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Impact of NaCl on morphological, photosynthetic pigments and biochemical changes in *Panicum sumatrense* Roth ex-Roem and Schult

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Abstract
Millets are small seeded cereal crops which face several abiotic constraints that lead to reduction in the yield. Salinity is considered to be the most important abiotic stress that limits the crop production. The current study deals with the impact of NaCl stress on growth and development of *Panicum sumatrense* (Little millet). The seeds were raised at different concentration of NaCl (0, 5, 10, 15, 20 mM) for 30 days. Under saline stress, morphological changes, pigments and biochemical levels determined to analyze the physiological and biochemical characteristics. In addition, there was a progressive increase in the levels of proline, glycine betaine, MDA and H_2O_2 activity from 0 to 20 Mm. statistically, there was a decrease in photosynthetic pigments, amino acid and starch with reduced leaf succulence under increasing salinity. Salt stress greatly influenced a significant reduction in the leaf area, fresh weight, dry weight, number of leaves and roots. From this study, little millet crops can be sustained at 5Mm salinity condition. It was concluded that these osmolytes play a key role in generating tolerance against salt stress.

Keywords: abiotic, salt stress, glycine betaine, MDA, osmolytes, little millet

Introduction
Abiotic stress is defined as any environmental factor which influences the optimal functioning of an organism [29]. Around the globe, 20% of total cultivated land and 33% of irrigated agricultural lands are aggravated by high salinity. Salinity stress is one of the most significant limiting factors in agricultural crop productivity [4]. Salinity and drought are among the major stresses that adversely affect plant growth and crop productivity. These constraints remain the primary causes of crop losses worldwide, reducing average yields by more than 50% [4, 31]. Salt stress alters various biochemical and physiological responses in plants, and thus affects almost all plant processes including photosynthesis, growth and development [14]. Salinity tolerance is defined as the ability of plants to continuously grow under salt stress conditions. Another major factor of salt tolerance mechanisms is the ability of plant cells to adjust osmotically and to accumulate organic solutes (proteins, sugar, amino acids, etc,). The accumulation of these compounds is not only important for cell osmoregulation but also for the protection of sub cellular structure and maintenance of protein structures [24].
Millets are the most important staple food for the millions of people around the globe. India is the largest producer of many kinds of millets, which are often referred to as coarse cereals. The nutritive value of millets is comparable to other staple cereals like wheat and rice. Some of the millets are nutritionally better than common cereals in protein, fat and mineral contents. There are several studies conducted on minor millets indicating utilization potential and variability including potential benefits in modern diets. Although little millet like any other millet is nutritionally superior to cereals, yet its utilization is limited. The major factor discouraging its cultivation and consumption is the drudgery associated with its processing. However, there is a need to restore the lost interest in millets due to its potential nutritional qualities and health benefits [20]. The effect of salinity appears to be dependent on the species and on the stage of the plant's development such as germination or vegetative growth [11]. Salinity limits the cultivation and yield of agricultural crops by reducing the ability of plant roots for uptake of water and nutrients, decreasing photosynthetic rates also [25]. Salinity enhances the production of reactive oxygen species, including superoxide anion, hydrogen peroxide and lipid peroxide in cellular membranes [27]. The present study is aimed to analyse the effect of NaCl on morphological and biochemical validations in *Panicum sumatranse.*

Materials and Methods
Collection of Seeds
Seed of *Panicum sumatrense* (Poaceae) were collected at Kolli Hills situated about 1200m mean sea level between 78ºE and 11ºN in the Namakkal district of Tamil Nadu in South India.

Plant materials and culture conditions
The seeds of *P. sumatrense* were sown equally in each pot filled with homogeneous mixture of garden soil containing red soil, sand and farmyard manure (1:2:1). The pots were continuously irrigated using tap water and maintained in the Botanical garden of PSG College of Arts & Science, Coimbatore.

