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EFFECT OF TRANSPIRATION RATE ON SODIUM ACCUMULATION IN RICE (*ORYZA SATIVA* L.) GROWN UNDER SALINE CONDITIONS

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Abstract

Uptake and transporting pattern of toxic ions to the shoot is a crucial response by plant under saline conditions affecting metabolic activities and thereby determines salt sensitivity/tolerance in rice. Experiments were conducted to compare the rate of transpiration and its effect on sodium accumulation in shoot of two inbred rice lines (IR55178 – 163 tolerant and IR55178 -104 sensitive differing in salt tolerance along with a salt tolerant check Shua-92) under different levels of salinity (0, 50 and 75 mM). The parameters studied indicated that transpiration rates were sharply decreased in response to salinity in both genotypes. While, sodium uptake continued to increase however, the tolerant genotype maintained transpiration rate quite satisfactorily and also exhibited ability to maintain higher K: Na ratio and low Na concentration in the xylem than the sensitive genotype. It is concluded that transport of sodium ions in the present study was negatively correlated with transpiration (r = -0.806).

Key words: Ion uptake, *Oryza sativa*, Salt stress, Transpiration and Xylem concentration.

Introduction

Soil salinity is one of the most adverse abiotic stresses that crops are faced, around the globe (Das *et al.,* 2015). This problem is of great concern in arid and semiarid areas of south and south East Asia where rice is an important staple crop (Iqbal *et al*., 2010). According to Munns & Tester, 2008; about 50% reduction in grain yield may occur at an electrical conductivity of 6 dS m^{-1}. It is reported that out of 137 m ha of rice cultivable land in Asia, about 30% is salt affected that hinders normal rice productivity (Anon., 2012; Mishra, 2004).

Generally the plant face the problem in water uptake, ion regulation, nutrient balance, stomatal control, CO_2 assimilation, and reactive species quenching mechanisms when grown in high salinity and thus reduce their growth (Munns & Tester, 2008). Sodium is generally the main toxic ion under such conditions which is taken up by the root system and transported to the shoot via transpiration stream consequently accumulating in aerial plant tissues (Tester & Davenport, 2003; Moller & Tester, 2007). Salt tolerance in grasses was found to be negatively correlated with shoot Na^+, Na^+/K^+ of tissue sap while positively linked with selectivity and potassium-use efficiency (Flowers and Colmer 2008; Marcum *et al.,* 1998). The uptake of essential minerals like K alter under higher Na^+/K^+ medium is closely linked with the transpiration mechanism of plant (Garcia *et al*., 1997; Tester & Davenport, 2003). The fundamental processes governing the relationship between water and ion flow are complex and not well understood. Very limited information are available about these regulatory processes and their significance in adaptation of rice plants to saline conditions (Kronzucker & Britto, 2011).Salt sensitivity varies with duration of exposure to salt and growth stages, especially in rice early seedling stage is considered vulnerable to salt. Therefore the present study was conducted at this stage to understand the pattern of transpiration over different time periods and its role in sodium uptake and K homeostasis of rice genotypes differing in their salt sensitivity.

Materials and Methods

Two inbred rice lines from IRRI, Philippines (IR55178 – 163 tolerant and IR55178 -104 sensitive) selected from our earlier studies (Shereen *et al*., 2005) along with a locally developed salt tolerant variety (Shua-92) were used. Seeds were sterilized in 1% NaOCl solution for 20 minutes and washed thoroughly three times with distilled water. Sterilized seeds were germinated in dark (four days) at 30°C in incubator and were further raised for seven days (photoperiod 16 h day/8 h night with 22 W m^2 light intensity). Seven days old seedlings were transplanted on perforated lids of plastic boxes containing 2.5 L of nutrient solutions adjusted at pH 5 (Yoshida *et al.*, 1976). Salinity treatments of NaCl (0, 50 and 75 mM) were imposed after 7 days of seedling acclimatization. Three boxes of each treatment were kept without plants to measure the evaporation rate. The mean transpiration rate was determined by gravimetric method (Udayakumar *et al*., 1998). The boxes were initially weighed with plants at the time of NaCl application and thereafter were weighed on daily basis to determine the loss in weight (by subtracting in the initial weight). Equal volume of nutrient solution was added to bring back to initial weight. Three harvests (five plants) were made at weekly interval. After every harvest, the remaining weight of each box was recorded and the solutions were renewed and weighed taken as the initial weight till the next harvest. The harvested plants were separated into root and shoot on single plant basis, rinsed twice with distilled water and blotted dry. Shoot and root fresh weights (FW) were recorded and samples were oven dried and analyzed for Na^+ and K^+ by flame photometer (Yeo & Flowers, 1983). Relative growth rate (RGR) was calculated using the formula (Hoffman & Poorter, 2002).

