American-Eurasian Journal of Agronomy 2 (2): 109-116, 2009
ISSN 1995-896X

Changes in Metabolites and Antioxidant Enzyme Activity of Three *Vigna* Species Induced by NaCl Stress

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Abstract: Three species of *Vigna* (*V. radiata, V. mungo* and *V. unguiculata*) were subjected to different doses of NaCl *Viz.,* 0 (Control), 50, 75, 100, 125 and 150 mM treatment to analyze the changes of biomolecules such as chlorophyll, reducing sugar, starch, protein, proline and an enzyme activity peroxidase in shoot and root at 15th day seedlings. Germination percentage, seedling growth, relative water content, relative growth rate and photosynthetic pigments were decreased with increasing concentration of NaCl treatment in all species of *Vigna*. While, metabolite solutes such as reducing sugar, starch, protein, proline content and an enzyme peroxidase activity were increased at increasing concentration of NaCl. in both shoot and root compared to respective control. This investigation demonstrated decreasing of growth parameters and phosynthetic pigments were more in green gram rather than black gram and cowpea with respective control due to low osmostic potential at intracellular level generated by NaCl stress. However, increasing of compatible solutes were more in cowpea rather than black gram and green gram that regulate the osmosis and maintain cytoplasm viscosity by conflict to equalize with Na^+ ions due to NaCl salinity. Among three *Vigna* species, *V. radiata* affects more compared to *V. mungo* and *V. unguiculata* regarding to decreasing growth parameters and photosynthetic pigments by NaCl salinity. While, metabolites were increased more in *V. unguiculata* rather than *V. radiata* and *V. radiata* at increasing NaCl treatment that indicated *V. unguiculata* is more tolerant than *V. mungo* and *V. radiata* for NaCl salinity.

Key words: Green gram, Black gram, Cowpea, Photosynthetic pigments, Bio-metabolites, Peroxidase, Salinity

INTRODUCTION

Legumes are considered as the major source of protein and dietary amino acid for man and farm animals [1]. Green gram (*Vigna radiata* L. Wilczek) is an important traditional legume crop the world over. It is of short duration, requires low inputs, yields highly and serves as an excellent source of protein as seed or sprout. Whereas, cowpea (*Vigna unguiculata* (L.) Walp.) is another important legume in tropical and semi-arid regions of the world. Black gram (*Vigna mungo* (L.) Hepper) is an important pulse crop occupying unique position in Indian agriculture. It is under cultivation in India is about 3.25 million hectares and an annual production is 1.45 million tons. Major obstacles to the growth and productivity of widely cultivated green gram, black gram and species of cowpea in arid and semiarid regions are the ever-increasing salinity and solidity of soils and the scarcity of good quality of irrigation water [2-3].

Salinity reduces the ability of plant to take water and this quickly causes reduction in growth rate along with a suite of metabolic changes. Physiological criteria are tissue ionic contents and photosynthetic rate [4-6]. As regard the chlorophyll content of the salinized plant, it is apparent that the chlorophyll content was reduced as a result of increasing salinity [7]. While, biochemical ones include qualitative and quantitative changes in proteins, fats and carbohydrate patterns [8,9]. Besides, salt induced osmotic stress as well as sodium toxicity trigger to the formation of Reactive Oxygen Species (ROS) such super oxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (O_2), which can damage mitochondria and chloroplast by disrupting cellular structure [10]. The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution, nutrient imbalance, specific ion effect or a combination of these factors [11]. In the pragmatic investigation, we carefully studied growth parameters,

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changes of photosynthetic pigments and bio-metabolites such as, reducing sugar, starch, protein, proline and an antioxidant enzyme-peroxidase activity in green gram black gram and cowpea influenced by NaCl stress.

MATERIALS AND METHODS

Collection of Seeds: The seeds of green gram, black gram and cowpea (variety vamban-1) were obtained from Pulse Research Station, Vamban, Pudukottai, TamilNadu, India. The experiments were conducted at the Department of Botany, Arignar Anna Government Arts College (Affiliated to Thiruvalluvar University), Villupuram, TamilNadu, India.

