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ASSESSMENT OF PLANT GROWTH RESPONSES AND PROLINE ACCUMULATION UNDER SALINITY STRESS IN THE SPECIES OF FAMILY MOLLUGINACEAE IN INDIAN THAR DESERT

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Abstract

Salinity is an abiotic stress factor that limits plant growth and development. It interferes with morphological, anatomical, physiological and biochemical characters of plants. Plants develop a plethora of mechanisms in response to abiotic stress; one of them is the synthesis and accumulation of ‘compatible solutes’ or ‘osmolytes’ in the cytoplasm for osmotic balance. It is found that osmolyte and especially proline accumulation represent a reliable tolerance detecting biochemical marker under salinity stress. Present study is aimed to investigate the plant growth responses and proline accumulation under various levels of salinity in the species of family Molluginaceae present in Western Rajasthan. The seeds of Glinus lotoides Linn., Mollugo nudicaulis Lamk. and Mollugo cerviana Linn. were sown in pots and after two weeks of cotyledonal emergence, seedlings were irrigated with various salinity levels (i.e. 0, 50, 100, 150 and 200 mM of NaCl concentration respectively). All experimental set ups were set in completely randomized block design. Plant growth response was determined in terms of several parameters (i.e. plant’s fresh weight, leaf area, shoot length, root length and S/R ratio). To determine salinity induced phenotypic damage in each accession, plant salinity tolerance (PST) scores and plant survival index (PSI) was estimated. It was observed that plant growth is adversely affected by salinity stress and showed inverse relation with proline accumulation in all the accessions. Proline accumulation increases significantly with increase in salinity level and recorded maximum at highest level of NaCl, i.e. 200 mM in all three species of family Molluginaceae. Interspecific and intraspecific correlation and multivariate cluster analysis revealed that the accessions of Glinus lotoides Linn. and Mollugo nudicaulis Lamk. are comparatively tolerant to NaCl stress while the accessions of Mollugo cerviana Linn. are comparatively susceptible to NaCl stress.

Key words: Proline, Osmolytes, Salinity stress, Stress marker, Molluginaceae.

INTRODUCTION

Salinity is one of the main restricting factors among abiotic stresses, affecting growth and productivity of plants in arid and semi-arid regions. (Li et al., 2011; Abdilaal et al., 2020). Due to the desert environment, Western Rajasthan also faces multiple stresses ((i.e. extreme aridity, high salinity, extreme temperature and deficiencies of nutrients in soil)) that make arid plant’s survival much complicated (Shannon, 1998; Poli et al., 2013). Plants inhabiting in this region are adapted to these stressful, complicated and adverse situations of life. The family Molluginaceae, also called as carpetweed family, includes several herbal weed species that are well represented in Indian Thar Desert. The genera Glinus Linn. and Mollugo Linn. have herbal and medicinal importance and three species i.e. Glinus lotoides Linn., Mollugo nudicaulis Lamk. and Mollugo cerviana Linn. are wide spreaded in Western Rajasthan but there is very little work on genetic diversity in relation to multiple stressors reported so far.

Salinity stress induces both osmotic stress and ionic stress (Ueda et al., 2004). The osmotic stress is triggered by low osmotic potential with limiting absorption of water from soil, and the ionic stress is caused by the over-accumulation of toxic salt ions within plant cells. These stresses, as well as the nutritional imbalance affect the physiological status and are responsible for deleterious effects to plant growth and development (Santos et al., 2001; Meringer et al., 2016). To cope up with these unfavorable environmental conditions, plants develop different protective mechanisms to preserve normal cellular metabolism and prevent injury to cellular constituents (Gratão et al., 2015; Kolupaev et al., 2016). Plants can accumulate excess ions, particularly in cell vacuoles, to reduce cellular osmotic potential. The continued accumulation of such ions will eventually lead to toxicity problems (especially when the ions are normally toxic e.g., excess Na+ or Cl-) and excess ions start to accumulate in the cell's cytoplasm (Asharf and Foolad, 2006; Bhauso et al., 2014).To counteract this toxicity, the cytoplasm accumulates compounds called Osmolytes or compatible solutes and achieve ion balance in the vacuoles, which do not interfere with normal biochemical reactions (Parida and Das, 2005; Chen et al.,2007; Gomes et al., 2011). Osmolytes are neutral, nontoxic and low molecular weight metabolites, which stabilize proteins and membranes as well as prevent denaturation at high salt concentrations (Hasegawa et al., 2000; Yancey, 2005). Even at low concentrations, osmolytes can prevent water loss and ion imbalance thereby reducing the intracellular concentration of salts (Burg and Ferraris, 2008).

Osmolytes accumulated in the cytoplasm of plant cells under salt stress include amino acids (e.g., proline, valine, isoleucine, aspartic acid and glutamate), sugars (e.g., glucose, fructose, sucrose and trehalose), sugar alcohols (e.g., mannitol, pinitol, sorbitol and inositol), quaternary ammonium compounds (e.g., glycine betaine and carnitine) and tetra hydro-pyrimidine (e.g., ectoine and hydroxyectoine) etc. (Hiroshi T, 2008, Sharma et al., 2019, Meena et al., 2019). The significance of each osmolyte varies between species and within species and under the environmental stress; however, the most commonly studied are proline (Kalsoom et al., 2016). Proline [L-pyrrolidine 2-carboxy acid (C5H9NO2)] is an essential amino acid for primary metabolism as a component of proteins (Szabados and Savouré, 2009). Proline accumulation in plants has been considered as one of the most widespread stress induced response, so its measurement would be an excellent stress detector (Lal et al., 2015). Several plants accumulate higher level of proline in contrast to other amino acids when exposed to drought or high salinity (Heidari et al., 2011; Gharsalah et al., 2016; Chun et al., 2018; Khalegi et al., 2019; Cao et al., 2020).

Therefore, this study is conducted to investigate the interspecific variability by comparing the proline content under various levels of salinity stress for species of Family Molluginaceae present in Indian Thar Desert.

MATERIAL AND METHODS

Plant material and seed germination

Seeds of Glinus lotoides Linn., Mollugo nudicaulis Lamk. and Mollugo cerviana Linn. were collected from various sites of Western Rajasthan. Then after seeds were cleaned by rinsing with distilled water and then surface sterilized by 0.1% $HgCl_2$ solution and again washed 5 times with distilled water. Furthermore the viability of seeds was confirmed by tetrazolium viability test using 0.1% solution of 2, 3, 5-tri-phenyl tetrazolium chloride (Porter et al. 1947; Wharton 1955; Verma et al. 2013). Only viable, mature, healthy seeds with uniform size were selected for germination experiments. The sterilized seeds were sown in earthen pots. After the emergence of cotyledons, seedlings were subsequently transplanted to plastic pots containing sterilized soil and farmyard manure (6:1) and watered with tap water as and when required.

