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Studies of morphological and biochemical parameters of *Oryza sativa* L. seedlings under salt stress

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ABSTRACT

A preliminary study of the manifold works done to apprehend the effect of salinity and the response of the crop in turn has been illustrated here. With respect to the devastating effect of salinity on this staple food crop, it is very important assimilate the knowledge of the response of the crop towards salinity. The germination percentage, Root and Shoot length, pigments and all biochemical parameters decreased with the increase in Salt Stress to rice seeds.The Low salt concentration has affected the biochemical parameters slightly, but increase in treatment decreased the parameters like Amino Acids (Root), Protein (Shoot) and DNA (Shoot). The Protein content in Root and RNA content in Shoot did not respond to the treatment of NaCl to rice seedlings. However, there was increased response of Protein (Root and Shoot), DNA and RNA (Root) contents. Overall, The salt stress has a little effect on biochemical parameters studied in the seedlings of *oryza sativa L.* This work is a minor attempt to summarize the salient contributions and breakthroughs made in this area in the course of understanding the response and thus the plant defence to fight salinity as a stress.

Keywords: Rice seedlings, Salt, Stress, Morphology, Biochemistry

INTRODUCTION

Rice is the best sources of protein and can prevent malnutrition in the protein hunger world. Rice is grown to maintain soil nitrogen and soil fertility level. It is affected by different biotic and abiotic factors that prevail in the micro and macro environment. An adverse effect of salinity is usually more at reproductive stage than any other stages in rice. Thus, Rice is placed in the group of salt sensitive crop (Rashid *et al.*, 2004). However, its productivity over the last few decades has been stagnant largely due to various biotic and abiotic stresses. Different abiotic stresses due to complex environmental conditions viz., UV rays, high or low temperatures, excess of moisture, drought, salinity, heavy metals, etc. caused extensive crop losses worldwide (Mittler, 2006). Effect of such stresses on crop production is difficult to determine on rational basis. Salinity is the major abiotic stress affecting the crop production worldwide. Among the abiotic stresses, salinity stress is more damaging that limits functional plant growth and yield worldwide. Saline soils possess an excess amount of soluble salt. Soil salinity can be characterized by soil EC (Electrical conductivity) value measured in unit deciSiemen /metre (dS/m).

Saha *et al.* (2010) stated that with the increase in the salt concentration, seed germination, fresh and dry weight, root and shoot length, photosynthesis, yield parameters affected and reduced drastically. Superoxide dismutase and catechol peroxidase activities increased under stress in both roots and leaves. But catalase activity showed an increase in roots and decrease in leaves. In these seedlings, the oxidative stress has been observed under salinity stress and the level of proline (osmolytes), H_2O_2 and malondialdehyde content were increased. Soil salinity has a great impact on the physiology and biochemistry of the plant that can greatly reduced plant growth and development. (Munns, 1993) stated that salinity reduces leaf growth, root growth, and decreases stomatal conductance which ultimately reduces vital processes like photosynthesis

MATERIAL AND METHODS

Experimental Plant

A native variety of *Oryza sativa* (L.) was used as the experimental plant seed were obtained from the Centre for Pulses Research Institute, Ratanpur, Berhampur (CPRI) with label Tejaswini. The selected species is popularly known.

TEST CHEMICAL

Sodium Chloride (NaCl) was used as test chemicals with molecular weight 58.41gm/mole. Different concentrations of salt solution were prepared by using Hoagland solution as the solvent.

SELECTION OF EFFECTIVE CONCENTRATION

For selection of effective concentration, a constant number of seeds were germinated and grown under different concentration of salt ranging from 0.128g to0.896g i.e 1dS/m to 10dS/m. The seeds germinate and grow well in Control, 1 dS/m (0.128g), 2 dS/m (0.256g) and 3 dS/m (0.384g). From 4 dS/m (0.512g) to 6 dS/m (0.768g) the seeds were germinated but growth is stunted. In 10 dS/m (1.28g) the growth was very little. Keeping a view on the above seed germination results, the Salt concentration were selected from 1dS/m to 10 dS/m for further studies.

GERMINATION STUDIES

For germination studies, Thermocol glasses (200ml) were surface sterilized with alcohol (Ethanol). The seeds were soaked for 24 hours seeds in water, then the seeds (10 per each glass) were placed on the top of the net fitted below the mouth of thermocol glasses. The glasses were then kept on the surface sterilized rack with 400 lux light and room temperature was maintained for germination of seeds. The percentage of germination of seeds for each of the treatment were recorded after 24 hours. Ten replicates were taken for each of control and treated salt concentrations to eliminate experimental error.