Salinity Treatment: The healthy, homogenous seeds of green gram, black gram and cowpea were subjected to surface sterilization with 0.01mM mercury chloride ($HgCl_2$) for 3 minutes with frequent shaking and then thoroughly washed with deionised water to remove the mercury chloride. The seeds were treated with 0 (control), 50, 75, 100, 125 and 150 mM concentrations of non-iodized sodium chloride (NaCl) immersion for 3 hours. After, the seeds were sown in petriplates in the laboratory and the pot. The pot culture was allowed to natural environment with 3 replications (soil mixture containing red soil, sand and farm yard manure at 1:1:1 ratio and irrigated with respective dose of saline water).

Growth Phase: The germination percentage was calculated at 7^{th} day in petriplates in the laboratory condition. For further study, the plant samples were collected at 15^{th} day from (DAS) pot culture and analyzed the growth parameter, biochemical estimations and an enzyme activity.

Biochemical Analyses: Photosynthetic pigments such as chlorophyll a and b content were estimated (663, 640) according to the method of Arnon [12]. Soluble sugar (reducing) was estimated at 520 nm according to Nelson [13] and pure glucose was used as standard. Starch content was estimated at 630 nm by the method of Hansen and Moller [14] and pure starch used as standard. Protein content was estimated at 640 nm according to the method of Lowry *et al. [15]* using bovine serum albumin as standard. Free proline was estimated at 520 nm according to the method of Bates *et al*. [16] and pure proline was used as standard.

Antioxidant Enzyme Assay: An enzyme peroxidase was estimated at 420 nm by the method of Chance and Maehly [17].

Statistical Analysis: Each treatment was analyzed and experiment was performed with 3 replications from petriplates and pot culture. The results are presented as means and compared and analyzed by one way ANOVA with standard deviation (±).

RESULTS AND DISCUSSION

Germination Percentage: The germination percentage was gradually decreased with regarding to increasing concentrations compared to control under salinity induced by NaCl in all three *Vigna* species at 7^{th} day of the present study. The highest reduction of germination was found at 150 mM treated population in the present study (Fig. 1). The increase in salinity not only decreased the germination but also delayed the germination initiation [18]. The germination percentage was affected more in green gram rather than black gram and cowpea with respective control. Inhibition or delay in germination under saline conditions is due to an osmotic effect [19,20] which limits the uptake of water during seed germination [21] by hindering of membrane or cytosolic enzymes and hormones [22]. It can be hypothesized that the presence of NaCl even at low concentrations, which is penetrating ions could have contributed to a decrease in the internal osmotic potential of germinating structures [23].

Seedling Growth: Growth of the seedling was retarded under salinity stress condition in all three *Vigna* species. Seedling height was significantly retarded in green gram compared to black gram and cowpea with respective control plants at increasing concentration of NaCl at 15^{th} day (Fig. 2). The growth retardation was severely affected at 150 mM NaCl concentration in all three genotypes however the reduction of seedling height was less in cowpea rather than black gram and green gram with respective control at increasing concentration of NaCl (Fig. 2). It is due to salts accumulating in transpiring leaves at the excessive levels, exceeding the ability of the cells to compartmentalize salts in the vacuole. There are two growth phases response to salinity. The first phase of growth reduction is quickly apparent and is due to the salt outside the roots. The growth reduction is presumably regulated by hormonal signals coming from the roots. Then, there is a second phase of growth