Stress treatments

After 2 weeks (when leaves were appeared on third node of shoot in Glinus lotoides Linn. and Mollugo cerviana Linn. whereas second whorl of radial leaves appeared in case of Mollugo nudicaulis Lamk.) pots were irrigated with various salinity levels (i.e. 50, 100, 150 and 200 mM of NaCl concentration respectively). Plant sample watered without any NaCl treatment was considered as control. To avoid salinity shock and to acclimatize seedlings to high NaCl concentrations, salinity stress was imposed by applying the saline irrigation water progressively. Each treatment was irrigated with the lowest NaCl concentration then with the next higher concentration until each treatment reached its considered irrigation concentration. Each treatment was triplicated and repeated three times.

Stress induced plant growth observations

All experimental set ups were set in randomized complete block (RCB) design to determine plant growth parameters (i.e. plant's fresh weight, leaf area and of shoot/root length). After 10 days of saline irrigation, the plants were removed from the pots along with the soil and were dipped in a bucket filled with water. They were moved gently to remove the adhering soil particles. Simultaneously, the fresh weight of each sample was noted and the length of root and shoot were measured. The leaf area was mapped and measured manually by using a graph sheet, in which the squares covered by a particular leaf were counted to note the leaf area. For normalizing the results, data were expressed as the relative reduction in comparison to the control using the following formula:

Relative reduction (%) = [1– (salinized/control) ×100]

Evaluation of plant salinity tolerance (PST)

A scoring procedure including number of plants showing the degree of salinity induced phenotypic damage in individual accession, we developed plant salinity tolerance (PST) score ranges 1-9 assigned on the basis of the standard evaluation system with few modifications (Gregorio et al. 1997; Kanawapee et al. 2012; Harsh et al. 2016) shown in Table 1.1.

Estimation of Plant Survival Index (PSI)

To find out the plant survival index, number of survived plants over saline treatments was recorded. Plants existing 2-4 green and healthy leaves at least considered as being survived while in which almost all leaves had dried or damaged were counted as non-survived plants. This data was used to estimate survival percentage.

PSI= No. of plant survived ÷Total no. of plant treated × 100

Table 1.1 Standard plant salinity tolerance index to evaluating salinity induced phenotypic damage. [Here, T-tolerant, MT-moderately tolerant, MS-moderately susceptible, S-susceptible, HS-Highly susceptible]

S.N.	Symptoms of salinity induced phenotypic damage	PST score	PST remarks
1.	Normal growth or nearly normal growth with a little degree of damage in leaves and shoot (most survived plants).	1	T
2.	Moderately retarded growth, leaf curling/rolling, yellowing and burning of margin/tip of some leaves.	3	MT
3.	Variously retarded growth, low height and most of leaves being damaged or drooped but youngest leaves still growing.	5	MS
4.	Almost succession of growth, stem lodging, old leaves totally dried and youngest one also start to wilt.	7	S
5.	Complete drying (non-survived plant).	9	HS

(Gregorio et al. 1997; Kanawapee et al. 2012; Harsh et al. 2016)

Determination of proline content

The mature leaves of 4-week-old plants were used to estimate proline content according to Bates et al. (1973). Leaf samples (0.5g) were homogenized in 5 ml of 3% aqueous sulfosalicylic acid and centrifuged at 1000 rpm for 10 minutes and filtered by Whatman paper No. 2. Supernatant was used, and equal volume of glacial acetic acid and ninhydrin solution (2ml. each) was added to it. The samples were incubated to 1000 C for 1 h on water bath for heating and the reaction was terminated in ice bath for cooling. After cooling, 4 ml of toluene was added to reaction mixture. Then after, mixture was vortexed for 20-25 seconds and kept for five minutes to develop chromophore layer. The toluene layer containing chromophore was aspirated from the aqueous phase and warmed to room temperature. The absorbance was read at 528nm on a spectrophotometer using toluene as a blank. The concentration of proline was determined by standard curve and calculated on a fresh weight basis using following formula-

Proline Content ($\mu mol^{-1} g^{-1}$FW) = [(Proline μg/ml) × (vol. of toluene) ÷ (MW of proline μg/μmole) ÷ (weight of sample/5)]

For normalizing the results, data were expressed as the relative increment in comparison to the control using the following formula:

Relative increment (%) = [(salinized/control)-1] ×100

Statistical Analysis

Each treatment was triplicated and repeated thrice. The values for various parameters of the plant growth responses were followed according to the agricultural statistical procedure given by Gomez and Gomez (1984) and compared with the standards of international seed testing association (ISTA 2015). The recorded experimental data were subjected to two-way analysis of variance (ANOVA) and Pearson correlation for each trait. Dunne's post-hoc test is used to determine the significant differences among treatments. The statistical analysis was performed using the data analysis tool of MS Excel (Microsoft Office Professional Plus 2010).

Multivariate cluster analysis was performed with the help of PAST 4.03 software.

RESULT AND DISCUSSION

Effect of salinity stress on plant growth

During the investigation of the effect of salinity on plant growth, all the accessions of Glinus lotoides Linn. and both the species of Mollugo Linn., it was observed that plants exposed to NaCl stress (0, 50, 100, 150 or 200 mM) showed significantly adverse effect on almost all the growth biomarkers like fresh weight, leaf area, length of the shoot and root and their ratio as compared to non-stressed control plants. The interpretations of tabulated data indicated that plant growth responses were successively reduced with increase in the level of sodium salt concentration in all accessions of the species of family Molluginaceae present in Indian Thar Desert (Table 1.2). The plant growth responses were significantly different between treatments of various levels of NaCl concentration showing P value less than 0.05 and there is significant phenotypic variability also noted among the species to cope up with salinity stress (Plate 1). The maximum decrease in plant growth response observed with the highest level of salinity, i.e. 200 mM, in all accessions of the species of family Molluginaceae present in Indian Thar Desert. Some researchers believe that the plant growth inhibition under salinity/sodicity stress may be due to some disturbed balance of enzymes through alteration in osmotic system (Katembe et al., 1998; Singh and Prasad, 2009).

Table 1.2: Effect of NaCl stress on plant growth parameters, proline content, plant survival and plant stress tolerance responses in plant species of family Molluginaceae present in Indian Thar Desert (Values with in parenthesis are percent decrement/ increment over control).

