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Research Article

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Elevated osmolytes accumulation helps in combating NaCl stress causing negative impacts on growth and metabolism of *Vigna radiata* (L.)

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ABSTRACT

Salinity stress is one of the main abiotic stresses that have a negative impact on the growth performance of green gram. The current study was carried out as a result to find out growth, and morpho-biochemical changes in *Vigna radiata* CO7 variety cultivated under NaCl stress treatments. The *V. radiata* CO7 variety was selected and the experiment was carried out in pot culture under varying NaCl concentrations viz., 0, 50, 75, 100, and 125 mM respectively to assess maximum tolerance range of the CO7 variety. The salt stress was given on 15^{th} days after sowing and sampling was done after 10 days of treatment on the 25^{th}, 35^{th}, and 45^{th} day respectively. Salt stress results in a steep decline in shoot length, biomass, chlorophyll contents a and b, and soluble protein contents with increased NaCl treatments on all sampling days. However, carotenoid contents, and compatible solutes including proline, Glycine-betaine, Amino acids and total soluble sugars contents were found to be upregulated under varying NaCl concentrations in *V. radiata* CO7 variety on all sampling days. Thus, increased carotenoid contents, and osmolytes, provide stress tolerance to *V. radiata* CO7 variety by maintaining the turgor pressure of cells and preventing further water loss under varying NaCl concentrations. Hence, this variety shows maximum surveillance at 75 mM and beyond this plant performance is restricted and further study is needed to access CO7 variety for a breeding program to enhance salt stress tolerance.

KEYWORDS: Compatible solutes, Mung bean, Growth, NaCl toxicity, Salinity stress, Pigments

INTRODUCTION

From the climatic perspective view, plants are counteracted by various abiotic stresses timely, such as salinity stress that results in a huge loss of production, plant performance and grain quality as well (Yadav *et al.*, 2020). However, anthropogenic activities including the use of groundwater for irrigation, excess inorganic fertilizers and pesticides, have worsened it further and this has created a great threat to the growing population, income of farmers, and acquiring food demand all over the world (Wang & Han 2007; Chen *et al.*, 2021). According to FAO, more than 800 million hectares of agricultural land are seriously affected due to salinity globally (Munns & Tester, 2008). It has been estimated that almost 52 million hectares of land in South Asia and approximately 6.73 million hectares in India are affected by salinity. The main reason is the use of poor-quality underground water for irrigation (32-84%) in various states of India (Jangir & Yadav, 2011), which hampers plant growth by creating secondary drought due inefficiency of plants to extract water and minerals from the soil.

Salinity imposes osmotic, ionic toxicity that leads to secondary stress (oxidative stress) (Ceccarini *et al.*, 2019), and affects plant growth, and metabolism directly through its potentially toxic effects and indirectly by the way of its osmotic effects and ionic stress (Qados, 2011). Oxidative stress also called second phase stress in which ROS are produced (O_2^-, H_2O_2, OH^-), that attack, lipids, proteins present in plasma-membrane and other organelle membrane, and nucleic acids used for cell processing and other activities (Das *et al.*, 2016). However, plants can somehow tolerate salinity by acquiring cellular physiological changes. The reduction of ROS through the action of the antioxidant system, which is composed of enzymatic and non-enzymatic compounds that maintain the cellular redox status (Tani *et al.*, 2019). Secondly, the implication of stress by buildup of different compatible solutes, including quaternary amino acid derivatives such as glycine-betaine, β-alanine-betaine, and proline found in various plants and act as defensive mechanisms by maintaining cell turgor (Nahar *et al.*, 2016). Among quaternary amino-acid derivatives, glycine-betaine and proline are readily available solutes produced in plants under various stress conditions (Mansour & Ali, 2017). There are also reports of additional soluble sugar buildup in plants as exposed to different Stressors (Murakeözy *et al.*, 2003). These osmotic solutes maintain osmotic equilibrium, regulate water inflow (reduce efflux) and enable turgor maintenance of plants under abiotic stresses (Chaparzadeh *et al.*, 2003).

Green gram is an essential summer-season pulse crop of the Fabaceae family, grown primarily for its protein-rich edible seeds. The great nutritional value of mung beans is well known contains about 55% - 65% carbohydrates, and are rich in minerals, proteins, fat, and vitamins. In addition, mung beans are the source of commerce worldwide. Notably, the impact of saline stress is evident in most of the crops utilized throughout the world. It is well known that leguminous crops contribute significantly to agricultural systems by supplying fixed nitrogen to plants from the soil through microorganisms mutualism (Valentine *et al.*, 2017). *Vigna radiata* has reported a salt-sensitive legume. Wherein the buildup of Na^+ and Cl^- has been reported to impact germination, decreased photosynthetic rate, reproductive phase, and ultimately causes the death of the plants (Hussain *et al.*, 2021). Moreover, the germination stage and early seedling stage are considered as sensitive phases in the life cycle of a plant (Munns & Tester, 2008). However, a scanty literature is available regarding salinity tolerance systems in CO7 varieties. Therefore, the investigation at various salt concentrations viz., 50, 75, 100, and 125 mM given to mung bean (CO7) variety in the current work to inquiry about its negative symptoms on physiological and biochemical aspects, and its maximum survival rate under varying NaCl doses as well.

