Pak. J. Bot., 39(7): 2527-2534, 2007.

PHYSIOLOGICAL RESPONSES OF RICE (*ORYZA SATIVA* L.) TO SALINE STRESS

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Abstract

A water culture experiment was conducted to study the physiological responses of salt tolerant and salt sensitive inbred lines of rice. Different growth and physiological attributes including ion uptake and synthesis of proline were studied after an exposure of two weeks salinity treatments (50 and 75mM NaCl along with non-saline control). The studies revealed that growth at early seedling stage was very sensitive. Data on ions uptake and proline contents of shoot revealed that in tolerant lines there was a less uptake of Sodium and higher production of proline contents in comparison to sensitive lines. A significant positive correlation was found between shoot Na and proline contents.

Introduction

Soil salinity is one of the most serious problems for irrigated agriculture, which drastically affect crop productivity throughout the world. High salinity causes both hyper osmotic and ionic stress, which results in alteration in plant metabolism including reduced water potentials, ionic imbalances and specific ion toxicity (Abdullah *et al*., 2002; Tester & DavenPort, 2003; Munns *et al*., 2006).

Plants have developed a number of adaptive characters against these stress factors that may cause alteration in different physiological processes and biochemical pathways to minimize the damaging effects of excess salts on cellular structure, function and several enzymatic activities. These adaptations may include ion compartmentation and osmoregulation. Plant cells accumulate proline and several other kinds of organic and inorganic solutes (i.e., nitrogen containing compounds like amino acids, QAC and polyamines, hydroxyl compounds like sucrose, polyols and oligosaccharides) as osmoprotectants to conserve osmotic stability and prevent damage. The effect of salt stress on proline accumulation is reported in many plant species (Yoshiba *et al*., 1997; Khan *et al*., 1999, 2000; Akram *et al*., 2007). The role of proline under stress has been a subject of controversy because it accumulate to a very high concentration under adverse saline conditions. Rice is widely documented as one of the most important crop sensitive to salinity. The role of proline accumulation in salt tolerance of rice is still unclear and contradictory (Gonzalez & Labrada, 1995; Yeo, 1998; Igarashi & Yoshiba, 2002).

The present study was carried out to compare the responses of salt tolerant and salt sensitive rice lines in their proline synthesis and to understand the relationship between sodium uptake and proline synthesis in shoot with reference to differential tolerance towards salinity.

Materials and Methods

Six inbred lines of rice along with locally developed salt tolerant variety, Shua-92 (check) (screened by Shereen *et al*., 2005 from a large number of rice lines obtained from IRRI, Philippines) initially selected as a salt tolerant and salt sensitive, were included in this study. The line numbers 12, 43, 96 and 104 were found to be tolerant and line numbers 2 and 64 were sensitive. Seeds sterilized with 1% commercial bleach were soaked and planted on a nylon netted frame (5 x 7″) fitted in plastic containers having a capacity of 2.5 liters culture solution (Yoshida *et al.*, 1976). These boxes were placed for germination at 35°C in an incubator under dark condition.

Seven days old seedling were transplanted and salinized with 50 and 75mM NaCl at the age of 14 days. The culture solution was renewed twice a week. Electrical conductivity of treatment solutions were maintained at the desired levels by topping up with culture solution during the entire period of study. After imposition of salinity treatments (0, 50 and 75mM NaCl) for the period of two weeks, three plants from each treatment were harvested. The shoot, root lengths and fresh, dry weights were recorded. The shoots were analyzed for sodium and potassium concentrations. The proline contents were analyzed by following Bates *et al*., 1973.

Results and Discussion

When the growth performance of these lines was compared under saline stress, it was observed that salinity caused a varying degree of reduction in seedling growth among these lines (Tables 1 & 2). There was no significant reduction in root and shoot lengths of all tested lines under different salinity levels except line number 64, where a slight reduction of 17% was observed in shoot length at higher level of salinity i.e., 75mM NaCl (Table 1).

Shoot and root fresh weights of all these lines decreased significantly as the levels of salinity increased from 50 – 75mM NaCl (Tables 1 & 2). At lower level (i.e., 50mM NaCl) of salinity comparatively less reduction in the fresh weights were observed. The higher salinity level (75mM NaCl) caused a significant reduction in seedling growth when compared with control. The line no. 12, 2 and 64 exhibited 55, 60 and 65% reduction, respectively, while the least reduction (-7%) in fresh weight was observed in line no. 96 (Table 1).