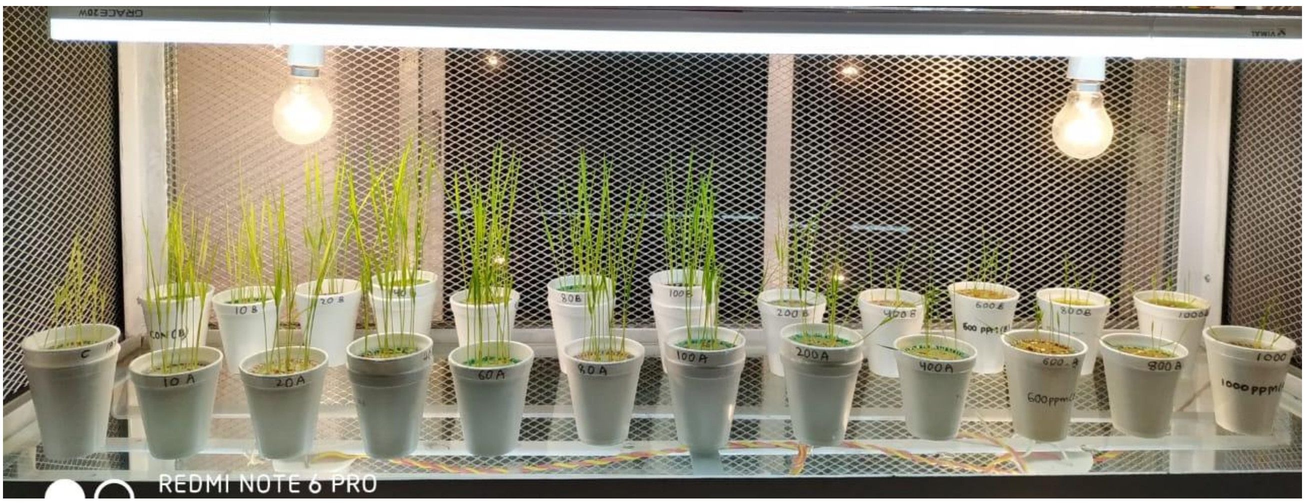

Plate No.-01: The germination of Rice seeds after Salinity stress for 7 days

MORPHOLOGICAL STUDIES

For morphological studies, the growth of the plant was evaluated by measuring the % germination, leaf emergence, shoot length and root length of seedlings on 7th day. Seed were allowed to grow till 7th day to measure the morphological parameters of rice seedlings. A 30cm scale was used for measurement of shoot and root length. Fresh and dry weights of the 7th days old seedlings were recorded in g. (in grams).

PIGMENT ANALYSIS

The pigment analysis was conducted using seven day old seedlings. The shoot and root portion of rice seedlings were separated and only the contents of shoot were taken for pigment analysis. Seedlings from each concentration were taken and leaves were plucked and weighted. The weighted leaves (100mg) were grinded in the mortar and pastel with aliquots of 80% Acetone (V/v). The homogenate was centrifuged at 3000 rpm for 10minutes. The supernatant was collected in a test tube and the residue was retreated with 80%Acetone and again subjected to centrifugation. Then supernatant was collected and pooled together. The supernatants of each concentration were poured in to the cuvette and its absorbance was measured at 645nm, 663nm, 666nm & 475nm for chlorophyll-a, chlorophyll-b, carotenoid and phaeophytin respectively. The various types of pigment contents were calculated following the formula of Arnon (1949 and 1956).

BIOCHEMICAL ANALYSIS

The biochemical parameters for Root and Shoot of 7days old seedlings like Amino Acids (Moore and Stein,1948), Protein (Lowry *et al.*, 1951), Carbohydrates/Sugar (Yoshida *et al.*,1972) and DNA and RNA (Schemider,1957) were estimated following standard quantitative methods.

ENZYME ACTIVITY

100 mg of the plant material was taken and grinded in the mortar and pastel with 5ml phosphate buffer P^{H} 7.0. The homogenate was centrifuged for 10-15 min at 15000 rpm. The supernatant was collected in a test tube. The collected supernatant was the source of Catalase, Peroxidase and Polyphenoloxidase. The volume makes up was made up to 10 ml with Phosphate buffer. In enzyme studies, Catalase, Peroxidase (POD) and Polyphenyl Oxidase (PPO) of shoot and root of 7-day old seedlings were analyzed (Kar and Mishra.1976). The enzyme activities was expressed in terms of in Absorbency Units (A_{420nm}).

TEST FOR PROLINE

100mg of plant material was grinded with mortar and pastel with 10ml of 3% Sulfosalycylic acid. The homogenate was centrifuged at 5000rpm for 15 min. The supernatant was collected and to 2ml supernatant, 2ml Ninhydrin solution and 2ml Glacial Acetic acid were added. The tubes were incubated for 1 hour at 100^{0} C. Then, the tubes cooled in ice bath. The formation of colour takes place by adding 4ml of Toluene. The absorbance was measured at 520 nm against Toluene.

RESULT

The present studies deals with salinity stress on the germination and other physiological parameters of *Oryza sativa L.* seedlings. The results are presented in graphs. (Fig. No. 1-11). During the experimental period the seeds of *Rice* showed 100% germination. There is no much variance in the percentage of germination. In general, decrease in germination has one of the manifestations of salinity stress.

At higher concentration of salt, there was no germination. Thus, it is concluded that beyond concentration 6dS/m stressed germination of seeds. The radicle emergence took place after 24 hours of germination of rice seeds. Thus, by the end of second day (including one day of soaking period), most of the seeds showed radicle emergency. For the next 48 hours there was a gradual growth of plumule took place. The length of root and shoot were measured after expansion of first two leaves and emerging second leaves. The growth of the seedlings is marked by increasing shoot and root length. Increasing the concentration there is gradual decrease in the seedling growth. Fig. No.2 shows a gradual decrease in the short length and root length with the increase in the salt concentration.

