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Effect of Salinity stress on Osmoregulation of Juvenile Fenugreek

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ABSTRACT
The environmental aenigma, such as salinity (soil or water) and drought are becoming acute obstacles for irrigation of vegetal crops across the globe, especially arid and semiarid regions. Fenugreek (Trigonella foenum graecum L.) leaves and seeds are used extensively as condiments in India. Also, it has multifarious medicinal chattels. The present research set insights on the effect of induced salinity during germination and early seedling growth on fenugreek. The studies involved four treatments of salinity including 0 mM (control), 50, 100 and 200 mM NaCl. The results showed that different treatments of salinity had considerable effect on the germination percentage, Plumule to Radicle Length Ratio, Seedling Vigor Index, Germination Velocity, Seedling, Plumule, Radical, Shoot and Root length, Shoot and Root fresh weight, Seedling, Plumule, Radical, Shoot and Root dry weight, Total Chlorophyll content, Glycine Betaine, Proline, Trehalose, Antioxidant scavenging activity and Total Protein of variety 1 and variety 2. The highest total chlorophyll content was found in variety 1. Germination percentage in both the varieties showed considerable decrease with increasing salinity up to 200 mM NaCl. This reduction was more in variety 2 as compared to variety 1. The seedling vigor index of both varieties was significantly inhibited by all salinity levels, particularly at 200 mM NaCl. Seed vigor is the sum total of all those properties that determine the activity and performance of seed lots having acceptable germination in a wide range of environments. Glycine Betaine, Trehalose was observed to increase with increasing NaCl concentration in both varieties. The Proline concentration reduced in variety 1, however was found to be high in variety 2. The result showed that antioxidants are responsive to salt stress. Variety 1 had a higher level of antioxidants and had greater resistance to different types of stress. Concomitant with a pattern of high-antioxidant enzyme activities, tolerant variety could be predicted in this study.
Key words: *Trigonella foenum graecum L., Osmoprotectants, Germination velocity, Seedling vigour index, Germination percentage, Plumule to Radicle Length Ratio.*

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INTRODUCTION

Salinity is one of the major factors limiting seed germination, plant growth and development as well as the quantity and quality of plant production in arid and semi-arid regions. Fenugreek (*Trigonella foenum graecum L.*), like other legumes, is a good source of dietary protein for consumption by humans and animals. Fenugreek is one of such plants whose leaves and seeds are widely consumed as a spice in food preparations, and as an ingredient in traditional medicine. It is a rich source of calcium, iron, â-carotene and other vitamins. (**1**).

Environmental stresses are the major force that governs the food production in the arid and semi-arid regions. Drought, high and low temperature, salinity and air pollution are most frequent abiotic stresses which are caused by various environmental factors. It is assumed that several abiotic factors in the soil, such as water stress, high soil temperature, salinity, nutrient deficiencies, alkalinity and acidity may limit growth, nodulation and nitrogen fixation of the legumes grown in the tropic's region. (**2**).

During the past four decades we have witnessed the doubling of the human population and a concurrent doubling of food production (**3)** Seed germination is the first stage in plant life cycle; and it is therefore one of the most critical phases that determine not only the degree of plant establishment in control habitats but also its ability to survive under stressful conditions **(4).**

Production and accumulation of compatible organic solutes in the cytoplasm acquire osmotic adjustment, in addition to several other cellular and molecular mechanisms. The major compatible solutes include proline and glycine Betaine are thought to function as osmoprotectants for protein (**5**)

Fenugreek is a flowering annual plant, with autonomous flowers. This crop is native to an area extending from Iran to northern India and widely cultivated in China, India, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, etc. (**6**). Salinity is one of the most serious agricultural problems worldwide. It is a key factor limiting plant growth and development not only by disrupting plant nutritional status and water uptake, but also by inducing an oxidative stress**.** In recent years, a vast majority of research has focused on identifying physiological, biochemical and molecular processes of plants that are affected by salinity. Indeed, the effects of salt stress depend on several factors, such as the severity of the stress, the plant growth conditions, the plant sensitivity and also the impact of combined stresses (**7**)**.** Both leaves and seeds should be included in normal diet, especially diet of growing kids, pregnant ladies, puberty reaching girls and elder members of family because they have hematinic (i.e., blood formation) value **(8**). The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline which are thought to account for many of its presumed therapeutic effects, inhibit cholesterol absorption and to help lower sugar levels (**9**).

Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses (antidiabetic, lowering blood sugar and cholesterol level, anticancer, anti-microbial, etc.), making stew with rice in Iran, flavor cheese in Switzerland, syrup and bitter run in Germany, mixed seed powder with flour for making flat bread in Egypt, curries, dyes, young seedlings eaten as a vegetable, etc. Beside this it is also used as roasted grain for coffee-substitute (in Africa), controlling insects in grain storages, perfume industries, etc. Fenugreek can be a very useful legume crop for incorporation into short-term rotation and for hay and silage for livestock feed, for fixation of nitrogen in soil and its fertility, etc. (**10**)

MATERIAL AND METHODS

Instrument, Chemicals, glassware's and plastic wares

For the experiment all instruments at the research facility were utilized *viz*. UV spectrophotometer (Shimadzu Pharm spec UV-1700), Ice flaker (Pooja Lab), Cold Centrifuge (R-V/Fm Plasto Crafts), Analytical weighing Balance (AUX 220), Refrigerator (4ºC and -20ºC from Pooja labs), pH meter (CL 54+), Monopan Balance, Magnetic Stirrer, Water Bath, Rocker, Autoclave. All the chemicals required for the assay and protein extraction were procured from Sigma-Aldrich Corporation, Sisco Research Laboratories Pvt. Ltd, HiMedia Laboratories Pvt. Ltd., Molychem Pvt. Ltd and S.D. Fine Chem Pvt. Ltd. All the glassware's and plastic wares were procured either from Borosil ® or Tarsons.

Plant Materials and seed treatment

Seeds of fenugreek were procured from Ratanshi Agro-Hortitech, Byculla, Mumbai, India. Two varieties of fenugreek i.e. variety 1 (Methi), and variety 2 (Kasuri Methi) were selected for the study. The seeds were rinsed thoroughly with tap followed by distilled water and imbibed for 48hrs, prior sowing for germination. For control the filter papers were moistened with distilled water, while the salt treatment had different concentrations of NaCl. Both the varieties of seeds (5-10) were treated with three concentrations of NaCl i.e., 50, 100, and 200 mM. Three weeks germinated plants were used for analysis of the effectors of salt stress. All the analysis was performed in triplicates.

Proline Estimation:

The levels of proline in the control and the treated samples were estimated according to Abdelhameed *et al.* (**11**). For estimation of standard proline (10-100mg/ml), an equal proportion of proline, ninhydrin acid and glacial acetic acid was incubated at 100ºC for 1 hr. The reaction was arrested in an ice bath and the chromophore was extracted with 4 ml toluene and its absorbance at 520nm was determined in a UV-Spectrometer.

0.1 gram of plant sample was homogenized in 5 ml of 3% sulphosalicylic acid and the residue was removed by centrifugation. One ml of extract was reacted with 1 ml of ninhydrin acid and 1 ml of glacial acetic acid for 1 hr. at 100ºC and the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene and optical density measured as described earlier. The amount of proline was determined from a standard curve and expressed as µmoles/gram of plant tissue.

Trehalose Estimation:

Trehalose was extracted according to method described by Clémence et. al. (**12**). Plant samples (1.0g) were mixed with 5 ml of 80% methanol and incubated at 85ºC in a water bath for 1 hr. Samples were then centrifuged at 5000 rpm for 5 minutes and the supernatant was collected. Further the supernatant was evaporated in an oven set at 85ºC overnight and dry residue dissolved in 2 ml of deionized water. For Trehalose quantification anthrone reaction was used (**8**). 0.5ml of each of Trehalose solution in deionized water was mixed with 5 ml of 66% sulphuric acid containing 0.05% w/v anthrone and incubated at 100ºC

for 15 minutes. After cooling the sample, the absorbance was measured at 620nm and compared to the standard curve of Trehalose.

Glycine Betaine Estimation:

Glycine Betaine was determined by following the Bardan et al. (**13**) method. Plant material (1.0g) was crushed in 10 ml of distilled water and filtered. After filtration 1 ml of extract was mixed with 1 ml of 2M HCl. 0.5 ml of this mixture was mixed with 0.2 ml of potassium tri-iodide. The contents were shaken and cooled in an ice bath for 90 minutes with intermittent shaking. Two ml of chilled distilled water and 20 ml of 1-2 dichloromethane (cooled at -20ºC) were added to the mixture. Of the two layers formed in a mixture, the upper aqueous layer was discarded and optical density of the organic layer was measured at 365nm. The concentration of the Betaine was calculated against the standard curve and was expressed as a unit of mg/g tissue.