Dry weights of these genotypes showed a decline when compared with their respective controls. All lines showed a slight reduction at lower level of salinity (50mM NaCl). However at higher salinity level (75mM NaCl) these lines exhibited differential response. The highest reductions in dry weights were observed in Line no. 64 and 12 (56 and 51%) while line no. 96, 43 and Shua-92 exhibited least reduction (10, 16 and 7% compared to their respective controls) and proved to be comparatively tolerant than the other lines (Table 1).

The effect of salt concentration on root growth was comparatively less severe than that observed on shoots growth (Table 2). The lower level of salinity (50mM) slightly affected root growth and a reduction of 9-26 % was observed among different rice lines with least reduction in line no. 104, 96 and Shua-92. The reduction in fresh weights of roots became more pronounced at 75mM NaCl where it varied from 14–61%. The Line no. 96 produced better root growth than Line no. 64. Dry weight of root when compared on the basis of percent reduction under different levels of salinity in comparison to their respective controls showed that dry weights reduced to varying degree among these lines. The highest reduction was observed in line no.64, where degree of reduction was increased from 35-67% at 50 and 75mM NaCl salinity. The least reduction was observed in Shua-92 and line no.96.

Table 1. Effect of salinity on shoot growth of rice at seedling stage

L. No.	**Shoot height (cm plant^{-1})**			**Fresh weights (g plant^{-1})**			**Dry weight (mg plant^{-1})**		
	Control	**mM NaCl**		**Control**	**mM NaCl**		**Control**	**mM NaCl**	
		50	**75**		**50**	**75**		**50**	**75**
2	20.33 (0.00)	20.26 (-0.3)	19.46 (-4)	0.88 (0.00)	0.46 (-48)*	0.35 (-60)	136.50 (0.00)	112.57 (-18)	86.30 (-37)
12	20.00 (0.00)	20.67 (+3)	17.23 (-14)	0.89 (0.00)	0.74 (-17)	0.40 (-55)	155.80 (0.00)	136.50 (-12)	76.43 (-51)
43	24.80 (0.00)	24.23 (-2)	24.17 (-3)	0.76 (0.00)	0.74 (-3)	0.53 (-30)	146.23 (0.00)	127.77 (-13)	123.13 (-16)
64	29.50 (0.00)	27.37 (-7)	24.50 (-17)	1.10 (0.00)	0.93 (-16)	0.38 (-65)	196.37 (0.00)	165.40 (-16)	85.77 (-56)
96	21.57 (0.00)	20.70 (-4)	21.77 (-1)	0.84 (0.00)	0.83 (-1)	0.78 (-7)	164.17 (0.00)	160.57 (-2)	148.43 (-10)
104	26.10 (0.00)	25.47 (-2)	25.17 (-4)	0.98 (0.00)	0.95 (-3)	0.65 (-34)	186.93 (0.00)	160.53 (-14)	119.70 (-36)
Shua-92	29.15 (0.00)	28.52 (-2)	27.82 (-5)	1.18 (0.00)	0.95 (-19)	0.94 (-20)	176.90 (0.00)	172.72 (-2)	164.34 (-7)
LSD at α 0.05	2.14			0.25			34.24		

*Figure in parenthesis indicates % increase (+) and decrease (-) over control.