Salt treatment and experimental design
20 days old, mature and healthy seedlings were selected for NaCl treatment. The preliminary experiments were carried out in *P.*

sumatrense at different concentrations of NaCl (5mM, 10mM, 15mM, 20 mM) in order to determine the viable range of salinities. The experimental plants treated with NaCl by soil drenching method up to 20mM were maintained in the experimental plot. The experimental yard was roofed with transparent polythene sheet at a height of 3m from the ground in order to protect the plants from rain. Sampling for various studies was taken on 30 days after NaCl treatment. Standard procedures were employed for the morphological studies and estimation of biochemical parameters [2, 3, 6, 7, 8, 12, 19, 22, 28, 35].

Results

The various morphological and biochemical parameters analyzed exhibited significant variations in the plants grown under salinity.

Morphological and Photosynthetic pigments variations

The plantlets grown under high salinity had stunted growth where the root and shoot length, number of leaves and roots, leaf area, fresh weight and dry weight demonstrated significant changes with constitutive increase in the salinity strata. The calculated results showed that salt stress significantly reduced morphological parameters. The photosynthetic pigments such as chlorophyll and carotenoid showed a significant decrease under increasing salinity.

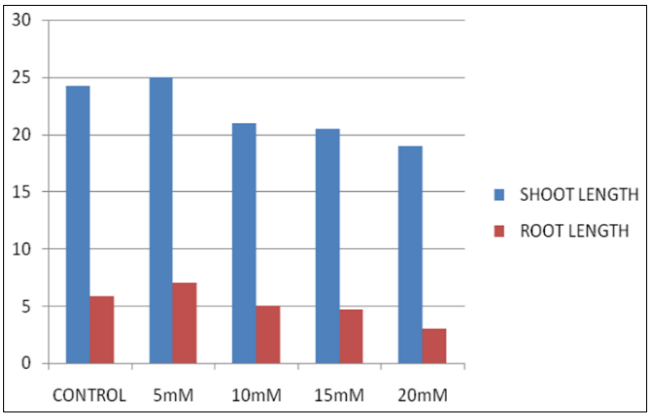

Fig 1: Effect of different concentration of NaCl on shoot and root length (cm) of *P. sumatrense*

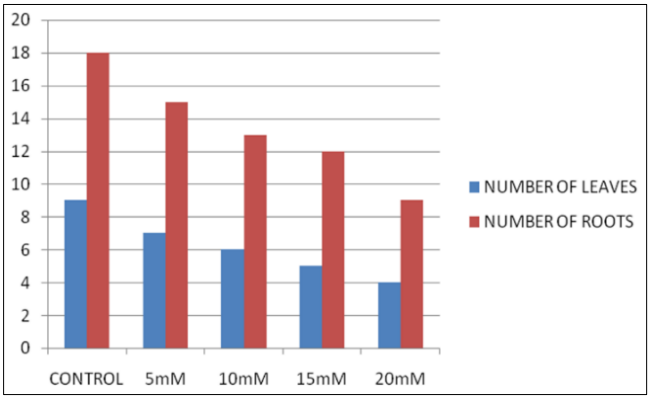

Fig 2: Effect of different concentration of NaCl on number of leaves and roots of *P. sumatrense*

