24.10	36.56	37.60
UM-90	67.21	55.76	53.18	26.12	30.84	32.16
RMt-305	67.32	65.63	57.97	22.82	27.91	28.95
RMt-354	70.79	51.83	51.00	19.96	27.49	29.72
RMt-143	73.86	64.94	56.12	18.83	26.60	30.18
Mean	68.47	59.87	54.59	22.33	29.25	31.89
		SEm±	CD (*P* =0.05)		SEm±	CD (*P* =0.05)
Stress(S)		0.48	1.39		0.45	1.29
Genotypes(G)		0.97	2.79		0.90	2.58
G x S		1.37	3.94		1.27	3.65

In the present investigation minimum accumulation of MDA in genotype UM-68 followed by Rmt-354 showed better stability of cell membranes than other genotypes at both stages.

Reactive oxygen species are known to damage cellular membranes by inducing lipid peroxidation (Devi *et al*., 1998) [15]. Membrane stability index may be used as parameter to estimate the cellular injury caused due to peroxidation of fatty acids of the membrane. In present study, the increased levels of MDA in stress condition indicated the membrane sensitivity/membrane damage due to water stress. Lower rate of increase of MDA in genotypes indicated better membrane strength. In the present study, the MSI were found maximum in genotypes UM-68 and Rmt-

305, thus indicating these to be putatively tolerant at both the stages. Maximum MSI values was observed in genotype UM68 and Rmt-305 at both stages, indicating their high tolerance to water stress. The results are supported by (Pant *et al*., 2014, Mittal *et al*., 2006, Mittal, 2010, Karmakar *et al*., 2014) [29, 33, 34].

The proline content varied from 44.09 to 54.24 mg 100 g $^{-1}$ fresh weight under non stress condition, while it varied from 58.32 to 71.75 mg 100 g $^{-1}$ fresh weight at midterm moisture stress stage. Likewise at terminal stage, proline content varied from 154.45 to 173.47 mg 100 g-1 fresh weight, The mean percentage of increase in proline content under drought was 18.2 per cent in comparison to control at both flowering and pod formation stage. Proline is one of the most common compatible osmolytes in drought stressed plants. The glycine Betain content varied from 2.11 to 2.82 mg g $^{-1}$ fresh weight under drought at flowering stage while at pod formation stage it varied from 3.36 mg g $^{-1}$ fresh weight to 3.77 mg g $^{-1}$ fresh weight. The present investigation showed increase in proline and GB content due to drought at both stages. The maximum increase was reported in UM-78 at flowering and UM-68 at pod formation stage. In order to maintain osmotic balance, specific types of organic molecules accumulated in the cytoplasm, which formed as compatible solutes e.g. proline, glycine betaine. These solutes do not impair normal physiological function even if accumulate at high concentrations. Beside osmoregulation, glycine betaine stabilizes the oxygen evolving activity of photosystem-2 at high level of abiotic stress. The major role of GB might be to protect membrane and macromolecules from damaging effects of stress (Sawahel, 2003) [45]. In the present study the proline and glycine betaine level increased in all genotypes subjected to water stress as compared to normal. Further, the levels were higher at terminal moisture stress as compared to midterm moisture stage in all genotypes indicated that pod formations stage to be a more respective stage in terms of cellular osmotic adjustment. Comparing performance of genotypes at two stages, UM-68, Rmt-305 were found to be tolerant. The genotypes accumulations of compatible solutes under water stress protect the cell from its adverse effect by osmotic adjustment of cytosol with that of vacuoles and external environmental condition.

Increase in proline content under limited moisture condition has been suggested due to enhanced synthesis of proline and stress induced decrease in incorporation of proline into proteins (Mishra *et al.,* 1995) [32]. In plants GB amongst many quaternary ammonium compounds is synthesized or found abundant mainly in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane there by maintaining photosynthetic efficiency. Several reports have shown that accumulation of GB under drought stress was found to be high in drought tolerant species (Mittal 2010) [34], Ranganayakulu *et al.,* 2015 Meena *et al.,* 2016) [31, 42]. Glycine betaine accumulates in response to stress in many crop plants, including sugar beet (*Beta vulgare*), wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor*). In these species, tolerant genotypes normally accumulate more GB than sensitive genotypes in response of stress. This relationship however is not universal (Ashraf and foolad, 2007) [6].