MATERIALS AND METHODS

Seed Collection

The mung bean CO7 variety seeds were provided by the Agriculture faculty (Department of Genetics and Plant Breeding), Annamalai University, Tamil Nadu.

Pot and Lab Experimental Design

For the pot experiment plants were raised in the Botanical Garden, and laboratory work was done at the Stress Physiology Lab, Department of Botany, from February - April (2022), Annamalai University, Tamilnadu.

Healthy seeds of the Mung bean (CO7) variety were surface sterilized with 0.1% sodium hypo-chloride for 3 minutes, followed by thorough washing with sterile water to remove traces. Then the 12 hours soaked seeds were blotted dry, and planted in plastic pots (Height = 12 cm and Inner diameter = 12.5 cm), filled with 1.5 kg of a homogenous mixture soil - red soil, sand, and farmyard manure in a ratio (1:1:1). Afterwards, plants were divided into 5 groups with three replicates (n=3) to each treatment and NaCl stress was imposed on 15th days after sowing (DAS) with five treatments (T0-T4) (Table 1). However, control plants were irrigated routinely with tap water. The plant samples were harvested for observations on days 25th, 35th, and 45th respectively for morpho-chemical analysis. The Figures 1 and 2 shows uproot and pot culture of mung beans.

Table 1: The treatment and its NaCl concentrations

S. No.	Treatments
1	T0 - (Control 0 mM NaCl)
2	T1 - (NaCl 50 mM)
3	T2 - (NaCl 75 mM)
4	T3 - (NaCl 100 mM)
5	T4 - (NaCl 125 mM)

Experimental Work

Morphological parameters

Five plants from each treatment were selected randomly to find the morphological traits root length, stem length, fresh weight and dry weight. The uprooted plants were cleaned, and then root length and shoot length were taken and expressed in cm plant^{-1}. Further, fresh weight of the plant was taken using an electronic balance (Model – DS-852J Series) and expressed in gm plant^{-1}. After taking fresh weight and recorded in g plant^{-1}, fresh weighted plant samples were oven-dried for 72 hours at 60 ºC to reach a constant dry weight and recorded in g plant^{-1}. The total leaf area per plant was also calculated by following the protocol previously described by Yoshida *et al.* (1972) and K (Kemps' Constant) was 0.66 for dicot leaves.

$$\text{Leaf Area (cm}^2\text{)} = k \times \text{length} \times \text{breadth}$$

Photosynthetic pigments

Pigment contents a and b were extracted from 0.5 g fresh leaves with 10 mL of 80% acetone at 4 ºC temperature in a pestle and mortar and then centrifugation was done at 2,500 rpm for 10 min at 4 ºC. The absorbance was taken at different wavelengths (nm) viz., 645, 663, and 480 in a Spectrophotometer (U-2001–Hitachi) using acetone as a blank, and calculated by the formula of Arnon (1949). Carotenoid contents were calculated by the formula of Kirk and Allen (1965), and denoted in mg g^{-1} fresh weight (FW).

Determination of protein, proline, glycine-betaine, free amino acids, and soluble sugars

Proline content was quantified by following the protocol of Bates *et al.* (1973). Fresh samples of 0.5 mg were extracted in a mortar and pestle with 10 mL of 3% aqueous 5-sulfosalicylic acid. Finally, the proline extract of 2 mL volume was taken, ninhydrin of 2 mL and glacial acetic acid of 2 mL were later added. Subsequently, the mixture was incubated for an hour at 100 °C in a water bath. The reaction was terminated into an ice bath to stop the reaction. Later, the toluene containing the chromophore (organic phase) was isolated from the liquid phase using a separating funnel and the optical density was measured at 520 nm in a UV-VIS spectrophotometer (Model-118, Systronic India Limited, Gujarat, India). The known proline was used as standard and the results were expressed in μg g^{-1} dry weight.

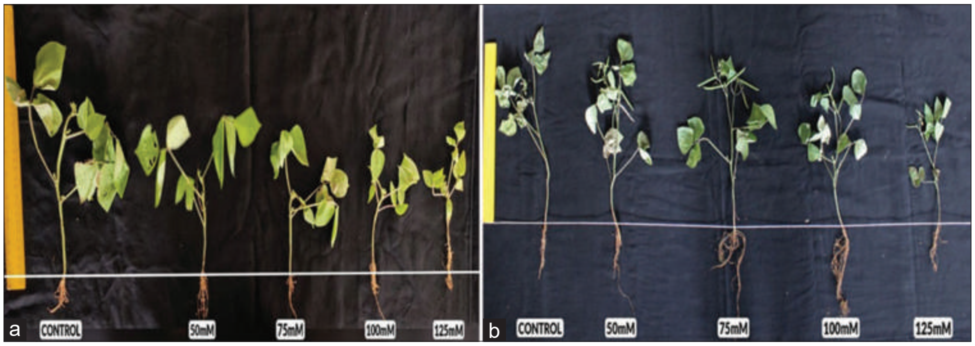

Figure 1: *Vigna radiata* L. Uproot (a) (25 DAS) and (b) (35 DA)