Values are mean of fifteen seedlings which were in triplicate (± values are SD)

Species	Treatments (conc. in mM)	Plant growth's parameters					Proline content ($\mu mol^{-1}g^{-1}FW$) (%)	PSI (%)	PST score	PST Remarks
		FW (g)	LA (cm^2)	SL (cm)	RL (cm)	S/R ratio				
***Glinus lotoide* Linn.**	Control	1.47 ± 0.03 (00.00)	3.01 ± 0.17 (00.00)	37.53 ± 2.03 (00.00)	14.82 ± 0.38 (00.00)	2.53 ± 0.15 (00.00)	0.71±0.02 (00.00)	100.00	1	T
	50	1.18 ± 0.11 (+19.73)	2.12 ± 0.27 (+29.57)	34.90 ± 1.05 (+07.01)	12.83 ± 0.86 (+13.43)	2.73 ± 0.23 (-7.91)	0.95±0.03 (33.80)	100.00	1	T
	100	0.81 ± 0.14 (+44.90)	1.32 ± 0.14 (+54.95)	32.22 ± 0.62 (+14.15)	10.83 ± 0.62 (+26.93)	2.98 ± 0.19 (-17.79)	1.52±0.04 (114.08)	77.78	3	MT
	150	0.52 ± 0.06 (+64.63)	0.80 ± 0.14 (+72.70)	26.30 ± 2.06 (+29.92)	7.98 ± 1.01 (+46.15)	3.32 ± 0.28 (-31.23)	2.10±0.03 (195.77)	55.56	5	MS
	200	0.46 ± 0.06 (+68.71)	0.46± 0.10 (+84.30)	21.08 ± 1.66 (+43.83)	4.48 ± 0.94 (+69.77)	4.83 ± 0.68 (-90.91)	2.68±0.03 (277.46)	33.33	7	S

	Control	1.45 ± 0.04 (00.00)	7.15 ± 0.15 (00.00)	23.50 ± 0.50 (00.00)	6.77 ± 0.25 (00.00)	3.48 ± 0.17 (00.00)	0.42±0.02 (00.00)	100.00	1	T
	50	1.17 ± 0.11 (+19.31)	4.68 ± 0.06 (+34.54)	19.50 ± 0.50 (+17.02)	6.17 ± 0.29 (+13.10)	3.16 ± 0.09 (+8.97)	0.64±0.06 (52.38)	100.00	1	T
***Mollugo nudicaulis* Lamk.**	100	0.72 ± 0.08 (+50.34)	3.58 ± 0.14 (+49.93)	17.33 ± 0.70 (+26.26)	5.40 ± 0.36 (+23.40)	3.21 ± 0.09 (+7.56)	1.09±0.12 (159.52)	77.78	3	MT
	150	0.53 ± 0.07 (+63.45)	1.51 ± 0.09 (+78.88)	15.33 ± 0.76 (+34.77)	4.00 ± 0.50 (+43.66)	3.86 ± 0.29 (-10.60)	1.44±0.26 (242.86)	44.44	5	MS
	200	0.40 ± 0.04 (+72.41)	0.08 ± 0.02 (+98.88)	13.00 ± 1.00 (+44.68)	3.00 ± 0.50 (+55.69)	4.39 ± 0.59 (-26.37)	2.00±0.24 (376.19)	22.22	7	S
	Control	0.98 ± 0.01 (00.00)	0.69 ± 0.03 (00.00)	27.03 ± 1.69 (00.00)	5.63 ± 0.39 (00.00)	4.80 ± 0.10 (00.00)	0.10±0.01 (00.00)	100.00	1	T
	50	0.85 ± 0.02 (+13.27)	0.52 ± 0.02 (+18.75)	19.93 ± 1.75 (+26.26)	4.47 ± 0.34 (+20.60)	4.46 ± 0.06 (+7.08)	0.18±0.02 (80.00)	88.89	3	MT
***Mollugo cerviana* Linn.**	100	0.68 ± 0.02 (+30.61)	0.27 ± 0.03 (+57.18)	15.49 ± 0.82 (+42.69)	3.12 ± 0.46 (+44.58)	5.02 ± 0.47 (-4.58)	0.36±0.06 (260.00)	66.67	5	MS
	150	0.57 ± 0.02 (+41.84)	0.17 ± 0.02 (+75.36)	12.02 ± 0.47 (+55.53)	1.70 ± 0.19 (+69.80)	7.12 ± 0.53 (-48.33)	0.70±0.15 (600.00)	33.33	7	S
	200	0.21 ± 0.01 (+78.57)	0.05 ± 0.02 (+92.75)	9.21 ± 0.53 (+65.93)	1.01 ± 0.15 (+82.06)	9.24 ± 0.96 (-92.50)	1.21±0.17 (1110.00)	0.00	9	HS

Plate 1: Phenotypic variability between Mollugo nudicaulis Lamk. [1], Mollugo cerviana Linn. [2] and Glinus lotoides Linn. at natural habitat.

The effect of NaCl stress on various plant growth parameters in the accessions of all three plant species of family Molluginaceae present in Indian Thar Desert. are as follows:

Fresh Weight- There is constant reduction observed in fresh weight of all the accessions under salinity stress (Fig.1.2, Table 1.2). After evaluation of recorded data, it was found that Mollugo cerviana

Linn. is light weighted species (since it is called as wire-stem/thread-stem carpetweed due to thin stem) as compared to both sister species and it shows highest relative reduction in fresh weight (78.57%) than Mollugo nudicaulis Lamk.(72.41%) and Glinus lotoides Linn. (68.71%) at highest salinity level (i.e. 200mM). Besides this, as we increased NaCl concentration level, these wire-stem carpetweed accessions showed stunted growth so that only 21.43% relative fresh weight was recorded at highest level of salt concentration (200 mM). At this stage almost all leaves drooped out, stem also became dry and bent downward to escape the stressed situation. It may be possible that this stress escaping strategy against the environmental stress be one of the reasons of wire-stem architecture of this herb (Plate 1). Here one point is also important that all three species of family Molluginaceae present in W. Rajasthan are ephemerals and survive for only 3-4 months in their natural habitat but the symptoms of phenotypic damage proves that Mollugo cerviana Linn. is highly sensitive species against salinity stress as compared to Mollugo nudicaulis Lamk. and Glinus lotoides Linn. The interspecific correlation analysis is also supporting this result (Fig. 1.6).