Table 2. Effect of salinity on root growth of rice at seedling stage

L. No.	**Root length (cm plant^{-1})**			**Fresh weights (g plant^{-1})**			**Dry weights (mg plant^{-1})**		
	Control	**mM NaCl**		**Control**	**mM NaCl**		**Control**	**mM NaCl**	
		50	**75**		**50**	**75**		**50**	**75**
2	16.60	17.10	15.40	0.50 (0.00)*	0.40 (-20)	0.39 (-22)	55.25	40.10 (-27)	34.67 (-37)
12	16.07	20.47	17.37	0.72 (0.00)	0.75 (4)	0.42 (-42)	66.87	70.86 (6)	40.50 (-39)
43	9.43	12.00	10.27	0.54 (0.00)	0.45 (-17)	0.26 (-52)	52.10	50.83 (-2)	38.20 (-27)
64	11.66	11.43	10.87	0.84 (0.00)	0.62 (-26)	0.33 (-61)	88.60	57.63 (-35)	29.63 (-67)
96	11.16	11.83	11.50	0.63 (0.00)	0.55 (-13)	0.54 (-14)	73.13	64.60 (-12)	57.10 (-22)
104	17.50	19.90	15.33	1.00 (0.00)	0.91 (-9)	0.69 (-31)	99.87	87.00 (-13)	54.87 (-45)
Shua-92	11.84	12.72	11.66	0.74 (0.00)	0.64 (-14)	0.61 (-18)	52.10	47.56 (-9)	41.24 (-21)
LSD at α 0.05	NS**			0.18			17.2		

*Figure in Parenthesis indicates % decrease (-) over control.
**Not-significant

The ions concentration calculated on unit dry weight basis showed that salinity caused an increase in the concentration of Sodium (%) in shoot and root of all rice lines (Table 3). This increase was dependent on concentration of salt in the medium. Under non-saline conditions the roots accumulated comparatively more Sodium than shoots. While under saline condition it was observed that all rice line exhibited comparatively less Sodium concentration in roots than that observed in shoots. The line no. 12, 43 and 96 (tolerant) accumulated less Sodium and exhibited less increase in Sodium concentration comparative to the control. The line no.64 (sensitive) accumulated higher Sodium concentration and exhibited a highest increase (187%) in comparison to their control. At lower level of salinity (50mM NaCl), shoot Sodium concentrations when compared to their respective controls, the minimum increase was observed in L–96 (118%) and maximum in L–64 (620%). At the higher level of salinity (75mM NaCl) maximum increase (820%) was observed in L–64 (salt sensitive) and minimum (251%) in L–12 (Tolerant).

The Potassium concentrations when calculated on a unit dry weight basis showed higher concentrations in shoot than in root. With the increase in Sodium concentration the Potassium concentration decreased in both root and shoot. This reduction was more pronounced in roots. The line no. 96, 43 and 104 have shown less reduction in root and shoot Potassium concentration at higher salinity level (Table 4). While line no. 43 exhibited a slight stimulation in K concentration at lower level of salinity (50mM NaCl) and least reduction in Potassium concentration was observed at 75mM NaCl. The reduction in K concentration was more pronounced in roots as well as in shoots of the sensitive lines (line no. 2 & 64).

Salinity damage is usually attributed to water deficit and excessive ion entry, which may further interfere with nutrient uptake. In the present study, the genotype with lowest Na^+ concentration produced comparatively more shoot growth (in term of fresh and dry weight). An inverse correlation (r= -0.6847) was observed between Sodium concentration and shoot growth indicated an association between ion selectivity and salt tolerance. Similar relationship between shoot dry matter and leaf Sodium was observed in many plant species (Lee *et al*., 2003; Munns & James, 2003; Zhu *et al*., 2004). The effect on growth was probably due to a better carbon balance in the genotypes with less Sodium (Munns *et al*., 2006).

A substantial body of information in literature indicates that the plant may not exhibit the same response / function under saline conditions as it does under non-saline condition. Numerous studies have shown that the K concentration in plant tissue is reduced as Na salinity or the Na^+/Ca ratio in the root media is increased (Lutts & Guerrier, 1995; Grattan & Grieve, 1999; Shin & Lee, 1999).

The maintenance of adequate levels of K^+ is essential for plant survival in saline habitats. It contributes to reducing the osmotic potential in root cells and facilitate solute transport process to sustain the overall water balance of the plant. Because plasma membrane of the root cortical cells have a high affinity for K^+ over Na^+ even though the degree of selectivity can vary quite drastically among species. Sodium transport from the environment into the cytoplasm of the plant cell is a passive process. It depends on the electrochemical potential gradient of Na^+ and the presence of Na- permeable channels in the plasma membrane, which allow Na^+ permeation. Under saline conditions salinity affects sterols and phospholipids composition of plasma membrane thereby inducing structural changes in bilayer lipid membrane. This causes depolarization of plasma membrane which affect regulation and selectivity of these channels making it more permeable to ions. Regulation and selectivity of such channel seems to be responsible for Na exclusion in many salt tolerant plants (Jacoby, 1999). This differential selectivity of plasma membrane may be a contributing factor in sensitivity/tolerance of these genotypes.