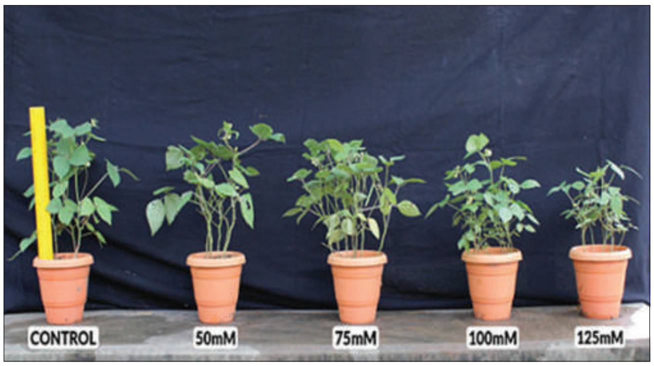

Figure 2: Effect of NaCl on morphology of *Vigna radiata* L. Pot Culture (25 DAS)

The protein concentration of unknown samples was measured by the calibration curve resulting from BSA sol. by reading the reaction mixture at 595 nm. The results were expressed as mg gm^{-1} fresh weight (FW) by the protocol of Bradford (1976).

The glycine betaine of plant samples was quantified by following the protocol of Grieve and Grattan (1983). The absorbance was read at 365 nm in a Spectrophotometer. The reference standard used was glycine betaine prepared in 1 N H_2SO_4, and used for estimating the glycine betaine of plant samples and the results were expressed in μg gm^{-1} dry weight.

Total free amino acids were quantified by following the protocol of Moore and Stein (1948). The optical density was read at 570 nm in a Spectrophotometer (U-2001–Hitachi). The leucine was taken standard and the results were expressed in mg gm^{-1} dry weight.

Soluble sugars (reducing and non-reducing) were estimated by a modified method of Nelson (1944). Non-reducing sugars were hydrolyzed to reducing sugar and total sugars were estimated. One millilitre of the extract was evaporated to dryness in a water bath. To the residue, 1 mL of sterile water and 1 mL of 6 N sulphuric acid were added. A volume of 1 mL fresh copper reagent and 1 mL of extract [prepared by mixing copper tartrate solution and copper sulphate solution (25:1 v/v)] were added. The mixture was heated in a Folin-Wu-tube with its mouth covered with a marble in a boiling water bath for 20 min., then cooled and 1 mL of arsenomolybdate reagent was added. The final volume of 20 mL was made using sterile water. The resultant blue colour was read at 520 nm in a spectrophotometer against the appropriate blank. The glucose was taken as standard and result was expressed in mg gm^{-1} dry weight.

Statistical Analysis

The data pertained to all the characters studied were based on statistical analysis using SPSS- 22 Version. Statistical analysis was performed for the mean of values (n=3) for three samples and (±) S.E in each group at significance $P \leq 0.05$ level.

RESULTS

Effect of NaCl Stress on Growth Attributes

Root length

With increasing NaCl concentrations, a significant increment in the root length of *V. radiata* cultivated in NaCl stress environment on all sampling DAS viz., 25, 35, and 45 DAS respectively. It is clear from Figure 3a, which shows that root length is slightly increased at 50 mM NaCl stress. However, a tremendous increase was noted with increasing NaCl concentrations (75 mM, 100 mM, and 125 mM) respectively. Further, the highest root length increase was found on 45 DAS for all NaCl treatments, it was 104% for 50 mM, 109% for 75 mM, 144% for 100 mM, and 154% over control for 125 mM respectively.

Stem length

A significant decline in the stem length of mung bean plants cultivated under different NaCl stress treatments was noted. However, the highest decrease in stem length was noted on 100 mM and 125 mM NaCl treatments on 45 DAS and it was 65% over control for 100 mM and 53% over control for 125 mM respectively (Figure 3b).

Leaf area

Growth-related characteristics, including the leaf area of *Vigna radiata* plants, was negatively impacted by salt-stressed treatments on all sampling DAS viz., 25, 35, and 45 DAS (Figure 4). However, with enhancement in NaCl doses, a

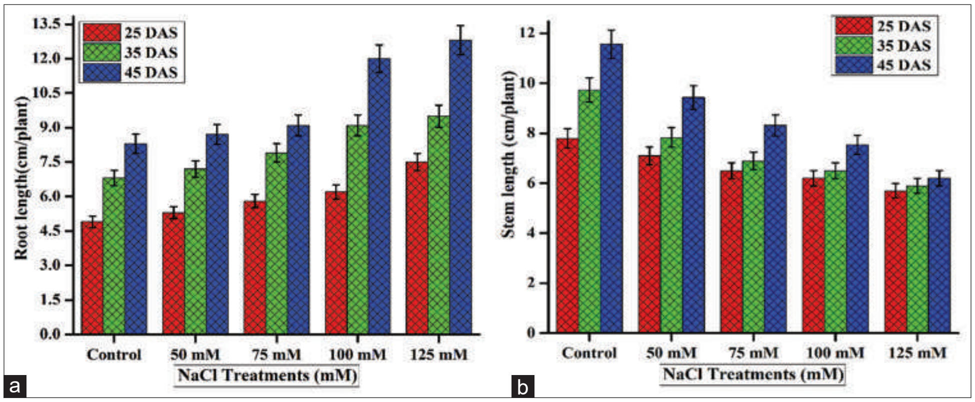

Figure 3: Effect of NaCl stress on (a) root length and (b) stem length of *V. radiata*. Values are the mean±SE of three replicates (n=3)

