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Full Length Article

Morphological and Physiological Evaluation of Korean Rice Genotypes for Salt Resistance

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ABSTRACT

Salinity either in the soil or irrigation water hampers the profitable crop production. This study was conducted to evaluate the response of rice genotypes to salt stress. Four rice genotypes (IR29, CHIEH-KENG44, CHING-YIN1 and RYKUU15) were sown in germination trays filled with soil especially formulated for rice and were then transferred to iron containers. Salt stress (100 m*M* NaCl) was imposed in equal increments of 25 m*M* per day. Salt stress caused substantial decrease in plant height, shoot length, root and shoot fresh and dry weights, leaf area, and fresh and dry weight of seedlings of all rice genotypes. Although the genotypes responded to salt stress differently, however, root length of IR29 and RYKUU15 was increased under salt stress. Salt stress increased the polyphenol contents and antioxidant activity of all rice genotypes, but maximum polyphenol contents were observed in CHING-YYEH1. Salt stress caused substantial decrease in K^+/Na^+ ratio owing to significant rise in Na^+ contents on the expense of K^+, but genotypes behaved differently in this regard. Minimum decrease in seedling fresh and dry weights was observed in rice genotype CHING-YIN1 owing to less Na^+ uptake under salt, which also helped in maintaining better K^+/Na^+ ratio. Although Na^+ content indicated a strong negative correlation, whereas K^+/Na^+ ratio had strong positive correlation with seedling fresh and dry weights. In conclusion, although salt stress decreased the growth of tested rice genotypes; genotype CHING-YIN1 was more resistant to salinity amongst all the genotypes owing to reduced Na^+content and greater K^+/Na^+ ratio. This study suggests that seedling fresh weight, tissue Na^+ contents and tissue K^+/Na^+ ratio may be used as markers in screening rice genotypes resistant to salt stress.

Keywords: Salinity; Rice genotypes; K^+/Na^+ ratio; Leaf area; Seedling fresh weight

INTRODUCTION

Salinity is a serious danger to agriculture of arid and semi-arid regions of the world owing to limited rainfall tied with high evapotranspiration demand due to prevailing high temperature (de Azevedo Neto *et al*., 2006). It is generally perceived that salt stress occurs only in arid- and semi-arid regions but in reality any climatic zone of the world is not free from this problem (Bhutta *et al.*, 2004; Rengasamy, 2006; Murtaza *et al.*, 2011). According to some estimates, more than 800 million hectares (Mha) of land of this globe is salt-affected, either by salinity (397 Mha) or sodicity (434 Mha) (FAO, 2005; Munns, 2005).

Among many salt contaminants, NaCl is the dominant in saline soils and is readily soluble in water to yield toxic ions like sodium (Na^+) and chloride (Cl^-). Being a smaller molecule, Na^+ is readily absorbed by the roots of higher plants and ultimately distributed in all plant parts to cause toxic ion damage, osmotic stress and imbalance nutrition in rice (*Oryza sativa* L.) plants (Siringam *et al*., 2009, 2011). Osmotic stress is linked with the accumulation of ions in soil solution whereas, nutritional imbalance and specific ion effect is connected with buildup of ions mainly Na^+ and Cl^- at toxic levels leading to hamper the absorption of other essential elements like calcium (Ca^{+2}) and potassium (K^+) etc. (El-Bassiouny & Bekheta, 2001; Munns *et al*., 2006). Toxic levels of Na^+ in plant organs damages membranes and organelles that results in growth reduction and abnormal development before plant mortality (Davenport *et al*., 2005; Quintero *et al*., 2007). According to bi-phasic model of salinity induced growth reduction in cereals proposed by Munns (1993), osmotic stress during 1st phase and ion toxicity during 2nd phase is responsible for growth reduction.

Salinity-induced osmotic stress alters the general metabolic processes and enzymatic activities leading to over generation of reactive oxygen species (ROS) to cause oxidative damage (Menezes-Benavente *et al*., 2004). These ROS produced in plants are highly toxic and cause damage to proteins, lipids, carbohydrates and DNA. Photosystems I and II of chloroplast, and complex I, ubiquinone and complex III of electron transport chain (ETC) in mitochondria are the major sites of their synthesis (Gill &

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Tuteja, 2010). According to Meloni *et al*. (2003), salt stress caused injury to cell membrane and enhanced membrane leakage in salt-sensitive rice cultivars. Plants display multigenic responses against salt stress, which involve osmotic and ionic homeostasis, and cell detoxification with the induction of antioxidant defense mechanisms (Zhu, 2001; Sairam & Tyagi, 2004). Higher buildup of polyphenols in plants under salt stress plays an important physiological role in salinity-induced oxidative damage. For instance, accumulation of polyphenols protected youngest leaves of maize from ROS-induced oxidative damages (Hichem *et al*., 2009).