Leaf area- Leaves are the food manufacturing unit as well as ornamental part of the plant, so they have too much physiological as well as morphological and anatomical importance. In nature, the genus Mollugo Linn. shows interspecific diversity in leaf morphology (Plate 1). Mollugo nudicaulis Lamk. has a tuft of radial leaves at the bottom of shoot while Mollugo cerviana Linn. have simple, linear leaves on a number of nodes throughout the stem and branches. Similarly Glinus lotoides Linn. has opposite, obovate leaves, cuneate at the base densely villous on both the surfaces and appear more than two at nodes throughout the stem and branches (Plate 1). It was noted that leaf area decreased constantly and damaged in case of both species of Mollugo Linn. as well as Glinus lotoides Linn. (Fig.1.1 and 1.2, Table 1.2). Gradual leaf burning at leaf apex as well as leaf margins and reduction in number of leaves at originating node were observed in the accessions of Mollugo nudicaulis Lamk. and Glinus lotoides Linn. whereas leaf curling was also shown by accessions of Mollugo cerviana Linn. against sequential levels of NaCl stress. There is a significant relative reduction in leaf area found in the accessions of Mollugo nudicaulis Lamk. (98.88%), Mollugo cerviana Linn. (92.75%) and Glinus lotoides Linn. (84.30%) at highest salinity level (i.e. 200 mM). The maximum reduction in leaf area was observed in the accessions of Mollugo nudicaulis Lamk. and only 1.12% area is left at highest level of salt concentration (200 mM) with maximum leaf burning response (Fig.1.1). It may be due to the radial pattern of leaves and highly condensed internodal part at base as compared to both the sister species. The stress responses in leaves are also observed in Glinus lotoides Linn. and Mollugo cerviana Linn. like leaf curling/burning/dropping symptoms started in basipital manner from lower nodes to upper nodes because they have long internodal architecture of stem. The interspecific correlation analysis is also supporting this result (Fig.1.6).

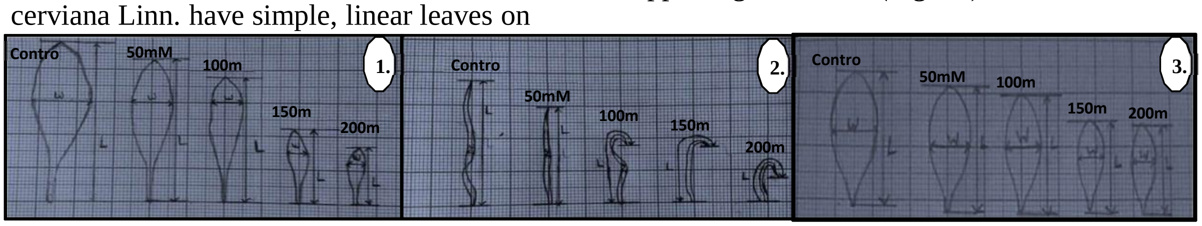

Fig 1.1: Leaf sketch of [1.] *Mollugo nudicaulis* Lamk. [2.] *Mollugo cerviana* Linn. and [3.]*Glinus lotoides* Linn. representing leaf growth response under NaCl stress (at different level of concentration i.e. 0, 50, 100, 150 and 200 mM respectively.)

Shoot-Root length and S/R ratio - It was observed that the shoot and root length reduced steadily with increase in salinity (Fig.1.4, Table 1.2). The reduction may be due to the toxic effect of NaCl. Several studies have suggested that the unbalanced nutrient uptake by the seedling/plant may be responsible for such reduction which may also be applied for present study (Jamil et al. 2005; Fariduddin et al. 2012; Ratnakar and Rai, 2012). There is a significant relative reduction in shoot as well as root length found in the accessions of Mollugo nudicaulis Lamk.(44.68% and 55.69%), Mollugo cerviana Linn.(65.93% and 82.06%) and Glinus lotoides Linn.(43.83% and 69.77%) at highest salinity level (i.e. 200 mM). It was also observed that the reduction in shoot length was more apparent than that of root length leading to an increased S/R ratio (Table 1.2). The interspecific correlation analysis is also supporting this result (Fig.1.6). It is observed that the accessions of Mollugo nudicaulis Lamk. and Glinus lotoides Linn. showed comparatively less reduction in plant growth regarding the above parameters as well as more PSI and less PST score under same salinity level (Table 1.2). It clearly indicates that, this genotype has made some interior mechanism to cope up with salinity stress. This kind of response makes it to more tolerant as well as more adapt against unfavorable environmental stress, whereas the accessions of Mollugo cerviana Linn. showed greater loss in plant growth regarding the above parameters as well as less PSI and more PST score under same salinity level. It means that, this genotype is unable to develop that level of defense to cope up with salinity stress. This kind of response makes it to more susceptible as well as more sensitive against unfavorable environmental stress (Plate 1 and Fig. 1.1).

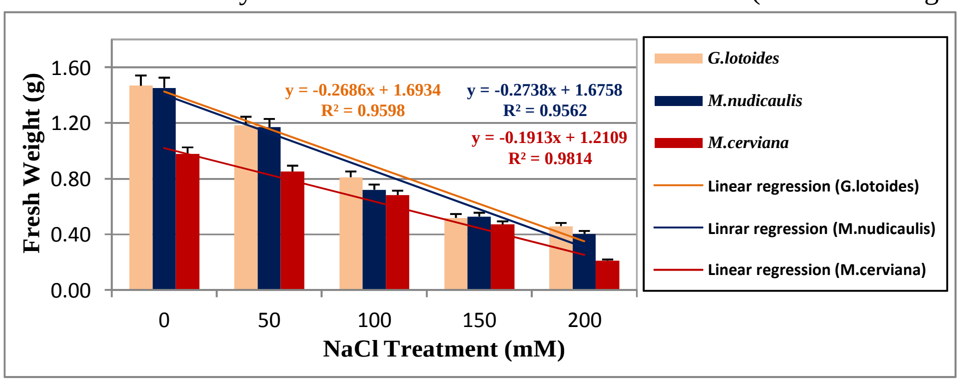

Fig. 1.2: Comparative salinity stress response on plant growth (Fresh Weight) in plant species of family Molluginaceae present in Indian Thar Desert in various NaCl concentrations

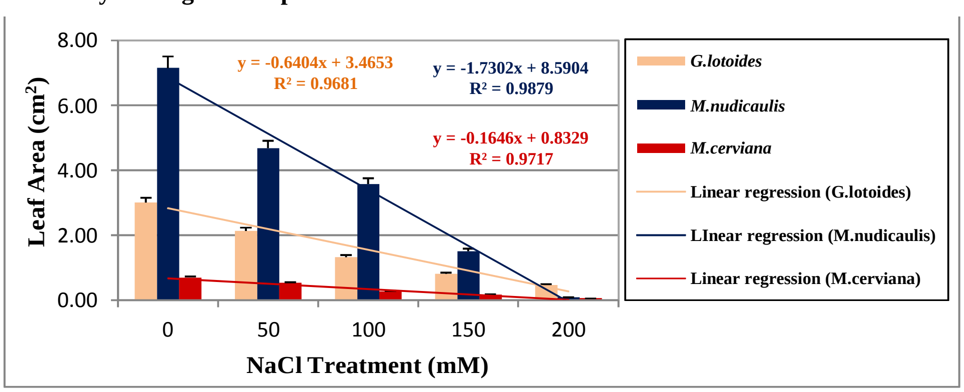

Fig. 1.3: Comparative salinity stress response on plant growth (Leaf area) in plant species of family Molluginaceae present in Indian Thar Desert in various NaCl concentrations