In shoot, there is a gradual decrease in the length as the concentration increases. In the 7th day the shoot length of control is 11.6 cm whereas, the length in 10dS/m salt concentration is least i.e. 3.2 cm. The root has same effects on increasing the concentration of salt. The control is having the highest root length and the length decreases with the increase in the salt concentration.

The contents of various pigments in leaf were estimated in the 7 days old seedlings. The root portions were separated and shoot portions were analyzed. The pigments studied were chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid and phaeophytin.

The estimation of chlorophyll was limited to the shoots only. The total chlorophyll contents were calculated taking different concentrations i.e. from 2dS/m to 10dS/m along with control solution.

The amount of pigments (Chl.-a, Chl.-b and Total Chl.) decreased with the increase in salt concentration (Fig. No.4).

The percentage of contents of carotenoid increased with 2dS/m in concentration of salt whereas the contents of phaeophytin decreased after 2dS/m. However, the percentage of contents of total carotenoid and phaeophytin of the seedlings of *Oryza sativa* increased.

The amounts of carotenoid don't have a linear relation with concentration of salt. There is a sharp decrease in the contents of carotenoid from 2dS/m concentration. Again, at 6dS/m concentration there is seen a sudden increase in the amount of carotenoid. After this sudden increase there is a sharp decrease.

PHAEOPHYTIN

The estimation of the pigments phaeophytin was limited to the shoots only. These were calculated taking different concentrations as that of chlorophyll. There is a sharp decrease in the contents of phaeophytin from 2dS/m concentration. Again, at 6dS/m concentration there is seen a sudden increase in the am

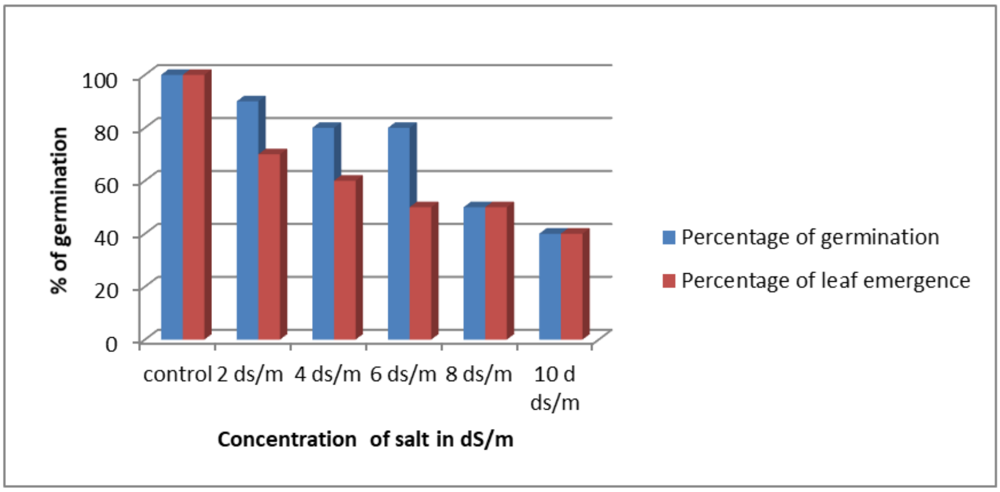

Figure 1: Effect of salinity stress on the % of seed germination and leaf emergence of *Oryza sativa*, L.

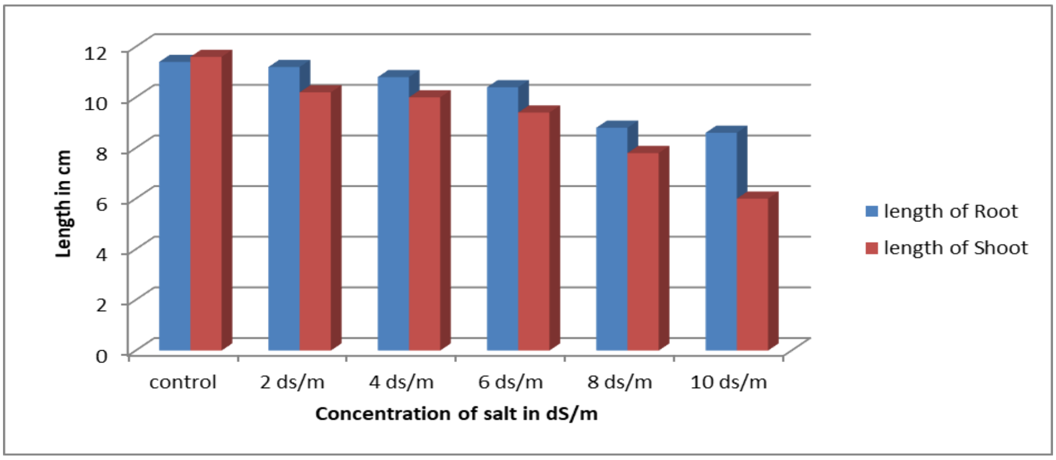

Figure 02 : Effect on shoot length and root length (in cm) of 7th day *Oryza sativa* seedlings under different concentration of salt.