Great genetic diversity for salt resistance amongst different genotypes of cultivated crops such as wheat (Sairam *et al*., 2005; Jafar *et al*., 2012), sugarcane (Akhtar *et al*., 2003), maize (Akram*et al*., 2007), canola (Farhoudi*et al*., 2012) and rice (Gurmani *et al*., 2006; Quinet *et al*., 2010) has been reported. Being moderately salt resistant, rice may be grown in salt-affected areas. Mass screening and physiological characterization of rice genotypes may help in improving resistance against salt stress. It was hypothesized that rice genotypes differ for their potential of salt resistance. This study was conducted to evaluate different rice genotypes, of Korean origin, under salt stress on morphological and physiological basis.

MATERIALS AND METHODS

Site description and experimental details: This experiment was conducted in a plastic house (with 22/16ºC day/night temperatures, respectively) at Department of Crop Science and Biotechnology, Dankook University, South Korea.

Sprouted seeds (25 in number) of rice genotypes IR29, CHIEH-KENG44, CHING-YIN1 and RYKUU15 were sown in germination trays (5 seeds in one hole) filled with artificial rice soil. Soil was composed of vermiculite, diatomaceous earth, clay, coco peat, charcoal and water-soluble humic acid having moisture contents 25±8%, bulk density 0.50±0.10 Mg m^{-2}, pH 5.4, EC 2 dS m^{-1}, ammonia nitrogen (NH_4-N) 350 ppm, and available phosphorous (P) 350 ppm. After achieving the constant emergence count, 15 rice seedlings were maintained in each replicate with three seedlings per hole. Germination trays were shifted in iron containers having 25 m*M* NaCl solution (salt stress) or tap water (control). Solution concentration was increased to 50, 75 and 100 m*M* NaCl on 16, 17, 18th day after sowing, respectively in salt stress treatment. The experiment was conducted in completely randomized design (CRD) with factorial arrangement having five replications.

Observations: On 23rd day after sowing, experiment was harvested to record observations. Immediately after harvest of experiment, root and shoot lengths, root and shoot fresh weight and seedling fresh weight of ten randomly selected seedlings from each replicate were taken and averaged. After that these samples were put in an oven at 70°C for 72 h to record root and shoot dry weight, and seedling dry weight. Leaf area of rice seedlings was measured at harvesting by a leaf area meter (Area Meter AM-200 ADC Bio-scientific limited).

One gram plant sample was dissolved in 10 mL of 80% methanol to prepare extract to estimate total polyphenols and antioxidant activities. Total polyphenols contents were determined by reacting phenolic compounds with phosphomolybdate blue using Folin–Ciocalteu's procedure (Shen *et al*., 2009).

Antioxidant activities of the extracts were measured by scavenging the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals in a process guided by its discoloration (Lee & Lee, 2004).

Sodium (Na^+) and potassium (K^+) contents (mg g^{-1} dry weight) of seedlings was determined from 0.5 g dried digested sample with flame photometer (Jenway PFP-7). After that K^+/Na^+ ratio was also computed.

Statistical analysis: The collected data was statistically analyzed according to Fisher's analysis of variance technique. Least significant difference test (LSD) at $P<0.01$ level was used to compare treatments means (Steel *et al*., 1997).

RESULTS

Rice genotypes IR29 and RYKUU15 respectively observed 16.00 and 12.30% increase, while genotypes CHIEH-KENG44 and CHING-YIN1 respectively observed 7.78 and 16.00% decrease in root length under salt stress (Table I). Salinity caused substantial decrease in shoot length of all rice genotypes with maximum decrease (42.40%) in genotype IR29 (Table I). Likewise, leaf area of all rice genotypes was markedly decreased under salt stress; and minimum reduction (56.62%) was observed in genotype CHING-YIN1 (Table I).

Likewise, salt stress decreased the root fresh and dry weights in all rice genotypes; however, minimum decrease was noted in genotype CHING-YIN1 in this regard (Table II). Moreover, salinity significantly decreased the shoot fresh and dry weights of all rice genotypes; however, genotypes differed in their response to salinity. Minimum decrease in shoot fresh and dry weights was observed in genotype CHING-YIN1 whereas genotype CHIEH-KENG44 observed maximum decrease in this regard (Table II).

Salt stress decreased the seedling fresh and dry weights of all rice genotypes (Table III). However, genotype CHING-YIN1 had minimum decrease in seedling fresh and dry weights, respectively (Table III). Salt stress increased the total polyphenols and antioxidant activityin all rice genotypes with varying degree (Table III). In this regard, maximum total polyphenols and antioxidant activity was observed in genotype CHING-YIN1 (Table III).

