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Studies on effect of salt stress on growth and biochemical compounds of *Arachis hypogea* l

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Abstract
The effect of varying levels of salinity on germination, seedling growth, and some metabolic parameters *viz*., total soluble proteins, free amino acids and nitrate reductase activity in *Arachis hypogea* L. was studied. Seeds of *Arachis hypogea* tolerated salinity up to 100mM during germination. Root elongation remains unaffected and tolerated all the concentrations of NaCl tested while the shoot elongation was reduced by salinity. Chlorophyll and carotenoid content declined with increasing concentration of NaCl tested. Total soluble proteins accumulated due to salinity whereas total free amino acid content decreased. Nitrate reductase activity although declined, in general, about 60% activity was left at the highest concentration (100mM) of NaCl used.

Keywords: *Arachis hypogea* l., biochemical compounds

Introduction
A plant faces two problems due to salt stress, one of obtaining water from a soil of negative osmotic potential and another of dealing with the high concentrations of potentially toxic sodium, carbonate and chloride ions (Salisbury and Ross, 1992). The effect of high concentrations of total salt in the soil is referred to as salinity.
Salinity is known to reduce the growth of glycophytes (salt-sensitive species). This reduction in growth may result from salt effects on dry matter allocation, ion relations, water status, physiological processes, biochemical reactions or a combination of such factors (Seemann and Critchley, 1985) Salinity and sodicity are the major factors restricting the economic and efficient utilization of available and resources and adversely affect the crop productivity. Under natural conditions, terrestrial higher plants encounter high concentrations of salts. An extensive problem in agriculture is the accumulation of salts from irrigation water. Millions of acres have gone out of production as salt from irrigation water accumulates in the soil. Evaporation and transpiration remove pure water from the soil, and this water loss concentrates solute in the soil. The poor quality of irrigation water (that is water containing more of salts) and no opportunity to flush out accumulated salts to a drainage system results in salt injuries to sensitive species (Taiz and Zeiger, 1991).Pea nut (Arachis hypogea L.,) is an important leguminous crop, cultivated extensively in Tamil Nadu. The aim of the present investigation is to study the changes in growth, protein, amino acid and nitrate reductase activity under NaCl salinity.

Materials and Methods
Plant Material
Seeds of Ground nut (Arachis hypogea L.) obtained from local seed store,Tiruchirappalli-20 for the present investigation were surface sterilized with 0.1% HgCl2 solution(w/v) for one minute and then thoroughly washed with tap water and rinsed with distilled water.

Acid washing of sand
Carefully mixed enough volume of concentrated sulfuric acid with dried sand in a plastic container using a glass rod and retained as such overnight. Washed the sand thoroughly with sample volume of tap water, rinsed with distilled water, air dried and stored in a clean container next day for further use.

Seed germination
Soaked the surface sterilized seeds for three hours in distilled water and of equal number transferred over washed sand in polythene bags.

Seedling development
Raised the seedlings under cool, white fluorescent light of 1500Lux in Jamal Mohamed College, Botany Department Laboratory for seven days.

Seedling treatment
Exposed the seedlings to NaCl of various milliMolar concentrations *viz*. 5, 10, 25,50 and 100 which also contained the Hoagland nutrient solution, the controls received only Hoagland solution. On 8th day seedling were analyzed for germination, shoot and root elongation, total soluble proteins, and free amino acids, and Nitrate reductase activity.

Seedling Analysis
The seedlings were analyzed for the following parameters on the 8th of treatment.
Percentage of Seed Germination. The shoot emergence was considered as seed germination.

Shoot and Root Elongation
The seedlings were uprooted carefully without any damage and the shoot and root length was measured using a scale.

Seedling fresh weight and dry weight
Fresh weight of the seedlings was measured in an electronic balance immediately after uprooting the seedlings.

Seedlings were them dried in a hot air oven at 60oC until a constant weight was reached.

Chlorophyll

Homogenized 250mg of fresh leaf segments thoroughly using pestle and mortar adding glass powder. Extracted the chlorophyll adding 4ml of 80% acetone to the homogenate. centrifuged the homogenate at 5000rpm for 5min. transferred the supernatant chlorophyll to another clean test tube. extracted the pellet again with 4ml of 80% acetone as above. Ensured the complete extraction by adding 2ml of the solvent to the pellet and repeated the process. Pooled all the supernatants together in the same tube and made the volume up to 10ml with the solvent. Read the absorbance at 645 and 663nmin the spectrophotometer against acetone blank. Calculated the amount of chlorophyll a, b and total chlorophyll (Arnon, 1949)