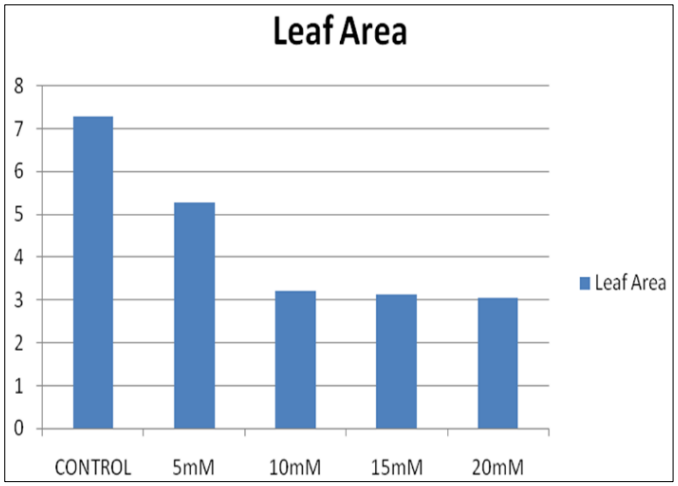

Fig 3: Effect of different concentration of NaCl on Leaf area of *P.sumatranse*

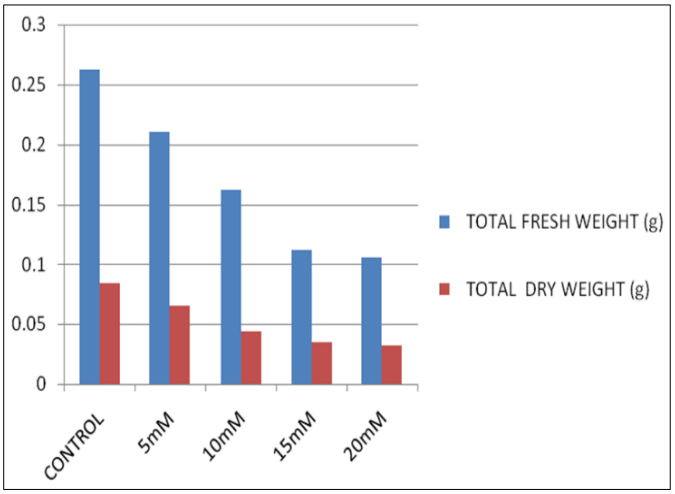

Fig 4: Effect of different concentration of NaCl on total fresh and dry weight (g) of *P. sumatranse*

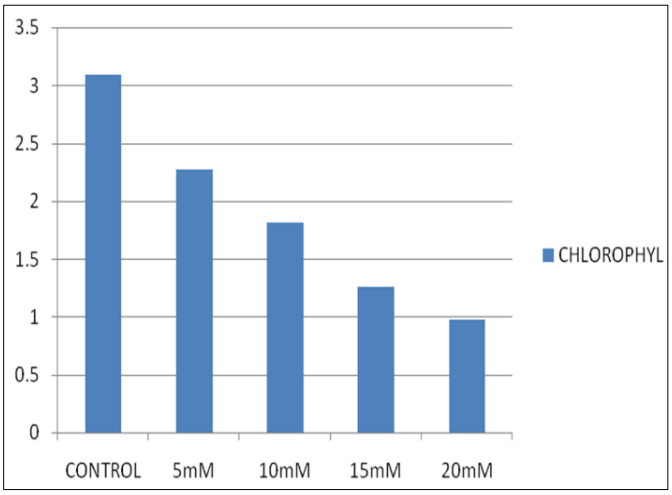

Fig 5: Effect of different concentration of NaCl on Chlorophyll content (mg/g/fr.wt) of *P. sumatranse*

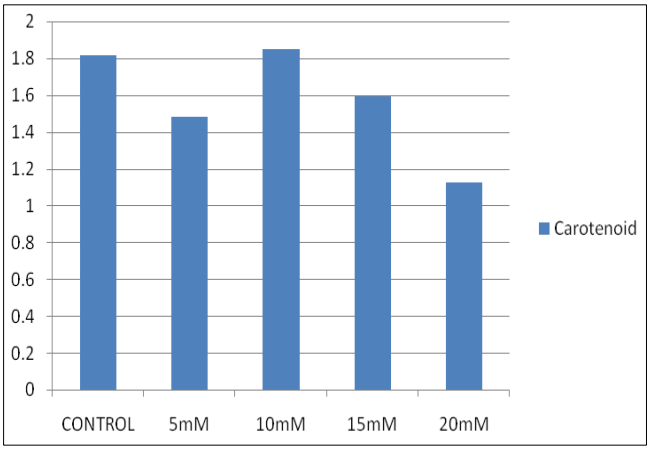

Fig 6: Effect of different concentration of NaCl on Carotenoid content (mg/g/fr.wt) of *P. sumatranse*

Biochemical analysis

Starch content

There were prominent changes observed in the starch content of *P. sumatranse* leaf, stem and root. Starch contents were decreased in the plants grown under severe salt stress. The relatively increased starch content was noted to be 0.699 in the plants grown in 5 mM salt concentration. Leaf tissues show more starch content when compared to stem and roots.