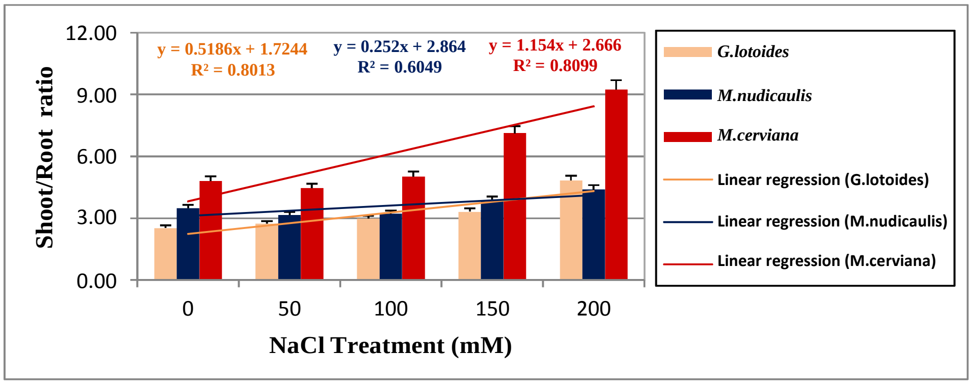

Fig. 1.4: Comparative salinity stress response on plant growth (S/R ratio) in plant species of family Molluginaceae present in Indian Thar Desert in various NaCl concentrations

Effect of salinity stress on proline content

To avoid cellular dehydration under stress, plants accumulate compatible solutes/osmolytes such as proline which act as low molecular weight chaperones (Gupta et al., 2013). Due to its zwitter ion character, proline accumulates in high concentration in cell cytoplasm under stress condition (Burg and Ferrari, 2008; Harsh et al. 2016). Proline accumulation in plants is the widely common defensive action against stress, so it proves an effective marker to detect stress induced responses in plants (Kapoor et al. 2013; Lal et al., 2015; Khalil et al. 2016).

The interpretations of tabulated data indicated that proline accumulation was progressively increasing with enhancement in a level of sodium salt concentration in both species of *Mollugo* Linn. (Table1.2). Proline content was significantly different between treatments of various levels of NaCl concentration showing P value less than 0.05 (Fig.1.5). It was observed that maximum increase was found with the highest level of salinity, i.e. 200 mM, in both the species of *Mollugo* Linn. as well as *Glinus lotoides* Linn. It was also found that the accessions of *Glinus lotoides* Linn accumulated more proline content (2.68±0.03 $\mu mol^{-1} g^{-1}$FW) and showed 277.46% relative proline increment at the highest level of salinity (200mM) as compare to control while *Mollugo nudicaulis* Lamk. accumulated some less proline content (2.00±0.24 $\mu mol^{-1} g^{-1}$FW) and showed 376.19% relative proline increment at the highest level of salinity (200mM) as compare to control. The accumulation of proline content by these both species is completely matched with plant growth parameters, PSI and PST scores at same salinity level and evidencing that proline is an effective biochemical market to detect salinity stress tolerance. In contrast of this the accessions of *Mollugo cerviana* Linn. accumulated least proline content (1.21±0.17 $\mu mol^{-1} g^{-1}$FW) and showed 1110.00% relative proline increment at the highest level of salinity (200mM) as compare to control (Fig.1.5, Table 1.2). This response also supports plant growth parameters, PSI and PST scores at same salinity level which has discussed earlier. These observations point towards the highest susceptibility and sensitivity of *Mollugo cerviana* Linn. against salinity stress.

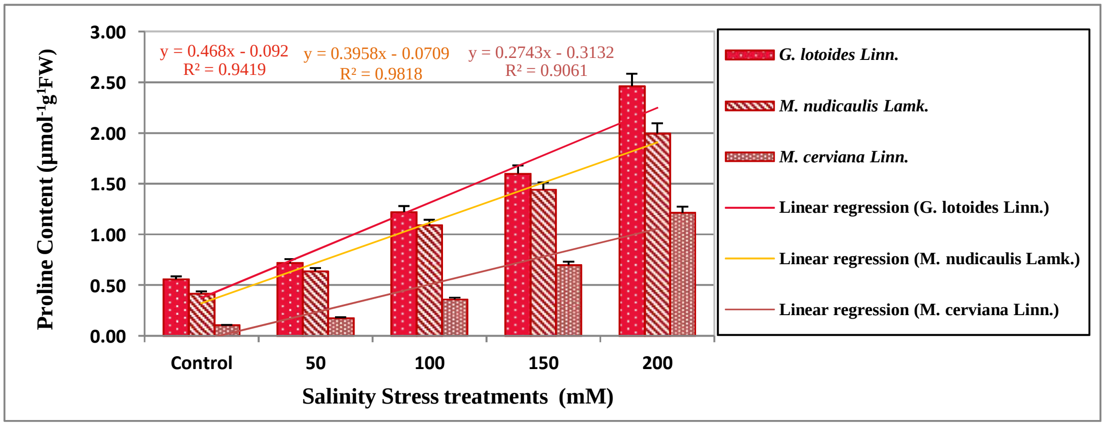

Fig. 1.5: Comparative proline accumulation under salinity stress by genera of Molluginaceae present in Indian Thar Dessert with their R2 values calculated by linear regression

Interspecific and intraspecific correlation analysis

Overall, the present study has clearly illustrated an enhancement in proline content along with increasing PST score and decreasing PSI. The plant survival as well as proline content was directly associated with level of tolerance to salinity stress as depicted by significant correlation with PST scores (Fig.1.5). Correlation analysis among all studied control as well as stress treatments revealed that each parameter was significantly correlated with NaCl stress tolerance (on the basis of PST scores) except S/R ratio in the accessions of *Glinus lotoides* Linn. (0.9294) (Fig.1.6.1), *Mollugo nudicaulis* Lamk. (0.8908) (Fig.1.6.2) and *Mollugo cerviana* Linn. (0.9000) (Fig.1.6.3). Correlation among the accessions of all three species was highest for Shoot length (0.9582) (Fig.1.6.4) and followed by PSI (0.8913) (Fig.1.6.4). The basal level of five plant growth parameters, PSI, PST and proline content was significantly correlated with the level in treated plant accessions suggesting genotype with higher level of osmolyte activity under stressed conditions.