Salt stress significantly decreased the seedling K^+ contents with simultaneous increase in Na^+ contents (Table IV). Rice genotypes RYKUU15 and IR29 had 547.49 and

Table 1: Influence of salt stress on root and shoot length, and leaf area of different rice genotypes

Treatments	Root length (cm)			Shoot length (cm)			Leaf area (cm^2 $plant^{-1}$)		
	Control	Salinity	Decrease over control (%)	Control	Salinity	Decrease over control (%)	Control	Salinity	Decrease over control (%)
IR29	6.00±0.12 c	6.96±0.26 b	16.00	12.50±0.51 c	7.20±0.60 e	42.40	11.06±0.29 a	3.12±0.42 e	71.79
CHIEH-KENG44	7.46±0.40 a	6.88±0.33 b	-7.78	19.72±0.52 a	13.97±0.84 b	29.16	11.61±0.74 a	3.18±0.19 e	72.61
CHING-YIN1	6.25±0.25 c	5.25±0.12 d	-16.00	19.78±0.55 a	12.40±0.30 c	37.31	9.96±0.74 b	4.32±0.12 d	56.62
RYKUU15	6.83±0.19 b	7.67±0.19 a	12.30	13.89±0.32 b	9.77±0.51 d	29.66	9.10±0.32 c	2.49±0.25 e	72.64
LSD at p 0.01		**0.44**			**0.94**			**0.77**	

Means not sharing the same letter within a column, for a factor, differ significantly from each other at p 0.01

Table 2: Influence of salt stress on root and shoot fresh and dry weights of different rice genotypes

Treatments	Root fresh weight (mg per seedling)			Root dry weight (mg per seedling)			Shoot fresh weight (mg per seedling)			Shoot dry weight (mg per seedling)		
	Control	Salinity	Decrease over control (%)	Control	Salinity	Decrease over control (%)	Control	Salinity	Decrease over control (%)	Control	Salinity	Decrease over control (%)
IR29	120.60± 1.75 e	96.66± 4.65 f	19.85	40.20± 0.58e	32.22± 1.55f	19.85	51.70± 2.12 f	31.53± 0.66 g	39.01	10.34± 0.42 f	6.31± 0.13 g	38.97
CHIEH-KENG44	184.44± 7.62 a	127.10± 1.95 e	31.09	61.48± 3.54 a	41.37± 0.65e	32.71	93.30± 2.94 a	53.09± 1.22 f	43.10	18.66± 0.59 a	10.72± 0.24 f	42.55
CHING-YIN1	178.33± 2.00 a	168.00± 2.57 b	05.79	59.44± 0.67a	56.00± 0.86b	5.78	81.88± 2.01 b	74.52± 2.15 c	08.99	16.38± 0.40 b	14.90± 0.43 c	09.04
RYKUU15	153.54± 3.10 c	138.99± 6.67 d	09.48	51.18± 1.03 c	45.66± 2.22 d	10.79	69.36± 1.42 d	57.05± 1.38 e	17.75	13.87± 0.28 d	11.41± 0.28 e	17.73
LSD p 0.01		**8.78**			**2.93**			**3.22**			**0.64**	

Means not sharing the same letter within a column, for a factor, differ significantly from each other at p 0.01

Table 3: Influence of salt stress on seedling fresh and dry weights, total polyphenols and antioxidant activity of different rice genotypes

Treatments	Seedling fresh weight (mg)			Seedling dry weight (mg)			Total polyphenols (mg GAE g^{-1} DW)			Antioxidant activity (IC_{50}; µg mL^{-1})		
	Control	Salinity	Decrease over control (%)	Control	Salinity	Decrease over control (%)	Control	Salinity	Decrease over control (%)	Control	Salinity	Decrease over control (%)
IR29	172.30± 2.32 f	128.20± 5.29 g	25.59	50.54± 0.61d	38.53± 1.68 e	23.76	52.19± 1.79 d	62.01± 2.48 a	18.82	154.50± 6.02 b	105.96± 1.16 e	31.42
CHIEH-KENG44	277.74± 13.50 a	180.70± 3.00ef	34.94	80.14± 4.12 a	52.09± 1.07 d	35.00	55.92± 2.88 c	58.55± 1.40bc	4.70	156.86± 1.48 b	125.17± 2.69 c	20.20
CHING-YIN1	257.63± 3.20 b	241.51± 3.45 c	6.26	73.38± 1.33 b	64.69± 1.96 c	11.84	48.71± 0.64 c	63.35± 0.74ab	30.06	191.95± 7.93 a	104.43± 1.71e	45.60
RYKUU15	222.8± 3.88 d	189.71± 3.56 e	14.89	65.05± 1.17 c	51.32± 0.72 d	21.11	55.36± 2.62 cd	61.40± 0.65ab	10.91	116.73± 6.08 d	92.97± 0.95 f	20.35
LSD p 0.01		**8.78**			**2.93**			**3.22**			**0.64**	

Means not sharing the same letter within a column, for a factor, differ significantly from each other at p 0.01

*GAE: Gallic acid equivalent, **IC_{50} is the concentration needed to inhibit activity of free radical below 50%

455.65% rise in Na^+ contents, respectively; whereas genotype CHING-YIN1 had minimum (244.35%) rise in Na^+ contents (Table IV). Moreover, K^+/Na^+ ratio was significantly decreased by salt stress in all genotypes, with varying degree, and minimum decrease was noted in CHING-YIN1 (Table IV).

