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Effect of short term salinity stress induced physiological, biochemical and histochemical changes of Green gram (*Vigna radiata* L.) roots

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Abstract
Soli salinity is one of the most severe limiting factor growth and germination. In the present study physiological, biochemical, root histochemical and antioxidant variables of salinity stress of green gram (*Vigna radiata*) under different concentration of NaCl (Control, 10, 25, 50, 75 and 100 mM). The results show that, the salinity stress significantly reduced the plant growth characteristics for example, shoot and root length, fresh and dry weight, chlorophyll 'a', 'b' and total chlorophyll content. Whereas, the free proline, reducing sugar, starch, protein, amino acid content, and catalase and peroxidase activities were increased while increasing concentration of NaCl. The level of root ROS accumulation always increased. Overall, these results strongly suggested that the increased concentration of NaCl reduced the plant growth and expressively affects the physiological, biochemical and antioxidant metabolism.

Keywords: salinity, seed germination, histochemical, biochemical and antioxidant

Introduction

Environmental stress, soli salinity is one of the most Severer factor for limiting plant growth, seed germination, plant development and yield [1]. In next 25 years, the increase of salinity or alkalinity in arable land is become predictable land loss around global level [2]. The saline pressure influences practically 10% of arable land and 24% of inundated land regions all through the world [3]. The saltiness instigated decrease in crops are basically because of various physiological and biochemical dysfunctions in plants that developed under such pressure [4]. Last few decades, the researchers are focusing on overcome the problem of salinity by employing a variety of strategies. There are various strategies to improve salt tolerant trait in crops by inducing chemical mutagens such as plant hormone, membrane stability and trace mineral nutrients seems to be an efficient, economical and a shot-gun approach.

The use of such chemicals resulted a generous increment in both development and yield of numerous harvests under saline conditions [5]. Salinity stress affects the photosynthetic pigment and inducing oxidative stress, which affect the thylakoid membrane and decreases the photosynthetic capacity in plant [6].

Green gram is a significant traditional legume crop of the world over and high nutritive value. *Vigna radiata* is one of the short duration and self-pollinated pulse crop which are edible and rich protein seeds. However, *Vigna radiata* is salt sensitive crop and salt stress reduced plant growth rate and biomass [7].

Reproducing of salt-tolerant genotypes can develop more productively than the ordinary assortments under saline pressure, Hence, the present investigation was subjected to induce various dose of NaCl in green gram to study the seedling growth, physiological, biochemical constituents and an antioxidant system.

Materials and method

Experimental design

The green gram seeds Vamban-3 was purchased from TNAU (Tamil Nadu Agricultural University, Coimbatore, India. The homogenous seeds were exposed to surface Disinfection with 0.1g Mercury Chloride ($HgCl_2$) for 3 min with fiery shaking and washed with deionized water. The seeds were placed in Petri plates and treated with different (10, 25, 50, 75, and 100mM) concentration of NaCl.

Seed germination

The seed Germination tries were done by uniformly circulating the seeds in a 9-cm Petri plate with two layers of filter paper, fifty seeds for each plate. Deferent focus NaCl were persistently treated with the Green gram seeds at seven-day, after seventh day count the number of germinated seeds. The germination parameters were measured permitting to the International Seed Testing Association rules (ISTA).

$$\text{Germination percentage is } G\% = \frac{n}{N} \times 100$$

Where "n" is the number of germinated seeds and "N" is the total number of total sown seeds.

The salinity tolerant index (STI) calculated for G% according to the equation:

$$\text{STI (G\%)} = \frac{\text{G\% under NaCl treatment}}{\text{G\% under control}} \times 100$$

Growth characteristics Plant height (cm)
The plant height was calculated on 7th day in control (untreated plants) and NaCl treated plants. Five plants were taken to measure the mean value for all the treated concentrations (10, 25, 50, 75 and 100 mM) and control.

Fresh and dry weight (g)
Seedlings were collected from triplicates of seedling height, shoot and root lengths were recorded. Ten seedlings of each replicates were taken from respective concentrations with control and the fresh weight (FW) was weighed then dried in an oven at 60ºC until relentless dry weight (DW) was obtained.

