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SALT STRESS INDUCED CHANGES IN GROWTH, PIGMENT, PROLINE AND NA^+ - K^+ CONTENT IN *ORYZA SATIVA* L.

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Salt stress reduced the germination rate of rice (*Oryza sativa* L.) seeds. An uniform decrease in root length was marked in case of both NaCl and $CaCl_2$ treatments compared to shoot growth except for a minor changes in NaCl treatment. An uniform decrease in fresh and dry matter content was seen for both the salts. An increase in Na^+ uptake was recorded with a decrease in K^+ content in the rice shoot. Salt stressed seedlings showed a decrease in chlorophyll and carotenoid content from control seedlings. For both the salts tested an uniform accumulation of proline was visible.

Keywords : Growth; K^+; Na^+; *Oryza sativa*; Pigment; Proline.

Introduction

Salt stress is an abiotic environmental stress that limits the growth and development of plants which can be seen through changes in plant's morphology, physiology and metabolism. Rice is a sub-tropical cereal crop which is generally considered as salt sensitive than later stage[1,2]. Salt stress is known to affect germination, growth, pigment content, mineral composition, ionic balance, proline accumulation and enzyme activities in various plants[3-9]. The major objective of the present study was to investigate the salt stress induced changes in germination, growth, pigment, proline content and Na^+ - K^+ accumulation in a salt sensitive traditional rice variety.

Materials and Methods

Dry graded uniform rice (*Oryza sativa* L. var. Beeroin) seeds were procured from Assam Agricultural University's Regional Rice Research Station, Karimganj, Assam and were surface sterilised with 0.1% mercuric chloride (w/v) for 10 min followed by three rinses in sterile distilled water. The seeds were germinated in petriplates containing Whatman No. 1 filter paper moistened with distilled water and under different (0, 0.5, 1, 2 and 3%) salt solutions and keep in BOD incubator at 25 ± 2°C.

On the 3rd day germination rate was calculated for both the salts (treated and untreated). The germinated seeds (untreated) were transferred to plastic glasses containing Yoshida solution and kept in a growth chamber under continuous white light. White light was provided by cool fluorescent white tubes (36 W Philips TLD) with a photon flux density of 52 $\mu Em^{-2}s^{-1}$ (PAR) under a 16-h photoperiod. On the 12th day, rice seedlings growing in Yoshida solution were given a treatment of NaCl and $CaCl_2$ (0, 0.5, 1, 2 and 3%) solution for 4h. After 4h, seedlings were rehydrated with fresh Yoshida solution and after 24 h i.e., on 13th day plants were taken. For different growth parameters, 13 days old seedlings growing in Yoshida solution alone and with various concentrations of NaCl and $CaCl_2$ were studied for root and shoot growth in terms of length. Triplet samples of uniform seedlings washed with the help of electronic balance and measurements were noted. Seedlings were kept in an oven at 80°C for72h and dry matter was determined. Both fresh and dry masses were expressed as biomass produced in mg/seedlings. Primary leaves and roots were sampled, oven dried and acid digested as per the methods of Humpries[10]. Na^+ and K^+ were estimated from the samples using a Flame Photometer (Systronics - India).

Analysis of pigments and metabolites was done on 13 days old seedlings growing in Yoshida solution and under different salt treatments. For the analysis of chlorophyll, carotenoid and proline, primary leaves of the seedlings were taken. The extraction of chlorophyll and carotenoid was done using