Antioxidant Assay:

DPPH Assay:

The free radical scavenging capacity of the extract was determined using DPPH (**14**) using ascorbic acid as standard extract were prepared by grinding 1 gm of plant sample with 10 ml extraction buffer in mortar-pestle. The sample was centrifuged at 4ºC, 10 minutes at 5000 rpm and the supernatant was used for analysis. Freshly prepared DPPH solution was taken in a test tube and extract (300 µl) was added to each test tube. The mixture was incubated in dark (30 min.) and the absorbance was read at 517 nm. Control sample was prepared containing the same volume without any extract and standard. Methanol was used as a blank. Percentage scavenging activity of the DPPH free radical activity was measured by following equation:

$$\% \text{ DPPH Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_o = Absorbance of control sample
A_1 = Absorbance of test sample

Protein Extraction:

The effect of salt treatment on all the varieties was done by estimating the protein estimation by method described by Lowry's (**15**) with Bovine Serum Albumin as standard. The amount of protein in the samples and the control was estimated by taking the absorbance at 650 nm against a blank.

Pigment Assay by Chlorophyll Extraction:

Leaf chlorophyll content of Fenugreek seedlings was calculated by Lichtenthaler (**16**). Chlorophylls a, b and total chlorophyll were extracted in 80% acetone, measured spectrophotometrically. The plant tissues were dried and grinded (1gm), mixed and homogenized with 20ml of 80% acetone. The samples were then centrifuged 5000 rpm for 5 min and supernatant was transferred to 100 ml of volumetric flask. The residue was further mixed with 20 ml of 80% acetone, centrifuged and supernatant transferred to the same volumetric flask. This process is repeated until the residue becomes colorless. The volume was made upto to 100 ml with 80% acetone. The solution was then measured at 645 and 663 nm. The concentration of chlorophyll was calculated against the standard curve and calculated according to the given formulae.

1) **Chlotophyll a (mg/g) =** $12.7\ (A_{663}) - 2.69\ (A_{645}) \times V/1000 \times W$

2) **Chlorophyll b (mg/g)** $= 22.9\ (A_{645}) - 4.68\ (A_{663}) \times V/1000 \times W$

3) **Total chlorophyll (mg/g)** $= 20.2\ (A_{645}) - 8.02\ (A_{663}) \times V/1000 \times W$

A = absorbance at specific wavelength
V = final volume of chlorophyll in 80% acetone
W = fresh weight of tissue extract

Germination Percentage

The experiment was terminated on the 21st day and data on plumule and radicle length (cm) and fresh weight of plumule, radicle (mg) and seedling vigour index were recorded. The data on plumule and radicle dry weight was recorded after drying in a hot air oven at 65ºC for 48 hours. The germination percentage was determined by using the following formula described by Fuller et al. (**17**).

Germination percentage = (Number of seeds germinated/ Total number of seeds sown) x 100

Plumule to Radicle Length Ratio (PRLR)

The plumule to radicle length ratio of seedling was calculated by the following formula (**18**).

Plumule to Radicle Length Ratio = Plumule Length/Radicle Length

Seedling Vigour Index (SVI)

The seedling vigour index was determined by multiplying the sum total of mean length of plumule and radicle of a seedling with germination percentage of the respective seedling by the following formula (**4**)

Seedling Vigor Index (SVI) = (RL+PL) x (GP)

Where RL= Mean radicle length, PL= Mean plumule length, GP= Germination percentage

Germination Velocity

The germination velocity (GV) was evaluated according to the formula of Saberali and Moradi (**19**).

$$GV = \frac{\text{Total number of seedlings}}{A1T1 + A2T2 + Ax\ldots. TX}$$

Where, A, is the number of seedlings emerging on a particular number of days (T) and subscripts 1, 2 … x is the respective number of germinated seeds per respective number of days after sowing of the seeds.

Statistical Evaluation

For all the experiments five plantlets per variety were analyzed and all the assays were carried out in triplicates. The results were expressed as mean ± standard deviation. The significance of the relation of induced salinity and the physiochemical responses observed was validated by performing analysis of variance, with an alpha value of less than 0.05 (**18**).