$$\mathbf{RGR} = \left(\overline{\ln W_2} - \overline{\ln W_1}\right)/(t_2 - t_1),$$

where $\overline{\ln W_1}$ and $\overline{\ln W_2}$ are the means of the natural logarithm-transformed plant weights.

Water use efficiency (WUE) was calculated on the basis water consumed to produce per gram FW of plant. Graph (WUE) was plotted on the basis of total water consumed and average FW (average of five plants) produced. Xylem concentrations (µM Na) were calculated by following the methods of Rozema *et al*., 1981. Data were statistically analyzed for analysis of variance (ANOVA) and Duncan multiple range test (DMRT) was applied for comparison among treatment means using computer software MSTAT-C.

Results

Effects of salinity on growth: Salinity reduced shoot fresh weight more than the root (Table 1). Genotype IR55178 -104 (sensitive) exhibited least reduction during 1st week while, at subsequent growth stages, drastic reduction in shoot fresh weight (59% and 65%) was observed at 50 mM and 75 mM NaCl, respectively. On the other hand, tolerant genotypes (IR55178 – 163 and Shua – 92) maintained good growth constantly at 50 mM NaCl throughout the growth period. The higher level of salt (75 mM NaCl) had deleterious effect here as well but these genotypes exhibited less reduction (53% and 60% respectively) compared to the sensitive one. Relative growth rate (RGR) decreased with the passage of time and also decreased with the increase in salinity. Drastic reduction in RGR was observed during second week of growth under both salinity treatments. The tolerant line IR55178 – 163 maintained their RGR throughout the growth period under salinity (Table 1).

Effects of salinity on ions and water relations: Salinity significantly increased sodium uptake in shoot. This increase was dependent on external concentration of salt in the medium and duration of exposure (Table 2). Among the lines , IR55178 – 163 accumulated less sodium and also exhibited less relative increase in shoot Na in comparison to IR55178 -104 at each stage of growth. The Na concentration in xylem (µM) has reflected differential sodium uptake ability of these genotypes (Table 2). Shua-92 maintained comparatively lower Na concentration in xylem up to 50 mM NaCl salinity while, the tolerant line IR55178 – 163 was found efficient in maintaining lower Na concentration at both level of salinity.

Table 1. Effects of salinity for varying growth period at seedling stage of rice.

Harvest/ Treatment	Root FW (g plant^{-1})			Shoot FW (g plant^{-1})			RGR (g g^{-1} FW day^{-1})		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
	IR55178 -104								
Control	0.51 (0)	1.01 (0)	2.05 (0)	0.70 (0)	1.55 (0)	2.58 (0)	0.132 (0)	0.106 (0)	0.103 (0)
50mM NaCl	0.42 (18)	0.59 (42)	1.12 (45)	0.66 (6)	0.63 (59)	1.26 (51)	0.116 (12)	0.020 (81)	0.076 (26)
75mM NaCl	0.39 (24)	0.45 (55)	0.93 (55)	0.61 (8)	0.55 (65)	1.04 (60)	0.099 (25)	0.006 (94)	0.064 (38)
	IR55178 – 163								
Control	0.63 (0)	1.25 (0)	2.07 (0)	1.1 (0)	1.93 (0)	2.95 (0)	0.150 (0)	0.075 (0)	0.075 (0)
50mM NaCl	0.62 (2)	1.14 (9)	1.7 (18)	0.72 (35)	1.17 (39)	1.95 (34)	0.088 (41)	0.065 (13)	0.074 (3)
75mM NaCl	0.59 (6)	0.89 (29)	1.06 (49)	0.75 (32)	1.05 (46)	1.38 (53)	0.087 (42)	0.054 (27)	0.045 (40)
	Shua –92								
Control	0.74 (0)	1.18 (0)	2.05 (0)	1.08 (0)	1.98 (0)	3.01 (0)	0.15 (0)	0.089 (0)	0.069 (0)
50mM NaCl	0.64 (14)	0.82 (31)	1.94 (5)	0.95 (12)	1.13 (43)	2.27 (25)	0.135 (10)	0.035 (60)	0.069 (0)
75mM NaCl	0.61 (18)	0.48 (59)	0.75 (63)	0.96 (11)	0.8 (60)	1.22 (59)	0.126 (16)	0.010 (89)	0.058 (16)
LSD at . □ 0.05	0.29			0.33			-		