Determination of relative water content (%)
Collect the all treated and control seedling and separate the (shoot and root independently) and dried at 70°C for 48 h. The dry loads and water substance (%) were resolved. The relative water content (RWC) was determined by following equation.

$$RWC(\%) = \frac{FW - DW}{DW} \times 100$$

Estimation of photosynthetic pigments
Chlorophyll 'a' 'b' substance were assessed by technique for [8] and carotenoids content was controlled by the strategy for [9]. The chlorophyll-a, chlorophyll-b and carotenoid substance were estimated at 663, 645 and 480 nm.

Biochemical analysis
The seven days old salinity treated and control and treated seedling were collected.
The sample were limitedly frozen in liquid nitrogen and stored at -80°C for used in further analysis.
The five hundred mg of sample were taken and estimate the reducing sugar following method of [10] using glucose as standard. Starch content was assessed by Clegg [11]. Protein was evaluated by following the technique depicted by [12] the BSA as standard. The free amino corrosive was evaluated by [13] glycine as standard. Proline was assessed by Sulphosalicylic acid method by [14].

Enzymatic antioxidant activity
Taken five hundred mg of frieze tissue was ground with 5ml ice-cold 50mM phosphate buffer (pH 7.0). the CAT activity was measured following by the method of [15]. Peroxidase activity was evaluated by the technique for [16].

Histochemical detection of cell death
Cell death assay was far-famed by the following method of [17]. The different concentration of NaCl treated 3 days old green gram seedling root tips were taken and stained with 0.5% of Evans Blue. After 15 min staining the root tips were washed several times with tap water blue cooler stained accumulated tips were identified and immediately tack photograph with help of Nikon digital camera.

Histochemical detection of H_2O_2 accumulation
The accumulation of H_2O_2 was detected by the following method of [18]. The different concentration of NaCl treated green gram seedling root tip were immersed with 0.5 mM DAB staining solution pH 4 for 30 min after staining the reaction was stopped seedling were washed with distil water, H_2O_2 accumulated green gram root tips were identified and immediately tack photograph with help of Nikon digital camera.

Statistical analysis
Every treatment was examined with three replications in Petriplate culture. The replicates variances between the NaCl treatments were analyzed by one-way ANOVA, consequence of variances between means were determined at $p \leq 0.05$ according to Tukeys HSD post hoc multiple range test by using SPSS software 16.1 ver.

Results and Discussion
Physiological and biochemical effects
Effect of NaCl on seed germination
Under saline condition the green gram seed germination efficiency and germination percentage was decreased with increasing NaCl concentration, compare to control Fig 1. The seedling germination was decreased at the highest concentration of NaCl treated plant in 100mM concentration compere to control (100mM 45 and control 97%). The results strongly confirm that adverse negative effects on seed germination under 100mM of NaCl level. Similarly, [19] and [20], informed that increasing salt concentration reduced the cowpea seed germination.

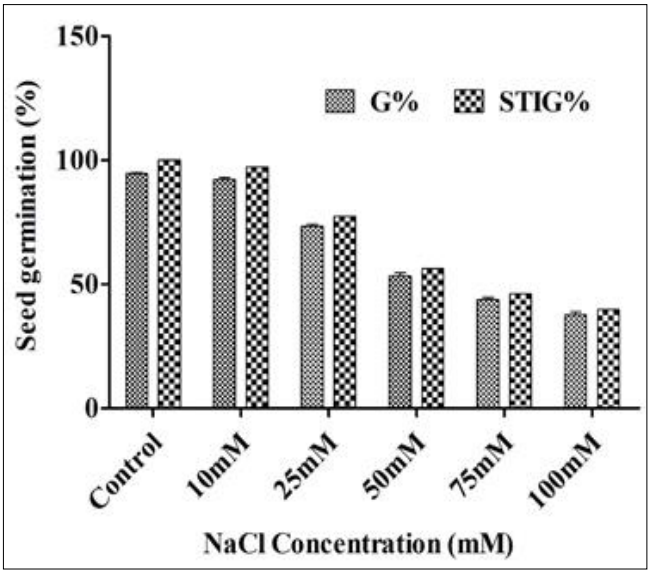

Fig 1: Effect of different NaCl concentration on green gram seed germination percentage (G%) and salt tolerant index (STIG %).