The proline concentration (μmole proline/g shoot F.wt.) of these lines were compared under different levels of salinity (0, 50, and 75mM NaCl) (Table 5). It was observed that proline increased with varying rate under different salinity levels (50, 75mM NaCl). This increase was dependent on the increase in shoot Sodium concentration as well as upon the inherent ability to synthesize proline (Wanichananan *et al*., 2003). A significant positive correlation exists between shoot Sodium and proline concentration (r = 0.722). The maximum increase in proline concentration was observed in tolerant line (L–96) and salt tolerant check (Shua-92) comparative to sensitive ones.

Table 3. Effect of salinity on Sodium concentration (%) in shoot and root of different rice (*Oryza sativa* L.) lines.

L. No.	Shoot Na (%)			Root Na (%)		
	Control	mM NaCl		Control	mM NaCl	
		50	75		50	75
2	0.66 (0.00)	2.01 (205)*	2.96 (348)	0.59 (0.00)	1.52 (158)	1.59 (170)
12	0.86 (0.00)	2.59 (201)	3.02 (251)	0.73 (0.00)	1.28 (75)	1.67 (129)
43	0.45 (0.00)	2.07 (393)	2.81 (569)	0.74 (0.00)	1.55 (110)	1.66 (124)
64	0.3 (0.00)	2.16 (620)	2.76 (820)	0.69 (0.00)	1.56 (126)	1.98 (187)
96	0.39 (0.00)	0.85 (118)	2.03 (421)	0.63 (0.00)	1.10 (75)	1.49 (137)
104	0.57 (0.00)	2.78 (388)	3.71 (551)	0.69 (0.00)	1.43 (107)	1.77 (157)
Shua-92	0.40 (0.00)	1.51 (277)	2.39 (498)	0.65 (0.00)	1.71 (163)	2.39 (268)
LSD at α 0.05	0.69			1.04		

*Figure in parenthesis indicates % increase (+) over control.

Table 4. Effect of salinity on Potassium concentration (%) in shoot and root of different rice lines.

L. No.	Shoot K (%)			Root K (%)		
	Control	mM NaCl		Control	mM NaCl	
		50	75		50	75
2	2.12 (0.00)	1.95 (-8)	1.44 (-32)	2.23 (0.00)	1.17 (-48)	1.09 (-51)
12	1.76 (0.00)	1.72 (-2)	1.43 (-19)	1.79 (0.00)	1.51 (-16)	1.02 (-43)
43	1.71 (0.00)	1.85 (+8)	1.66 (-3)	1.22 (0.00)	1.00 (-18)	0.62 (-49)
64	2.46 (0.00)	2.13 (-13)	1.65 (-33)	1.46 (0.00)	0.88 (-40)	0.61 (-58)
96	2.07 (0.00)	1.96 (-5)	1.83 (-12)	1.57 (0.00)	1.05 (-33)	0.98 (-38)
104	1.91 (0.00)	1.86 (-3)	1.46 (-24)	1.60 (0.00)	1.06 (-34)	1.01 (-37)
Shua-92	2.19 (0.00)	1.98 (-10)	1.70 (-22)	2.02 (0.00)	1.50 (-26)	1.00 (-50)
LSD at α 0.05	0.30			0.34		

*Figure in parenthesis (-) indicates % decrease over control.

When the responses of these lines were compared at higher salinity level (75mM) in relative terms (% increase in Na and proline under salinity in comparison to control) on the basis of ion uptake and related it with proline production. It was observed that in tolerant line (L.No.96), there was less uptake of Sodium ion (420% increase in comparison to control) and more proline production (1973% increase in comparison to non-saline treatment). While in sensitive line (L.No.64), there was 1706% increase of

Table 5. Effects of salinity on proline (μmol g^{-1} F.Wt.) in different rice (*Oryza sativa* L.) lines.