RESULT AND DISCUSSION

Salinity, an abiotic stress, has been observed to induce toxicity on plants and reduce the seed germination along with crop yield significantly (**20**). In present study, NaCl induced toxicity was gauged in two varieties of fenugreek**.** The research herein focuses on metabolic changes in three Osmoregulators under induced NaCl stress, along with overall changes in phenome as well as the sustenance of this vegetal species. Primary effects are ionic toxicity and osmotic stress. Ionic toxicity occurs because high concentrations of Na^+ and Cl^- in the cytoplasm of cells disturb several biochemical and physiological processes, and osmotic stress is induced by the lowering of the water potential causing turgor reduction and cellular water loss. Secondary effects of NaCl stress include inhibition of K^+ uptake, membrane dysfunction and generation of reactive oxygen species in the cells. Previous studies have also reported that excess of NaCl induces an ionic and metabolic imbalance, which eventually diminishes the nutritional quotient and medicinal chattels (**21**).

Effect of NaCl on mean performance, Germination Percentage, Plumule to radical ratio

It was observed that all the characters varied along the NaCl gradient. The value was maximum in the control and minimum at the highest concentration of NaCl for most of the parameters evaluated for both the plant varieties. A severe inhibition of plant growth and development, damaged membranes, ion imbalances have been recorded in different plant species also (Fig: 1**)**. This can be attributed to flux variations ensued by Na^+ and Cl^- accumulation, enhanced lipid peroxidation and increased production of reactive oxygen species like superoxide radicals, hydrogen peroxide and hydroxyl radicals (**22**).

Similarly, a deceleration in the germination percentage was recorded for both the varieties 1 and 2, with the increase in the osmotic stress (from 0 to 200 mM) with a maximum germination recorded at 0 mM of NaCl and minimum at 200 mM. Furthermore, the length of the seedling, shoot and plumule length ratio was recorded high in variety 2 as compared to variety 1, in such that ratio of control was scored to be 5.3 of variety 2, had maximum PRLR values as linked to all the different concentrations of NaCl. (Fig: 2). The varieties demonstrated significant differences for plumule length with varying concentration of osmotic stress. The plumule length was scored to be decreasing with increasing concentrations of NaCl. The seedling length was also decreased with increasing osmotic stress. It was highest in control and then decreased in 50 mM, 100 mM and 200 mM. Mean growth of the plumule length was highest in untreated plants and was observed to reduce in direct proportion to NaCl concentrations, with minimum growth at 200 mM. A similar response to abiotic stress was reported earlier in fenugreek **(23)** in spinach (**24**) and in oat **(25).**

The PRLR is a derived character, which reveals a differential comeback in different NaCl concentrations. In variety 1 the ratio increased at 50 mM and decreased in control followed by 100 mM and 200 mM. The plumule dry weight was highest in control then decreased in 50 mM followed by in 100 mM then abnormally increased at 200 mM. In case of radicle dry weight; there was also a reduction recorded with increase in osmotic stress. It was highest in 100 mM and 200 mM and was reduced at control and 50 mM concentrations. The shoot dry weight was highest in control, and decreased proportionally at 50 mM, 100 mM and 200 mM. Similarly, the root dry weight was highest in control, and reduced in accordance with increase in NaCl induced stress at 50, 100 and 200 mM. A similar response was observed in Variety 2, w.r.t.

PRLR, PDW, RDW, shoot and root dry weight. However, variety 2 was not as efficient as variety 1 in ameliorating the NaCl induced osmotic stress (Fig. 1).

Effect of NaCl induced Stress on SVI

Vigor testing does not only measure the percentage of viable seed in a sample, but it also reflects the ability of those seeds to produce normal seedlings under less than optimum or adverse growing conditions similar to those which may occur in the field. Generally, seeds start to lose vigor before they lose their ability to germinate. In both the varieties the SVI decreased with increased NaCl concentration. It was maximum in control, then progressively decreased at 50, 100 and 200 mM (Fig: 3). A similar observation was reported earlier in fenugreek (**23**, **26**), in spinach (**27**) and in oat (**25**). Germination percentage, plumule length and radicle length showed a positive and significant correlation with vigor index, therefore SVI also reduced in significant proportion to growth parameters observed at seed germination stage.