Values in parenthesis are relative decrease under salinity in comparison to their non saline control

Table 2. Effect of salinity on transpiration and sodium concentrations in different rice genotypes.

Harvests	1st week			2nd week			3rd week		
Treatment mM NaCl	Na mg/g d.wt	Transpiration Rate(gH_2O/P)	Xylum concentration (µM Na)	Na mg/g d.wt	Transpiration Rate(gH_2O/P)	Xylum concentration (µM Na)	Na mg/g d.wt	Transpiration Rate (gH_2O/P)	Xylum concentration (µM Na)
					IR55178 -104				
Control	5.48 (0.0)	6.78 (0.0)	0.39 (0.0)	2.92 (0.0)	7.92 (0.0)	0.90 (0.0)	4.06 (0.0)	10.44 (0.0)	0.83 (0.0)
50	18.58 (239)	4.72 (-30.4)	2.71 (605)	29.11 (897)	3.81 (-51.9)	9.44 (944)	36.24 (793)	1.62 (-84.5)	32.67 (3823)
75	19.52 (256)	2.65 (-60.9)	6.10 (1483)	36.19 (1139)	2.46 (-68.9)	17.45 (1830)	43.82 (979)	1.08 (-89.7)	49.66 (5864)
					IR55178 – 163				
Control	4.88 (0.0)	6.36 (0.0)	0.55 (0.0)	5.28 (0.0)	7.97 (0.0)	0.97 (0.0)	6.60 (0.0)	11.99 (0.0)	1.42 (0.0)
50	12.90 (164)	4.14 (-34.9)	3.08 (462)	27.63 (423)	4.88 (-38.8)	9.26 (850)	34.34 (420)	1.99 (-83.4)	24.19 (1606)
75	18.24 (274)	3.35 (-47.3)	4.74 (765)	32.59 (517)	4.03 (-49.4)	10.71 (998)	33.10 (402)	1.22 (-89.8)	37.78 (2565)
					Shua –92				
Control	4.03 (0.0)	7.92 (0.0)	0.44 (0.0)	5.85 (0.0)	8.98 (0.0)	1.11 (0.0)	9.30 (0.0)	13.47 (0.0)	1.74 (0.0)
50	15.09 (274)	6.26 (-21.0)	2.22 (403)	31.91 (252)	6.02 (-33.0)	7.07 (536)	32.73 (252)	2.70 (-80.0)	22.36 (1182)
75	23.87 (492)	3.19 (-59.7)	6.69 (1417)	35.71 (364)	3.28 (-63.5)	13.25 (1091)	43.11 (364)	1.21 (-91.0)	45.44 (2504)
LSD for genotypes & Treatments *weeks at α 0.05	2.141	0.50	0.916						
	3.709	0.873	1.587						

Values in parenthesis are relative increase/decrease (+/-) under salinity

The ability to exclude Na from the shoot and maintaining K accumulation was reflected by the ratio of K/Na in shoot (Fig. 2). Significant decline in K/Na ratios under salinity with varying intensities was observed. There was comparatively less reduction in IR55178 – 163 throughout the growth period. At 50 mM NaCl K/Na ratio in Shua-92 and IR55178 – 163 was twice as that of found in IR55178 – 104.