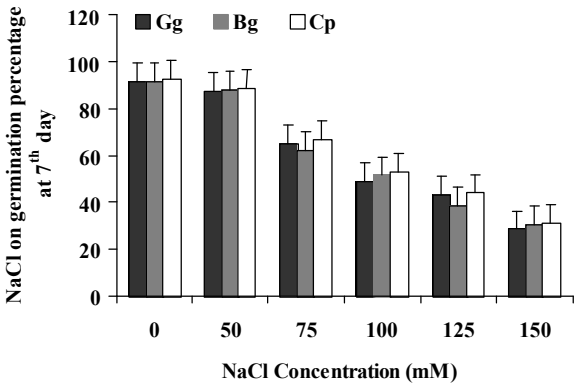

Fig. 1: Effect of NaCl on germination percentage at 7th day

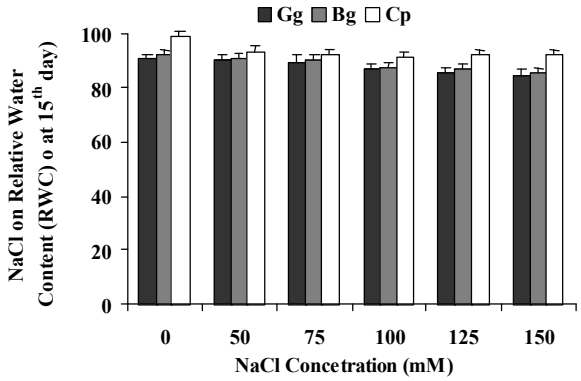

Fig. 3: Effect of NaCl on Relative Water Content (RWC) of seedling at 15at day

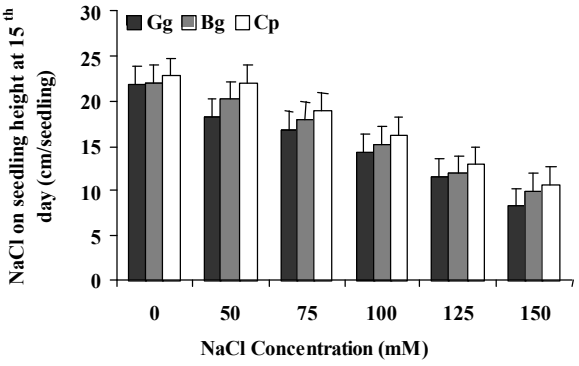

Fig. 2: Effect of NaCl on seedling height at 15th day (cm/seedling)

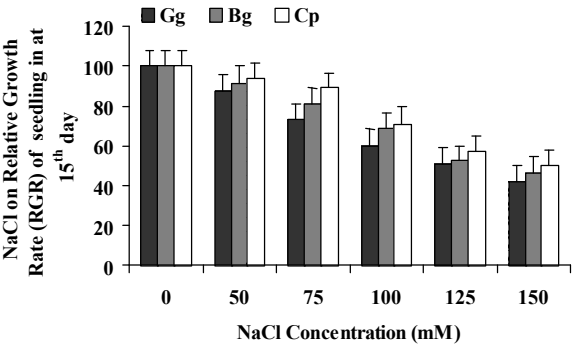

Fig. 4: Effect of NaCl on Relative Growth Rate (RGR) of deedling at 15th day

reduction which takes time to develop and results from internal injury [24]. A disturbance in mineral supply, either an excess or a deficiency, induced by changes in concentrations of specific ions in the growth medium, might directly affected growth [25-27]. This study showed green gram was more sensitive than black gram and cowpea and affected more by NaCl stress and reduce the seedling growth at 15th day. This is agreement with reports showed decrease in growth parameters by NaCl stress [28].

Relative Water Content (RWC) and Relative Growth Rate (RGR): Relative water content and relative growth rate were significantly lowered with increasing concentrations of NaCl compared to control in all three genotypes. Where as, green gram had highest RWC and RGR following black gram and cowpea (Fig. 3, 4). This could be attributed to the competition of Na^+ with the uptake of K^+ resulting in a K^+ /Na^+ antagonism [29]. The present results are in line with findings with NaCl [30,31] regarding to reduction of water content percentage due to increasing NaCl salinity. It is due to deficiency of water supply to the cells by increasing Na^+ ions in cytoplasm compete with K^+ ions led to lowering osmotic potential in cell cytoplasm of all three genotypes.