Amino acids

At the elevated levels of NaCl there was a decrease in the contents of amino acids in leaf, stem and roots. It showed a gradual decrease in the amino acid contents upto 20 mM. However the highest value obtained in the leaf was 1.624 less than that to the control. The similar trend was also seen in the stem and root tissues. In all treatments, the roots showed lower levels of amino acids when compared to leaf and stem.

Protein

Protein contents were increased in the leaf, stem and root with increasing NaCl concentration. At higher concentration, salinity gradually increased the protein, even upto 20 mM NaCl concentration. The leaves showed more protein than the stem and root.

Proline

In *P.sumatranse*, an increase in proline content was recorded in the leaf, stem and root. An increase in free proline content in stem and root under salt stress were recorded which increased with all regimes of NaCl stress possessing the values of 2.673 and 2.171 respectively. Leaf accumulated higher proline when compared to stem and root under salt stress

Glycine betaine

There was a remarkable increase in the accumulation of glycine betaine with increasing salinity upto 20 mM. When compared the glycine betaine content in both stem and root, the increase was not as prominent as in roots where the values are recorded as 1.623 in roots and 1.611 in shoots. Thus, in all treatments the leaf showed higher levels of glycine betaine content when compared to shoot and root.

Lipid peroxidation

The little millet irrigated with different concentration showed a marked change in the Lipid peroxidation activity. The activity of hydrogen peroxide increased significantly with increasing NaCl at different concentration. The increase of H_2O_2 activity in treated plants was accompanied by elevated lipid peroxidation as evidenced by the change in Malondialdehyde levels. Salt stress caused increased activity of Malondialdehyde and H_2O_2 activity with increasing NaCl concentration than the root and shoot tissues. The highest recorded MDA and H_2O_2 content was 1.842 and 0.81 respectively in the plants grown in 20 mM concentration on 30DAT.

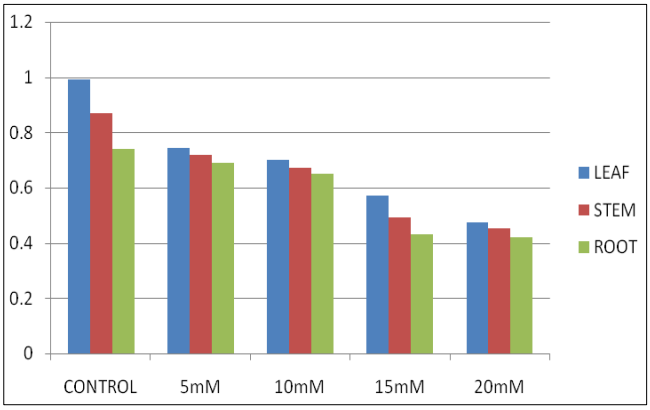

Fig 7: Effect of different concentration of NaCl on Starch content (mg/g/fr.wt) of *P. sumatranse*

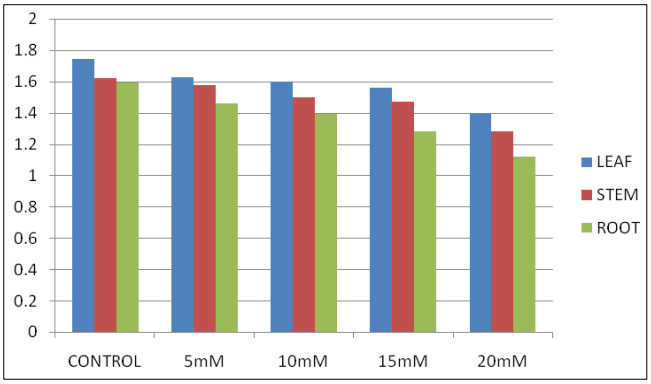

Fig 8: Effect of different concentration of NaCl on Aminoacid content (mg/g/fr.wt) of *P. sumatranse*