Total Chlorophyll and carotenoids pigments (Fig.4) of fenugreek leaves were significantly decreased under limited moisture stress as compared with normal plants. The mean percentages of decrease were 12.37 per cent and 9.65 per cent in chlorophyll and carotenoids pigments, respectively at both stages. The photosynthetic pigments decline was observed with the advancement of stage in all genotypes. Among the genotypes the maximum content was reported in UM-68 followed by Rmt-305 under limited moisture condition at both stages. The high chlorophyll content under limited moisture condition indicates that their photosynthetic apparatus is able to resist adverse condition due to water stress. Gupta *et al.,* (2000) [22] reported that drought in variably reduced chlorophyll content but its reduction was lower in tolerant genotypes (Dwivedi *et al.,* 2018) [17]. Carotenes are responsible for scavenging singlet oxygen and hence their comparatively higher level in UM-68 and Rmt-305 suggest their tolerance to drought at cellular level. It is inferred that higher level of their pigments under drought might have kept these genotypes in privileged situation.

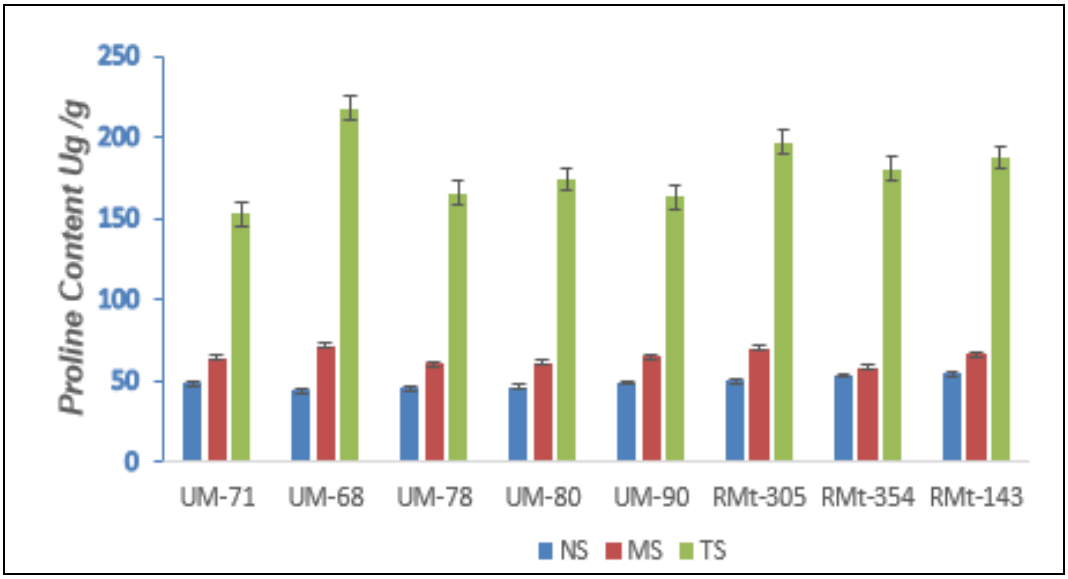

Fig 2: Effect of limited moisture on proline content of fenugreek genotypes at midterm and terminal moisture stress condition

