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SALINITY INDUCED METABOLIC CHANGES IN RICE (*ORYZA SATIVA* L.) SEEDS DURING GERMINATION

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Abstract

Six inbred lines of rice exhibiting differential tolerance to salinity were exposed to 0, 50, 75, 100 and 200mM NaCl for 24, 48, 72 and 96 h. The salinity induced metabolic changes (solute leakage, K efflux and α-amylase activity) were studied during germination. Germination of rice seeds was not affected by NaCl concentration less than 100mM. At higher salinity levels (100 & 200mM NaCl), a delay of 3-6 days in germination was observed. In the present study, comparatively higher values of solute leakage were observed in those lines in which germination was comparatively affected more adversely (sensitive). Sodium chloride reduced α-amylase activity in germinating rice seeds to varying degree even at low NaCl concentrations (50 & 75mM), where germination was not affected greatly. The tolerant lines exhibited higher enzymatic activity than the sensitive ones.

Introduction

Salinity is one of the most important agricultural constraint that hampers crop productivity throughout the world including Pakistan (Ashraf & Foolad, 2007; Mehmood *et al*., 2009). According to an estimate, 1/3 of irrigated land has been affected by salinity and saline area is increasing each year. In Pakistan alone, 40,000 hectares of arable land is lost annually due to salinity (Ahmad *et al*., 2006; Ashraf *et al*., 2008; Mehmood *et al*., 2009).

Rice (*Oryza sativa* L.), one of the important cereal crops, ranks second in consumption after wheat. This crop is regarded as a salt sensitive especially at young seedling stage, where varying degree of mortality occurs at 50mM NaCl in solution culture and in most salt sensitive varieties 50% of the population may die within ten days of salinization at the age of 14 days (Flowers & Yeo, 1981).

Under salinity, plant has to face both osmotic and ionic stresses which ultimately cause reduction in growth (Munns & Tester, 2008). In the presence of high salt concentration in the medium, osmotic potential is negative enough to cause water to diffuse out of tissue. It has been reported that stress environment affects membrane selective efficiency in germinating seed (Kawano *et al*., 2009; Lodhi *et al*., 2009), which ultimately results in excess absorption of toxic ion. Although ion absorption facilitates the osmotic adjustment, but may lead to ion toxicity, which in turn hampers enzymatic activity and nutritional balance (Ashraf *et al*., 2002; Ahmad *et al*., 2006; Acharya *et al*., 2008). Alpha amylase is an important key enzyme in carbohydrate metabolism that plays an important role in starch degradation in germinating seeds and mobilization of reserved material required for the growth of young seedling (Chen *et al*., 2006). Since the integrity of membrane and regulation of enzymatic activity is of particular importance especially under stress in retaining cell metabolite during the period before normal metabolic activity is resumed in developing seedlings, so considering this, experiments were conducted to study the effects of salinity on membrane permeability by determining solute leakage concentration in the growth medium and α-amylase activity in germinating rice seeds.

Materials and Methods

Two sets of experiments were conducted separately, one for solute leakage and germination responses and other for assaying α-amylase activity of germinating seeds. The six inbred rice lines (selected previously as salt tolerant and salt sensitive on the basis of their differential tolerance to salinity by Shereen *et al*., (2005) from a large number of rice lines obtained from IRRI, Philippines) alongwith locally developed salt tolerant variety Shua-92 (Check) were included in this study. The experiments were conducted in germinators (Naqvi *et al*., 1994). Seeds were sterilized in 1% NaOCl solution for 20 minutes and washed thoroughly three times with distilled water and were planted over the nets in germinators containing treatment solution with different concentrations of NaCl (0, 50, 75, 100 and 200mM) for germination and solute leakage studies and at 0, 50 and 75mM NaCl for determining α-amylase activity. These germinators were covered with polyethylene sheets to minimize evaporation. The whole setup was then placed in complete darkness in an incubator at temperature 30/28°C day and night cycle.