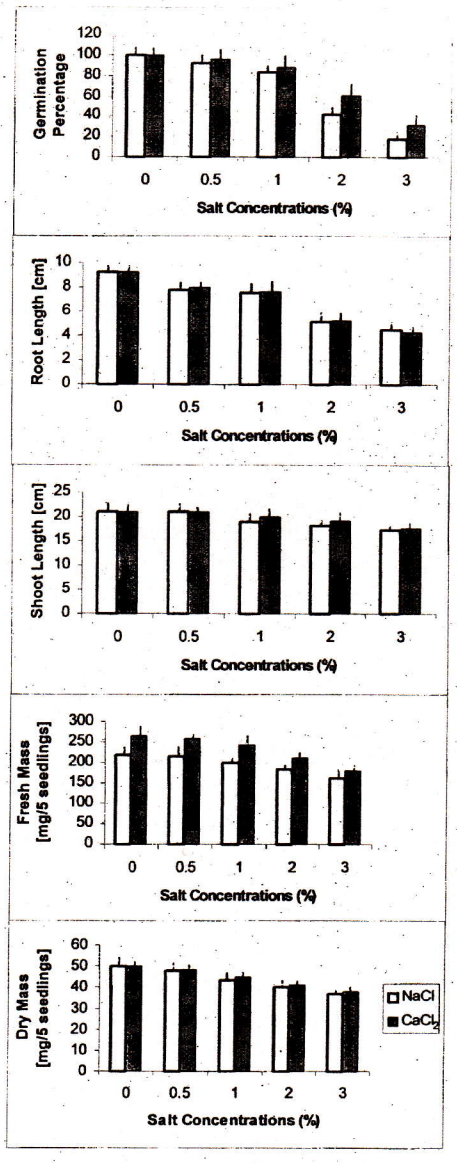

Fig. 1. Changes in germination percentage, root length, shoot length, fresh mass and dry mass of 13 days old rice seedlings subjected to salt treatments. Data presented are mean ± SE.

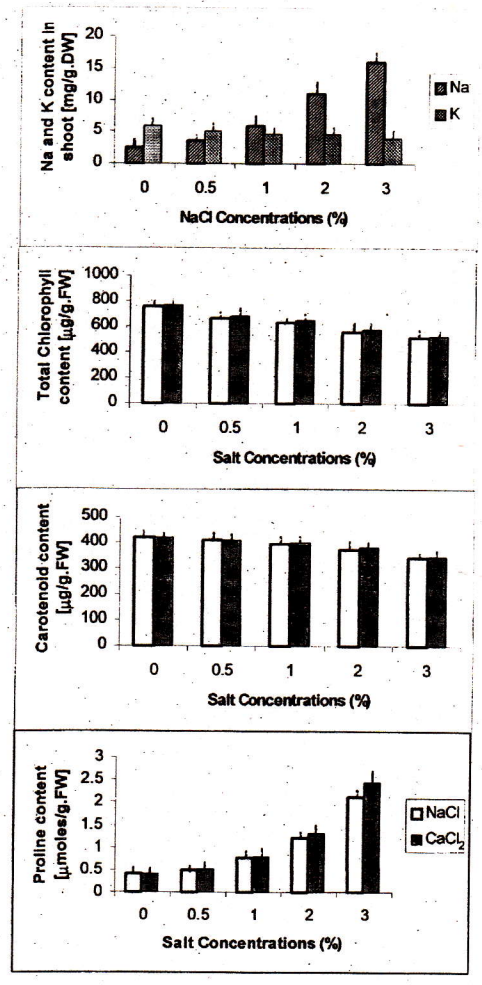

Fig. 2. Changes in Na^+ - K^+ content, total chlorophyll, carotenoid and proline content of 13 days old rice seedlings subjected to salt treatment. Others same as Fig. 1.

80% cold alkaline acetone[11]. The chlorophyll and carotenoid content was expressed as µg/g fresh weight of sample. Primary leaves (both treated and untreated) were homogenised with 3% aqueous sulfosalicylic acid and centrifuged at 3,000 g for 10 min. Proline was estimated as per method of Bates *et al*[12]. Each experiment was done in triplicates and data represent mean ± SE.

