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Physiobiochemical basis of seedling vigour, Na+/K+ ratio and total dry matter production for salinity tolerance in rice (*Oryza sativa*)

P SENGUTTUVEL[1], C VIJAYALAKSHMI[2], K THIYAGARAJAN[3], J R KANNANBAPU[4] and B C VIRAKTAMATH[5]

Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641 003

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Soil salinity is a major problem in world agriculture that limits crop productivity. More than 800 million hectares of land throughout the world are salt affected and accounts 7% of world's total area. Of the current 230 million hectare of irrigated land, 45 million ha (20%) are salt affected (FAO 2008). Studies conducted at IRRI, indicated that rice (*Oryza sativa* L.) is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage, becomes sensitive during pollination and fertilization and then becomes increasingly more tolerant at maturity. Characteristics of a salt-tolerant variety include Na^+exclusion, K^+/Na^+ discrimination, retention of ions in the leaf sheath, tissue tolerance, ion partitioning into different-aged leaves, osmotic adjustment, transpiration efficiency, early vigor and early flowering leading to a shorter growing season, the latter increasing water use efficiency (Colmer *et al.* 2005).

Rice is an important crop and experimental evidence suggests that most rice varieties exhibit salt sensitivity, but that varietal differences tend to manifest themselves only at moderate salt concentrations, that is, 50mM NaCl (Yeo and Flowers 1986). An approach to enhancing salt resistance through selection of parents on the basis of physiological traits and the subsequent combining of these traits, in a process termed pyramiding was advocated by many rice workers. Difference in vigor among rice cultivars accounted for much of the variation in their survival of salinity (Yeo and flowers 1986). Vigorous growth may also have a dilution effect; one tall, salt tolerant landraces had the same net transport of Na^+ through its roots as a semidwarf susceptible line, but had much lower shoot Na^+ concentration (Yeo and Flowers 1986). Numerous abnormalities were noted in rice due to salt injury as stunted growth, rolling of leaves, white leaf tips, blotches in leaves, drying of older leaves, poor root growth and reduced survival (Ponnamperuma and Bandyopadhya 1980).The present study was carried out at seedling stage under glass house condition, which is a very sensitive phase of crop growth with the objective of verifying the effects of salinity on seedling vigour of three weeks old seedlings, sodium and potassium accumulation in leaves and shoots and total dry matter accumulation under saline and non-saline condition.

Single seedling per hole was planted with a total of 11 seedlings per test entry. Twenty four rice genotypes, 24 hybrids and FL 478 were selected for this investigation and the study was undertaken during 2008-2009. Among the genotypes mentioned above, IR29 (susceptible), Pokkali and FL 478 (tolerant) were used as checks. Seeds of each genotype were sown on sterilized sand media. After 5 days the seedlings were transferred to the Thermocole sheets placed on plastic trays of 12L capacity. Each thermocole sheets had 77 holes (11 × 7) rows. Three replications were used per test entry in a randomized complete block design. The thermocole sheets were first floated on the nutrient solution until the seedlings were 14 days old. The salinity treatments were imposed thereafter. Two levels of salt stress were introduced corresponding to 60 and 120 mM of NaCl, while the nutrient solution without NaCl served as control. The culture solution was renewed weekly and the pH adjusted daily to 5.5. The seedlings were evaluated for visual salt injury SES score and then harvested 7 days after the salt treatment. The Na^+ and K^+ content and were quantified in the seedlings.

The visual salt injury (SES) score was recorded on 3, 7, 10 and 13 days after salinization based on modified standard evaluation score (SES) of visual salt injury (Gregorio *et al.* 1997) during early seedling stage. Sodium and potassium

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[1]Research scholar (e mail : Senguttuvel@gmail.com), Centre for Plant Breeding and Genetics. (Present address:Scientist, Directorate of Rice Research, Rajendranagar, Hyderabad 500 030, [2]Professor (vijicv@yahoo.co.uk), Department of Plant Physiology, [3]Director (ktrajancpbg@gmail.com), Centre for Plant Breeding and Genetics, [4]Professor (kannanbapu@rediffmail.com), Centre for Plant Breeding and Genetics, [5]Project Director (bcviraktamath123@rediffmail.com), Directorate of Rice Research, Rajendranagar, Hyderabad, Andhra Pradesh 500 030

content was assessed in triple acid extract (9:2:1 parts of HNO_3:H_2SO_4:$HCLO_4$) extract using Flame Photometer (Model 21 Systronics) and expressed as mg/g on weight basis (Jackson 1967).Three highly salt tolerant genotypes, viz. IR 74802, IR 73104, IR 72593 selected from experiment I were utilized for study. The genotypes FL 478 and IR 29 served as tolerant and susceptible checks respectively.