Correlation analysis indicated strong negative relationship between seedling Na^+ contents and seedling fresh and dry weights (Table V). However, strong positive association of K^+/Na^+ratio with seedling fresh and dry weight, and root fresh weight was observed (Table V). Moreover, correlation of total polyphenols and antioxidant potential with seedling fresh and dry weights, and root fresh weight was non-significant (Table V).

DISCUSSION

Salt stress significantly decreased the growth of all rice genotypes, although to a varying degree (Tables I-III). Decrease in seedling fresh and dry weights was primarily due to increase in Na^+ contents and decrease in K^+/Na^+ ratio (Table IV). This has also been indicated by strong negative correlations between seedling Na^+ contents and seedling fresh and dry weights, and positive association of K^+/Na^+ ratio with seedling fresh and dry weights (Table V). Although polyphenols and antioxidant potential were

Table 4: Effect of salinity on seedling K^+ and Na^+ contents, and K^+/Na^+ ratio of different rice genotypes

Treatments	K^+ contents (mg g^{-1})			Na^+ contents (mg g^{-1})			K^+/Na^+ ratio		
	Control	Salinity	Decrease over control (%)	Control	Salinity	Increase over control (%)	Control	Salinity	Decrease over control (%)
IR29	16.14±0.39 c	14.28±0.33 e	11.52	3.54±0.05 e	19.67±0.42 a	455.65	4.56±0.13 c	0.73±0.03 e	83.99
CHIEH-KENG44	15.43±0.53 cd	12.70±0.70 f	17.69	2.48±0.24 f	11.87±0.62 c	378.63	6.26±0.67 b	1.07±0.10 de	82.91
CHING-YIN1	15.11±0.34 d	13.13±0.21 f	13.10	2.30±0.09 f	7.92±0.50 d	244.35	6.58±0.38 b	1.66±0.11 d	74.77
RYKUU15	19.17±0.33 a	17.18±0.38 b	10.38	2.19±0.21 f	14.18±0.33 b	547.49	8.81±0.98 a	1.21±0.05 de	86.27
LSD p 0.01		**0.73**			**0.62**			**0.78**	

Means not sharing the same letter within a column, for a factor, differ significantly from each other at p 0.01

Table 5: Correlation between different traits of rice genotypes under salinity (n = 5)

Variables	Seedling fresh weight	Seedling dry weight	Root fresh weight
Seedling Na^+ contents	-0.92*	-0.97**	-0.80^{ns}
K^+/Na^+ ratio	0.99**	0.95*	0.98**
Total polyphenols	0.10^{ns}	0.04^{ns}	0.16^{ns}
Total antioxidant activity	0.01^{ns}	0.00^{ns}	0.09^{ns}

** = significant at p 0.01, * = significant at p 0.05, ns = non-significant

increased under salt stress (Table IV), absence of any significant correlation of total polyphenols and antioxidant potential with seedling fresh and dry weights (Table V) indicated that generation of ROS was not the only reason for salt-induced decrease in rice growth. Salt-induced osmotic stress (Bandeoglu *et al*., 2004), altered metabolism, inability of apoplastic acidification and lack of turgor seemed to be the possible reasons of salinity-induced decrease in rice growth (Munns & Tester, 2008); increase in Na^+ uptake also contributed towards this (Munns *et al*., 2006).

A minimum decrease in seedling fresh and dry weights was observed in genotype CHING-YIN1 (Table III). The same genotype also maintained higher leaf area under salinity (Table I) and K^+/Na^+ ratio (Table IV). Decrease in Na^+ uptake and increase in uptake of K^+ are amongst the important indicators of salt resistance (Marschner, 1995; Hu & Schmidhalter, 1997). The ability of plants to limit Na^+ transport to shoot is important for the maintenance of growth rates and protection of the metabolic process in elongating cells from the toxic effect of Na^+ (Razmjoo *et al*., 2008).

In conclusion, salt stress decreased the growth of tested rice genotypes; genotype CHING-YIN1 was more resistant to salinitythan other genotypes owing to decrease in Na^+ accumulation and better K^+/Na^+ ratio, which helped in growth maintenance. Seedling fresh weight, K^+/Na^+ ratio and Na^+ contents may be used as markers in screening rice genotypes resistant to salt stress.

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