The rate of transpiration was higher under non-saline conditions and significantly decreased with salinity at each stage of growth. Comparatively higher transpiration was observed in Shua-92 and IR55178 – 163 under both non saline and saline conditions. These two genotypes behaved significantly different from sensitive IR55178 -104 during initial two weeks. While, the rate of transpiration reduced drastically at 3rd week of growth, where more than 80% reduction was observed in all lines with no significant differences among tolerant and sensitive genotypes (Table 2).

Pronounced differences in terms of water use efficiency were observed among the genotypes. The IR55178 – 163 and Shua-92 was found efficient (consumed comparatively less water to produce per gram shoot fresh weight) at each stage of growth under saline as well as under non-saline conditions (Fig. 1). The relationship between rate of transpiration with other parameters (Table 3) have shown shoot fresh weight was significantly positively correlated (r = 0.646).Whereas significant negative correlation was observed with Na contents (r = -0.806) and xylem sodium concentration (r = -0.701).

Discussion

Salt tolerance is a complex phenomenon, involves interaction of morphological, physiological, biochemical and genetical traits (Ashraf, 2009; Tatar *et al*., 2010). An important feature of salt tolerance is the restricted entry of Na^+ in plants (Moller *et al*., 2009). A conceptual model was developed relating transpiration driven sodium uptake with differential varietals tolerance (Asch *et al.,* 1997; Asch 2011). Assuming this hypothesis rice genotypes differing in salinity tolerance were tested for their responses. Rice is generally regarded as a salt sensitive especially at young seedling stage, where varying degree of mortality occurs only at 50 mM NaCl salinity and in most salt sensitive varieties 50% of the population may dies within ten days of salinization at the age of 14 days. The results of present study also demonstrated varying degree of growth reduction when exposed to different salinity levels. The higher level of salt (75 mM NaCl) had drastic reduction in shoot fresh weight. Extreme sensitivity and subsequent death in most of the rice varieties at seedling stage at 8 dS m^{-1}(80 mM) are also reported earlier (Suriya *et al*., 2005; Evangelista *et al*., 2013).Results of the present study agree with earlier findings. Salinity may affect growth indirectly by decreasing the rate of photosynthesis or by preventing the growth factors from reaching the growing regions (Ashraf, 2009; Kanwal *et al*., 2011). Transplantation of rice seedlings to saline environment, temporarily disturbs, the balance between water supply and water loss from the leaves. As a result, water contents in leaves reduced and consequently the cell turgor. Subsequently there will be closing of stomata which follow reduction in transpiration and photosynthesis resulting in stunted plant growth.

Table 3. Correlation (r) between transpiration and sodium accumulation in rice shoot.

	Transpiration	Shoot Na	Xylem Na
Shoot Na	-0.806**	----	----
Xylem Na	-0.701 **	0.824**	----
Shoot FW	0.646 **	-0.321*	-0.092