The seedlings were developed as by growing on sand culture and transplanted on two leaf stage by single seedling per hole on the thermocole and floated on the yoshida solution and grown until 35 days. Similar to experiment I, except the salinity treatment period, after 14 days, salinity was imposed and extended upto 14 days (instead of 7 days as in earlier experiment I). The visual salt injury (SES) score was recorded on 3, 7, 10 and 13 days after salinization. The salinity treatments were imposed 21 onwards using two salinity levels with EC of 60 and 120 mM. The treatments were replicated thrice and 2 such sets were developed. The seedlings from one set were harvested 14 days after initiating salinity stress for enzyme analysis, i.e. at 35 days after sowing, the plants were harvested for Na^+ and K^+ content and total dry matter production. Leaves were scored basipetally with leaf number 3 being the oldest leaf and leaf 7 being the youngest leaf.

The TDMA was calculated by adding the dry weights of leaf shoot and root and mean values worked out. It is expressed in g/plant. The mean data of selected plants for each genotype per replication on dry weight basis were subjected to analysis of variance for all the characters appropriate for randomized complete block design (Panse and Sukhatme 1954). The

Table 2 Modified SES for visual salt injury and Na^+: K^+ content (mg/g) in shoot of rice genotypes at 7 days after salinization

Genotypes	SES Score				Na/k content		
	Control	60mM	120mM	Mean	Na	K	Na^+/K^+ ratio
IR 66401-2B-6-1-3	1.0	2.3	4.3	2.53	3.75	4.53	0.83
IR 69997-AC-3	1.0	2.3	3.8	2.37	3.57	4.23	0.84
IR 73104-B-1-1-3-2-1	1.0	1.8	3.0	1.93	3.18	4.65	0.68
IR 74105-3R-2-2	1.0	2.8	4.0	2.60	4.02	4.35	0.92
IR 74095-AC-38	1.0	3.3	4.0	2.77	3.77	4.22	0.89
IR 74099-3R-5-3	1.0	3.3	4.3	2.87	3.86	4.16	0.93
IR 72400-B-3-2-2-1-2	1.0	3.5	4.0	2.83	3.94	4.26	0.92
IR 72579-B-2R-1-3-2	1.0	2.8	4.3	2.70	3.49	4.10	0.85
IR 74802-3R-7-1-2	1.0	1.8	3.0	1.93	3.20	4.60	0.70
IR 71896-3R-8-3-1	1.0	3.3	4.0	2.77	3.99	4.1	0.97
IR 69992-AC-2	1.0	3.5	4.3	2.93	3.86	4.30	0.90
IR 72593-B-19-2-3-1	1.0	1.8	3.0	1.93	3.10	4.60	0.67
IR 72580-B-4-1-1-1-2	1.0	3.0	3.8	2.60	3.76	4.23	0.89
IR 29	1.3	4.8	6.8	4.30	4.40	4.50	0.98
IR 64	1.0	3.8	4.5	3.10	3.90	4.45	0.88
TRY 2	1.0	3.0	3.8	2.60	3.80	4.62	0.82
CSR 23	1.0	2.8	3.5	2.43	3.66	4.35	0.84
TKM 11	1.0	2.0	3.5	2.17	3.75	4.21	0.89
MI 48	1.3	5.3	5.8	4.13	3.92	4.25	0.92
JGL 384	1.3	3.8	5.3	3.47	3.83	4.36	0.88
GMS 47	1.3	3.8	5.5	3.53	3.92	4.25	0.92
GMS 52	1.3	3.6	4.5	3.13	3.86	4.20	0.92
CO 43	1.0	2.5	4.5	2.67	3.57	4.10	0.87
Pokkali	1.0	1.8	3.0	1.93	3.13	4.62	0.68
FL 478	1.0	1.8	3.0	1.93	3.25	4.68	0.69
Mean	1.06	2.98	4.14		3.70	4.35	0.85
Significance							
Genotypes		0.0733***			0.0616	0.082	0.0137
Salinity		0.0253***			0.1751***	0.233***	0.039***
Genotype × Salinity		0.126***			9.3	4.7	11.7
CV		1.9			0.0616	0.082	0.0137

