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Research Paper

Morphological and Biochemical Behaviour of Rice Seedlings Due To Effect of Calcium Salts On Salinity Stress

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ARTICLE DETAILS

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ABSTRACT

Soil salinity is one of the major abiotic stress that affects plant growth and rice production globally as well as in the Sunderban areas of West Bengal. The study comprises comparison of the effect of the combined action of sodium chloride (NaCl) and three types of calcium salts *viz.* calcium chloride ($CaCl_2$), calcium sulphate ($CaSO_4$) and calcium carbonate ($CaCO_3$) on three rice varieties ('Chinsurah Nona-2', 'Dudherswar' and 'Pratiksha') both morphological and biochemical estimations *i.e.* shoot growth, chlorophyll content, starch and reducing sugar content. The results show maximum recovery of shoot growth in 10 mM $CaSO_4$ with 200 mM NaCl treatment set with respect to seedlings stressed with only 200 mM NaCl *i.e.* 32% recovery in variety 'Chinsurah Nona-2' and 10.5% recovery in variety 'Pratiksha', whereas 'Dudherswar' variety shows 100% recovery. In case of chlorophyll content in the following sets, 5 mM $CaCl_2$, 15 mM $CaSO_4$ and 10 mM $CaCO_3$ are showing best recovery. Similarly, starch and reducing sugar content 10 mM $CaSO_4$ and 15 mM $CaCO_3$ sets show best results against 200 mM NaCl.

1. Introduction

Rice is considered as a major food crop across worldwide and indeed India is one of the largest rice producing countries in the world (ranking 2nd rice producing country globally after China). Rice plant (*Oryza sativa* L.) is a salt sensitive crop. Soils becomes saline when electric conductivity of the soil is greater than 4 decisiemens per meter(dS/m), that is equivalent to 40 millimolar (mM) aqueous NaCl solution(Manishankar *et al*. 2018). In coastal region, the concentration of sodium ion in soils is observed to range from ~100 mM to 2380 mM sodium ion(Flowers 1985). Most crops can be able to stay alive or not to produce seeds or fruits under growth condition in the above said range. Therefore soil salinity is a major factor for limiting yield throughout the coastal areas of Africa and south Asia and south-eastern Asia (Flowers and Yeo 1981).

The Sunderban area (coastal area of the Bay of Bengal) of West Bengal in India is facing this issue. However, Rice genotypes show ample variations in salt tolerance (Sahi *et al.* 2006). Rising sea levels, salt accumulation, erosion and human activities lead to increase the salinity in the rice fields rapidly (Maclean *et al*. 2002). Salinity effects on rice both in morphological and physiological ways (Khatun and Flowers 1995). There are reports on the influence of sodium salt (mainly NaCl) stress on overall plant growth (Coca *et al.* 2023). Morphological changes in plant like reduced root development, leaf tillering, leaf rolling, chlorosis etc. were studied and analyzed the results. Plant intakes water for their growth from soil. This is occurring through osmosis. But osmosis depends on osmotic pressure which is controlled by dissolved salts in water. Decreased water content inside the plant is noticed because of upper concentration of electrolytes and lesser osmotic potential. This provokes physiological changes like photosynthesis inhibition, closure of stomatal (mainly to preserve water in side cell) etc. An accumulation of Na^+ salts also amplifies pH and alkalinity of soil. Reactive oxygen species (ROS) are produced by the plant during photosynthesis and respiration. Stress situations lead to the formation of ROS in higher amount, causing cell death (Gupta and Huang 2014). Calcium ion has been reported to restrict the entry of sodium ion into the plant cells (Kader and Lindberg, 2008), (Hussain et al. 2010) and improve the soil physical

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conditions (Qadir et al. 2001). This study comprises comparison of the effect of the combined action of sodium chloride (NaCl) and three types of calcium salts *i.e.* calcium chloride ($CaCl_2$), calcium sulphate ($CaSO_4$) and calcium carbonate ($CaCO_3$) on three rice varieties ('Chinsurah Nona-2', 'Dudherswar' and 'Pratiksha') by both morphological and biochemical estimations.

2. Materials and Methods

Three varieties of rice seeds, 'Chinsurah Nona-2' (CN2), 'Dudherswar' (DDH) and 'Pratiksha'(PAT), locally cultivable at salted soils of Gosaba (Sunderban) were collected from Gosaba Rice Research Centre, Gosaba, South 24-Parganas, West Bengal.