** = Significant @1% prob., * = Significant @ 5% probability

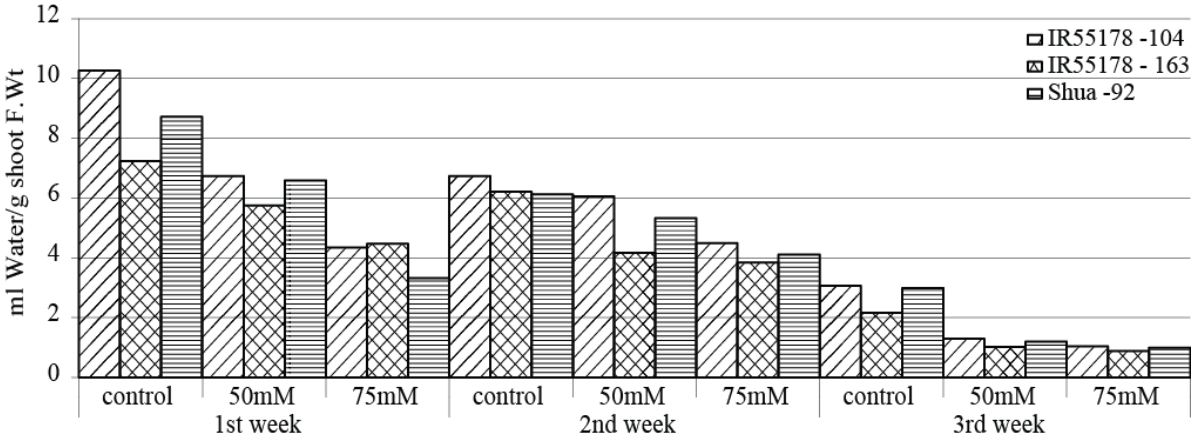

Fig. 1. Effect of salinity on WUE (g H_2O consumed/g shoot F.Wt) at 1st, 2nd and 3rd weeks of growth.

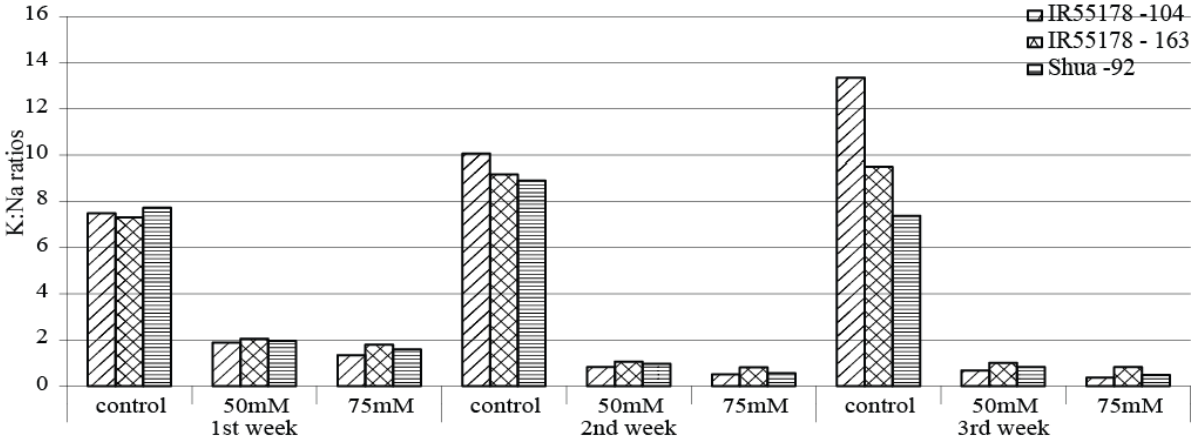

Fig. 2. Effect of salinity of potassium sodium ratios in shoot at different levels of salinity.

Besides several other factors there is substantial evidence for the physiological role played by abscisic acid and reduced supply of cytokinin in regulation of stomatal aperture (Hu *et al*., 2006; Nishiyama *et al*., 2011).In the present studies extreme reduction in transpiration rate was observed at high salinity level (i.e. 75 mM NaCl). The reduction in transpiration rate might be due to increased abscisic acid (ABA) concentration in plant organs and transport fluids and thereby strongly inhibit transpiration by causing loss of potassium from guard cells, which in turn creates closure of stomata (Hu *et al*., 2006; Nishiyama *et al*., 2011).

The fundamental processes governing the relationship between water and ion flow through roots are complex and not well understood. A negative relationship (r = -0.806) between the rate of transpiration and shoot sodium was observed in the present study where, transpiration decreased sharply with the increase in salinity and duration of exposure while, the sodium uptake continued to increase. Munns (1985) demonstrated that in barley NaCl does not move passively with the transpiration stream, neither its movement entirely independent of it over certain range of transpiration. The data of transpiration rates of these lines indicated almost similar relative reduction at 3rd week under salinity while the relative increase in sodium concentration in shoot of sensitive line was about two to three times more than that of tolerant genotypes. This indicates that differences in uptake of sodium may have involved different uptake regulatory mechanism or the existence of some alternative pathway as it was observed that salt tolerant genotypes (IR55178 – 163 and Shua-92) maintained low Na concentration in xylem. This could be due to reduced uptake into the root stele, recovery of Na from the basal xylem and sequestration into parenchyma