Effect of NaCl on Seedling Growth and Development after NaCl Induced Osmotic Stress

In variety 1, the seedling fresh weight was highest at 100 mM, whereas it was comparatively lower in control and at both 50 and 200 mM. The shoot's fresh weight was also highest at 50 mM. The root fresh weight also demonstrated a similar pattern in variety 1. On the contrary, for variety 2 the parameters appear to decelerate with increasing concentration, although a variation was recorded at 100 and 200 mM in some growth parameters being analyzed in this study (Fig 4). A similar response was reported by Morant et al. **(28)** in *Triticum dicoccum farrum* and Secale *cereale* cv. Petkus and tricales T300. Reduction in seedling development due to induced osmotic stress can be also attributed to increase in osmotic potential across the root zone which might have led to physiological and phenetic changes. A substantial variation in fresh weight and dry biomass was also observed in both the varieties understudy (Fig. 4). Previous studies have reported that biomass accumulation might be considered as an indicator of crop tolerance to NaCl stress (**29**). The percent decrement observed in this study is thus an upshot of elevated induced NaCl stress. Also, transpositions in enzymatic processes through some interactions of salts, some organic substances might have a deleterious effect on seedling growth and development. Moreover, crop reduction due to salinity is generally related to the osmotic potential increase at the root–zone soil solution which leads to certain physiological changes and substantial reduction in productivity.

The growth response in the treated and control seedlings was also gauged by calculating the total chlorophyll (A & B) content in both the varieties (Table 1). The amount of chlorophyll is observed to decrease in control for variety 1, with increasing concentration of Nacl, indicating the deleterious effect of NaCl for plant growth. However, for variety 2, the amount reduced in comparison to control at 50- and 100-mM treatment, but upsurge was recorded at 200 mM, probably an mitigation strategy was in process of adaptation for this variety. The chloroplast is the source of powerhouses and sites of reactive oxygen species (ROS) generation within plant cells. Chlorophyll is involved in maintenance of a fine balance between energy linked functions and control of ROS production (**30**). Reactive oxygen radicals abate membrane fluidity selectivity and chlorophyll degradation. Thus, chlorophyll loss is thought to be indications of oxidative damage induced under presence of excess of NaCl. (**31**). Total protein analysis revealed that with increase in NaCl concentration, the content of protein increased in variety 1, while decreased in variety 2 (Fig. 5). A crucial stratagem that has developed to play a crucial regulatory role in the growth and development of plants under salt stress is the accumulation of various kinds of soluble proteins (**32**). Thus, from the basic parameter analyzed herein, the variety 1 appears to adapt more better than variety 2 under osmotic stress being studied.

Effect of NaCl on Osmoregulators of both varieties

Salinization of the soil fends off plants from ingesting water, which results in an ionic imbalance that results in ionic toxicity, and produces osmotic stress. Osmoregulators like proline, glycine betaine, trehalose, have been reported, not only to lower the cytoplasmic osmotic potential, but also facilitate water absorption, and scavenge reactive oxygen species molecules. Thus, plants modulate (either activate or deactivate) their expression and synthesis so that they can tolerate salt stress (**33**).

In present study, variety 1 exhibited a decrease in the expression of proline with increase in the NaCl concentration. On the other hand, accumulation was observed in variety 2 in proportion to increasing NaCl concentration. The concentration of proline was observed to be three times higher in the plants grown at 100 mM salinity compared to the 50 mM and 200 mM of variety 2. Analysis of trehalose, demonstrated an abatement in concentration in comparison to control in both the varieties. However, the trehalose content was comparatively higher in variety 2 than variety 1. Therefore, the osmoprotectants mechanism was interrupted, reducing the trehalose content in plant**s** under study. An augmentation of glycine betaine was observed in both the varieties of fenugreek understudy (Fig. 6). Proline plays a key role in osmoregulation in plants subjected to hyperosmotic stresses, primarily drought and salinity stress. Accumulation of proline is a means of adaptation to abiotic stress and has been observed in rye grass for the first time (**34**). Glycine