2.1 Experiment Designing and general Morphological

Seeds were grown with Hoagland media (Hoagland &Arnon 1950). The 15 days old seedlings were treated with 200 mM of NaCl solutions with the three different calcium salts-$CaCl_2$, $CaSO_4$ and $CaCO_3$ in 5 mM, 10 mM and 15 mM concentration. Rice seedlings from each concentration were randomly selected after seven days for the measurements of shoot length, biochemical estimations and estimations of antioxidant activities.

2.2 Estimations of Chlorophyll content

Chlorophyll content was measured by using the method of Arnon (1949) at 645 nm and 663 nm and calculated using the following equation: Chlorophyll a: $12.7(A_{663}) – 2.69(A_{645})$ and Chlorophyll b: $22.9(A_{645}) – 4.68(A_{663})$.

2.3 Estimations of Carbohydrates

Reducing sugars were extracted with hot 80% ethanol, then estimated by using Dinitrosalicylic acid reagent calorimetrically at 540 nm wavelength and starch was extracted with perchloric acid, then estimation with 'Anthrone reagent' (Miller1972).

3. Results and Discussions

3.1 Chlorophyll content and Shoot growth

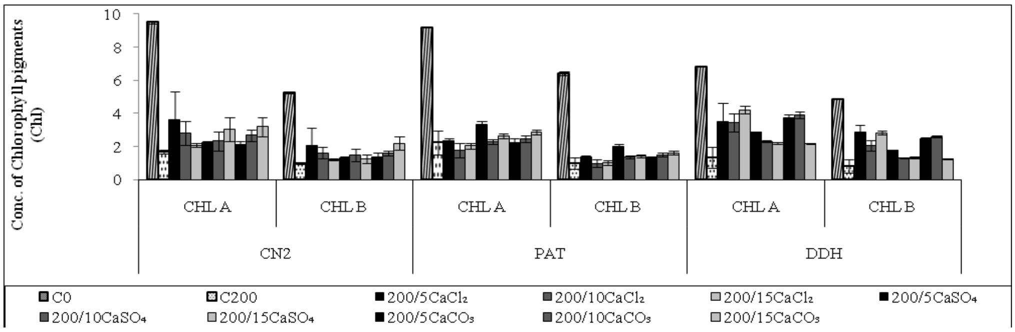
The Chlorophyll *a* content was reduced by 82%, 75% and 80% and Chlorophyll b content was reduced by 81%, 84% and 83% in CN2, PAT and DDH variety respectively in the salt stress set (200 mM NaCl conc.). The best recovery of Chlorophyll a was found in the following treatment sets *i.e.* 5 mM $CaCl_2$ (1.1 times), 5mM $CaSO_4$ (0.48times) and 15 mM $CaCl_2$ (2.1 times) in CN2, PAT and DDH variety respectively with respect to the. On the other hand, best recovery of Chlorophyll b was found in the following treatment set of 15mM $CaCO_3$ (1.1 times), 5mM $CaSO_4$ (1.1 times) and 15 mM $CaCl_2$ (2.4 times) in CN2, PAT and DDH variety respectively with respect to stress control set. Similar results have been found by Lutts *et al.* (1996) and Tahjib *et al.* (2018) where $CaCl_2$ treatments at 5 mM significantly increased Chl-a, Chl-b, and Chl-(a+b) contents under salt stress conditions in early vegetative stages of rice seedlings. Gaballah *et al.* (2007) showed that $CaCO_3$ helped to increase chlorophyll content in sesame plants.