Effect of NaCl on shoot and root length of green gram at 7th day (cm / seedling)
The impact of NaCl on stem and root development was gradually decreased at increasing NaCl concentration compared to control at 7th day in the concentration (1.4 and 2.4 cm) 100 mM NaCl treated Fig 2a. when compared with control. The decreased length in root and shoot might be due to NaCl which affects the permeability in the plasma membrane and increases the influx of external ions and efflux of cytosolic solutes in plant cells. On the other hand, NaCl causes hardening of cell wall [21], which decreases in water conductance of the plasma lemma using reduction in plant height [22].

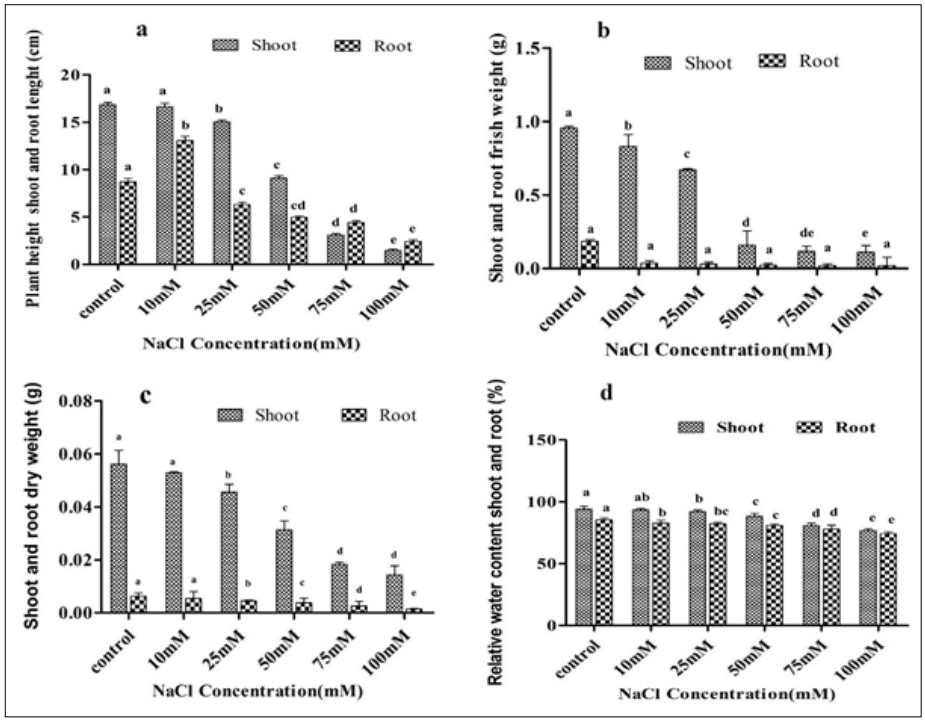

Fig 2: Effect of different NaCl concentration on green gram of 7th day seedlings (a) Plant height shoot and root length (b) shoot and root fresh weight (c) shoot and root dry weight (d) relative water content. Values are given as mean ± SEM of three replicates. Within each graph mean followed by same letter is not significant different, varies latter significant different $p< 0.05$.

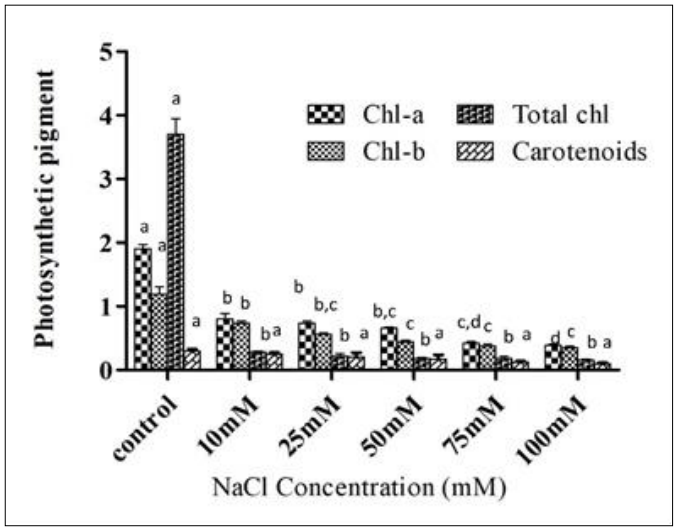

Fig 3: Effect of NaCl different concentration on photosynthetic pigment chlorophyll a, b, total chlorophyll and carotenoid content at 7th days seedling. Values are given as mean ± SEM of three replicates within each graph mean followed by same letter is not significant different, varies latter significant different $p <$