Results and Discussion

There is a gradual decrease in the germination rate with the increase in the salt concentration upto 1.0% compared to control and less than 50% has been observed

in 2.0% NaCl. 3.0% showed a maximum toxicity where almost 80% seeds could not germinate. In case of $CaCl_2$ treated samples, similar results were observed with less toxicity as compared to NaCl treated rice seeds. LC_{50} in NaCl is 2.0% and LC_{50} in $CaCl_2$ is 3.0% treated rice seeds was observed. Results showed a uniform decrease in seed germination rate with a maximum in NaCl as compared to $CaCl_2$. Similar results for various other plants have been reported by several other workers[9,13-15]. There is a gradual decrease in the root length of the rice plants subjected treated with NaCl and $CaCl_2$. The effect of the salts on the roots is toxic where the length of the root has been reduced to almost half compared to control. However, the $CaCl_2$ treated samples were affected more than that of NaCl treated rice plants. Root length decreased in both the salts whereas a slight increase in shoot growth was recorded. There is a decrease in 0.5% in the shoot length of NaCl treated plants compared to control followed by a gradual increase with the increase in the salt concentration. Similar result was observed in $CaCl_2$ treated rice plants but more severely in 3.0% concentration (Fig. 1). A gradual increase in the dry weight with the increase in the salt concentration was observed in 13 days old NaCl treated plants. A similar result was observed in $CaCl_2$ treated plants. The dry weight was reduced to almost one forth of the fresh weight. The effect of salt stress on the performance and growth are mediated through decrease in stimulating conduction and photosynthesis. It is known that the degree to which the growth is reduced by salinity differs greatly with species and then cultivar within a species[16]. Fresh and dry masses though decreased with a slight increase in some of the concentrations showing a modulatory role as reported elsewhere[17].

Figure 2 illustrates the changes in the Na^+ and K^+ content in the shoot of 13 d old rice seedling under differnt concentration of NaCl. There is an increase in the Na^+ ion content with the increase in the salt concentration with the maximum of at 3.0%. The K^+ content decreased with the increase in the NaCl concentration compared to control. The increase in Na^+ content with a decrease in K^+ content in shoot tissues suggested a better Na^+ uptake which disturbed ionic balance as seen for other salt sensitive rice varieties[18-20]. Higher salinity disturbs Na+ : K^+ ratio in the plant, which impairs the proteins metabolism of the plant. It has been reported that varieties of rice avoid the sodium toxicity by better potassium levels in the tissue but the reults showed a relative decrease along the salinity gradient[21,22] proving the cultivar to be a salt sensitive one. The NaCl treated leaf tissues showed a decrease in the chlorophyll and carotenoid content with the increase in the salt concentration. 1.0% NaCl treated plants showed a sharp increase followed by a decrease in 2.0% and 3.0%. Similar results were seen for $CaCl_2$ treated seedling. Total chlorophyll showed a uniform decrease in NaCl and $CaCl_2$ treatments with the increasing concentrations for seedling of both the ages. Similar reports are there for various other plants[6,23-24]. Carotenoid content decreased uniformly in salt stressed plants, suggesting an inhibition of photosynthetic efficiency in salt sensitive rice plant[24]. Decreased chlorophyll synthesis or chlorophyll molecules breakdown, rate of light reaction in photosynthesis, decreased rate of ribulose bisphosphate carboxylase - oxygenase (RUBISCO) enzyme activity has been reported under salt stress[25]. There is an uniform increase in the proline content compared to control in both the 13 and 14 days NaCl treated leaf tissues with a maximum in 3.0% NaCl. 14 days old plants contain higher proline than the 13 days old seedlings. Similar result was noticed in $CaCl_2$ treated rice leaves. An increase in proline content under both NaCl and $CaCl_2$ salinity suggested an osmoprotection to rice seedling under salt stress as reported for other plants as proline act as an osmoprotectant by providing non-toxic sinks for carbon and nitrogen preservation[18,26,27]. Increasing proline level is considered to help the cells in

osmoprotection as well as in regulating the redox potential, scavenging hydroxy radicals in the protection against denaturation of various macromolecules[28].

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