From the first set of experiment, solute leakage in the treatment solution was determined after the exposure of treatment for 48, 72 and 96 h by measuring OD at 280nm in Hitachi Spectrophotometer (Model 150-20). Potassium efflux was also determined in leachates by using Flame Photometer. From set number 2 after 72 h, the germinating seeds were analyzed for α-amylase activity by following the method of William & Peterson (1973). The whole experiment was repeated thrice. The data were analyzed statistically by using ANOVA and DMRT was applied for comparison among treatment means using computer software MSTAT-C.

Results and Discussion

All rice lines showed more than 80% germination up to 75mM NaCl at 72 h (Table 1), while at higher salinity levels, these lines germinated after a delay of 3-6 days in 100 and 200mM NaCl, respectively. At 100mM NaCl, the germination in most of the lines was only slightly affected (7–19%) except line no. 2 where a reduction of 41% was observed. At the higher salinity level (200mM NaCl), pronounced differences among these lines were observed. The highest germination (80%) was observed in check variety (Shua–92) and least in line no. 2 (38%), while among the tested lines, highest germination (77%) was observed in L-43 and L-104. On the basis of these results it can be concluded that rice plant is fairly tolerant at germination stage as also reported by Lee *et al.,* (1999).

While estimating solute leakage at different time periods, it was observed that leakage of solutes was about the same in all rice lines at 48 h growth period. The leakage of solutes constantly increased with time and became maximal at 96 h (Table 2). However, no significant differences were observed among the treatments upto 75mM NaCl at 72 h (data not shown). At 96 h, a pronounced increase in solute leakage was observed with the increase in salinity treatment, when comparison was made with their respective controls. The least increase was observed in Shua–92 at each level of salinity and highest in line no. 2 and 64. In the present study, comparatively high values of solute leakage were observed in those lines (line nos. 2, 12, 64, 96) in which germination was comparatively affected more adversely at 200mM NaCl.

The data of K effluxes under different salinity treatments (50 and 75mM NaCl) at 72 h have shown highest K efflux in sensitive lines (line no.2 followed by line no. 64 and line no.12), while the least increase was found in check variety (Shua-92). The tolerant lines (L-96, L-104, and L-43) have shown comparatively less increase in K efflux than the sensitive lines (Table 3).

A decrease in activity of ∝-amylase was observed in a salt concentration dependent manner (Table 4). When the enzymatic activity was compared on the basis of relative reduction in comparison to their respective control, significant differences were observed among different lines. At lower level of salinity (50mM NaCl), all tolerant lines showed only a slight reduction in ∝- amylase activity. The sensitive lines (L-2 & L-64) exhibited a reduction of 10-15% even at lower level of salinity. At 75mM NaCl salinity, the maximum reduction (36%) in activity of ∝- amylase has been observed in line no. 64 (sensitive) followed by line no. 2 (28%). Among the tolerant lines, the line no. 96 exhibited a least reduction (6%).

Table 1. Effect of salinity on germination % of different rice lines.

L. No.	Salinity level (mM NaCl)				
	Control	50	75	100	200
2	98	97	98	58	38
12	95	96	87	82	65
43	92	88	88	85	77
64	95	90	85	82	65
96	100	100	92	81	53
104	100	98	100	87	77
Shua–92	98	95	90	85	80

LSD at $p<0.05$ = 7.66

Table 2. Effect of salinity on solute leakage in rice lines at 96 h.

L. No.	Salinity level (mM NaCl)				
	Control	50	75	100	200
2	0.598 (0)	0.671 (12)*	0.838 (40)	0.975 (63)	1.013 (69)
12	0.543 (0)	0.571 (5)	0.682 (26)	0.823 (52)	0.865 (59)
43	0.512 (0)	0.559 (9)	0.647 (26)	0.758 (48)	0.780 (52)
64	0.538 (0)	0.534 (-1)	0.638 (19)	0.738 (37)	0.865 (61)
96	0.615 (0)	0.671 (9)	0.678 (10)	0.715 (16)	0.945 (54)
104	0.498 (0)	0.485 (-3)	0.566 (14)	0.653 (31)	0.758 (52)
Shua-92	0.512 (0)	0.471 (-8)	0.543 (6)	0.660 (29)	0.710 (39)

LSD at $p<0.05$ = 0.16

* Figures in parentheses indicate % increase (+) and decrease (–) over control.

Table 3. K effluxes (ppm) under different levels of salinity.