Effect of NaCl on fresh and dry weight in green gram at 7th day (g)

The effect of NaCl showed gradually decreased fresh and dry weight of root and shoot, respectively with increasing concentration when compared to control Fig 2b and c. The maximum reduction was recorded in 100 mM NaCl (FW- 0.111 and 0.019; DW- 0.014 and 0.002g) treated shoot and root compared to control (FW- 0.955 and 0.184; DW- 0.056 and 0.006g).

The present result depicts that the impact of osmotic pressure, leads hurtful impacts to plants during the succulent seedling state and the higher iron uptake Similarly, the present outcome shows that the root, shoot, and leaf length of NaCl-treated seedlings decreased with the increasing NaCl concentration in excess of control [23].

Relative Water content and relative growth rate (%)

The effect of NaCl on relative water content and growth rate were decreased at 7th day with an increasing concentration when compared to the control Fig 2d and Fig 4a. The highest reduction was recorded in 100mM NaCl treated shoot and root (70.66 and 74.20 percent) which could be accredited to the competition of Na^+ with uptake of K^+ resulting in a K^+/Na^+ antagonism. This might happen by the deficiency of supply to the cells by increasing Na ions in cytoplasm with K^+ ions lower the osmotic potential in cell cytoplasm as evidenced in earlier report [24].

Photosynthetic pigments

The effect of NaCl on Chlorophyll a & b, total Chlorophyll and carotenoids of green gram at 7th day (mg/g fr.wt). The

effect of NaCl on Chlorophyll a, b and a/b ratio, carotenoids in 7th day showed decreased content, respectively with increasing concentrations when compared to control (1.192, 0.112, and 3.703, 0.299 mg/g fr.wt) Fig 3. The maximum reduction was recorded in 100mM NaCl (0.39, 0,294 and 0.150, 0.097 mg/g fr.wt). Likewise, the salinity also induces waning of protein pigment-lipids complex by the concealment of the particular catalyst that is liable for amalgamation of green colors [25]. Similar report was recorded in decrease of total chlorophyll and carotenoid content in *Vigna unguiculata* due to effect of sodium chloride [26].

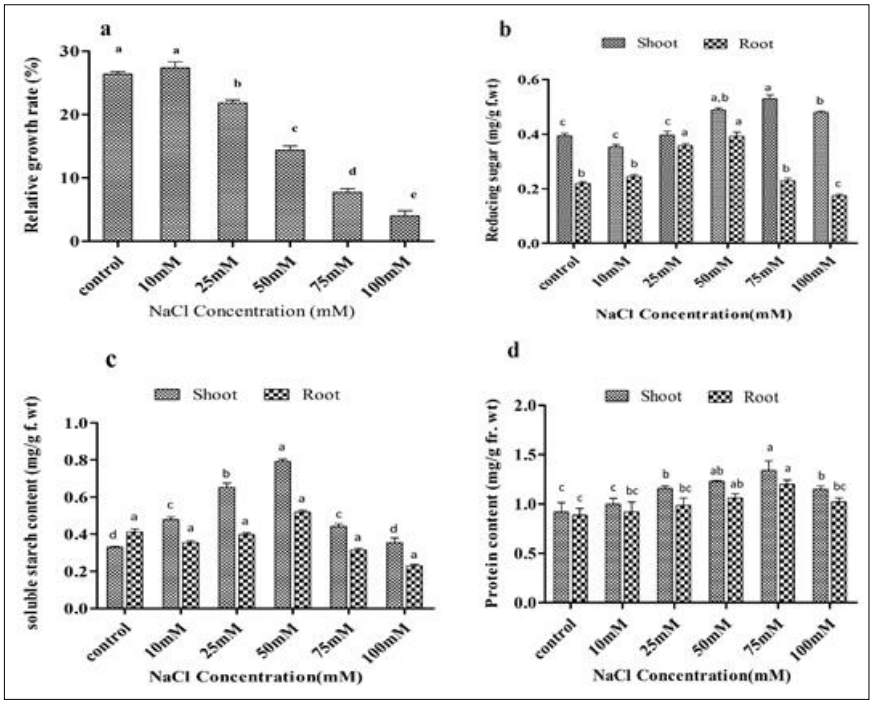

Fig 4: Effect of NaCl different concentration on (a) relative growth rate (b) shoot and root reducing sugar (c) shoot and root soluble starch (d) protein content in shoot and root at 7th Days seedling. Values are given as mean ± SEM of three replicates. Within each graph mean followed by same letter is not significant different, varies latter significant different $p < 0.05$.