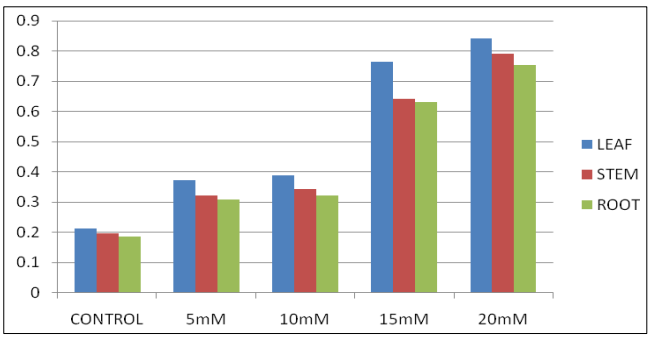

Fig 9: Effect of different concentration of NaCl on Protein content (mg/g/fr.wt) of *P. sumatranse*

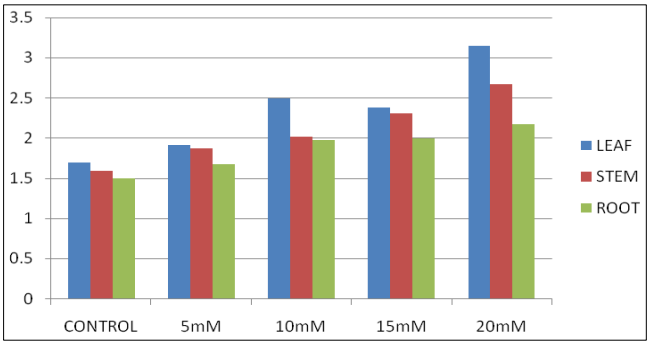

Fig 10: Effect of different concentration of NaCl on Proline content (mg/g/fr.wt) of *P. sumatranse*

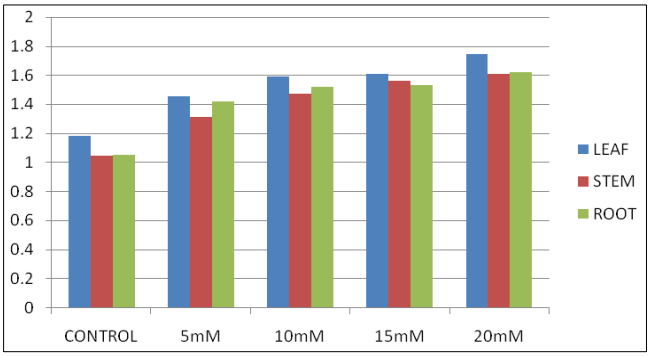

Fig 11: Effect of different concentration of NaCl on Glycine betaine content (mg/g/fr.wt) of *P. sumatranse*

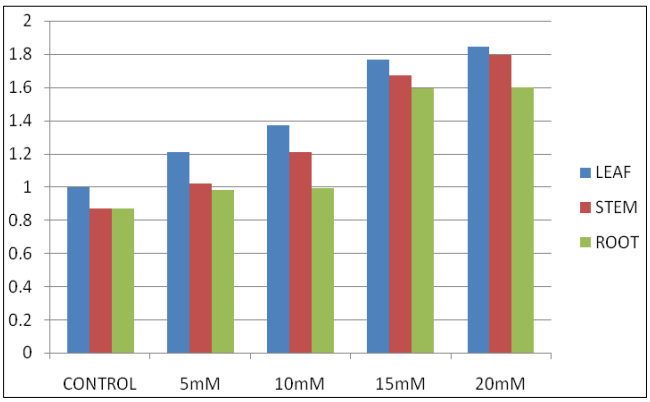

Fig 12: Effect of different concentration of NaCl on Malondialdehyde (mg/g/fr.wt) content of *P. sumatranse*