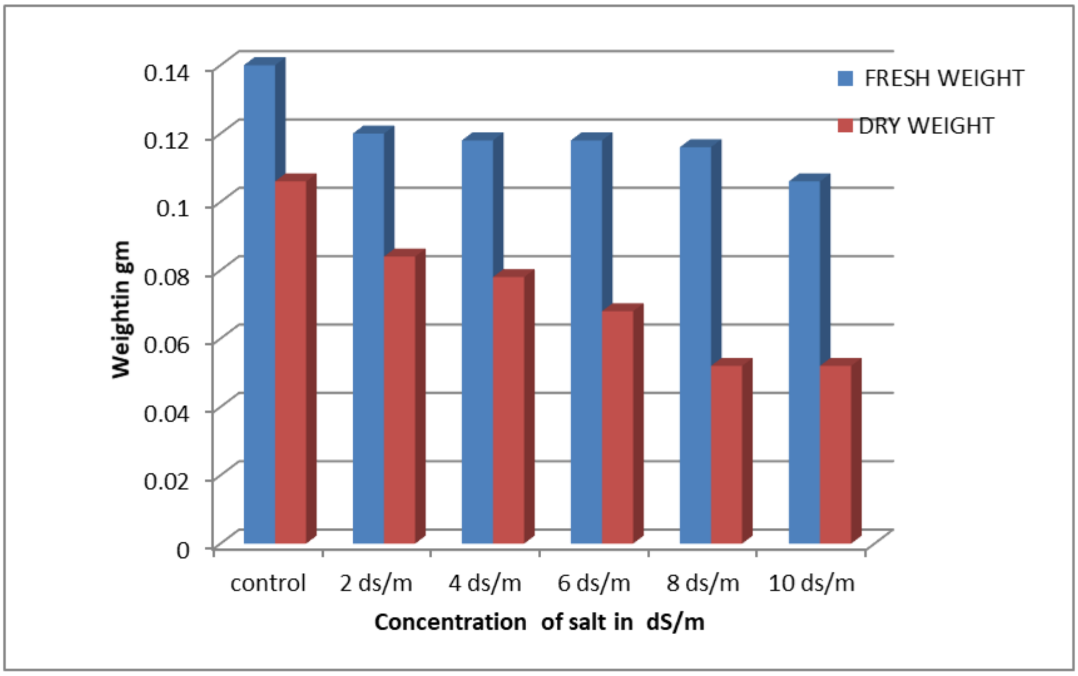

Figure 3: Changes infresh weight and dry weight of the root of *Oryza sativa,* L. after Salt stress