Effects of NaCl on reducing sugar content of green gram at 7th day (mg/g fr.wt)

The impact of NaCl on lessening sugar in Seventh day demonstrated an expansion with expanding fixation, when contrasted with control.

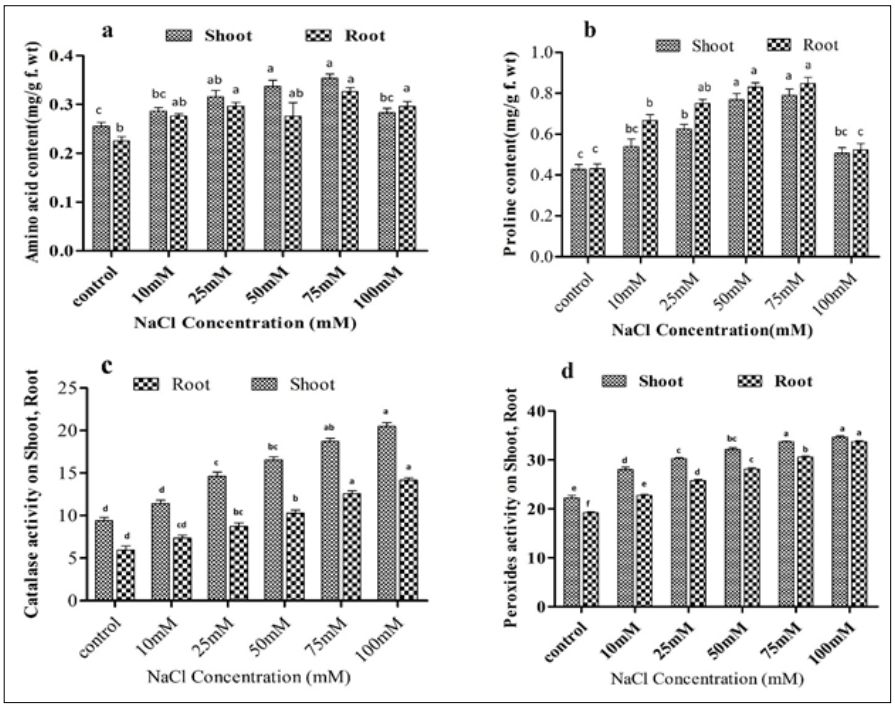

Fig 5: Effect of NaCl different concentration on (a) amino acid content (b) proline content (c) antioxidant enzyme catalase (d) peroxides activity of 7th days seedling. Values are given as mean ± SEM of three replicates. Within each graph mean followed by same letter is not significant different, varies latter significant different $p < 0.05$.

Fig 4b. The most extreme paces of increment in the lessening sugar were recorded in 75mM NaCl of shoot and root (0.529 and 0.229 mg/g fr.wt) and contrasted with control (0.393 and 0.218 mg/g fr.wt). The amassing of dissolvable starches in plants has been accounted for as a reaction to saltiness or dry season worry in sense, the starches, for example, sugars and starch were aggregating under salt pressure [27].

Starch content of green gram at 7th day (mg/g fr. wt)

The impact of NaCl on starch content at seventh day indicated an expansion with expanding focus when contrasted with control.

.Fig 4c. The greatest substance of starch was recorded in 50mM NaCl treated seedling shoots and roots (0.79 and 0.59 mg/g fr.wt) contrast with control (0.329 and 0.413 mg/g fr.wt). Comparative reports have discovered that the absolute solvent sugars get expanded in salinized plants when contrasted with control *Vigna ungiculata* [26].