L. No.	**Proline concentration (μmol g^{-1} F.Wt.)**		
	Control	**mM NaCl**	
		50	**75**
2	1.17 (0.00)	6.45 (451)*	13.46 (1050)
12	0.77 (0.00)	4.20 (446)	5.23 (579)
43	0.86 (0.00)	6.58 (665)	9.81 (1041)
64	0.70 (0.00)	6.64 (849)	12.64 (1706)
96	0.78 (0.00)	8.88 (1039)	16.17 (1973)
104	1.28 (0.00)	7.27 (468)	12.00 (838)
Shua-92	0.67 (0.00)	7.37 (1000)	14.16 (2013)
LSD at α 0.05	2.98		

*Figure in parenthesis indicates % increase (+) over control.

proline production at 75mM NaCl. Besides this high rate of proline production; the rate of increase in Sodium was twice (820% increase) of the tolerant lines (420% increase). Due to which, this line was unable to adjust osmotically and suffered to a greater extent. Line no. 43 & 104 exhibited intermediary response in proline production It was evident from these results that L–96 was efficient in producing comparatively higher quantity of proline to cope with toxic effects of Na in the shoots.

Salt injury in rice involves water stress. In the presence of high salt concentration in the soil solution the osmotic potential is negative enough to cause water to diffuse out of the tissue into the surrounding solution, unless the water potential in the tissue is at least as negative as in soil solution and ideally it must be more negative than that of the surrounding solution if the tissues are to absorb water and survive. One way to overcome this problem would be for the cells simply to accumulate salts to the same or higher concentration as those found outside plants. But this does not happen due to the fact that salts such as NaCl, denature the enzymes and these cannot be tolerated in the cytoplasm itself. Many plants that tolerate various kinds of salt and water stress do so by synthesizing in their cytoplasm the compounds that can exist at high concentration without denaturing enzymes essential for metabolic process of life. These organic compounds are known as compatible solutes. Many plants accumulates compatible osmolytes such as sugar, alcohol and proline or glycine betaine when they are subjected to drought or salinity stress (Yoshiba *et al*., 1997; Khan *et al*., 1999, 2000). Considerable research has been conducted to characterize the accumulation of proline, a compound known to contribute to the osmotic adjustment and tolerance of plants exposed to unfavorable stress (Yoshiba *et al*., 1997; Aziz & Khan, 2000, 2001 and 2003). There are many reports regarding variation in genotypic resistance to environmental stress. This response differs between cultivars adapted to certain growth condition, as well as within the species more or less tolerant to salinity or drought. Earlier studies have reported that higher accumulation of proline in salt sensitive varieties (MI-48 & Perla) than the salt tolerant Pokkali and IR-42 (Gonzalez & Labrada, 1995). Most of the research carried out with rice varieties subjected to salt stress showed higher level of proline in tolerant than in susceptible genotypes. Igarashi & Yoshiba (2002) reported less proline accumulation in salt sensitive rice variety (IR-28) than DGWG (salt tolerant). However, Wanichananan *et al*., (2003) reported that among the salt tolerant rice lines, the genotype Leuang Tang Mo accumulated 8 times more proline in shoot than when grown in normal soil.

Major differences in osmotic adjustment through proline accumulation were also found in crops cultivars despite identical water potentials implying genetic variations (Heuer, 1999). An increase in the proline content may be associated with either enhanced biosynthesis, with stimulated proline oxidation or an impaired protein synthesis, and thus providing several possible physiological functions. These functions include water potential balance between cytoplasm and surrounding environment. Proline may affect the solubility of various proteins, thus protecting against denaturation under water stress conditions, contributes to the maintenance of cell growth through protective effects on photosynthetic pigment under condition of increased ion concentration (Hare & Cress, 1997; Iyer & Caplan, 1998; Igarashi & Yoshiba, 2002; Xiong *et al*., 2002; Ashraf & Foolad, 2007).

The accumulation of proline in response to stress indicates that its synthesis is a non-specific response to decreased H_2O potential. However it can be concluded that, in general, proline accumulation is specific to genotypes. The different rate of proline accumulation in response to salt stress indicate genetic variability for its *denovo* synthesis and this may be used as a physiological selection criteria for inducing salt tolerance.

Acknowledgement

We are thankful to Mr. Saqib Ali Memon (NIA, Tandojam) for composing and typing the manuscript.

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(Received for publication 14 February 2006)