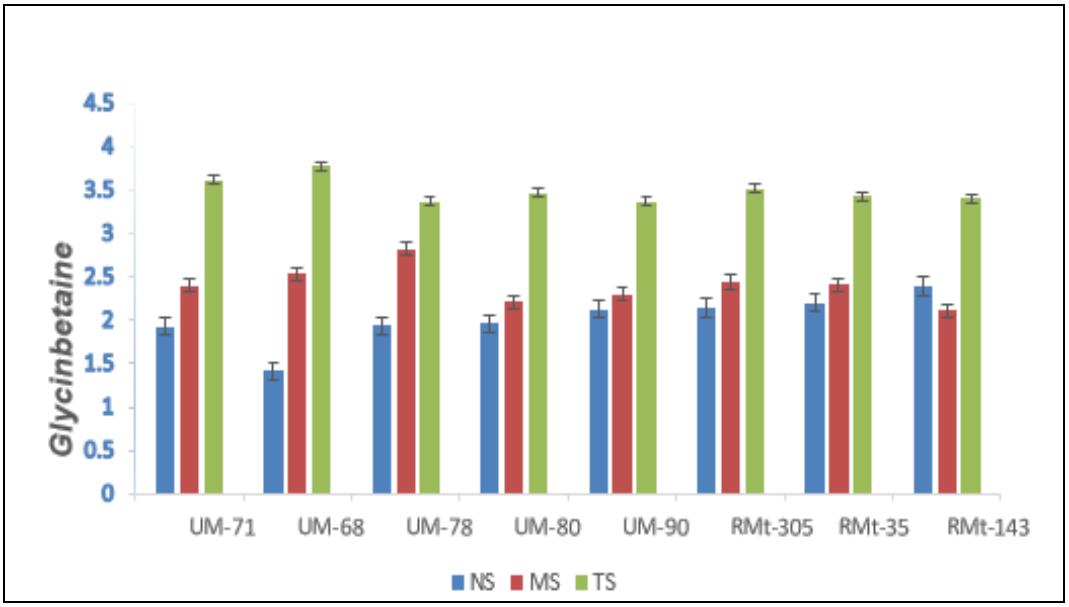

Fig 3: Effect of limited moisture on glycine betaine content of fenugreek genotypes at midterm and terminal moisture stress condition

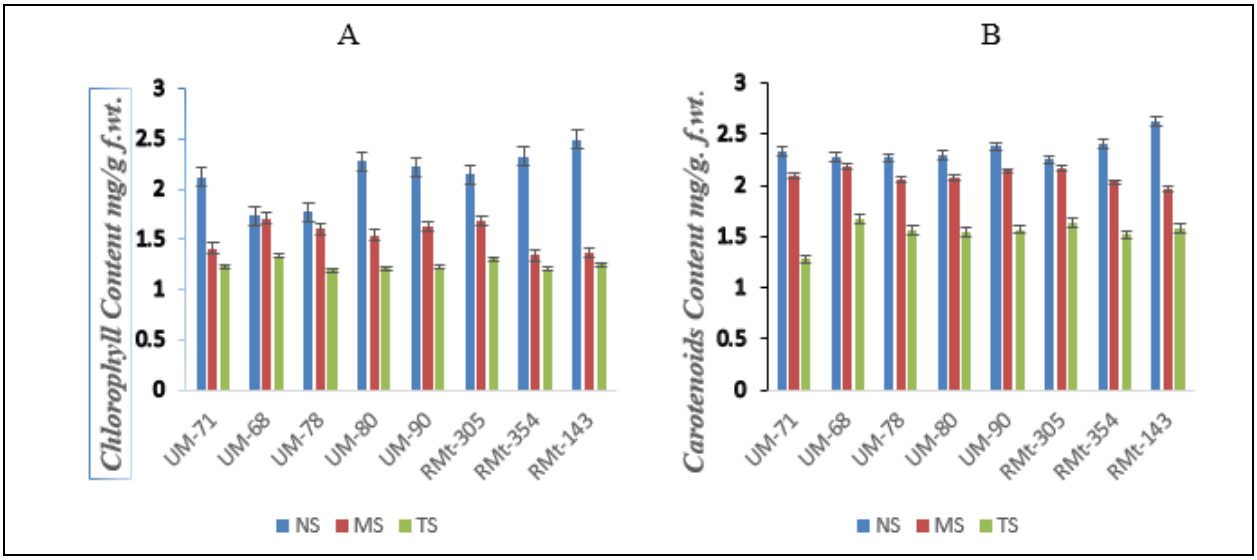

Fig 4: Effect of limited moisture on a Chlorophyll and b Carotenoid content of fenugreek genotypes at midterm and terminal moisture stress conditio