Protein content of green gram at 7th day (mg/g fr. wt)

The impact of NaCl focus expanded the protein content at seventh day with expanding fixation when contrasted with control Fig 4d. The most extreme protein substance of shoot and root was recorded in 75mM NaCl (1.34 and 1.19 mg/g fr.wt) focus contrast with control (0.919 and 0.885 mg/g fr.wt). Normally, the proteins which amass in plants under saline conditions may give a capacity type of nitrogen that reutilized later [28], was prove in the present investigation.

Amino acid content of green gram at 7th day (mg/g fr.wt)

The impact of NaCl on amino corrosive substance at seventh day indicated an expansion with expanding focus when contrasted with control Fig 5a. The greatest pace of increment in the amino corrosive substance was noted in 75mM NaCl (0.353 and 0.326 mg/g fr.wt) focus contrast with control (0.255 and 0.225 mg/g fr.wt). In a few plants, the amino corrosive digestion assumes a crucial job in the reaction to salt pressure, the amino acids could ensure cell macromolecules, keeps up the osmotic adjust and furthermore free radicals searching under salt pressure conditions. Affecting of amino corrosive digestion may be an essential methodology of improving salt resistance to grain crops [29].

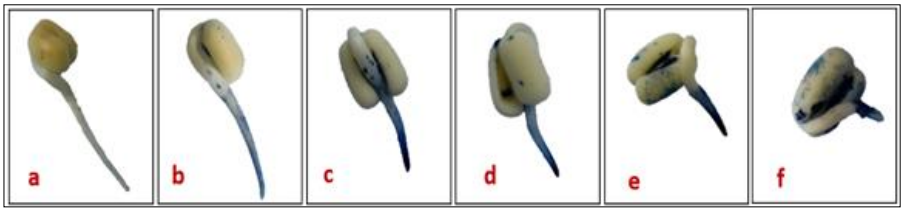

Fig 6: Effect of NaCl different concentration induced cell death Evuans blue (a) Control (b) 10 mM NaCl (c) 25 mM NaCl (d) 50 mM NaCl (e) 75 mM NaCl (f) 100 mM NaCl, activity of 3 days seedling.

Proline content of green gram at 7th day (mg/g fr.wt)

The proline content at 7th day was increase with increasing the NaCl concentrating when compared to control Fig 5b. Maximum amount of proline content of shoot and root was recorded at 75mM NaCl treated when compared to control (0.789 and 0.847 mg/g fr.wt). Minimum amount of proline accumulation noted in control shoots and roots (0.427 and 0.430 mg/g fr.wt). Proline accumulation is an important factor which has been evolved to stabilize the growth under drought or salinity stress in many plant species. The role of proline as discussed earlier, could evident the present report which protects the subcellular structures and mediating osmotic adjustment in stressed condition [29].

Antioxidant enzymes

Effect of NaCl on catalase activity of green gram at 7th day (mg/g fr.wt)

Salt treatments from (10 – 100 mM) NaCl induced significantly increased catalase activity in increasing concentration of green gram at 7th day Fig 5c. The higher catalase activity of shoot and root was recorded under salt stress at the concentration of 100mM NaCl. The lower level of catalase activity content in shoots and roots were recorded in control plants after 7th days (9.38 and 5.93 mg/g fr.wt). Higher activity of CAT would decrease the H_2O_2 level in cell and increase the immovability of membranes as well as the CO_2 fixation where several enzymes of the calvin cycle within chloroplasts are very sensitive to H_2O_2. Under salt stress condition the catalase (CAT) activity at 19% wheat seedlings were increased [30].

Peroxidase activity of green gram at 7th day (mg/g fr.wt)

Treatment with NaCl (10–100 mM) in green gram shoot and roots exhibited a significant increase in Peroxidase activity when compared to control. A higher peroxidase activity in shoots and roots compared to control was observed after 7th days at 100 mM NaCl (34.65 and 33.67 mg/g fr.wt) Fig 5d. Minimum Peroxidase activity contents in shoots and roots were recorded in control plant after 7th days of treatment (22.24 and 19.21 mg/g fr.wt). The salt stress significant increase in the peroxidase activity was reported by several researchers in *Vigna radiata* [31].