betaine, as an osmolyte, helps in lowering the osmotic potential of the cell and thus prevents movement of water from the cell, as well as compatible solutes. Thus its augmentation assures prevention of denaturation of macromolecules like enzymes/proteins (**35**). The total protein content analysis exhibited an accumulation of proteins, probably to stabilize the cellular environment under osmotic stress (Fig 6.).
A natural mechanism for living beings is regular synthesis of ROS in any biological pathways, but when ROS are produced in excess then the condition leads to oxidative stress. Plants are severely affected by oxidative stress, if ROS are accumulated to large extent. The major damage to some cellular components caused by ROS are lipids, proteins, carbohydrates and nucleic acids. Scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and the ferric reducing ability of the fractions was measured using the FRAP (ferric reducing antioxidant power) assay. The antioxidant activity of plants under varying stress conditions was expressed as percentage inhibition of DPPH. A high antioxidant activity i,e. ROS scavenging potentials were screened to be in variety 1 as compared to variety 2 (Fig 6). Oxidative stress defenses involve enzymatic antioxidant mechanisms such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and non-enzymatic antioxidants as phenolics, flavonoid, alkaloids and osmoregulators like proline, glycine betaine etc. (**31**). An increase in activity of these osmoregulators in the plant varieties being analyzed in present study thus indicate metabolic changes occurring in them to sustain the induced oxidative stress by NaCl.

Statistical Analysis:

The result of single factor ANOVA on effect of salt concentration on morphometric characters for fenugreek variety 1 depicts the variance of one-way ANOVA when applied to see the difference between the salt concentration and it reveals the significance impacts of salt concentration ($P<0.01$). $F = 87$ $P_{value} = 7.63$, E-39, $\alpha = 0.05$. Further for the variety 2 the morphometric characters, the one-way analysis of variance when applied to see the difference between the salt concentration, reveals the significance of salt concentration ($P<0.01$). $F = 281$ $P_{value} = 1.39E-55$, $\alpha = 0.05$. The results of single factor ANOVA on effect of salt concentration on fenugreek on osmoprotectants, total protein, total chlorophyll and ASA for variety 1, which depict the significance impact of salt concentration ($P<0.01$). $F = 153$ $P_{value}= 2.08E-17$, $\alpha = 0.05$. Considering the result of single factor ANOVA on effect of salt concentration of fenugreek on osmoprotectants, total protein, total chlorophyll and ASA for variety 2, which depict the significance impact of salt concentration ($P<0.01$). $F = 50$, $P_{value}= 6.01E-12$ $\alpha = 0.05$. For all the experiments three plantlets per land variety were analyzed and all the assays were carried out in triplicates. The results were expressed as mean ± standard deviation. The significance of the relation of induced salinity and the physiochemical responses observed was validated by performing analysis of variance, with an alpha value of less than 0.05. Apart from osmotic stress, NaCl is responsible for the generation of ROS in cells that lead to oxidative stress. The generation of ROS is due to imbalance between the production and scavenging machinery of ROS.

Table 1: Amount of Chlorophyll in fenugreek at different concentrations of NaCl

Concentration (mM)	**Chlorophyll content of variety 1 (mg/g)**			**Chlorophyll content of variety 2 (mg/g)**		
	Chl a	**Chl b**	**Total Chl**	**Chl a**	**Chl b**	**Total Chl**
Control	0.590	0.257	0.844	0.024	0.045	0.0715
50	0.512	0.193	0.705	0.021	0.041	0.0679
100	0.405	0.134	0.521	0.034	0.019	0.0546
200	0.391	0.151	0.509	0.022	0.0025	0.437