Photosynthetic Pigments: Salinity drastically affects photosynthesis due to decreasing chlorophyll content and commonly showed adverse effects on membrane stability [32,33]. Salinity reduced the chlorophyll a and b content with increasing NaCl concentrations compared to control of green gram, black gram and cowpea (Fig. 5). Increasing salinity decreased chlorophyll content in plants [34,35]. Salinity caused decreases in phototsynthetic pigment contents and photosystem II electron transport activity in plants [36]. Reduction in pigments content and photosynthetic activity under stress [37]. Reactive oxygen species cause chlorophyll degradation and membrane lipid peroxidation, reducing membrane fluidity and selectivity [38] due to NaCl salinity. The highest reduction of photosynthetic pigments was recorded at 150 mM NaCl concentration in all genotypes

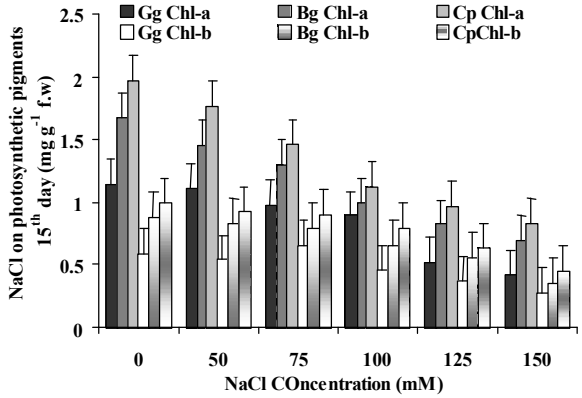

Fig. 5: Effect of NaCl on photosynthetic pigments at 15 th day (mg g-[1] f. Wt)

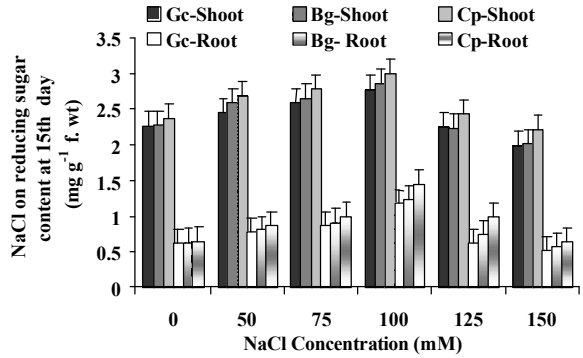

Fig. 6: Effect of NaCl on reducing sugar content at 15th day (mg g-[1] f. Wt)

(Fig. 5) however; the reduction of pigments was more in green gram rather than black gram and cowpea. The reduction of photosynthetic pigment in the present study might have been degradation of chlorophyll by chlorophyllase and reactive oxygen species generated during photorespiration under salinity. Salt induced osmotic stress as well as sodium toxicity trigger to the formation of reactive oxygen species (ROS) such superoxide (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (O^{2-}), which can damage mitochondria and chloroplast by disrupting cellular structure [39]. It is attributed to a salt-induced weakening of protein-pigment-lipid complex and due to the suppression of the specific enzyme which is responsible for synthesis of green pigments [40] or increases chlorophyllase enzyme activity [41].

Reducing Sugar and Starch Content in Shoot and Root: Carbohydrates such as sugars (glucose, fructose, sucrose and fructans) and starch accumulate under salt stress

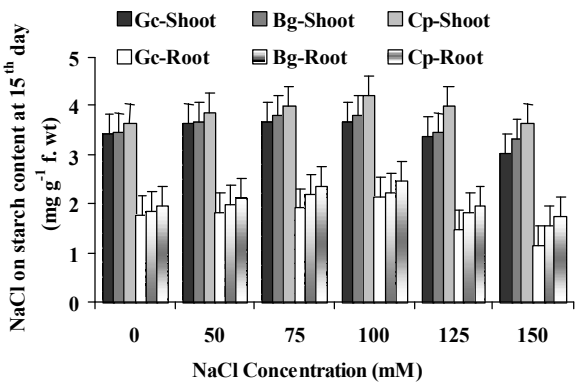

Fig. 7: Effect of NaCl on starch content at 15th day (mg g-[1] f. Wt)