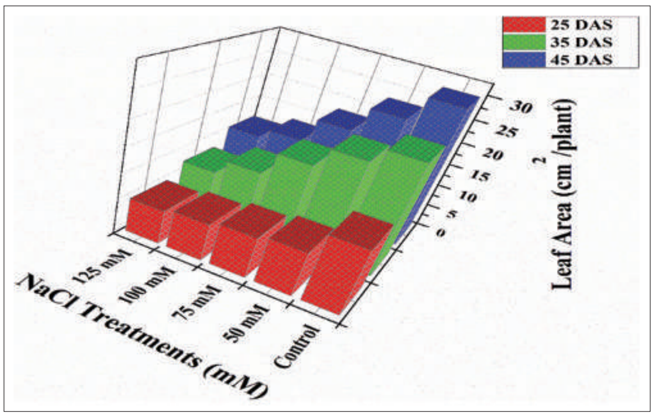

Figure 4: Effect of NaCl stress on leaf area of *V. radiata* (Values are the mean±SE (n=3) three replicates)

significant decline in leaf area was noticed on 45 DAS and it was 84, 67, 50 and 44% over control for 50 mM, 75 mM, 100 mM, and 125 mM NaCl treated mung bean plants respectively.

Effect of NaCl Stress on Biomass

Fresh weight and dry weight

Biomass is an indicator of growth performance in plants, especially when subjected to NaCl salt stress. Growth-related characteristics, including the fresh weight of green gram plants, were adversely impacted by salt-stress treatments on all sampling DAS viz., 25, 35, and 45 DAS respectively (Figure 5a). However, with enhancement in NaCl doses, a significant reduction in fresh weight was noticed for 50 mM, 75 mM, 100 mM, and 125 mM NaCl stress and it was 87, 77, 67 and 60% over control respectively on 45 DAS.

The reduction in dry biomass of *V. radiata* plants was severely affected by rising salt-stress treatments on all sampling days (Figure 5b) in unstressed plants. However, with increasing NaCl concentrations, a profound reduction was noticed in dry biomass at all NaCl treatments viz., 50, 75, 100, and 125 mM respectively. However, the highest decrease was noted on 45 DAS in all NaCl treatments and it was 70, 51, 38, and 32% over control respectively.

Effect of NaCl Stress on Chlorophyll and Carotenoids Contents

Chlorophyll a

Under increased NaCl treatments a tremendous decline in chlorophyll contents both chlorophyll a and b was observed. The maximum decrease in Chl. a was found on 45 DAS in all NaCl treated plants viz., 50, 75, 100 and 125 mM and it was 79, 66, 58 and 47% over control respectively (Table 2).

Chlorophyll b

Similarly, a sharp decrease in the chlorophyll b content was observed on all sampling DAS viz., 25 DAS, 35 DAS, and 45 DAS respectively (Table 2). The highest recorded decrease was noted on 45 DAS and among different NaCl treatments 100 mM and 125 mM treated plants showed more decrease in chlorophyll b pigments and it was 70 and 63% over control respectively on 45 DAS.

Carotenoid contents

Increased salt concentrations cause a tremendous increase in carotenoids for all sampling days Viz., 25, 35, and 45 DAS in NaCl treated plants than unstressed plants (Table 2). However, 45 DAS treated plants showed more upsurge in the carotenoid contents in all NaCl treated plants and it was noted 103% over control for 50 mM, 114% over control for 75 mM, 157% over control for 100 mM, and 171% over control for 125 mM respectively on 45 DAS.

Biochemical Contents

Proline (Pro)

Similarly salt stress increased proline content in both the shoot and root of *V. radiata* with increased NaCl levels on all sampling days. It is shown from Figure 6a and b that there was a direct proportionality between the proline contents and upsurging NaCl stress concentrations of *Vigna radiata*. However, this increased amount was found higher in both shoot and root on 45 DAS respectively in comparison to control and it was 138, 146, 149 and 176% over control for 50, 75, 100, and 125 mM NaCl