Carotenoids

Homogenized 200 mg of fresh leaf segments thoroughly using pestle and mortar adding glass powder. extracted the chlorophyll adding 4ml of 80% acetone to the homogenate. centrifuged the homogenate at 5000rpm for 5min.transferred the homogenate chlorophyll to another clean test tube. extracted the pellet again with 4ml of 80% acetone as above. Ensured the complete extraction by adding 2ml of the solvent to the pellet and repeated the process. Pooled all the supernatants together in the same tube and made the volume up to 10ml with the solvent. Read the absorbance at 645and 663nm in spectrophotometer against acetone blank. Calculated the absorbance due to carotenoids. (Sawhney and Singh, 2000)

Total soluble proteins

Homogenized thoroughly a known amount of leaf tissue with mortar and pestle by adding 5ml of distilled water. Filtered the homogenate and the filtrate centrifuged. Discarded the pellet. Saved the supernatant and the volume measured. Mixed 0.5 ml of the supernatant with 0.5ml of 10% Trichloro acetic acid and allowed to stand for 1hour in ice. The protein precipitated. Centrifuged the protein precipitation and dissolved it in 0.5ml 0.1Nsodium hydroxide. Added 5ml of alkaline copper mixture to the protein solution. after 10minutes added rapidly 5ml of 1:1 diluted Folin- Phenol reagent and mixed immediately. Read the absorbance at 700nm in Bausch and Lomb Spectronic - 20 after 20minutes. Calculate the protein using a standard graph prepared with bovine serum albumin. (Lowry *et al*.1951)

Free amino acid

Homogenized a known amoun of leaf tissue with 5ml of 80% methanol in an ice cold morter and pestle. Filtered the homogenate and the filtrate centrifuged at 5000rpm for 10minutes. Collected the supernatant and added equal volume of petroleum ether.after 30minutes, the supernatant separated into two distinct layers. Collected the lower methanol phase containing amino acid and measured the total volume. Pipetted out 0.1mo of sample into a clean test tube and 0.1ml of 80% phenol added. Kept the sample in boiling water bath for 10minutes. Added 2.0ml of 5% ninhydrin reagent after cooling and again kept in water bath for 10minutes and the purple coloured complex deveooped was made up to 10ml with 60% ethanol. Read the absorbance at 575nm in spectrophotometer. Calculated the amino acid content from the standard graph prepared with a mixture of amino acids. (Sawhney and Singh, 2000)

Nirtrate reductase activity

Fresh leaves were used for the assay. cut the leaves into 2mm segments with a sharp blade on ice in diffused light incubated the leaf segments of 100mg fresh weight in glass vials containing 2.0ml of incubation medium composed of 0.1M KH2PO4, KNO3, pH7.5. KMOH, 0.1% V/V Triton X 100,1%V/V n-Propanol. the vials incubated with leaf tissues were kept in dark for 1hour at room temperature. (Jaworski, 1971)

Nitrite

At the end of one hour incubation an aliquot of 0.2ml of the incubation medium was pipetted out into a test tube and the volume made up to 2ml with distilled water. To this 1ml of 1% sulphanilamide in 3N HCL and 0.02% N-(1-napthyl) ethylene diamine dihydrochloride were added. Mixed the contents thoroughly. After 15minutes the pink coloured complex was read at 540 nm in a spectrophotometer. Nitrate reductase activity was calculated from the amount of nitrite released in the incubation medium. This was calculated using the standard graph for nitrite.

Results and discussion

The germination percentage of Arachis hypogea L. seeds to various concentrations of NaCl salinity are shown in Figure 1. The final germination percentage was unaffected up to 25mM NaCl and above these values a slight inhibition was recorded. According to Zidan and Elewa (1995) the tolerance in rate of germination was more or less due to constant values of water content. However Dell Aqualla (1992) pointed out that the decrease in final germination percentage was always with a decrease in water absorption.

The germination tolerance at low salinity level and the inhibition at higher values of the present study may be attributed to that of the above authors. A differential effect of NaCl salinity was observed on the shoot and root length of kidney bean seedlings (Fig 2). The shoot length reduction was found to be gradual with an increase of salinization level and especially under higher concentrations the retardation was considerable over the3 control. Conversely the root lenth remained more or less unaffected whatever salinization level used and the reason for this differential response is unknown. A gradual reduction of seedling fresh weight and dry weight was found with increasing concentration of NaCl tested (Fig. 3). Effect of various concentrations of NaCl on total chlrophyll, chlorophyll a and chlorophyll b are shown in Fig.4. the chlorophyll content also showed a decreasing trend with increasing concentration of salinity in Arachis hypogea L. Carotenoid content showed a sharp decline within increasing concentration of NaCl treatment. The decline was shown to 50% at 10mM and 75% at 100mM NaCl (Fig.5). A general increasing trend in the content of soluble protein over the control has been observed in the seedling of cow pea. however the soluble protein content progressively decreased with increasing salinity levels(Fig 6). Same way, free amino acids content enhanced at 50 and 100mM NaCl (Fig 7). The higher protein content in the NaCl stressed seedling may be due to the synthesis of additional stress proteins. Synthesis of several new proteins in response to NaCl salinity has been reported by several authors.