[42], playing a leading role in osmoprotection, osmotic adjustment, carbon storage and radical scavenging. The reducing sugar and starch content were increased in both shoot and root with increasing NaCl treatments compared to control of green gram followed by black gram and cowpea (Fig. 6 and 7). Accumulation of sugar and starch were more in shoot rather than root in all three *Vigna* species. The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity or drought, despite a significant decrease in net CO_2 assimilation rate [43]. The accumulation of sugar and starch was more at 100 mM NaCl concentration in cowpea than black gram and green gram of the present study (Fig. 6 and 7). The accumulation of starch under abiotic stress has been reported previously [44] and it is tempting to speculate that starch synthesis from sucrose play a role in moderating the hyper-osmotic condition. Total soluble carbohydrates increased in salinized plants compared with control [44] that ability for water absorption under salt stress [45]. This indicates the carbohydrate metabolism was altered to adjust the osmosis in shoot and root at increasing concentrations of NaCl however; it was decreased at higher doses such as 125 and 150 mM due to severe salinity.

Protein Content in Shoot and Root: Proteins that accumulate in plants under saline conditions may provide a storage form of nitrogen that is re-utilized later [46] and may play a role in osmotic adjustment. In the present investigation, protein content was increased in shoot and root with increasing NaCl in cowpea followed by black gram and green gram compared with respect control plants. Between shoot and root, former one had more

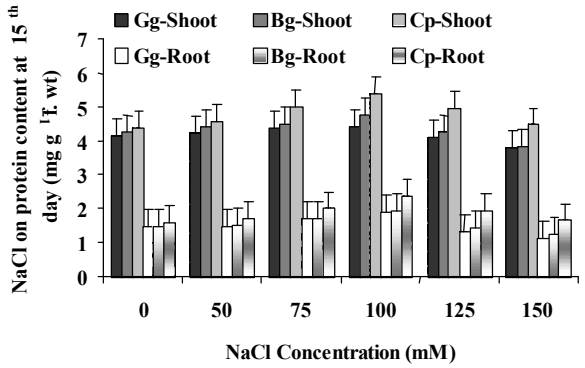

Fig. 8: Effect of NaCl on protein content at 15th day (mg g-1 f. wt)

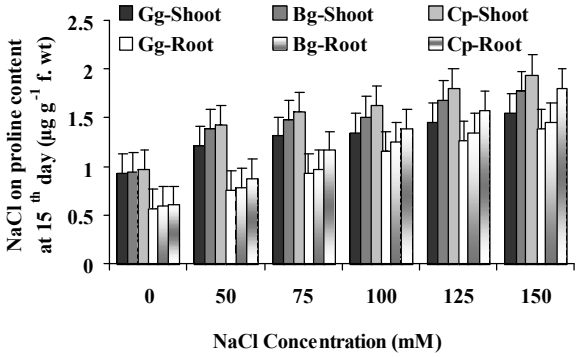

Fig. 9: Effect of NaCl on prolein content at 15th day (µg g-1 f. wt)

protein than later at lower concentrations in all species at lower concetratiions. However, at higher concentration (150 mM), protein content was reduced in all three species due to severe salinity (Fig. 8). A higher content of soluble proteins has been observed in salt tolerant cultivars [47]. They may be synthesized *de novo* in response to salt stress or may be present constitutively at low concentration [48]. The synthesis of protein was more at 125 mM induced by NaCl. Comparatively, cowpea accumulated more content of protein than black gram and green gram with respective control. It has been concluded that a number of proteins induced by salinity are cytoplasmic which can cause alterations in cytoplasmic viscosity of the cells [34].