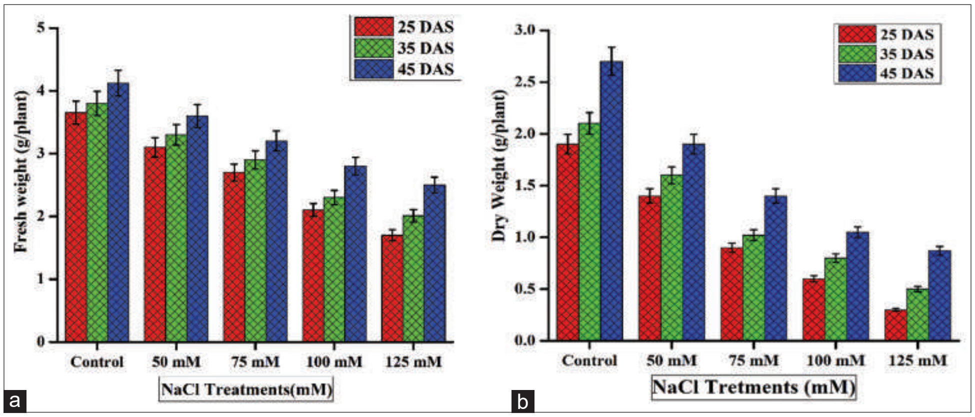

Figure 5: Effect of NaCl stress on (a) fresh weight and (b) dry weight of *V. radiata*. Values are the mean±SE (n=3) three replicates

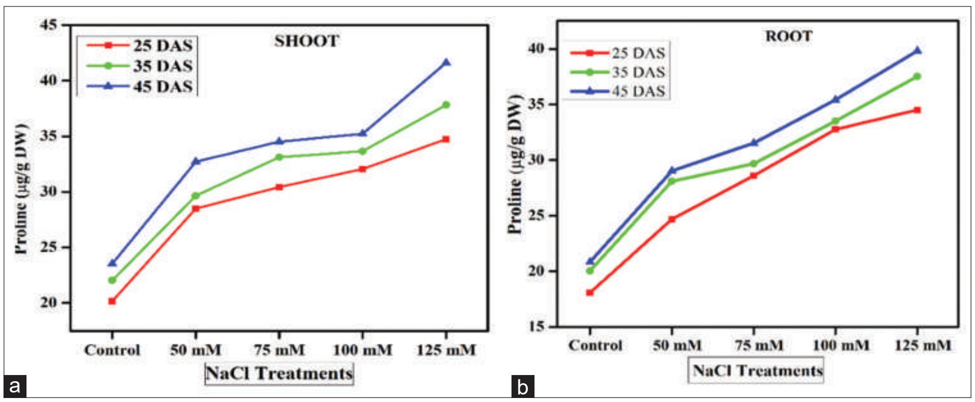

Figure 6: Effect of NaCl stress on Proline content on a) shoot and b) root of *V. radiata*. Values are the mean±SE (n=3) three replicates

Table 2: Effect of NaCl stress on chlorophyll (a, b) and Carotenoid contents of *V. radiata*

DAS	Control	50 mM	75 mM	100 mM	125 mM
Chlorophyll a (mg g^{-1} FW)					
25 DAS	0.152±0.006	0.116±0.004	0.095±0.004	0.081±0.003	0.062±0.002
35 DAS	0.162±0.006	0.126±0.005	0.105±0.004	0.091±0.003	0.072±0.003
45 DAS	0.172±0.007	0.136±0.005	0.115±0.002	0.101±0.004	0.082±0.003
Chlorophyll b (mg g^{-1} FW)					
25 DAS	0.152±0.006	0.116±0.004	0.106±0.004	0.065±0.002	0.015±0.001
35 DAS	0.169±0.006	0.127±0.005	0.121±0.005	0.086±0.003	0.032±0.001
45 DAS	0.174±0.007	0.139±0.006	0.134±0.005	0.122±0.004	0.109±0.004
Carotenoids (mg g^{-1} FW)					
25 DAS	0.147±0.006	0.153±0.006	0.161±0.001	0.165±0.005	0.170±0.005
35 DAS	0.155±0.007	0.158±0.004	0.164±0006	0.167±0.006	0.182±0.007
45 DAS	0.159±0.007	0.164±0.006	0.182±0.005	0.251±0.004	0.273±0.004

The values are the mean±(S.E) (n=3) three replicates

treatments in shoots respectively. However, in case of roots, the increase recorded was 137% for 50 mM, 144% for 75 mM, 169% for 100 mM, and 182% for 125 mM on 45 DAS respectively.

Protein

It is apparent from Figure 7a and b that the protein content decreased progressively in the shoots and roots of *Vigna radiata* with increased salt concentrations than those of control plants on all sampling days viz., 25, 30, and 45 DAS. This decrease was observed higher on 45 DAS in comparison to non-stressed plants, and was 68, 61, 54, and 35 % over control noted in 50 mM, 75 mM, 100 mM, and 125 mM respectively in shoots. Similarly, a negative correlation was also found between increased NaCl concentrations and protein content of the roots. When compared with control this decrease was noted higher on 100 and 125 mM, on 45 DAS respectively and was observed 60% and 23% over control respectively.

Glycine betaine (GB)

The glycine betaine was also increased progressively in all organs (shoots and roots) of *Vigna radiata* with the rise in salt concentrations compared with control plants on all sampling days viz, 25, 35, and 45 DAS respectively. A positive trend was found between increased NaCl concentrations and GB content of both parts on all sampling days. When compared with control this increase was noted higher on 45 DAS and it was 90, 149, 164, and 189% over control respectively (Figure 8a & b).

Similarly, in the roots of V. *radiata* glycine betaine also showed a positive trend with increased NaCl concentrations on all sampling days. When compared with control this increase was noted higher on 45 DAS and it was 103%, 110%, 138%, and 155% over control respectively for 50 mM, 75 mM, 100 mM, and 125 mM NaCl treatments.