Drought is known to accompany with the formation of reactive oxygen species (ROS) including singlet oxygen (O^{-}), superoxide radical (O_2^{-}), hydrogen peroxide (H_2O_2) and hydroxy radical (OH^{-}). are symptoms of cellular injury due to drought (Mittler 2002). Super oxide radical is regularly synthesized in chloroplast and mitochondria and some quantities are also produced in micro bodies.

Table 2: Effect of limited moisture on superoxide dismutase and catalase activity at midterm and terminal moisture stress condition

Genotypes	Superoxide dismutase (unit/mg f.wt.)			Catalase (mM/min/ mg $^{-1}$ f.wt.)		
	NS	MS	TS	NS	MS	TS
UM-71	3.19	3.21	3.48	40.19	48.00	49.03
UM-68	2.56	3.76	4.12	34.24	62.06	64.58
UM-78	2.82	2.86	3.06	36.42	43.50	43.48
UM-80	2.73	2.94	3.35	37.22	44.45	43.89
UM-90	3.16	3.26	3.66	41.94	50.48	48.69
RMt-305	3.11	3.75	3.77	40.49	61.09	61.84
RMt-354	3.22	3.51	3.73	43.75	51.63	55.35
RMt-143	3.28	3.33	3.61	51.15	52.25	57.45
Mean	3.01	3.33	3.60	40.68	51.68	53.04
		SEm+	CD (P =0.05)		SEm+	CD (P =0.05)
Stress(S)		0.05	0.16		0.49	1.41
Genotypes(G)		0.11	0.31		0.98	2.81
G x S		0.15	0.44		1.38	3.97

SOD is usually considered as first level defence against oxidative stress. The scavenging of superoxide radical by superoxide dismutase (SOD) results in the production of H_2O_2, which is removed by catalases and peroxidases. In our study the activity of SOD and CAT measured in fenugreek genotypes showed enhanced activity under limited moisture condition, however with variable Magnitude at both flowering and pod formation stages. The catalase activity increased significantly under drought condition at both flowering and pod formation stages. The increment in catalase under the limited moisture condition as compared to control was 27.04 per cent and 30.38 per cent respectively at both stages.

Table 3: Effect of of limited moisture on number of pods/plants, no. of seeds /pod and test weight of fenugreek genotypes at midterm and terminal moisture stress condition

Genotypes	Number of pods/plants			No. of seeds /pod			Test weight		
	NS	MS	TS	NS	MS	TS	NS	MS	TS
UM-71	43.89	37.38	27.2	13.79	10.70	8.90	13.24	10.79	8.95
UM-68	43.77	42.27	38.3	12.70	12.23	11.3	12.10	12.06	10.88
UM-78	39.26	33.87	31.4	12.85	10.25	8.25	13.38	10.91	8.68
UM-80	40.10	35.01	31.1	12.01	10.38	8.38	13.53	11.03	9.03
UM-90	49.86	38.37	28.2	12.70	11.17	9.17	10.81	11.32	9.32
RMt-354	48.98	38.71	28.9	13.96	12.19	10.19	14.01	11.80	9.80
RMt-305	50.53	33.24	33.5	14.25	10.02	8.02	10.81	9.86	7.86
RMt-143	51.93	38.53	32.5	15.26	11.36	9.36	13.89	11.42	9.42
Mean	46.04	37.17	31.38	13.44	11.04	9.20	12.72	11.14	9.24
	SEm±	CD (*P* =0.05)		SEm±	CD (*P* =0.05)		SEm±	CD (*P* =0.05)	
Stress(S)	0.54	1.55		0.20	0.57		0.34	0.99	
Genotype(G)	1.08	3.11		0.40	1.14		0.69	NS	
G x S	1.53	4.40		0.56	1.61		0.97	NS	