The leaf nitrate reductase activity was inhibited by increasing concentrations of NaCl. The inhibition was found to be gradual. About 60% activity was noted even with higher values (100mM) of NaCl used (Fig.8). Inhibition of nitrate reductase activity in response to salt stress is in accordance with the previous reports on some other crop species (Srivastava, 1980; Rao and Gnanam,1990) [5, 2, 10].

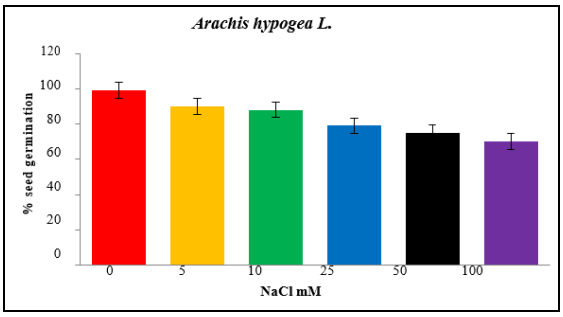

Fig 1: Effect of NaCl on germination of seeds of *Arachis hypogea L*

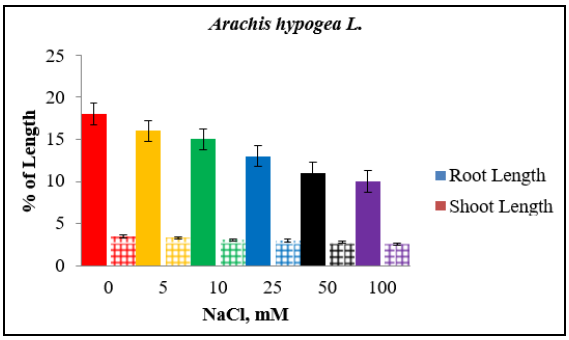

Fig 2: Effect of NaCl on shoot and root elongation of *Arachis hypogea L*

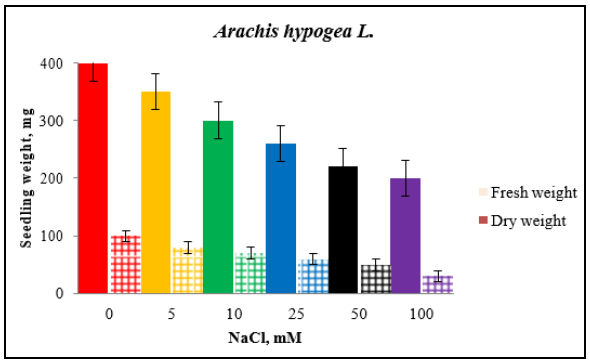

Fig 3: Effect of NaCl on seedling fresh weight and dry weight of *Arachis hypogea L*