Free Amino acids (AA)

Figure 9a and b showed an increased amino acid contents in both the parts (root and shoot) of NaCl-treated green gram plants progressively with increased salt doses than non-stressed plants on all sampling days. However, the highest upsurge was observed on 45 DAS and it was 109, 113, 117, and 126 percent over control in shoots respectively, and in roots it was 103, 106, 118 and 122 percent over control respectively on 45 DAS. NaCl stress upsurged amino acid contents in all parts of mung bean plants on all sampling days with increased NaCl treatment concentrations.

Total soluble sugars (TSS)

It is clear from Figure 10a and b that the soluble sugar contents increased progressively in all parts (root and shoot) of V. *radiata* with increased salt doses than non-stressed plants. This higher increase was observed on 45 DAS respectively, in comparison with control and was noted 106% over control for 50 mM, 114% over control for 75 mM, 122% over control for 100 mM and 128% over control for 125 mM respectively. However, in roots it was noted 109% over control for 50 mM, 120% over control for 75 mM, 135% over control for 100 mM, and 151% over control for 125 mM respectively. Total soluble sugar contents were higher at all growth stages in both the shoots and roots of V. *radiata* on all sampling DAS under different NaCl stress concentrations.

DISCUSSION

Soil salinity has an impact on every trait of the plant including plant height, leaf canopy, and yield as well and this is evident especially legumes which are prone to salinity stress (Mir & Somasundaram, 2021b). Thus, the goal of the current study is to find out the tolerance index of V. *radiata* cultivated under different NaCl stress treatments.

Effect on Morphological Traits

In this study, sodium chloride stress caused a drastic reduction in plant growth attributes. However, a slight enhancement in the root height with increased NaCl concentrations on all sampling days was recorded. This increase could be possible because roots try to uptake water and minerals for growth.

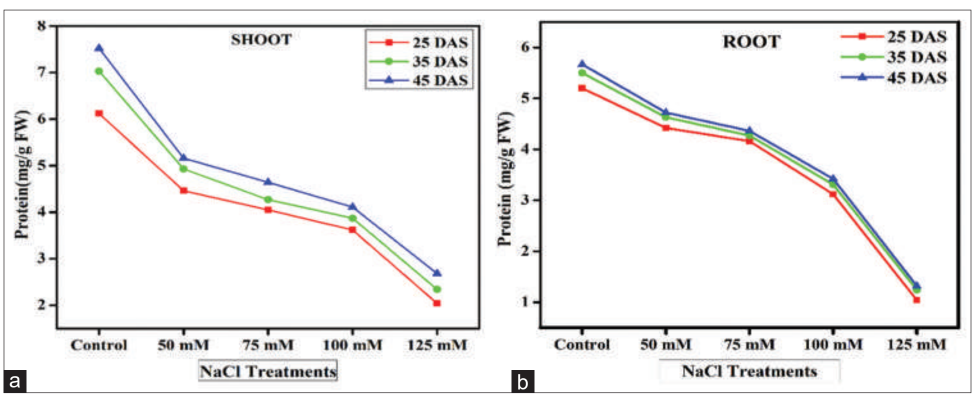

Figure 7: Effect of NaCl stress on Protein content on a) shoot and b) root of *V. radiata*. Values are the mean±SE (n=3) three replicates

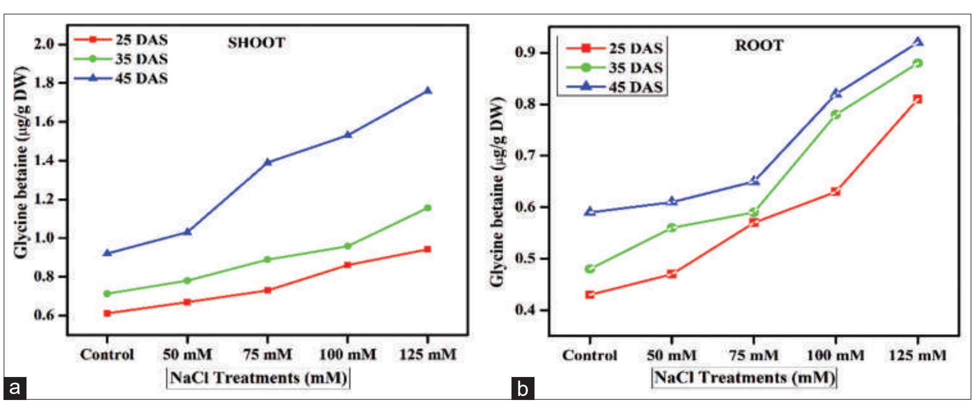

Figure 8: Effect of NaCl stress on Glycine betaine on a) shoot and b) root of *V. radiata.* Values are the mean±SE (n=3) three replicates