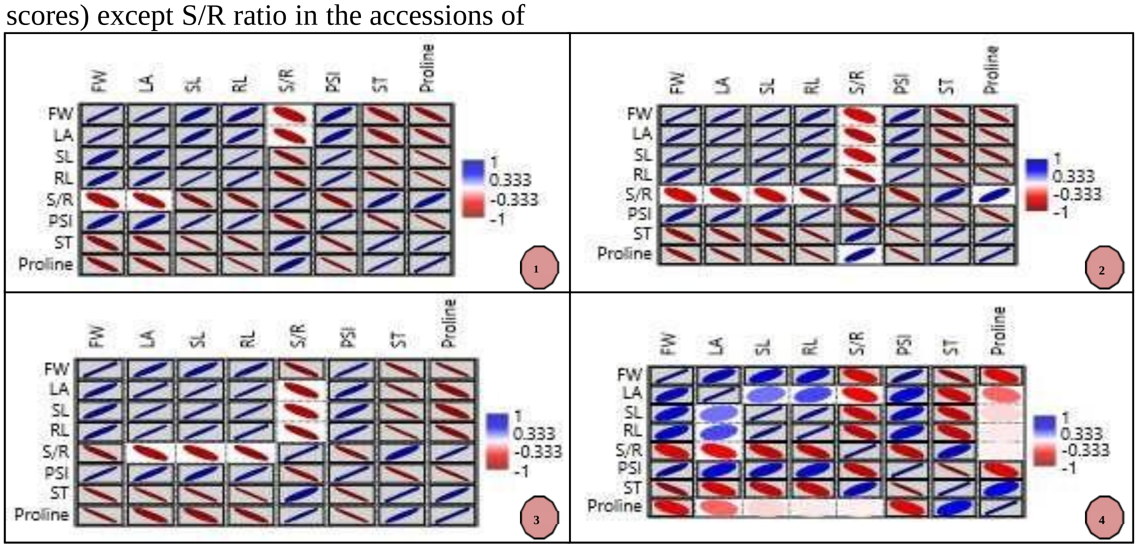

Fig. 1.6 Intraspecific correlation among various parameters related to salinity tolerance in the accessions of Glinus lotoides Linn. (1), Mollugo nudicaulis Lamk. (2), Mollugo cerviana Linn. (3) under NaCl stress and Interspecific correlation overall accessions (4). [Significant values (<0.05) are boxed]

The interspecific correlation among various accessions of the all three species of Molluginaceae was also significant at the level $p<0.05$. Tolerant and moderately tolerant plant accessions showed higher PSI and comparatively low osmolyte activity than susceptible types of accessions. At highest salinity stress level (200mM) of NaCl, the susceptible type of accessions of *Glinus lotoides* Linn. and *Mollugo nudicaulis* Lamk. showed non-significant association with the control, Moderately susceptible and higher susceptible types of accessions of *Mollugo cerviana* Linn. This correlation analysis reveals that intraspecific association among the accessions of all three species of family Molluginaceae present in Indian Thar Desert under salinity stress are more correlated as compare to interspecific association (Fig.1.6).

Multivariate cluster analysis

It was performed on the basis of plant growth parameters (FW, LA, SL and RL), proline content, plant survival index (PSI) and plant salinity tolerance (PST) scores due to high correlation coefficients among these characters. All accessions of all three species of family Molluginaceae present in Indian Thar Desert under salinity stress were classified into five clusters as shown in Fig.1.7. The clusters were generated based on plant growth parameters and PSI from the highest to the lowest while proline content and PST score from the lowest (more tolerant) to the highest (more susceptible).The group of plant accessions were categorized in five groups (Group with 80-100% PSI and PST score 1; Group with 60-80% PSI and PST score 3; Group with 40-60% PSI and PST score 5; Group with 20-40% PSI and PST score 7; Group with 0-20% PSI and PST score 9) and remarked as salinity tolerant cluster (T), moderately tolerant cluster (MT), moderately susceptible cluster (MS), susceptible cluster (S), highly susceptible clusters (HS) respectively (Fig.1.7)

Cluster I: 'Cluster of salinity tolerant groups of plant accessions (T)' - It is divided into two sub categories: I-A and I-B. The first category [I-A] included total five groups of plant accessions in which two were of groups of *Mollugo nudicaulis* Lamk. (MN-0: Control plants; MN-50: Plants treated with 50mM NaCl), two were of groups of *Glinus lotoides* Linn. (GL-0: Control plants; GL-50: Plants treated with 50mM NaCl) and one was the group of *Mollugo cerviana* Linn. (MC-0: Control plants) whereas the second category had only one group of *Mollugo cerviana* Linn. (MC-50: Plants treated with 50mM NaCl). It was observed that GL-50 group responded nearest hierarchal neighborhood with non-stressed groups (control) of itself as well as both species of *Mollugo* Linn. and also with MN-50 group. The plants of GL-50 group showed relatively less reduction in plant growth in terms of fresh weight (19.73%); leaf area (29.57%); shoot length (07.01%); root length (13.43%) and S/R ratio (-7.91) as compare to other groups of plant accessions. Although a little degree of damage in leaves and partial shoot retarding were observed in plants but besides this, all of them survived successfully with 100% PSI and remained tolerant with PST score-1 against the stress treatment of 50mM NaCl. Interpretation of tabulated data are evident that the relative increment in proline content (33.80%) in leaves than control is the critical supporting factor for increasing tolerance capability of this group to cope up with NaCl stress level up to 50mM.

The plants of MC-50 group had 88.89% PSI with PST score 3 and showed 13.27%, 18.75%, 26.26%, 20.60% and 07.08% more relative reduction in plant growth in terms of fresh weight, leaf area, shoot length, root length and S/R ratio respectively as compare to prior level of stress treated plants (control group) therefore it remained intermediate neighbor among I-A and II group of cluster.

Cluster II: 'Cluster of moderately tolerant groups of plant accessions (MT)' - It was included two groups of plant accessions in which one was the group of *Glinus lotoides* Linn. (GL-100: Plants treated with 100mM NaCl) and other was the group of *Mollugo nudicaulis* Lamk. (MN-100: Plants treated with 100mM NaCl). Both groups had 77.78% PSI and PST score-3. The plants of GL-100 group showed 44.90%, 54.95%, 14.15%, 26.93% and -17.79% more relative reduction in plant growth in terms of fresh weight, leaf area, shoot length, root length and S/R ratio respectively as compare to prior level of stress treated plants (GL-50 group). The plants of MN-100 group showed 50.34%, 49.93%, 26.26%, 23.40% and 7.56% more relative reduction in plant growth in terms of fresh weight, leaf area, shoot length, root length and S/R ratio respectively as compare to prior level of stress treated plants (MN-50 group). The more relative increase was found in proline level (114.08% and 159.52% respectively) in this cluster as compare to cluster I. Interpretation of tabulated data is indicating that the accessions of *Mollugo nudicaulis* Lamk. (MN-100 group) shows moderately tolerant symptoms earlier than the accessions of *Glinus lotoides* Linn. (GL-100 group). It means that the accessions of the genera *Mollugo* Linn. are more sensitive against NaCl stress than the accessions of the genera *Glinus* Linn.