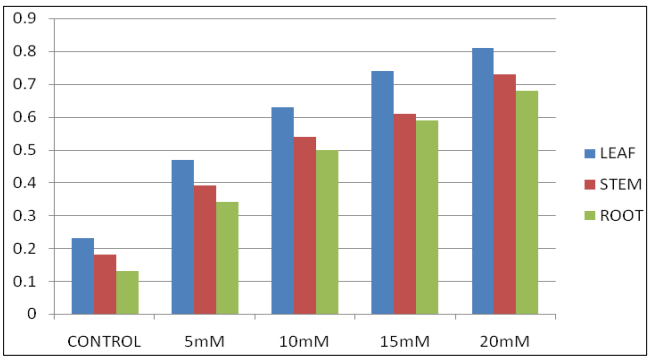

Fig 13: Effect of different concentration of NaCl on Hydrogen peroxide (mg/g/fr.wt) content of *P. sumatranse*

Discussion

The effect of salinity is highly diverse and depends on large number of factors such as multiple biochemical and morphological parameters. In *P. sumatranse*, the root and shoot growth was reduced to a great extend at increased salinity levels. This results are in agreement with the studies on the salinity effect in pearl millet (*Pennisetum glaucum*) where the root and shoot length of the pearl millet decreases with the increase in the salinity level [5]. As the saline concentration increased, there was a reduction in leaf area. Similar results were obtained seen in Oat (*Avena sativa)* where the root and leaf area were adversely affected due to NaCl treatments when compared to control [13].

The plants treated in the salinity showed relatively low percent of fresh and dry weight in accordance with the results reported on *Setaria italica* [17, 18]. The reduction in growth parameters with increasing salt concentrations is due to the limited supply of metabolites to the growing tissues as the metabolic productivity is significantly reduced at high salt concentration which is either due to low water uptake or toxic effect of salt [30, 21]. High levels of salinization reduced the photosynthetic pigments such as chlorophyll and carotenoid. The results are closely related to the results of [23], where the reduced chlorophyll contents at higher salinity. The carotenoid content of foxtail millet was higher in control compared to the different concentration of NaCl i.e., 25 mM, 50 mM, 75 mM and 100 mM. Increasing application of NaCl decreased the starch contents in the NaCl treated plants in comparison with the control. The results of *Glycine max*, there was an increase in sucrose content and decrease in Starch content [26]. Corresponding results were reported in *Vitis vinifera*, that there was a decrease in sucrose [9]. The *P. sumatranse* seedlings irrigated with 20 mM concentration of NaCl showed the higher protein content compared to control and other concentration. There was an increase in protein concentration with increasing salt concentration in *Vicia faba* [16]. In *P. sumatranse* grown under salinity stress, there was a remarkable increase in the proline content than other amino acids. In Poaceae proline accumulation is one of the common characteristics in many monocotyledons under saline conditions [15]. Proline, which occurs widely in higher plants, accumulates in larger amounts than other amino acids in salt stressed plants. Although in barley seedlings, NaCl stress did not affect proline accumulation [34]. The high salt tolerance of *P. sumatranse* is positively associated with accumulation of Glycinebetaine in whole plant. Glycinebetaine (GB) accumulates in response to salt stress studied in *Sorghum* [32]. The results obtained in *Ceriops roxburghiana*, the glycine betaine content was also increased significantly at all concentrations of NaCl [10]. The activity of hydrogen peroxidase increased significantly in *P. sumatranse* with increasing NaCl of different concentration.

The increase of H_2O_2 activity in treated plants was accompanied by elevated lipid peroxidation as evidenced by the change in Malondialdehyde levels.

Salt stress caused increase in H_2O_2 content of *Setaria italica* leaves, indicating that salt stress could cause damages to the integrity of the cellular membrane and to cellular components that were sensitive to oxidative stress [1]. Salt stress in Soybean (*Glycine max*), the hydrogen peroxide concentration of leaf tissue was significantly increased with increasing salinity [33]. Thus there is a positive correlation between the osmolytes and salt stress.

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