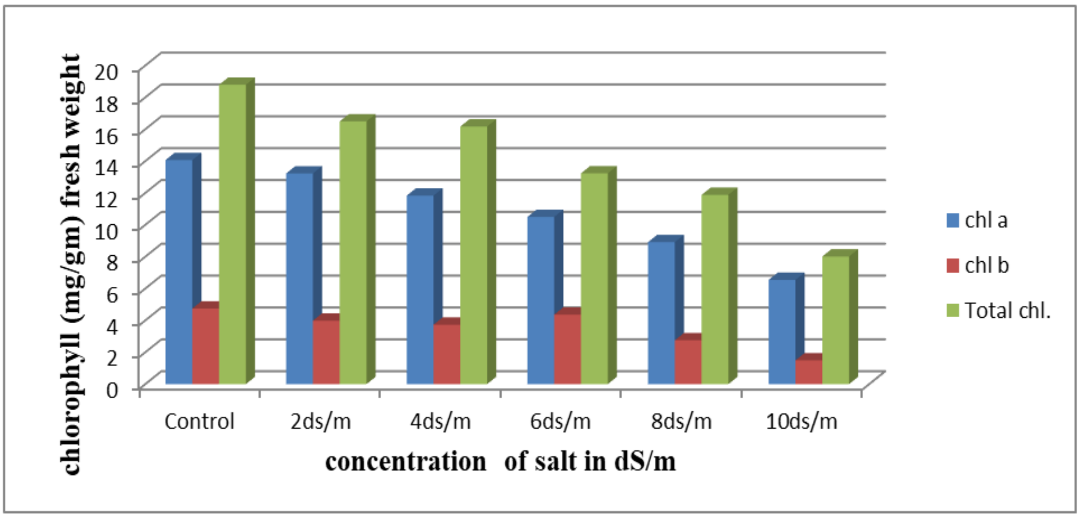

Figure 4: Changes in leaf pigment in *Oryza sativa,* L. after Salt stress

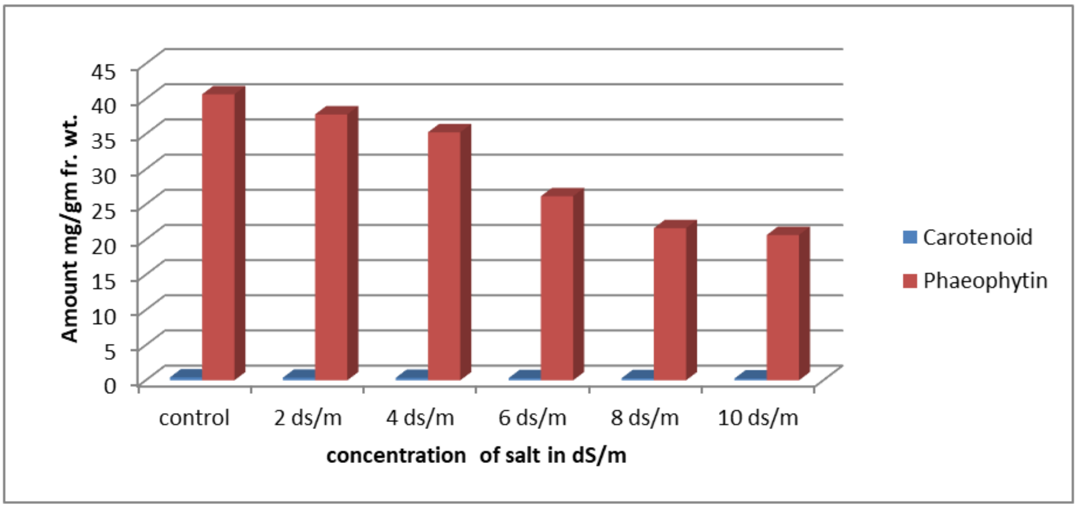

Figure 5: Changes in carotenoid and phaeophytin contents of *Oryza sativa,* L. after Salt stress

STUDIES OF CHANGE IN BIOCHEMICAL CONTENTS

The contents of various biochemical parameters were estimated using 7 days old seedlings. The roots and shoots were analyzed separately. The parameters studied were sugar, amino acid, protein, nucleic acid i.e. DNA & RNA.

The contents of sugar and amino acid in root are maximum in control i.e. 15.50g and 77.89g respectively. The contents of both parameters decreased with the increase in concentration of salt. Least amount of protein is found in the seedlings of control i.e. 53.54g and highest protein is found in the seedlings of 8dS/m and 10dS/m concentrations.

The amount of amino acid in shoot gradually decreases with increase in concentration of salt that means control has highest amount of amino acid. Similarly, for sugar, control has highest amount of sugar but there are two sudden increases in sugar content at concentration 2dS/m and 6dS/m concentrations. However highest amount of protein is found in the seedlings of 10dS/m concentration and lowest in 2dS/m concentration.

The contents of amino acid and sugar decreased with increase in concentration whereas the protein contents show variations in root of Oryza sativa. The content of amino acid goes decreasing with increase in concentration in shoot of the seedlings while sugar and protein show sudden increase and decrease in their amount. However, the amount of protein and sugar are more in shoot while amino acid is more in root.

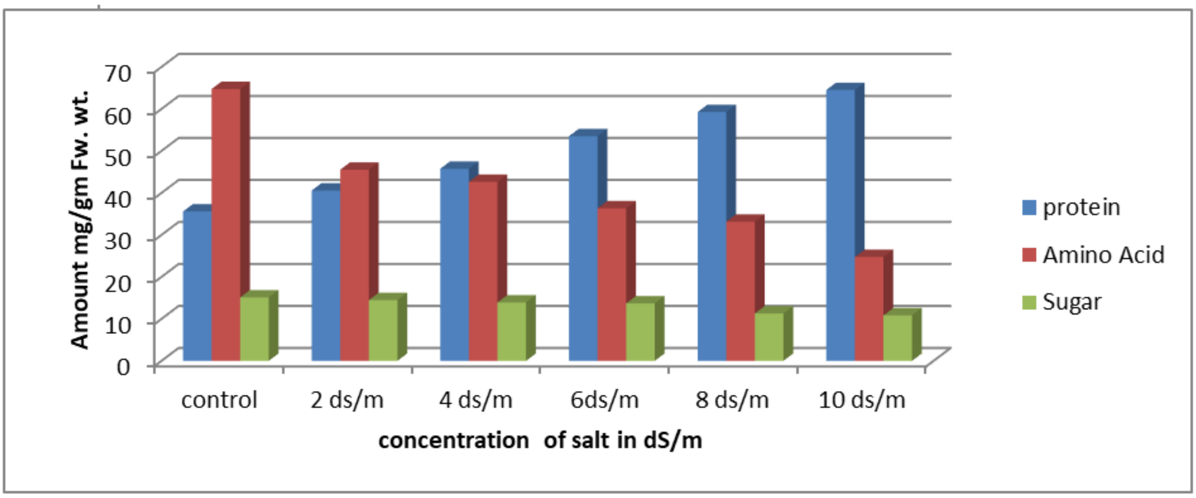

Figure 6: Changes in Biochemical parameters (Protein, Amino acids and Sugar) in root of 7days old rice seedlings under Salt stress