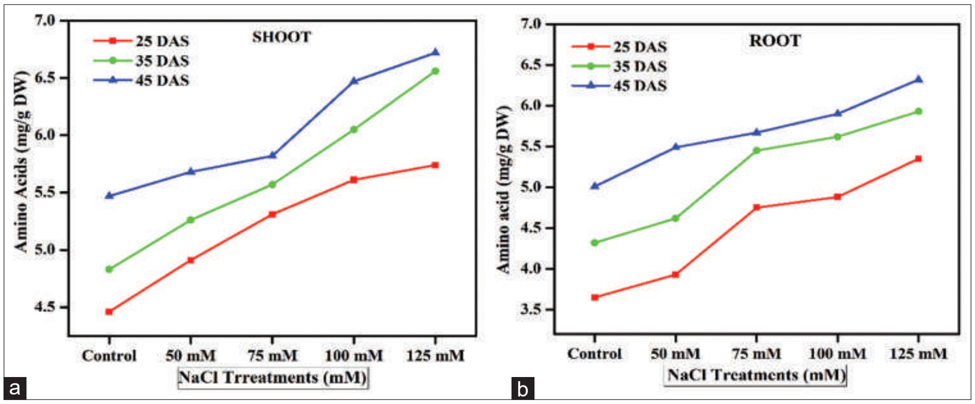

Figure 9: Effect of NaCl stress on free amino acid content on a) shoot and b) root of *V. radiata*. Values are the mean±SE (n=3) three replicates

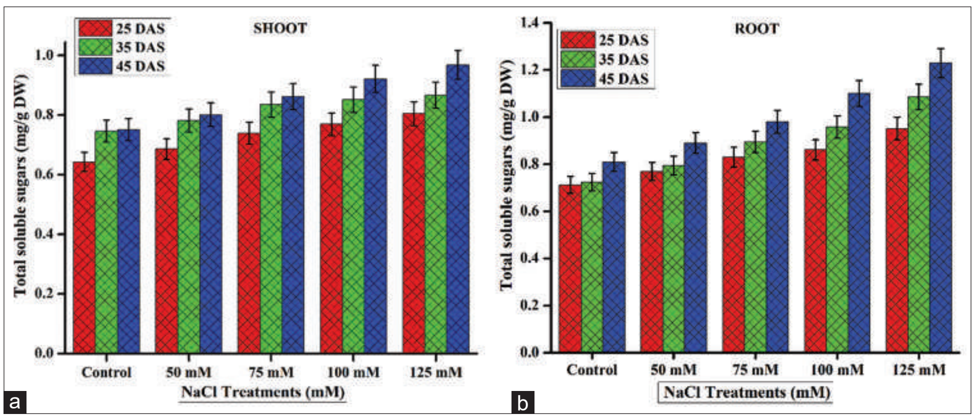

Figure 10: Effect of NaCl stress on Total soluble sugar contents on a) shoot and b) root of *V. radiata*. Values are the mean±SE (n=3) three replicates

However, a reduction in shoot length was seen more at 100 mM and 125 mM NaCl exposure at 45 DAS.

Our results agreed with the previous results documented by Mir and Somasundaram (2020, 2021a) and Noreen *et al.* (2021), who observed a drastic reduction in morphological traits of plants when treated with NaCl stress treatments. The decrease in plant growth is attributed to the NaCl stress-induced suppression of cell expansion and damage to the root architecture which restricts plants' capacity for water uptake and minerals absorption and their subsequent utilization (Kamran *et al.*, 2020; Naeem *et al.*, 2010).

Decreased leaf area under NaCl treatment was noticed in V. *radiata* (Hussain *et al.*, 2021) and canola cultivars (Naheed *et al.*, 2021). The leaf area represents a measure of plant growth, which can be affected by salt stress since, maximum leaf area results in enhancing photosynthetic activity. The aerial parts of plants are more prone to external stress and especially at the vegetative stage salinity anxiety lessened cell turgor, photosynthetic rate and together such factors interfere with wall properties and thereby diminishing leaf area (Aryendu *et al.*, 2022a). High salinity exhibited a decrease in total leaf area in the tolerant variety of Mung bean was reported by (Sehrawat *et al.*, 2015). Similar results of decreased leaf area were found in *Asteriscus maritimus* (Rodrıguez *et al.*, 2005) and *Punica granatum* (Liu *et al.*, 2019).

Fresh Weight and Dry Weight

Fresh mass and dry mass (Figure 5a & b) of V. *radiata* plants gradually decline with the upsurging of NaCl doses compared to unstressed plants. Because salinity-caused overproduction of ROS results in an imbalance of minerals, and inhibition of enzymatic activities, which significantly affects the cellular components and causes a drastic decline in biomass production (Alzahrani & Alaraidh, 2019; Kumar *et al.*, 2021). Plant growth reduction and less dry matter under salinity has been well addressed in several crops including *Salvinia auriculata* (Gomes *et al.*, 2017) and *Hordeum vulgare* (Noreen *et al.*, 2021).