Seedlings shoot growth was decreased 55%, 49% and 55% in CN2, PAT and DDH variety respectively in 200 mM NaCl with respect to control (normal tap water).The results show maximum recovery of shoot growth was found in 15 mM $CaSO_4$ treatment set 32% in CN2 variety, with respect to the seedlings stressed only with 200 mM NaCl; whereas, the shoot growth of DDH variety was markedly restored in 10 mM $CaCO_3$ set (100%). This result matches the findings of Lutts *et al*. (1996) and Tahjib *et al*. (2018) where salinity decreased growth of rice leaves and they have also found that shoot length can increase through supplementation with $CaCl_2$ to the salt-treated seedlings. Ornami and Hammes (2006) have found that $CaSO_4$ showed stronger ameliorative effect than $CaCl_2$ during the growth rate study of salt-stressed amaranth. Salty soil in coastal area decreases chlorophyll substance inside the plant. This is because of interruption of the photosynthetic path and development of oxidative stress. Reactive oxygen species (ROS) like superoxide ($O_2^{\bullet-}$), hydroxyl radicals (•OH), singlet oxygen, hydrogen peroxide (H_2O_2) etc. which are generated due to oxidative stress, destroys mainly chloroplasts. Moreover, electron transport chain can be interrupted by the salinity stress and this also intensifies ROS generation and as a result more chlorophyll degradation occurs. Calcium salts minimize uptake of Na^+ ions by the root of plant and this decreases toxicity of Na^+ in saline environment. Calcium ions boost the action of enzymes which are engaged in Calvin cycle. Thus efficiency of enzyme enhances rate of photosynthesis. So formation of chlorophyll occurs more rapidly. Water content inside the plant which is one of the key factor for perfect photosynthesis condition; is improved in presence of Ca^{2+} by amplifying hydraulic conductivity of root of a plant. So the said reasons can mitigate the adverse negative impact.

3.2 Carbohydrates content

The reducing sugar accumulation was increased 31%, 1.9 times and 1.2 times in CN2, PAT and DDH variety respectively in stressed set seedlings. The considerably best decrease in reducing sugar accumulation was found in the treatment set of 15 mM $CaSO_4$ (36%), 5 mM $CaCO_3$ (75%) and 5 mM $CaCl_2$ (88%) in CN2, PAT and DDH variety respectively, with respect to the200 mM NaCl-treated seedlings. Similar result was found by Hakim et al. (2014) in the rice variety MR52 and in Pokkali variety. On the contrary, starch content was decreased 16.6 times, 1.9 times and 56%in CN2, PAT and DDH variety respectively content in stressed set. However, the starch accumulation was significantly restored in the treatment set of 10mM $CaCl_2$ (5.1 times), 5mM $CaCO_3$ (6.3 times), and 10mM $CaSO_4$ (6.1times) in CN2, PAT and DDH variety respectively, with respect to stressed set. The findings of Amirjani (2011) also revealed that starch concentration decreased in rice seedlings under salt stress. Dubey and Singh (1999) showed that accumulation of sugars along with other compatible solutes contributes to an osmotic adjustment under salt stress and probably this high storage helps in basal metabolism under stress (Hurry et al. 1995). According to Krapp and Stitt 1995 starch may not play any important role in salt tolerance but metabolic alteration may cause reduction in starch content under salt stress.

The solubility of NaCl or inorganic salts is higher in water than carbohydrates. Moreover, monosaccharides are generally more soluble than disaccharides, oligosaccharides and polysaccharides considering their molecular weight. Therefore, less soluble carbohydrates in water are deposited rapidly than more soluble carbohydrates. Also solubility of carbohydrates in normal water is more than that for salty water. When plants are growing in salty soils, the aqueous portion inside the plant contains more inorganic salts than that for plants growing in normal soils. Thus deposition of comparatively soluble carbohydrate occurs rapidly under salt stress. Calcium ion is engaged in diverse physiological courses. These are controlled enzyme regulation, cell signalling etc. These may impact carbohydrate metabolism. Thus, though initial accumulation of carbohydrate was observed in salty soils, but decrease in carbohydrate accumulation was found upon mixing calcium ion in salinity condition.



Results of experiments of CN2, PAT and DDH. Different combinations of NaCl and Ca salts are marked as (C0- neither NaCl nor Ca salts added, C200-only 200mM of NaCl is applied, T1- 5mM of $CaCl_2$, T2- 10mM of $CaCl_2$, T3- 15mM of $CaCl_2$, T4- 5mM of $CaSO_4$,10mM of $CaSO_4$, 15mM of $CaSO_4$, 5mM of$CaCO_3$,10mM of $CaCO_3$ and 15mM of $CaCO_3$)(standard deviations are shown as bars)

4. Conclusions

The results showed that rice variety Dudherswar (DDH) and Pratiksha (PAT) are very susceptible to NaCl than salt tolerant variety Chinsurah Nona-2 (CN2).This study showed that exogenous Calcium can alter the salt stress in rice seedlings and also reveals that application of $CaCO_3$ and $CaSO_4$ are better than $CaCl_2$ to combat salinity for the cultivation of rice.

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