Cluster III: 'Cluster of moderately susceptible groups of plant accessions (MS)'- It is divided into two sub categories: III-A and III-B. The first category [III-A] contained single group of plant accessions of *Mollugo cerviana* Linn. (MC-100: Plants treated with 100mM NaCl). MC-100 group showed 66.67% PSI with PST score 5. This group had 30.61%, 57.18%, 42.69%, 44.58% and -04.58% more relative reduction in plant growth in terms of fresh weight, leaf area, shoot length, root length and S/R ratio respectively as well as more relative increase in proline level (260.00%) than the intermediate tolerant accession of same species (MC-50). The second category [III-B] included two groups of plant accessions in which one was the group of *Glinus lotoides* Linn. (GL-150: Plants treated with 150mM NaCl) while other was the group of *Mollugo nudicaulis* Lamk. (MN-150: Plants treated with 150mM NaCl). Both groups were ranging within 40-60% PSI and PST score-5. The plant accessions of the MN-150 group of plant accessions showed 44.44% PSI and 63.45%, 78.88%, 34.77%, 43.66% and -10.60% more relative reduction in plant growth in terms of fresh weight, leaf area, shoot length, root length and S/R ratio respectively as well as more relative increase in proline level (242.86%) than the moderately tolerant accession of the same species (MN-100). Similarly the plant accessions of the GL-150 group of plant accessions showed 55.56% PSI and 64.63%, 72.70%, 29.92%, 46.15% and -31.23% more relative reduction in plant growth in terms of fresh weight, leaf area, shoot length, root length and S/R ratio respectively as well as more relative increase in proline level (195.77%) than the moderately tolerant accession of the same species (GL-100). Interpretation of tabulated data is indicating that the accessions of *Mollugo cerviana* Linn. showed moderately susceptible symptoms at low level (100mM) of NaCl stress and more relative proline increment (260%) than the accessions of *Mollugo nudicaulis* Lamk. (at 150mM concentration level of NaCl stress and 242.86% relative proline increment) and the accessions of *Glinus lotoides* Linn. (at 150mM concentration level of NaCl stress and 195.77% relative proline increment) therefore it remained intermediate neighbor among III-B and II group of cluster. (It supports that the accessions of *Mollugo cerviana* Linn. are more sensitive against NaCl stress than the accessions of *Glinus lotoides* Linn. and *Mollugo nudicaulis* Lamk.)

Cluster IV: 'Cluster of susceptible groups of plant accessions (S)'- It was classified into three groups of plant accessions in which one was the group of *Glinus lotoides* Linn. (GL-200: Plants treated with 200mM NaCl), second was the *Mollugo nudicaulis* Lamk. (MN-200: Plants treated with 200mM NaCl) and third was the group of *Mollugo cerviana* Linn. (MC-150: Plants treated with 150mM NaCl). All groups were ranging within 20-40% PSI and PST score-7. The plants of GL-200 group had 33.33% PSI and showed 68.71%, 84.30%, 43.83%, 69.77% and -90.91% more relative reduction in plant growth in terms of fresh weight, leaf area, shoot length, root length and S/R ratio respectively as compare to moderately susceptible plants of the same species (GL-150 group). The plants of MN-200 group had 22.22% PSI and showed 72.41%, 98.88%, 44.68%, 55.69% and -26.37% more relative reduction in plant growth in terms of fresh weight, leaf area, shoot length, root length and S/R ratio respectively as compare to moderately susceptible plants of the same species (MN-150 group). The plant of MC-150 group had 33.33% PSI and showed 41.84%, 75.36%, 55.53%, 69.80% and -48.33% more relative reduction in plant growth in terms of fresh weight, leaf area, shoot length, root length and S/R ratio respectively as compared to the moderately susceptible plants of the same species (MC-100 group). The more relative enhancement was found in proline level (277.46%, 376.19% and 600.00% respectively) in this cluster as compare to cluster III. These data clearly indicated that the accessions of *Mollugo cerviana* Linn. showed susceptibility at lower level of salinity with higher proline content (150mM; 600%) as compare to the accessions of *Glinus lotoides* Linn. at higher level of salinity with lower proline content (200mM; 277.46%) as well as the accessions of *Mollugo nudicaulis* Lamk. (200mM; 376.19%). It proves that the accessions of *Mollugo cerviana* Linn. are more susceptible against NaCl stress than the accessions of other two species.

Cluster V: 'Cluster of highly susceptible groups of plant accessions (HS)'- It had a single group of *Mollugo cerviana* Linn. (MC-200: Plants treated with 200mM NaCl). MC-200 ranges within 0-20% PSI and PST score-9. The plants of MC-200 group had 00.00% PSI and showed 78.57%,92.75%, 65.93%, 82.06% and -92.50% more relative reduction in plant growth in terms of fresh weight, leaf area, shoot length, root length and S/R ratio respectively as compare to the susceptible plants of the same species (MC-150 group) that leads to complete dryness with more relative proline (1110%). This group showed highest degree of damage due to salinity stress. The recorded data pointing that MC-200 had least PSI which is a strong indication of higher susceptibility of the accessions of *Mollugo cerviana* Linn. as compared to the accessions of *Glinus lotoides* Linn. and *Mollugo nudicaulis* Lamk. against NaCl stress.

Table 1.3: Genetic distance matrix representing genetic diversity among various accessions of the all three species of family Molluginaceae present in Indian Thar Desert based on plant growth response, proline accumulation, PSI and PST under salinity stress.

Accessions	GL0	GL50	GL100	GL150	GL200	MN0	MN50	MN100	MN150	MN200	MC0	MC50	MC100	MC150	MC200
GL0	0.0000	0.0310	0.2621	0.4944	0.7268	0.0375	**0.0090**	0.2065	0.4462	0.6759	0.0788	0.1888	0.3230	0.4735	0.7313
GL50		0.0000	0.2310	0.4634	0.6957	0.0685	0.0401	0.1755	0.4152	0.6449	0.1098	0.2198	0.3540	0.5045	0.7003
GL100			0.0000	0.2323	0.4647	0.2995	0.2711	0.0556	0.2048	0.4139	0.3409	0.2102	0.2702	0.4208	0.5493
GL150				0.0000	0.2324	0.5319	0.5034	0.2879	0.1223	0.2074	0.5732	0.4425	0.2618	0.3383	0.4669
GL200					0.0000	0.7642	0.7358	0.5203	0.2806	0.1249	**0.8056**	0.6749	0.4942	0.2558	0.3844
MN0						0.0000	**0.0284**	0.2440	0.4836	0.7134	0.0413	0.1514	0.2855	0.5084	0.7687
MN50							0.0000	0.2155	0.4552	0.6850	0.0698	0.1798	0.3139	0.4800	0.7403
MN100								0.0000	0.2397	0.4694	0.2853	0.1546	0.2147	0.3652	0.5248
MN150									0.0000	0.2298	0.5250	0.3943	0.2136	0.2160	0.3445
MN200										0.0000	0.7547	0.6240	0.4434	0.2050	0.2595
MC0											0.0000	0.1307	0.3114	0.5498	**0.8101**
MC50												0.0000	0.1807	0.4191	0.6794
MC100													0.0000	0.2384	0.4987
MC150														0.0000	0.2603
MC200															0.0000