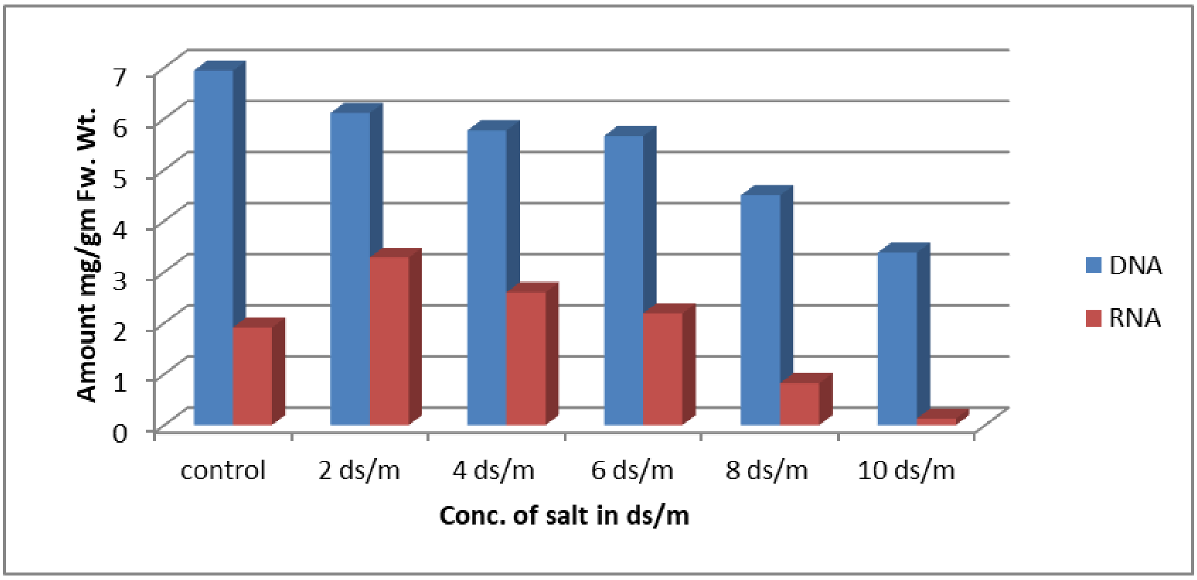

Figure 7: Changes in DNA and RNA content in Root of 7days old *Oryza sativa* seedlings after Salt stress

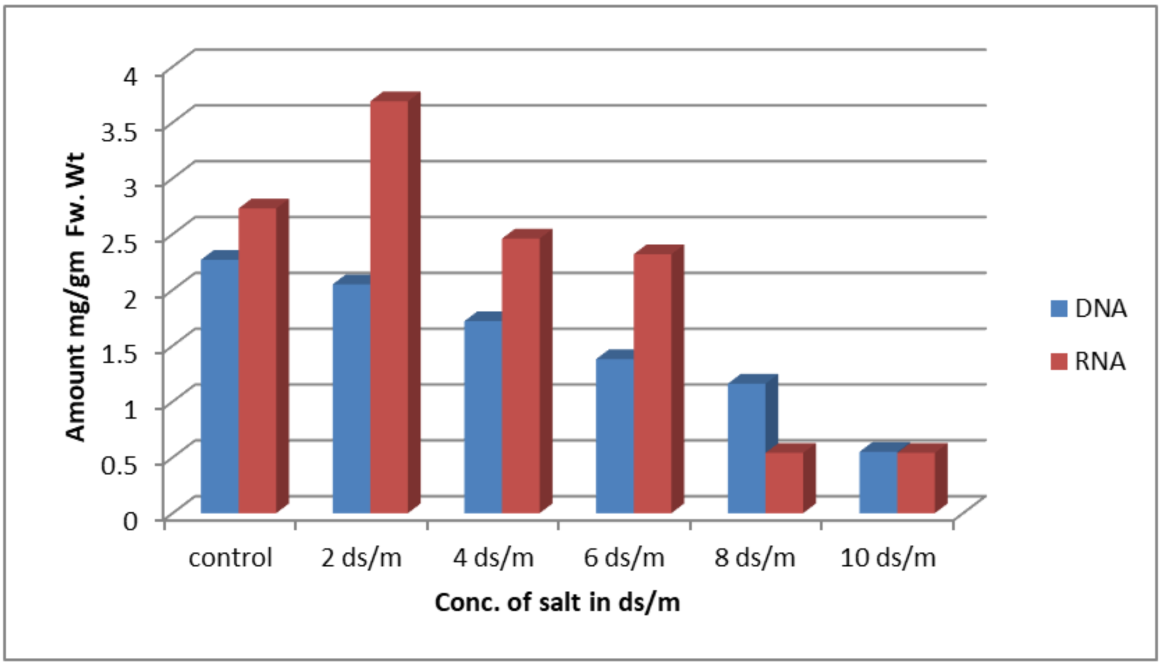

Figure 8: Changes in DNA and RNA content in Shoot of 7days old *Oryza sativa* seedlings after Salt stress