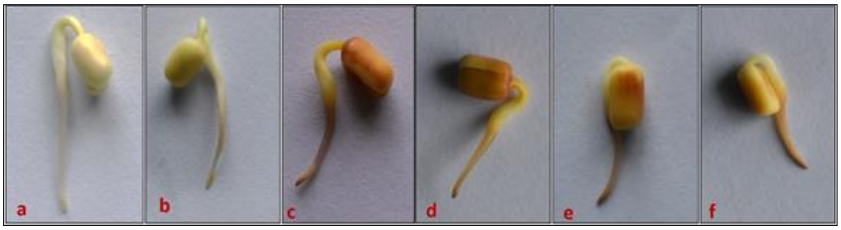

Fig 7: Effect of NaCl different concentration induced ROS accumulation H_2O_2 detection of DAB staining (a) Control (b) 10 mM NaCl (c) 25 mM NaCl (d) 50 mM NaCl (e) 75 mM NaCl (f) 100 mM NaCl, activity of 3 days seedling.

Histochemical assay cell death and H_2O_2 accumulation

Histochemcial cell passing affirmed the loss of layer trustworthiness in plasma film. Evan's blue test is notable to check the honesty of the plasma layer. It can just enter through a crumbled plasma film brought about by various pressure fixation. Control roots didn't show Evan's blue take-up while fixation Nacl treated root tips progressive expanded take-up of Evan's blue stain and in light of an expansion in NaCl focus, an expanded power of the blue shading was likewise watched (Fig.6).

Treatment with NaCl invigorates the endogenous H_2O_2 collection level. While expanding the NaCl fixation it expanded the degree of H_2O_2 the elevated level of H_2O_2 expanded at100mM NaCl focus (Fig. 7). In their investigation, [32] revealed that mung bean plant aggregates that more elevated level of ROS including H_2O_2.

Conclusion

The growth characteristics such as seedling growth at 7th day and water content were significantly decreased with increasing concentration of NaCl treated seedlings. The reduction of seed germination seedling growth and water content were significantly reduced in 100 mM concentration of NaCl. Chlorophyll a, b and total chlorophyll and carotenoids content were showed the decreasing of pigment content with increasing concentration of NaCl treatment due to degradation of pigment by salinity. Pigments were significantly reduced at 75mM concentration of NaCl. Biochemical metabolites such as reducing sugar and starch were increased with increasing concentration use of, not under NaCl delete due to adjust the osmosis in the cellular environment. The reducing sugar and starch content were significantly increased at 75mM concentration of NaCl. The present study elucidates green gram was tolerated under NaCl salinity up to 50mM concentration by altering the metabolism to detoxify the harmful molecules. It confirmed the High level of NaCl concentration increased ROS level and induced membrane damage of root cell.

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Conflicts of interest

No conflicts of interest were reported by the authors.