Under non stress condition the highest super oxide dismutase at midterm stage was noted in Rmt-143 (3.28 units /mg f.wt.) closely followed by Rmt-354 (3.22 units /mg f.wt.) and UM-71 (3.19 units /mg f.wt.) and at terminal moisture stage highest super oxide dismutase was noted in genotype UM-68 (4.12 units /mg f.wt.) closely followed by Rmt-305 (3.77 Units /mg f.wt.) and Rmt-354 (3.73 units /mg f.wt.). Under the drought condition the highest super oxide dismutase activity was noted in genotype UM-68, followed by Rmt-305 and Rmt-354 at both stages. Genotype x treatment interaction was also found significant.

The highest catalase at non stress was noted in Rmt-143 (51.15 mmol/min/ mg.f. wt) closely followed by Rmt -354 (43.15 mmol/min/ mg.f. wt) and UM-90 (41.94 mmol/min/ mg.f. wt) and at terminal moisture stress highest catalase was noted in UM-68 (64.58 mmol/min/ mg.f. wt.) followed by Rmt-305 (61.84 mmol/min/ mg.f. wt) and Rmt-143 (57.45 mmol/min/ mg.f. wt). Genotype x treatment interaction was also found significant.

Table 4: Effect of of limited moisture on seed yield of fenugreek genotypes at midterm and terminal moisture stress condition

	Seed yield (g/plant)				
Genotypes	NS	MS	% reduction in yield	TS	% reduction in yield
UM-71	7.22	6.38	11.63	5.5	23.82
UM-68	7.11	7.02	1.26	5.8	18.42
UM-78	6.95	6.16	11.36	4.6	33.76
UM-80	7.18	6.15	14.34	4.3	40.11
UM-90	7.09	6.24	11.98	5.4	23.83
RMt-354	6.71	6.14	8.49	5.2	22.50
RMt-305	7.25	6.70	7,58	5.7	21.37
RMt-143	7.53	6.50	13.67	5.0	33.59
		SEm±	CD (*P* =0.05)		
Stress(S)		0.09	0.27		
Genotype(G)		0.05	0.14		
G x S		0.13	0.38		

It showed that the enzymatic antioxidant system was operational in all fenugreek genotypes. These results are supported by (Pant *et al.,* 2014, Mittal 2010 and Karmakar *et al.,* 2014) [29, 34] in fenugreek. Among the genotypes the highest activity was noted in UM-68 followed by Rmt-305 under drought condition. Catalase and peroxidase, are the most important enzymes involved in intracellular level of H_2O_2. They convert H_2O_2 into H_2O along with regeneration of NADP thus helping the plant under stress conditions (Sairam *et al.,* 1997 and Agarwal *et al.,* 2017) [3, 44].

Yield is the most important character for a crop. However, the yield contributing parameters are different in cereals, seed spices and pulses. All tested genotypes showed a reducing trend in number of pods plant $^{-1}$ number of seed per pod and test weight under moisture stress condition. In non stress condition number of pods /Plant ranged from 39.26 (UM-78) to 51.93 (Rmt-143). Minimum number of pods /plants 33.24 was observed in Rmt 143 while maximum was recorded in UM-68 at midterm and terminal moisture stress condition. The studied genotypes showed