*** Significant at $P < 0.001$ %.

analysis was done using software CROPSTAT.

The visual salt injury score recorded on the 7th day after salinization increased as salt concentration increased (Table 2). In control, all genotypes performed well with a mean SES of 1.06. At 60mM concentration, the genotypes, viz.. IR 73104, IR 72593, IR 74802, Pokkali and FL 478 recorded a score of 1.8, while the genotype IR 29 had a score of 4.8. When the molar concentration increased to 120 mM, the genotypes IR 73105, IR 74802, IR 72593, Pokkali and FL 478 had the lowest score of 3.0 while IR 29 recorded a visual salt injury score of 6.8. The SES plays a key role in assessment of genotypes for stress tolerance. Irrespective of the rice hybrids, the average SES was 1.0 (control), 2.2 (60mM) and 3.9 (120mM) (Table 3). There is no variation among rice hybrids in control. Whereas, marked difference was noticed in 60 mM and 120mM concentration. In 60mM salt concentration, IR 79156 A / IR 72593-B-19-2-3-1, IR 79156 A / IR 74802-3R-7-1-2 and IR 79156 A / CSR 23 had the least SES of 1.3, followed by IR 79156 A / IR 73104-B-1-1-3-2-1, with score of 1.8. The hybrid IR 70369 A / IR 66401-2B-6-1-3 recorded highest score of 3.3. In case of 120mM salt stress. IR 68888 A / IR 66401-2B-6-1-3 recorded highest score of 5.3 followed by IR 70369 A / IR 66401-2B-6-1-3 with 5.0, whereas, IR 68897 A / IR 73104-B-1-1-3-2-1, IR 68897 A / IR 74802-3R-7-1-2, IR 68897 A / IR72593-B-19-2-3-1 and IR 79156 A / CSR 23 had least SES of 3.3. The shoot sodium concentration of the young rice seedlings at 120mM salt stress ranged between 3.10 (IR 72593-B-19-2-3-1) to 4.40 (IR 29). The general mean of the genotypes was 3.70 and sixteen genotypes exceeded (IR 66401, IR 74105, IR 74099, IR 72400, IR 71896, IR 69992, IR 72580, IR 29, IR 64, TRY 2,TKM 11, MI 48, JGL 384, GMS 42 and GMS

Table 3 Modified SES for visual salt injury and Na^+: K^+ content (mg/g) in shoot of rice hybrids at 7 days after salinization