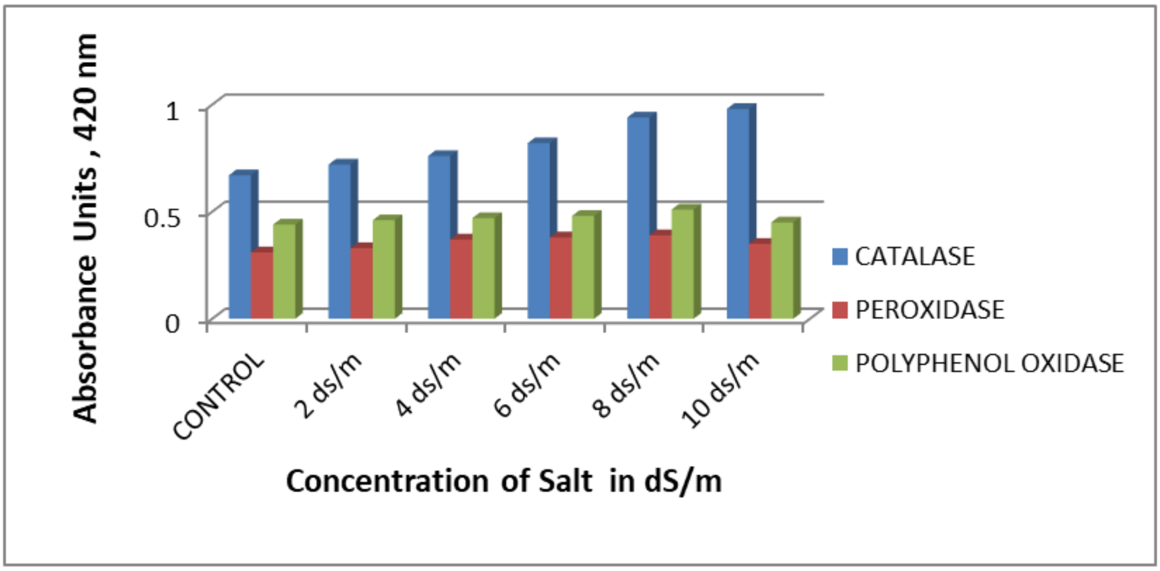

Figure 9: Changes in Enzyme Activity in 7days old *Oryza sativa* seedlings after Salt stress

The maximum DNA content in shoot is found in control and 2dS/m concentration while least amount is found in 10dS/m concentration. The amounts of DNA decrease at 10dS/m and again there is a rise at 2dS/m concentration. After this there is a sharp decrease in the contents upto 6dS/m concentration where a slight increase is found. Then again, the contents decrease at 10dS/m concentration. Maximum amount of RNA present in 2dS/m concentration while least is 10dS/m.

In root the amount of DNA goes on decreasing gradually with increase in salt concentration i.e. control has the maximum content and 10dS/m concentration has lowest contents of DNA. Maximum amount of RNA is found in the concentration 2dS/m while least amount is found in 8dS/m and 10dS/m.

ENZYME CONTENT

Effect of salinity stress on Oryza sativa, the enzymes which were estimated by following tabular form. The enzyme catalase, Peroxidase and Polyphenoloxidase goes in a particular range of the calculation.

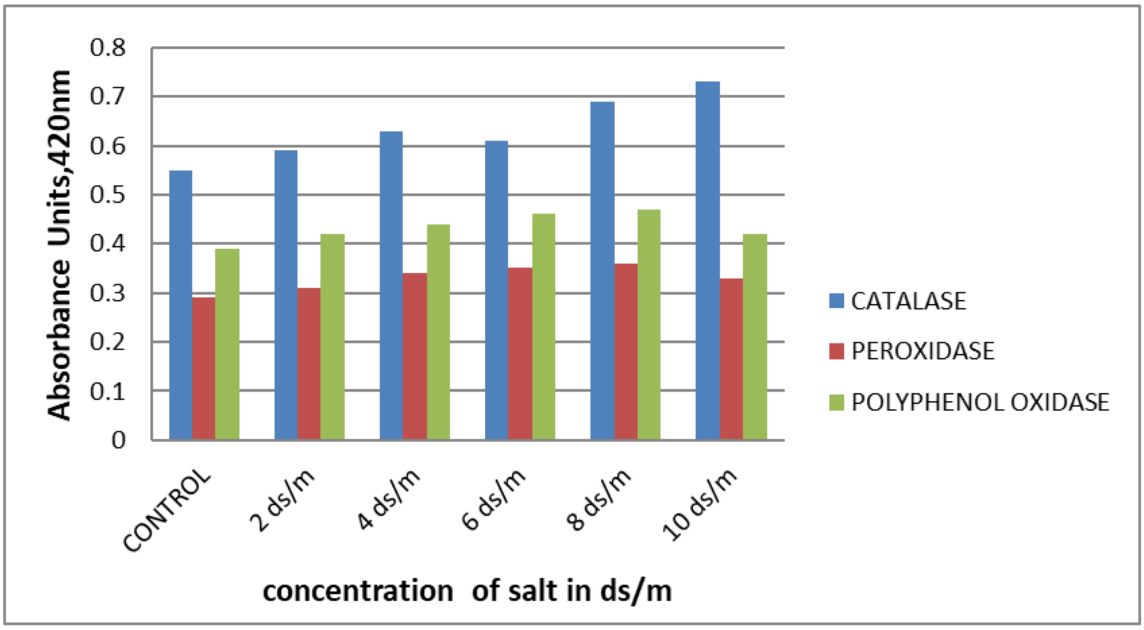

Figure 10: Changes in Enzyme Activity in 7days old shoots of *Oryza sativa* seedlings after Salt stress