L. No.	Salinity level (mM NaCl)		
	Control	50	75
2	5.50 (00)	10.16 (85)*	11.50 (109)
12	9.00 (00)	13.91 (55)	15.78 (75)
43	7.00 (00)	11.33 (62)	11.91 (70)
64	6.50 (00)	11.00 (69)	11.17 (72)
96	9.42 (00)	15.75 (67)	15.83 (68)
104	9.50 (00)	13.33 (40)	14.00 (47)
Shua-92	5.50 (00)	7.00 (27)	8.66 (58)

LSD at $p<0.05$ = 2.09

* Figures in parentheses indicate % increase (+) over control.

Table 4. Effect of salinity on α-amylase activity in different rice lines.

L. No.	Salinity level (mM NaCl)		
	Control	50	75
2	5.68 (0)	4.79 (-15.67)	4.08 (-28.17)
12	5.21 (0)	5.42(+4.03)*	4.69 (-9.98)
43	4.62 (0)	4.22 (-8.66)	3.47 (-24.90)
64	6.13 (0)	5.53 (-9.79)	3.93 (-35.89)
96	5.18 (0)	4.95 (-4.44)	4.87 (-5.98)
104	5.35 (0)	5.12 (-4.29)	4.76 (-11.02)
Shua-92	5.97 (0)	5.63 (-5.69)	5.43 (-9.04)

LSD at $p<0.05$ = 0.73

*Figures in parentheses indicate % increase (+) and decrease (–) over control.

Discussion

Germination is a complex phenomenon involving many physiological and biochemical changes that lead to the activation of embryo. Salinity induces numerous disorders in seeds during germination. Firstly, it reduces the imbibition of water because of lower osmotic potential of the medium (Almansouri *et al*., 2001; Munns & Tester, 2008) and secondly it causes mineral imbalances and toxicity (Rajendran *et al*., 2009). Cell membranes are the major sites for controlling active and passive solute fluxes, and thus membranes may be of special importance to plants for regulating the ion uptake (Munns & Tester, 2008). Mineral imbalances of the saline environments often affect the structure and chemical composition of bilayer lipid membrane and may thereby affect membrane selective ability to transport, solutes and ions inwards and also becomes leaky to solutes they contain (Cushman, 2001; Lodhi *et al*., 2009). This was also observed in the present study that solute leakage and K efflux in germinating seeds increased. This increase was dependent upon the differential permeability of seed coat and the toxicity of salt, which showed variation in the membrane potential of germinating seedlings under salinity stress. Furthermore, an inverse relation was also observed between solute leakage and germination of seeds. The rice lines which have minimum leakage of solutes exhibited comparatively more germination at higher salinity (100 and 200 mM NaCl) than those rice lines in which the leakage of solutes was maximum. Kawano *et al*., (2009) relates the reduced membranes selectivity with decrease in generation of ATP at electron transport level, which results in increased membrane permeability and solute leakage.

At the onset of germination, synthesis of enzymes and changes in the metabolic pattern are initiated but salt stress either alters it or does not permit the synthesis of specific metabolite required for seed germination (Pattanagul & Thitisaksakul, 2008; Kaya *et al*., 2008; Zapata *et al*., 2004; Acharya *et al*., 2008). Mobilization of seed reserves, which occurs during early seed germination, is crucial because it supplies substrate for proper functioning of different metabolic processes that are essential for growth of the embryonic axis. Changes in growth during initial stages due to salinity would therefore depend on differences in translocation of assimilates from the seed and later on other assimilatory process. Although the rice lines in this study showed tolerance at germination stage. However, it was noted that inhibitory effects of salinity started at this stage as observed from reduction in activity of α-amylase in germinating seeds under salinity. Similar inhibitory effects of NaCl on enzymatic activity were observed in different crops (Almansouri *et al*., 2001; Ashraf *et al*., 2002; Marambe & Ando, 2008). This reduction in enzymatic activity may be one of the factors for reduced assimilate transportation and reduction in growth at seedling stage.

In conclusion, α-amylase activity in germinating seed represents an important factor contributing to seedling development and vigor, which is an important agronomic trait. Unraveling these mechanisms may provide important information regarding how to improve the seedling vigor under salinity stress.

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