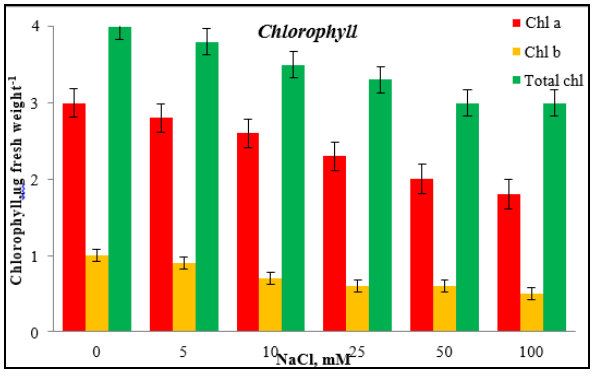

Fig 4: Effect of NaCl on Chlorophyll content of *Arachis hypogea L*

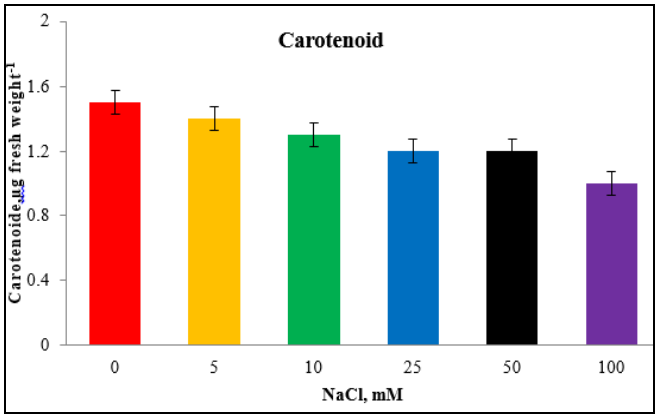

Fig 5: Effect of NaCl on Carotenoid content of *Arachis hypogea L*

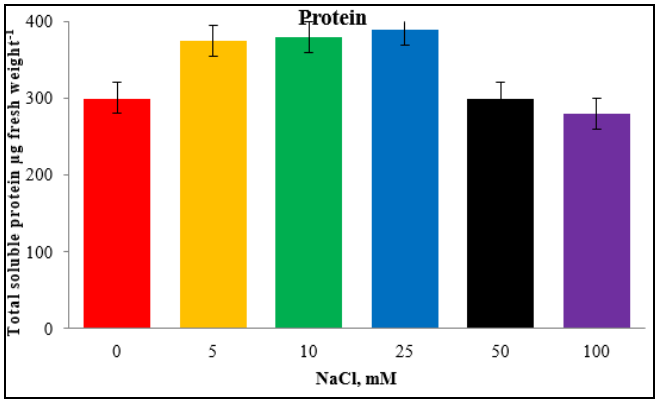

Fig 6: Effect of NaCl on total soluble protein content of *Arachis hypogea L*

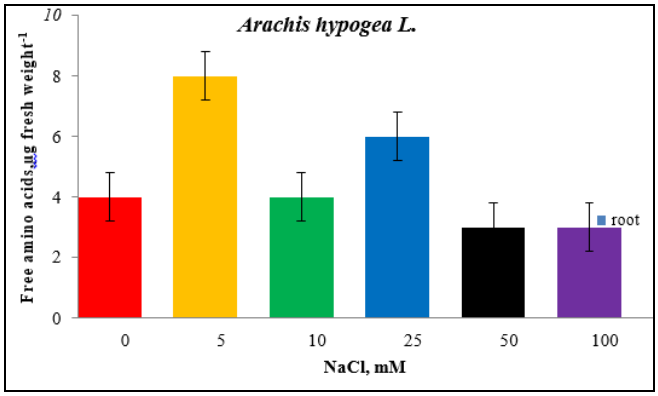

Fig 7: Effect of NaCl on free amino acid content of *Arachis hypogea L*

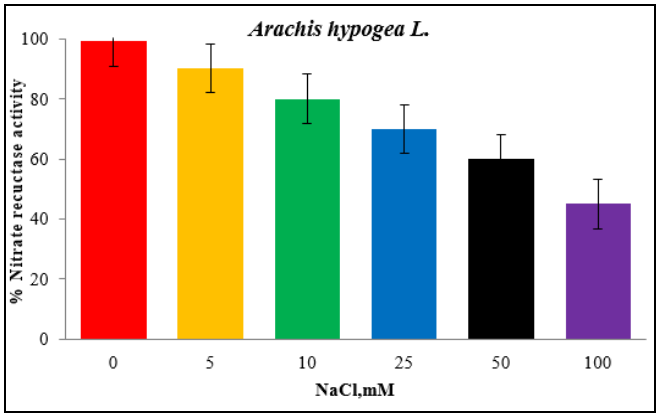

Fig 8: Effect of NaCl on nitrate reductase activity of *Arachis hypogea*

Summary
The effect of varying levels of salinity on germination, seedling growth, and some metabolic parameters *viz*., total soluble proteins, free amino acids and nitrate reductase activity in Arachis hypogea L. was studied. Seeds of Arachis hypogea tolerated salinity up to 100mM during germination. Root elongation remains unaffected and tolerated all the concentrations of NaCl tested while the shoot elongation was reduced by salinity.
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Reference
1. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of biological chemistry,1951:193:265-275.
2. Rao RK, Gnanam A. Inhibition of nitrate and nitrite reductase activities by salinity stress in Sorghum vulgare. Phytochemistry,1990:29(4):1047-1049.
3. Ritambhara GRS, Kumar, Dubey. change in activity of enzymes by NaCl salinity in rice. indian plant physiol, 1994, 38.
4. Jennings BD. The effects of sodium chloride on higher plants. Biological reviews,1976:51(4):453-486.
5. Srivastava HS. Regulation of nitrate reductase activity in higher plants. Phytochemistry,1980:19(5):725-733.
6. Sharma S. Studies On Growth, Water Reiafionsand Distribution of Na+, K+ And Other Ions In Wheat Under Shor-Term Exposuresto Salinity.
7. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology, 1949:24(1):1.
8. Jaworski EG. Nitrate reductase assay in intact plant tissues. Biochemical and biophysical research communications,1971:43(6):1274-1279.
9. Khan MG. Nitrate and nitrite reductase activities in soybean plants raised with saline water. Indian Journal of Plant Physiology,1996:1:128-129.
10. Rao RK, Gnanam A. Inhibition of nitrate and nitrite reductase activities by salinity stress in Sorghum vulgare. Phytochemistry,1990:29(4):1047-1049.
11. Kimball SL, Salisbury FB. Plant development under snow. Botanical Gazette,1974:135(2):147-149.