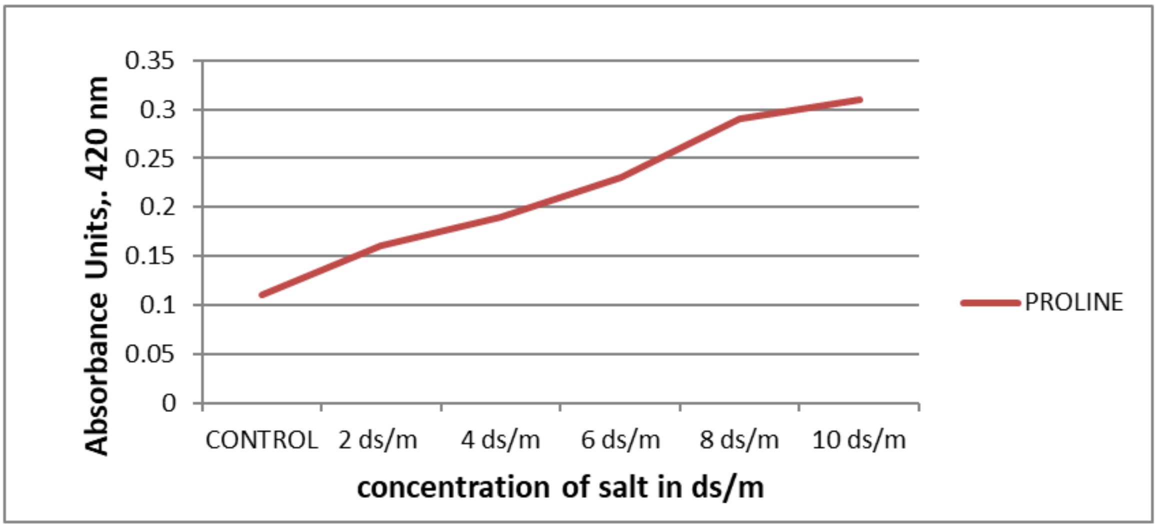

Figure 11: Changes in Proline Activity in 7days old seedlings of *Oryza sativa* seedlings after Salt stress

DISCUSSION

In plants, the most critical stage during seedling growth is the seed germination that determines effective crop establishment and production. Increasing salinity stress levels during rice bean seed germination significantly reduced the germination and seedling performance. Salinity adversely affects the plant growth at all stages, particularly at seedling stages, which dramatically reducing the percentage of germination and leaf emergence (Munns *et al.*,2002). Many earlier reports have shown that a significant reduction in shoot and root lengths in rice seedlings caused by salt stress was ameliorated by providing an induction treatment (with small dose of NaCl) prior to exposure to the lethal concentration. The percent survival was relatively more and reduction in recovery growth was appreciable in pre-treated plants over non treated plants, which indicates that pre-treatment has an assimilative effect in acclimation process as reported earlier in rice.

In our study, it was found that as the seeds were not pre-treated, there was significant decrease in percent of germination, percentage of leaf emergence, root length and shoot length, dry and fresh weight of root and shoot indicating even the sub-lethal dose is sensitive to the seedling. It was also noticed that increasing in concentration of salt has a inhibitory effect on chlorophyll-a and b, total chlorophyll, carotenoid, pheophytin content in the shoot. Salinity has its own effects on the plant growth and development right from the germination stage. In the present study, all recorded parameters decreased compared to control plants as Na Cl concentration increased except protein (shoot and root). The reduction of plant growth under salinity was due to the effect of salinity on the different vital activities of plants, such as declined enzymatic activity, metabolism, cell division and photosynthesis. Growth inhibition under salt stress which may be due to the diversion of energy from growth to maintenance respiration. Increasing salinity concentration levels leads to an increase on the absorbance of some essential elements that activated the action of some enzymes, which were essential for the protein synthesis (Mayer, 1973).

In the nucleic acid study, we found a negative co-relation in case of DNA with the increase of salt concentration but a positive co-relation in case of RNA both in root and shoot as an indication of essential increase of protein synthesis.

In enzymatic studies it was seen that in case of all the three enzymes i.e. catalase, peroxidase and polyphenol oxidase, there was a positive correlation except peroxidase and polyphenol oxidase where there was a negative correlation.

In proline content it was observed that there was a positive correlation of proline content to that of Salt concentration and it is may be due to the resources that its serve as osmotic regulator, (Pollad and Wyn Jones, 1979) or a protector of enzyme denaturation (Paleg *et al.*, 1984) or stabilizer of some macro molecules or molecular assemblies (Schwab and Gaff, 1990) and reservoir of nitrogen and carbon sources (Fukutaku and Yamada,1984).

The mechanisms by which protectants regulate activities of enzymes are enhanced and are undoubtedly interesting and demand insightful studies in rice plant. The clear mechanism of defence and signal transduction pathways is still in dark. Furthermore, the exact dose, duration and proper methods of application of exogenous protectants in rice plant under salt condition are be studied more precisely for complete elucidation of mechanism of protection.

The result explains that germination and growth significantly decreased with higher salinity levels. Reduced growth in rice under salt stress may attributes to more flowers shedding, reduced photosynthetic efficiency to fill the developing seeds, which may lead to reduced number of seed or plant and dry matter yield of individual seed and shattering of the seed. But in a few cases, like tolerant lines PSBRc 48,50,84,86,88,NSIKc 106,CRS 10 ,23,27,30 etc. no matter what the stress was, the plants put forth appreciable penicle yield, might attribute to better pollen fertility and translocation of photosynthates towards the penicle and seed components. Whereas in susceptible lines, salinity stress resulted in shrivelled seeds and impaired penicle setting leading to reduced penicle number and seed yield (Wahid, 2004)

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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