cells bordering the transpiration pathway or recovery and recycling in the phloem (Asch *et al*., 1997; Tester & Davenport 2003). If sodium is excluded effectively from the root xylem, there may be possibilities of existence of one solute-reflecting barrier through which all incoming solutes are screened before they enter the xylem stream. This will be possible only if there are significant apoplastic bypasses in this tissue. The existence and uptake of Na through such apoplastic bypasses have been proposed by Garcia *et al*. (1997). They reported in rice that 5.5% of total water transpired, moving through a bypass pathway from root to shoot and could account for all the sodium transported to the shoots. Ochiai & Matoh, (2002) and Krishnamurthy *et al*. (2011) also reported bypass pathway (apoplastic or transpirational) for carrying more salt in species that are very poor excluders, such as rice. On the basis of these findings it may be concluded that transport of ions was not totally dependent on the rate of transpiration.

References

Anonymous. 2012. FAOSTAT. (available at: www.faostat.fao.org/).

Asch, F. 2011. Crop Water Stress Management Modeling concept for salt uptake in rice-getting closer CIMAC 20011 7-9 Feb, Hohenheim.

Asch, F., M. Dingkuhn, K. Dorffling and K.M. Miezan.1997. A conceptual model for sodium uptake and distribution in irrigated rice, In: *Applications of System Approaches at the field level*. (Eds.): Kropff, M.J., P. Teng, P.K. Agarwal, J. Bouma, B.A.M. Bouma, J.W. Jones, H.H. VanLar Dordrecht. Kluver Academic Publishers, pp. 201-217.

Ashraf, M. 2009. Biotechnological approaches for improving plant salt tolerance using antioxidants as a marker. *Biotechnol. Adv*., 27: 84-89.

Das, P., K.K. Nutan, S.L.S. Pareek and A.Pareek. 2015. Understanding salinity responses and adopting omics based approaches to generate salinity tolerant cultivars of rice. Front. *Plant Sci*., 09. http://dx.doi.org/10.3389/fpls.00712.

Evangelista, F.C., R.R. Aldemita and L.B. Ungson. 2013. Responses of rice (*Oryza sativa* L. LX278) calli and seedlings to salinity treatment: Towards the development for salt tolerance. *Philippine Science Letters*, 6(1): 84-89.

Flowers, T.J. and T.D. Colmer. 2008. Salinity tolerance in halophytes. *New Phytol.,* 179(4): 945-963.

Garcia, A., C.A. Rizzo, J. Ud-din, S.L. Bartos, D. Senadhira, T.J. Flowers and A.R. Yeo. 1997. Sodium and potassium transport to the xylem are inherited independently in rice, and the mechanism of sodium potassium selectivity differs between rice and wheat. *Plant Cell Environ.*, 20: 1167-1174.

Hoffman, W.A. and H. Poorter. 2002. Avoiding bias in calculations of relative growth rate. *Ann. Bot*., 90(1): 37-42.

Hu, H., M. Dai, J. Yao, B. Xiao, X. Li, Q. Zhang and L. Xiong. 2006. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proceeding National Academy of Science*, 103(35): 12987-12992.

Iqbal, M.M., M.A. Gohee and A.M. Khan. 2010. Facing the food challenge under climate change threats to land resources through increased salinization. In: *Global forum on salinization and Climate Change*. (Ed.): R.P. Thomas. GFSCC 2010 FAO, UN.

Kanwal, H., M. Ashraf and M. Shahbaz. 2011. Assessment of salt tolerance of some newly developed and candidate wheat (*Triticum aestivum* L.) cvs using gas exchange and chlorophyll fluorescence attributes. *Pak. J. Bot*., 43(6): 2693-2699.

Krishnamurthy, P., K. Ranathunge, S. Nayak, L. Schreiber and M.K. Mathew. 2011. Root apoplastic barriers block Na^+ transport to shoots in rice (*Oryza sativa* L.). *J. Exp. Bot.,* 62(12): 4215-4228.