Genotypes	SES Score				Na/k content		
	Control	60mM	120mM	Mean	Na	K	Na^+/K^+ ratio
IR 68897 A × IR 66401-2B-6-1-3	1.0	2.5	3.8	2.4	3.75	4.42	0.85
IR 68897 A × IR 73104-B-1-1-3-2-1	1.0	2.5	3.3	2.3	3.40	4.70	0.72
IR 68897 A × IR 74802-3R-7-1-2	1.0	2.5	3.3	2.3	3.56	4.60	0.77
IR 68897 A × IR72593-B-19-2-3-1	1.0	2.0	3.3	2.1	3.33	4.25	0.78
IR 68897 A × CSR 23	1.0	2.3	4.3	2.5	3.65	4.62	0.79
IR 68897 A × Pokkali	1.0	2.3	3.3	2.2	3.40	4.25	0.80
IR 68888 A × IR 66401-2B-6-1-3	1.0	2.8	5.3	3.0	3.52	4.25	0.83
IR 68888 A × IR 73104-B-1-1-3-2-1	1.0	2.8	4.3	2.7	3.62	4.53	0.80
IR 68888 A × IR 74802-3R-7-1-2	1.0	2.0	4.3	2.4	3.50	4.70	0.74
IR 68888 A × IR 72593-B-19-2-3-1	1.0	2.0	4.0	2.3	3.44	4.40	0.78
IR 68888 A × CSR 23	1.0	2.5	4.0	2.5	3.62	4.39	0.82
IR 68888 A × Pokkali	1.0	2.0	3.8	2.3	3.00	4.60	0.65
IR 70369 A × IR 66401-2B-6-1-3	1.0	3.3	5.0	3.1	3.76	4.20	0.90
IR 70369 A × IR 73104-B-1-1-3-2-1	1.0	2.5	4.3	2.6	3.10	4.70	0.66
IR 70369 A × IR 74802-3R-7-1-2	1.0	2.5	4.0	2.5	3.12	4.65	0.67
IR 70369 A × IR 72593-B-19-2-3-1	1.0	2.0	3.8	2.3	3.30	4.80	0.69
IR 70369 A × CSR 23	1.0	2.3	4.8	2.7	3.50	4.45	0.79
IR 70369 A × Pokkali	1.0	2.0	4.0	2.3	3.40	4.70	0.72
IR 79156 A × IR 66401-2B-6-1-3	1.0	2.5	4.8	2.8	3.32	4.80	0.69
IR 79156 A × IR 73104-B-1-1-3-2-1	1.0	1.8	3.5	2.1	3.05	4.60	0.66
IR 79156 A × IR 74802-3R-7-1-2	1.0	1.3	3.5	1.9	3.30	4.80	0.69
IR 79156 A × IR 72593-B-19-2-3-1	1.0	1.3	3.5	1.9	3.00	4.68	0.64
IR 79156 A × CSR 23	1.0	1.3	3.3	1.8	3.10	4.55	0.68
IR 79156 A × Pokkali	1.0	1.5	3.8	2.1	3.25	4.87	0.67
Mean	1.0	2.2	3.9		3.37	4.56	0.74
Significance							
Genotypes			0.0938 ***		0.0480	0.0446	0.0141
Salinity			0.0331 ***		0.135***	0.125***	0.040***
Genotype × Salinity			0.162 ***		6.1	4.2	8.1
CV			2.4				

*** Significant at P < 0.001

57) the general mean. (Table 2). In case of the hybrids, the range varies from 3.00 (IR 79156 A / IR 72593-B-19-2-3-1 and IR 68888 A / Pokkali) to 3.76 (IR 70369 A / IR 66401-2B-6-1-3). The general mean of the hybrids was 3.37 and thirteen hybrids exceeded the general mean (3.37). The shoot potassium concentration of rice seedlings at 120mM salt stress ranged between 4.1 (IR 71896-3R-8-3-1) to 4.68 (FL 478). The general mean of the genotypes was 4.35 and 18 genotypes exceeded the general mean (Table 3).

The mean potassium level ranges with an average of 4.56, with nine hybrids with a parentage (IR 68897 A combination of IR 66401, IR 72593 and Pokkali, IR 68888A combinations of IR 66401, IR 72593 and CSR 23, IR 70369 A combinations of IR 66401 and CSR 23, IR 79256 A combinations of CSR 23 respectively) have least value than grand mean. The hybrid IR 70369 A / IR 66401-2B-6-1-3 recorded the lowest value (4.2), whereas, the hybrid IR 79156 A / Pokkali had a highest potassium content (4.87) (Table 3).

The ratio between the shoot sodium concentration and shoot potassium concentration was also calculated for all the rice genotypes in order to classify them into salt tolerant, moderately tolerant and salt susceptible lines (Table 2). The Na^+/K^+ ratio values should be lower for salt tolerant lines and higher for salt susceptible lines. The genotypes, FL 478, Pokkali (salt tolerant) and IR 29 (salt susceptible) were used as checks. In the present study, range for Na^+/K^+ ratio varies from 0.67 (IR 72593-B-19-2-3-1) to 0.98 (IR 29).