(Highest and lowest genetic distances are highlighted)

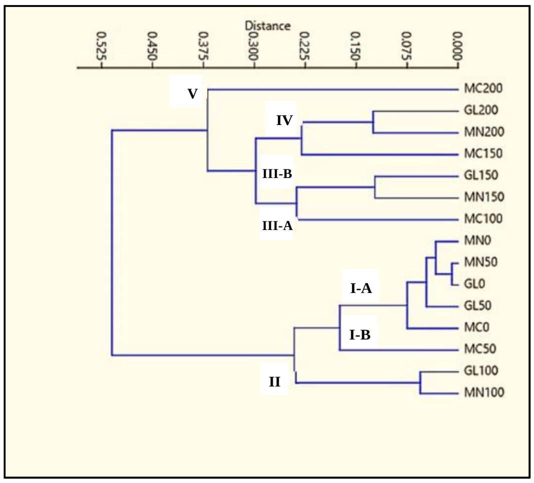

Fig. 1.7: UPGMA derived multivariate clusters of various accessions of the species of Molluginaceae present in Indian Thar Desert under salinity stress representing inter-specific variability based on plant growth parameters, proline content, PSI and PST- [I] Salt tolerant, [II] Moderately tolerant, [III] Moderately susceptible, [IV] Susceptible, [V] Highly susceptible. [Here GL- *Glinus lotoides* Linn.; MN-*Mollugo nudicaulis* Lamk.; MC-*Mollugo cerviana* Linn.]

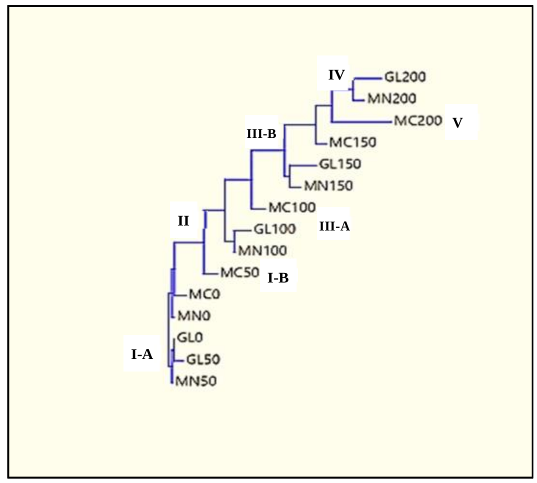

Fig. 1.8: UPGMA derived neighbour linkage dendrogram of various accessions of the species of Molluginaceae present in Indian Thar Desert under salinity stress representing inter-specific variability based on plant growth parameters, proline content, PSI and PST- [I] Salt tolerant, [II] Moderately tolerant, [III] Moderately susceptible, [IV] Susceptible, [V] Highly susceptible. [Here GL- *Glinus lotoides* Linn.; MN-*Mollugo nudicaulis* Lamk.; MC-*Mollugo cerviana* Linn.]

A two-way ANOVA indicated significant effect of salinity ($P<0.05$) and population ($P<0.05$) on the proline content in plant leaves (Table 1.4). The relative decrement in plant growth parameters and PSI as well as relative increment in proline content in the accessions of *Glinus lotoides* Linn., *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. under various levels of salinity stress revealed that the intra specific and interspecific variations in the proline accumulation in all three species contributed to the adaptation of plants in salt stress represented by high F-values and significant P-values (Table 1.5)

Table 1.4: Results of ANOVA (Two-way analysis of variance without replication) for proline accumulation under salinity stress by the species of *Molluginaceae* present in Indian Thar Desert

Species	Source of Variation	df	MS	F	P-value
***G. lotoides* Linn.**	Between Control and Stress (Salinity)	2	0.004	44.68***	0.0000456****
	Within Treatment (Population)	4	1.976	22380.00****	0.0000000****
***M. nudicaulis* Lamk.**	Between Control and Stress (Salinity)	2	0.124	8.29**	0.0112400**
	Within Treatment (Population)	4	1.197	80.25***	0.0000017****
***M. cerviana* Linn.**	Between Control and Stress (Salinity)	2	0.042	5.92**	0.0264800**
	Within Treatment (Population)	4	0.621	86.74***	0.0000013****

Here, **df**: degree of freedom; **MS**: mean square; ********: very high significant test; *******: high significant test; ******: significant test.

Table 1.5: Intraspecific and interspecific variation in proline content accumulated by the species of *Molluginaceae* present in Indian Thar Desert under salinity stress

Source of Variation	df	MS	F	P-value
Intraspecific variation	2	1.471	39.76**	0.0000698****
Interspecific variation	4	1.191	32.20**	0.0000558****

Values were obtained as a result of ANOVA (Two-way analysis of variance without replication)

CONCLUSIONS

In the light of the above result, it is concluded that:

- On the basis of growth parameters, the results shows variability in salt response within and between *Glinus lotoides* Linn., *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. genotypes, depending on the salinity level. The maximum decrease in plant growth response observed with the highest level of salinity, i.e. 200 mM, in all three species species of Molluginaceae present in IndianThar Desert.
- Salinity stress impacts significantly negative effect on plant growth and survival, but sensitivity differed for each species. The accessions of *Glinus lotoides* Linn. and *Mollugo nudicaulis* Lamk. showed comparatively less reduction in plant growth regarding plant growth parameters as well as more PSI and less PST score under same salinity level than the accessions of *Mollugo cerviana* Linn.
- Proline content was significantly different between treatments of various levels of NaCl concentration showing P value less than 0.05. It was resulted that maximum increase was found with the highest level of salinity, i.e. 200 mM, in all three species of Molluginaceae present in Indian Thar Desert.
- The result shows that the accessions of *Glinus lotoides* Linn. and *Mollugo nudicaulis* Lamk. were accumulated more proline and showed more relative increment in proline content at the highest level of salinity as compare to the accessions of *Mollugo cerviana* Linn.
- The interspecific and intraspecific correlation as well as multivariate analysis revealed that the accessions of *Glinus lotoides* Linn., and *Mollugo nudicaulis* Lamk. are comparatively tolerant to NaCl stress while the accessions of *Mollugo cerviana* Linn. are comparatively susceptible to NaClstress.

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