Proline Content in Shoot and Root: Accumulation of free proline under abiotic stress has been reported for several plants [36-38]. Proline is frequently involved in osmotic protection in higher plants and has been reported to be associated with salt tolerance. Proline content was increased with increasing NaCl treatments even at higher

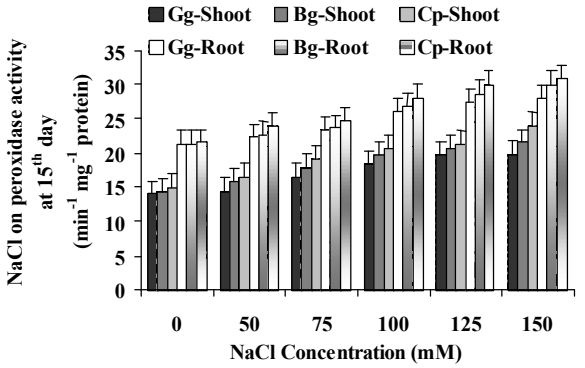

Fig. 10: Effect of NaCl on peroxidase activity at 15th day (min-1 mg-1 protein)

concentration (150mM) compared to control cowpea, black gram and green gram in the study. Accumulation of proline was more in shoot rather than root however; significant increase of accumulation was more in root compared to shoot (Fig. 9) in all species. A considerable increase was observed in proline levels during germination period in the seedling of *Phaseolus vulgaris* subjected to NaCl treatment. Accumulation of proline was more in root than shoot due to roots directly contact with NaCl impregnated soil sphere. Increased amount of proline is considered to be an indication of tolerance to salt stress because proline is thought to function either as an osmoregulator and a protector of certain enzymes [41-44].Among three *Vigna* species, proline was accumulated more in cowpea than black gram and green gram in NaCl concentrations of this study (Fig. 9).

Peroxidase Activity in Shoot and Root: The present study revealed peroxidase activity was increased in shoot and root with increasing NaCl concentrations in cowpea, black gram and green gram compared to respective control. The peroxidase activity was more in root than shoot (Fig. 10). The consistent increase in peroxidase activity was in shoots even during severe salt-stress and its differential regulation for stress tolerance than catalase [28-32]. Differences in protective enzyme activities are known for a number of species. A large number of studies on various species indicated that salt stress alters the amount and the activities of the enzymes involved in ROS scavenging [36-39]. This is probably dictated by the wide range of metabolic processes in which peroxidases are known to be involved such as lignin biosynthesis and formation of isodityrosine bridges that are believed to crosslink structural protein molecules, in addition to antioxidative

activity. Among three *Vigna* species, peroxidase activity was more in cowpea rather than black gram and green gram at increasing NaCl concentration. In tolerant plant species, POX activity was found to be higher, providing protection against the oxidative stress. Increase of POX activity was reported in fox-tail millet. In the present investigation, increased peroxidase activity might have been scavenged reactive oxygen species generated by photorespiration and â-oxidation of fatty acid under NaCl salinity was more in cowpea rather than black gram and green gram

CONCLUSION

In this pragmatic investigation, salinity decreased seedling growth and photosynthetic pigments at 15th day due to decreasing osmotic potential level in root and shoot led to decreasing photosynthesis in all three *Vigna* species. However, physiological system regulate the osmotic potential by increasing synthesis of reducing sugar, starch, protein at 100 and 125 mM NaCl of all *Vigna* species, higher dose 150 mM showed decreasing trend of these biomolecules due to acute salinity of NaCl. In mean while, osmoregulator proline was increased even at higher doses and regulate osmosis and cytoplasm viscosity. Because, proline may contribute to osmotic adjustment at the cellular level, may acts as an enzyme protectant and stabilizing the structure of macro-molecules and also acts as a major reservoir of energy and nitrogen for utilization upon exposure to salinity. An antioxidant enzyme enhanced its activity with increasing concentrations of NaCl on *Vigna* species, indicates that peroxidase scavenged the reactive oxygen species generated due to photorespiration and â-oxidation of fatty acid under NaCl salinity. Among three *Vigna* species, increase of biomolecules and peroxidase activity were more in cowpea compared to black gram and green gram. Hence from this investigation, it infers that cowpea was more tolerant than black gram and green gram and strives to modulate its physiological function to become accustomed in NaCl salinity condition rather than black gram and green gram respectively.

ACKNOWLEDGEMENT

Authors are expressing our gratitude to the Head of the Department of Botany, Arignar Anna Govt. Arts College, Villupuram and the authorities of Thiruvalluvar University, TN, India for their support to this research work.

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