Kronzucker, H.J. and D.T. Britto. 2011. Sodium transport in plants: a critical review. *New Phytol.,* 189: 54-81.

Marcum, K.B., S.J. Anderson and M.C. Engelke. 1998. Salt gland ion secretion: A salinity tolerance mechanism among five zoysa grass species. *Crop Sci*., 38: 806-810.

Mishra, B.2004. Present status issues and future strategies for increasing quality rice production and export. In: *National Symposium on "Strategies for enhancing export of quality rice"*. NBPGR, New Delhi, and pp. 1-16.

Moller, I.S. and M. Tester. 2007. Salinity tolerance of Arabidopsis: A good model for cereals Trends. *Plant Sci.*, 12: 534-5

Moller, I.S., M. Gilliham, D. Jha, G.M. Mayo, S.J. Roy, J.C. Coates, J. Haseloff and M. Tester. 2009. Shoot Na^+ exclusion and increased salinity tolerance engineered by cell type–specific alteration of Na^+ transport in *Arabidopsis*. *The Plant Cell,* 21: 2163-2178.

Munns, R. 1985. Na^+, K^+ and Cl^- in xylem sap flowing to shoots of NaCl treated barley. *J. Exp. Bot*., 36: 1032-1042.

Munns, R. and M. Tester. 2008. Mechanism of salinity tolerance. *Ann. Rev. Plant Bio.*, 59: 651-681.

Nishiyama, R., Y. Watanabe, Y. Fujita, D.L. Tien, M. Kojima, T. Werner, R. Vankova, K.Y. Shinozaki, K. Shinozaki, T. Kakimoto, H. Sakakibara, T. Schmülling and L.S. Tran. 2011. Analysis of cytokinin mutants and regulation of cytokinin metabolic genes reveals important regulatory roles of cytokinins in drought, salt and abscisic acid responses, and abscisic acid biosynthesis. *The Plant Cell,* 23(6): 2169-2183.

Ochiai, K. and T. Matoh. 2002. Characterization of the Na^+ delivery from roots to shoots in rice under saline stress: Excessive salt enhances apoplastic transport in rice plants. *Soil Sci. Plant Nutr*., 48(3): 371-378.

Rozema, J., H. Gude, F. Bijl and H. Wesselman.1981. Sodium concentration in xylem sap in relation to ion exclusion, accumulation and secretion in halophytes. *Acta Botanica Neerlandica*, 30: 309-311.

Shereen, A., S. Mumtaz, S. Raza and M.A. Khan. 2005. Salinity effects on seedling growth and yield components of different inbred rice lines. *Pak. J. Bot*., 37(1): 131-139.

Suriya, A.D., N. Supapoj, A. Vanavichit and T. Toojinda. 2005. Screening and selection for physiological characters contributing to salinity tolerance in rice. *Kasetsart J. Nat. Sci*., 39: 174 - 185.

Tatar, O., H. Brueck, M.H. Gevrek and F. Asch. 2010. Physiological responses of two Turkish rice (*Oryza sativa* L.) varieties to salinity. *Turk. J. Agr. & Fores*., 34(6): 451-459.

Tester, M. and R. Davenport. 2003. Mechanism of salinity tolerance: Na tolerance and Na transport in higher plants. *Ann. Bot*., 91: 503-527.

Udayakumar, M., R.C.N. Rao, G.C. Wright, G.C. Ramaswamy, R.S. Ashok, G.C. Gangadhar and I.S.A. Hussain. 1998. Measurement of transpiration efficiency in field conditions. *J. Plant Physiol. & Biochem*., 1: 69-75.

Yeo, A.R. and T.J. Flowers. 1983. Varietal differences in the toxicity of sodium ions in rice leaves. *Physiolgia Plantarum*, 59: 189-195.

Yoshida, S., D.A. Forno, J.H. Cock and K.A. Gomez. 1976. Laboratory manual for physiological studies of rice, 3rd edition, The International Rice Research Institute, Manila Philippines.

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