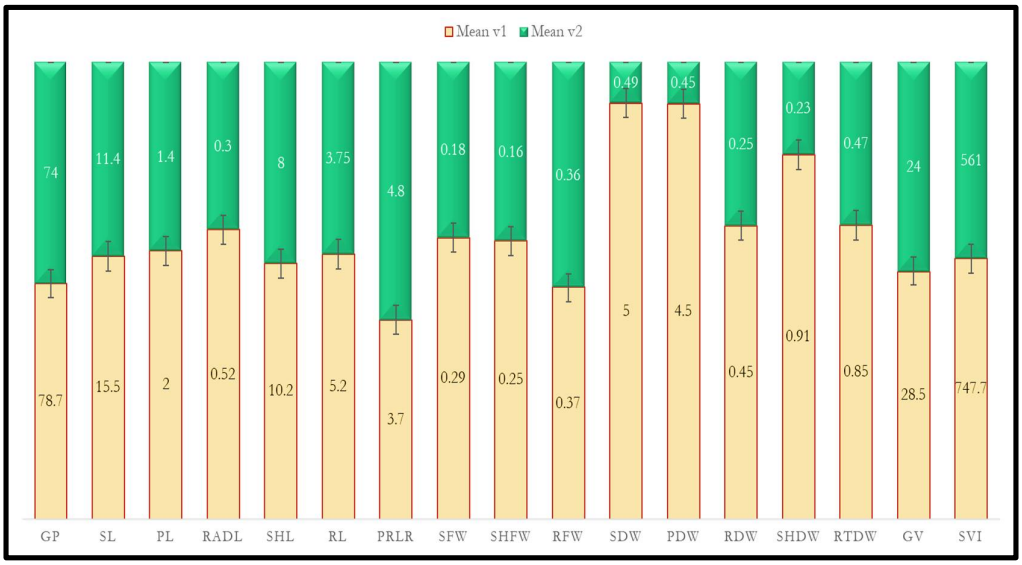

Figure 1: Graphical representation of morphometric characters of Mean value for both varieties

Figure 2:Impact of different salt concentration germination

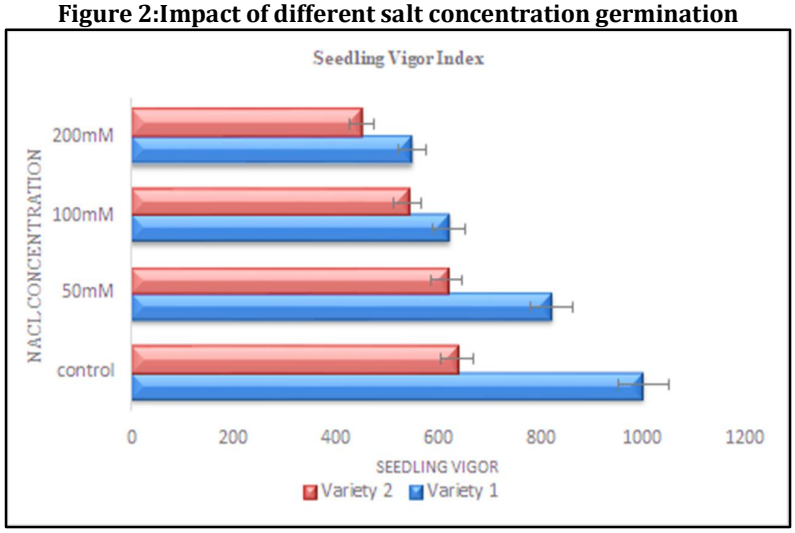

Figure 3: Graphical representation of seedling vigor index of both varieties

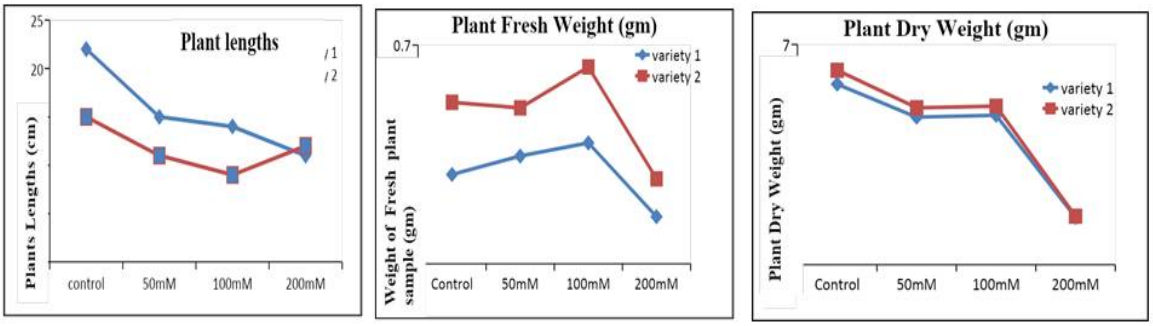

Figure 4: Graphical representation of Fenugreek plant length (cm), fresh and dry weight (gm) under different salinity level of NaCl Concentration of both Varieties