References

1. Jaleel CA, Gopi R, Manivannan P, Panneerselvam R. Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress. Turkish Journal of Botany,2007:31(3):245-51.
2. Wang W, Vinocur B, Altman A. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta,2003:218(1):1-4.
3. Pessarakli M, Szabolcs I. Soil salinity and sodicity as particular plant/crop stress factors. InHandbook of Plant and Crop Stress, Fourth Edition,2019:6:3-21. CRC press.
4. Munns R, Tester M. Mechanisms of salinity tolerance. Annual Review of Plant Biology,2008:2(59):651-81.
5. Kaya C, Tuna AL, Okant AM. Effect of foliar applied kinetin and indole acetic acid on maize plants grown under saline conditions. Turkish Journal of Agriculture and Forestry,2010:34(6):529-38.
6. Radhakrishnan R, Baek KH. Physiological and biochemical perspectives of non-salt tolerant plants during bacterial interaction against soil salinity. Plant physiology and biochemistry,2017:1(116):116-26.
7. Ghosh S, Mitra S, Paul A. Physiochemical studies of sodium chloride on mung bean (*Vigna radiata* L. Wilczek) and its possible recovery with spermine and gibberellic acid. The Scientific World Journal, 2015, 1-8.
8. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology,1949:24(1):1.
9. Kirk JT, Allen RL. Dependence of chloroplast pigment synthesis on protein synthesis: effect of actidione. Biochemical and Biophysical Research Communications,1965:21;21(6):523-30.
10. Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. J. biol. Chem,1944:153(2):375-80.
11. Clegg KM. The application of the anthrone reagent to the estimation of starch in cereals. Journal of the Science of Food and Agriculture,1956:7(1):40-4.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry,1951:193:265-75.
13. Moore S, Stein WH. Photometric ninhydrin method for use in the chromatography of amino acids Journal of Biological Chemistry,1948:176:367-88.
14. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil,1973:39:205-207.
15. Chandlee JM, Scandalios JG. Regulation of Cat1 gene expression in the scutellum of maize during early sporophytic development. Proceedings of the Natl Acad Sci Lett,1984:81:4903-4907.
16. Chance B, Maehly AC. [136] Assay of catalases and peroxidases,1995:764-775.
17. Panda KK, Achary VMM, Phaomie G, Sahu HK, Parinandi NL, Panda BB. Polyvinyl polypyrrolidone attenuates genotoxicity of silver nanoparticles synthesized via green route, tested in *Lathyrus sativus* L. root bioassay. Mutat Res-Gen Tox En,2016:1(806):11-23.
18. Singh KL, Chaudhuri A, Kar RK. Superoxide and its metabolism during germination and axis growth of *Vigna radiata* (L.) Wilczek seeds. Plant Signalling and Behaviour,2014:9(8):29278.
19. Thiam M, Champion A, Diouf D, Oureye SYM. NaCl effects on in vitro germination and growth of some senegalese cowpea (*Vigna unguiculata* (L.) Walp.) cultivars. ISRN biotech, 2013.
20. Zahedi SM, Ansari NA, Azizi M. The study of the effect of salinity stress on the germination and the initial growth of cowpea (*Vigna unguiculata* L. Walp). Journal of Agricultural Technology,2012:8:2353-2372.
21. Nabil M, Coudret A. Effects of sodium chloride on growth, tissue elasticity and solute adjustment in two *Acacia nilotica* subspecies. Physiologia Plantarum,1995:93:217-24.

22. Cramer GR. Kinetics of maize leaf elongation: II. Responses of a Na-excluding cultivar and a Na-including cultivar to varying Na/Ca salinities. Journal of Experimental Botany,1992:43:857-64.
23. Dumbroff EB, Cooper AW. Effects of salt stress applied in balanced nutrient solutions at several stages during growth of tomato. Bot Gaz,1974:135:219-24.
24. Arulbalachandran D, Sankar Ganesh K, Subramani A. Changes in metabolites and antioxidant enzyme activity of three *Vigna* species induced by NaCl stress. American-Eurasian Journal of Agronomy,2009:2:109-16.
25. Strogonov BP, Kabanov VV, Lapina LP, Prykhodko LS. Structure and function of plant cells under salinity conditions. Nauka Publishing House Moscow, 1970.
26. Taffouo VD, Kouamou JK, Ngalangue LM, Ndjeudji BA, Akoa A. Effects of salinity stress on growth, ions partitioning and yield of some cowpea (*Vigna unguiculata* L. Walp.) cultivars. International Journal of Botany,2009:5:135-43.
27. Parida A, Das AB, Das P. NaCl stress causes changes in photosynthetic pigments, proteins, and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. J Plant Biol,2002:45:28-36.
28. Huang WL, Liu LF. Carbohydrate metabolism in rice during callus induction and shoot regeneration induced by osmotic stress. Botanical Bulletin of Acad Sin,2002:43:107-113.
29. Yasar F, Ellialtioglu S, Yildiz K. Effect of salt stress on antioxidant defense systems, lipid peroxidation, and chlorophyll content in green bean. Russian Journal of Plant Physiology,2008:55:869-873.
30. Zhang S, Gan Y, Xu B. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. Frontiers in Plant Science,2016:7:1405.
31. Jisha KC, Puthur JT. Halopriming of seeds imparts tolerance to NaCl and PEG induced stress in *Vigna radiata* (L.) Wilczek varieties. Physiology and Molecular Biology of Plants,2014:20:303-12.
32. Nahar K, Hasanuzzaman M, Rahman A, Alam M, Mahmud JA, Suzuki T, Fujita M. Polyamines confer salt tolerance in mung bean (*Vigna radiata* L.) by reducing sodium uptake, improving nutrient homeostasis, antioxidant defense, and methylglyoxal detoxification systems. Frontiers in Plant Science,2016:7:1104.