reduction trend in number of seed per pod during both the stress condition i.e. midterm and terminal water stress condition. Under control condition the maximum no.of seed per pod was recorded in Rmt-143 while at midterm and terminal moisture stress condition maximum was recorded in UM-68 followed by Rmt-354. The test weight varied from 10.81 g to 13.89 g under control condition.The maximum test weight was recorded in Rmt 354 under control condition while at moisture stress it was observed maximum in UM-68 followed by Rmt-354.The genotypic variation in seed yield varied from 6.71 to 7.53 g per plant under non stress, while under midterm stress it varied from 6.15 to 7.02 g per plant and at terminal moisture stress it varied from 3.9 to 5.8 g per plant.Genotype UM-68 had least reduction in yield under water stress condition (18.42 %) followed by the genotype Rmt 305 (21.37 %). Maximum Reduction was found in genotype UM-80 (40.11 %) indicated that this genotype is most susceptible to water stress (Table 4). These variations in yield were found attributed to the variation in yield contributing parameters vis-a-vis existence of drought tolerance mechanism at cellular and molecular level. Genotypes variability in fenugreek has been reported previously by many researchers. Acharya *et al*., (2008) and Saxena *et al*., (2017) [2, 46] observed considerable variability among fenugreek genotypes. Genotypes differ in morphology, growth habits, biomass and seed production capability.

The relative performance of genotypes in drought stressed and non-stressed environment can be used as an indicator to identify drought resistant varieties for drought prone environments. Several

Drought indices have been suggested on the basis of a mathematical relationship between yield under drought conditions and non-stressed conditions. These indices are based on either drought resistance or drought susceptibility of genotypes (Raman *et al* 2012). Drought tolerance is expressed by the stress susceptibility index (SSI). Various results were obtained based on the screening of genotypes grown under optimal and dry conditions. As a measure of stress susceptibility, based on SSI, genotypes having different drought tolerance level were determined. Stress susceptibility (<1) is synonymous of high stress resistance. In the present investigation the Drought susceptibility index (DSI), Drought tolerance efficiency and drought tolerance index were measured in different genotypes under midterm and terminal moisture stress conditions. In the present study genotype UM-68 and Rmt 305showed lower SSI and higher DTI as compared to other genotypes at both stages. The genotypes with lower values (<1) of DSI are more stable in both the condition and are suitable for use in drought resistance breeding.

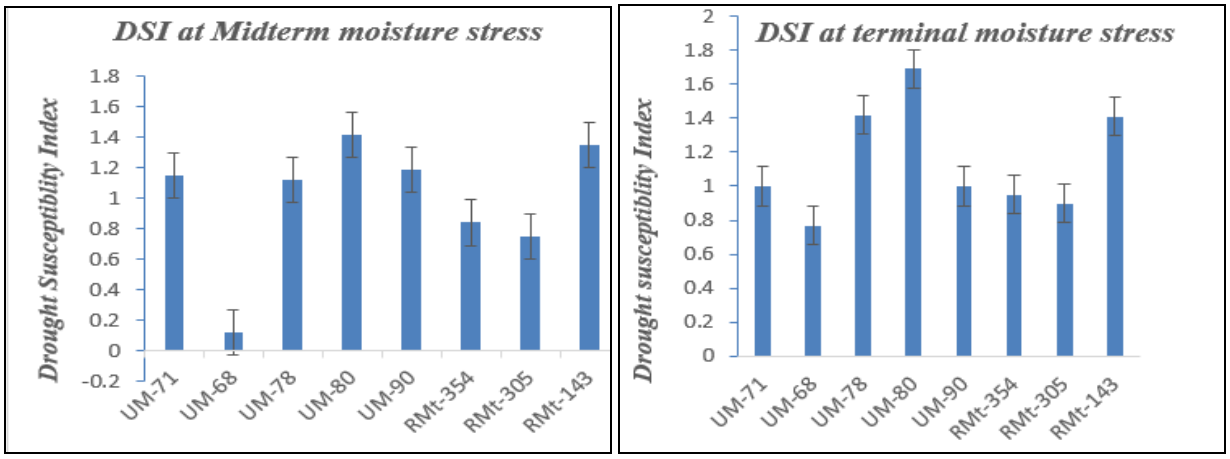

Fig 4: Effect of limited moisture on Drought Susceptibility Index of fenugreek genotypes at midterm and terminal moisture stress conditions