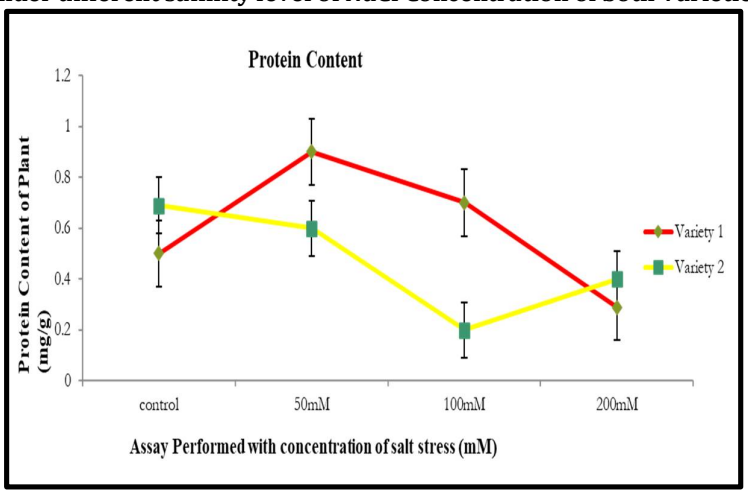

Figure 5: Estimation of protein content in seedlings of Fenugreek of both the varieties.

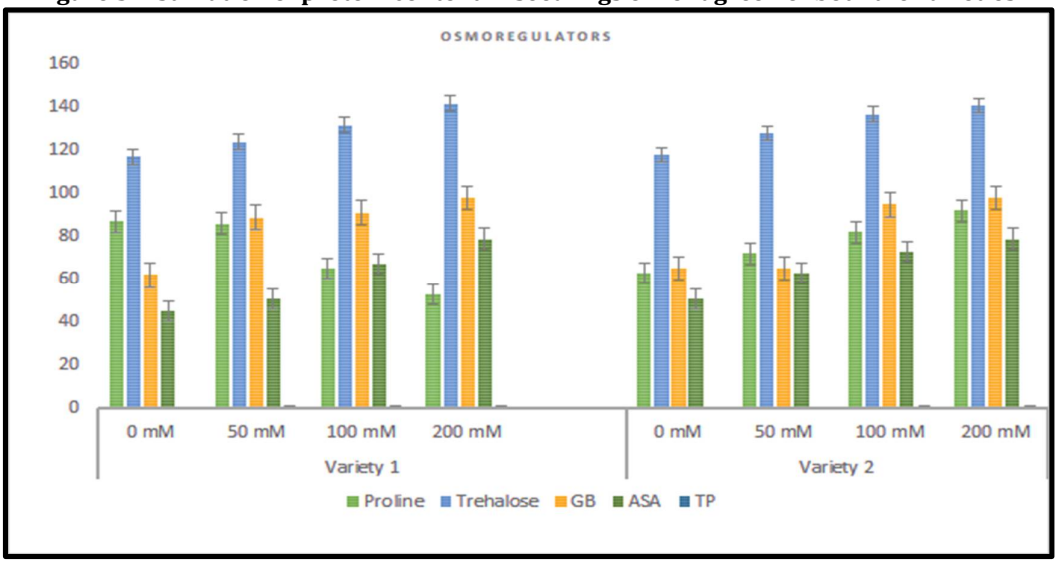

Figure 6: Effect on Osmoregulators response in both varieties of fenugreek under induced NaCl stress

CONCLUSION

In this study, two fenugreek varieties (variety 1 & 2) were analyzed for their NaCl susceptibility under laboratory conditions. Biochemical parameters, osmoprotectants, antioxidants, non-enzymic activities, morphological parameters were studied under non-saline (control) and induced saline stress conditions (50, 100 and 200 mM). The seedling vigor and other growth parameters were observed to demonstrate the

deleterious effect of induced osmotic stress. Glycine betaine and trehalose were observed to be strong osmoprotectants and their accumulation in NaCL stressed plants is a primary defense response to maintain the osmotic pressure in a cell. In this study the osmoregulator were observed to be protecting the protein integrity and thereby increasing the activity of many enzymes balancing the homeostasis within these plant systems. Salinity injures cell membranes and increases solute leakage. The effects of NaCl on membrane leakage are counteracted by $Ca2^{+}$. Collapse of mesophyll cells is a feature of NaCl toxicity. Between antioxidant non enzymes, variety 1 showed higher scavenging activity than in variety 2. These strong levels of ROS scavenging activity in variety 1 was the cause for tolerance against salinity stress. Apart from the osmotic stress, induced salt stress is also responsible for the generation of ROS in cells that lead to oxidative stress. These ROS are highly reactive and can alter the normal cellular metabolism through oxidative damage to membrane, protein and nucleic acid. The observed responses are thus themselves part of effective ways to improve and protect the plants under study from the adverse effects of soil salinization.

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Conflict of Interest: All authors have no conflict of Interest.

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