Photosynthetic Pigments

Our results regarding the reduction in Chlorophyll a, b content (Table 1) with rising NaCl concentrations agreed with the results earlier reported for common bean (Cokkizgin, 2012) and cowpea (Mir & Somasundaram, 2021a). The diminish in pigments (Chlorophyll a, b) are linked with ROS toxicity led by excess salt ions buildup in the cell resulting in the breakdown of chlorophyll molecules, disruption of essential elements uptake including Mg^{2+}, K^{+}, and thereby upregulating the catabolic activity of chlorophyll degrading enzyme known as chlorophyllase (Bulgari *et al.*, 2019; El-Beltagi *et al.*, 2020). However, our results (Table 1) showed an upsurge in carotenoid contents of V. *radiata*. Carotenoids are group of antioxidants formed under stressful conditions in the chloroplast and thereby protect the pigment system (PSII,I) against harmful environmental factors by scavenging ROS (Ramel *et al.*, 2012). Carotenoids are known to function as collectors of light energy for photosynthesis and also dissipate excess heat thereby, prevents PSII and PSI from damage which are very prone to salinity lead primary and secondary stress.

Biochemical Constituents

Salt stress increased proline content significantly in both parts of the V. *radiata* CO7 variety. However, a direct proportionality between the proline contents of root, shoot, and rise in the NaCl treatments than non-stressed ones was noted. Our results agreed with previous studies done by Mansour *et al.* (2005) in *Zea mays*, Kaya *et al.* (2010) in corn, and Aryendu *et al.* (2022b) in peanut respectively, reported an enhancement in proline content under NaCl stress environment. Recently, in sugarcane under various salinity circumstances, Chiconato *et al.* (2019) showed a linearity in upsurge soluble proline with increment of NaCl dosages (0 mM, 40 mM, 80 mM, and 160 mM). Under diverse abiotic stressors, osmolytes accumulation, particularly proline is essential for maintaining cellular turgor (Farooq *et al.*, 2015; Negrão *et al.*, 2017). In addition, "osmolytes have hydrophilic nature that helps to replace water by bonding at the surface proteins and membranes, which clearly explains their role as osmo protectants and as "Chaperones".

The soluble protein contents were reduced in response to saline stress and the effect was determined higher at increased stress conditions on all sampling days than control ones. The decline in protein content under salinity results from less availability of amino acids and the denaturation of enzymes required for protein synthesis *Mentha pulgeium*. Similarly, Khosravinejad *et al.* (2009) with their study on *Hordeum vulgare* seedlings, and Alharby *et al.* (2019) on *Vigna radiata* genotype observed reduced protein content when subjected to sodium chloride stress. Moreover, proteins serve as prime factors for enzymatic activities.

Glycine betaine content was found increased in all parts of V. *radiata* L. on all sampling days exposed to NaCl doses. Our results are in line with the previous results done by Farhangi-Abriz and Torabian (2017) on bean seedlings, and Akram *et al.* (2020) and Sardar *et al.* (2023) on Broccoli varieties respectively. Wherein authors recorded a significant upsurge in Glycine betaine under NaCl treatment than plants grown under normal conditions. Similarly, according to (Tuteja *et al.*, 2012), GB shields the extrinsic PSII complex proteins from salt stress, protecting the photosystem II complex. Similarly, a slight increase in glutathione content under all NaCl levels of stress was reported in common bean (Sofy *et al.*, 2020).

A prominent upsurge in the amino acids was observed in both roots, and shoots of V. *radiata* under varied NaCl concentrations than in non-stressed plants. Our results are in line with the previous results observed on sunflowers by Rady *et al.* (2011), and on flax plants by Mervat and Ebtihal (2013), wherein they concluded that salt stress possibly operate as an activator in the building of amino acids.

Soluble sugar contents were significantly upsurged gradually in all parts of plants treated with varied NaCl concentrations on all sampling days. With a rise in NaCl salinity levels, there were considerable, steady increases in soluble sugar contents. Further, increased soluble sugars were obtained in sunflowers (Rady *et al.*, 2011), broccoli and cauliflower, (Giuffrida *et al.*, 2012), and (Sardar *et al.*, 2023) in broccoli plants under NaCl salinity conditions. When glycophytic plants are exposed to primary stress (osmotic stress), the buildup of sugars, among compatible solutes are the major solutes implicated in osmotic balance (Amini & Ehsanpour, 2005). Sugars operate as osmoprotectants. Generally, soluble sugars protect the membrane's phospholipids by causing the cytoplasm to form glass cover (Crowe *et al.*, 1988).

CONCLUSION

In the current work, sodium chloride treatments cause a great reduction in morphology and biochemical traits of NaCl-stressed green gram plants under varied NaCl concentrations given @ 0, 50, 75, 100, and 125 mM, analysed on 25^{th}, 35^{th}, and 45^{th} days respectively. A tremendous decrease in growth, pigments - chlorophyll a & b, and protein content was recorded in all NaCl-stressed plants relative to unstressed plants. However, on the other side an upsurge in carotenoid contents and osmolytes accumulation such as proline, glycine-betaine, amino acids, and soluble sugars has shown linearity with increasing NaCl doses. Thus, increasing osmolytes plays a frontline role in salt stressed plants by maintaining the osmotic potential of cells, quenching ROS, and act as an adaptive mechanism supporting NaCl stress tolerance. Further, the CO7 variety shows maximum tolerance at 75 mM and therefore, this variety can be explored in saline soil. However, further studies are required to ascertain the stress tolerance nature of *Vigna radiata* CO7 variety on a molecular basis for the breeding program.

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