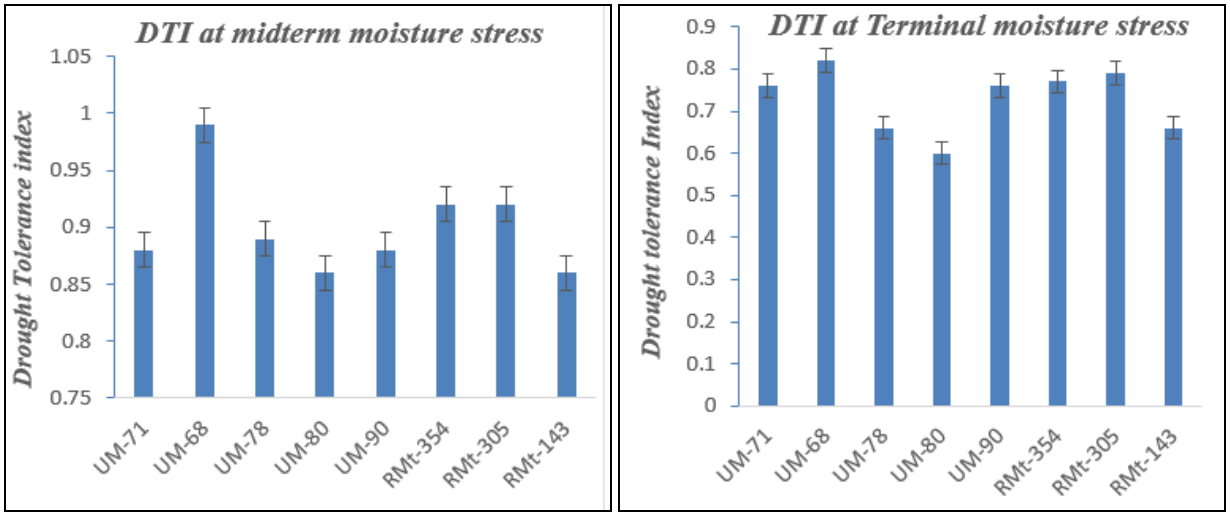

Fig 5: Effect of limited moisture on Drought Tolerance Index of fenugreek genotypes at midterm and terminal moisture stress conditions

Table 5: Overall performance of fenugreek genotypes for all the characters under limited moisture stress conditions

Character	Desired level	Genotypes							
		UM-71	UM-68	UM-78	UM-80	UM-90	Rmt-305	Rmt-354	Rmt-143
RWC	Highest		++				++	+	+
MSI	Highest		++				++	+	+
Chlorophyll	Highest		++		+		++	++	+
Carotenoids	Highest		++				++		+
MDA	Lowest		++				++	++	++
Proline	Highest		++				++		+
GB	Highest	++	++	++				+	+
SOD	Highest		++				++	+	+
CAT	Highest		++				++	+	+
DSI	Lowest		++	++				++	
DTI	Highest		++				++	++	
Economic Yield			++					+	+
No.of Pods/plant		++					++	++	+
No. of seeds/pod		++					++	++	++
Test weight		++	+					++	
Over all ranking			1				2	3	

+ Character showed superiority (highest/lowest) for either normal or late sown condition

++ Character showed superiority at both (MS &TS) limited moisture stress conditions

The tolerant genotypes of fenugreek could be screened out on the basis of overall performance of genotypes under limited moisture conditions. A critical evaluation of data showed that genotype UM-68 has higher RWC, MSI, photosynthetic pigments, SOD, CAT, proline, DTI, GB and lower MDA and DSI followed by genotype Rmt 305and Rmt-354, whereas genotype Rmt 143 showed superiority for most of the character under non stress condition.

From the present study if may be conducted that physiological traits like relative water content (RWC), membrane stability index, chlorophyll content, carotenoids content, proline and glycine betaine,DSI and DTI are important physio-biochemical traits under drought condition. The antioxidant defence system in terms of superoxide dismutase and catalase works in fenugreek at cellular level to protect the plants from oxidative damage. In the present investigation, UM-68, Rmt-305 and Rmt-354 were found the best suited genotypes under drought stress. These genotypes supposed to carry the drought tolerant genes and may be used for further drought tolerance breeding.

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