In hybrids, the mean value of 24 hybrids was 0.74., among them thirteen hybrids performed well and it was below the grand mean. The hybrid IR 79156 A / IR 72593-B-19-2-3-1 had the lowest Na^+/K^+ ratio of 0.64, followed by IR 68888 A / Pokkali (0.65), IR 70369 A / IR 73104-B-1-1-3-2-1(0.66) and IR 79156 A / IR 73104-B-1-1-3-2-1 (0.66) respectively. While, IR 70369 A / IR 66401-2B-6-1-3 recorded the highest Na^+/K^+ ratio of 0.90 (Table 3).

The analysis of visual salt injury in five rice genotypes was carried out by SES method (adapted from IRRI) and found highly significant Table 4. The visual salt injury score was done on 3rd, 7th, 10th and 13th days after salinization. The range varies from 1.0 (control) to 9.0 (120mM in IR 29). In control, all the genotypes recorded a score of 1.0 in all the days of observations and in case of IR 29 it is 1.25. In 60mM, the value varies from 1.0 (FL 478, IR 74802, IR73104 and IR 72593) to 7.0 (IR 29) at 13 day after salinization. In 120mM, value varies from 2.25 (IR74802, IR73105 and IR 72593) to 9.0 (IR 29) at 13th day after salinization. Among the genotypes FL 478, IR 72593, IR 73104 and IR 74802 scored least on all days of the salinization except IR 29.Salinity resulted in substantial increase in salt concentration in leaves, shoot and root of all rice genotypes. Under salt stress, the sodium concentration in individual leaves was the highest in leaf number 4 (oldest) and then decreased linearly across cultivars in newly formed leaves (Table 6). On the other hand, K^+ is

Table 4 Modified SES for visual salt injury of rice genotypes

Genotype	Days after salinization															
	3				7				10				13			
	Control	60mM	120mM	Mean	Control	60mM	120mM	Mean	Control	60mM	120mM	Mean	Control	60 mM	120mM	Mean
FL 478	1.00	1.00	1.00	1.00	1.00	1.50	3.00	1.83	1.00	2.00	3.75	2.25	1.00	2.50	4.50	2.67
IR 74802	1.00	1.00	1.00	1.00	1.00	1.50	3.00	1.83	1.00	1.75	3.50	2.08	1.00	2.25	4.25	2.50
IR 73104	1.00	1.00	1.00	1.00	1.00	1.50	3.00	1.83	1.00	1.75	3.50	2.08	1.00	2.25	4.25	2.50
IR 72593	1.00	1.00	1.00	1.00	1.00	1.50	3.00	1.83	1.00	1.75	3.50	2.08	1.00	2.25	4.25	2.50
IR 29	1.25	2.50	3.50	2.42	1.25	4.75	6.50	4.17	1.25	5.25	7.00	4.50	1.25	7.00	9.00	5.75
Mean	1.05	1.30	1.50	1.28	1.05	2.15	3.70	2.30	1.05	2.50	4.25	2.60	1.05	3.25	5.25	3.18
Genotype		0.077***				0.092***				0.133***				0.141***		
Salinity		0.059***				0.071***				0.103***				0.109***		
Genotype × Salinity		0.134***				0.159 ***				0.231***				0.244***		
CV		2.5				2.7				3.3				3.2		

*** Significant at $P < 0.001$

Table 5 TDMP, shoot and root dry matter (g) and R/S ratio of rice genotypes^{+} at 14 days after salinization

Genotype	Total dry matter				Shoot dry matter				Root dry matter				R/S ratio			
	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean
FL 478	29.73	22.76	20.92	24.47	19.52	14.20	13.80	15.84	10.21	8.56	7.12	8.63	52.31	60.28	51.59	54.73
IR 74802	30.55	27.20	24.03	27.26	19.65	17.50	16.10	17.75	10.90	9.70	7.93	9.51	55.47	55.43	49.25	53.38
IR 73104	34.50	29.30	24.84	29.55	21.00	18.80	16.24	18.68	13.50	10.50	8.60	10.87	64.29	55.85	52.96	57.70
IR 72593	36.37	31.98	27.54	31.96	22.25	19.62	17.89	19.92	14.12	12.36	9.65	12.04	63.46	63.00	53.94	60.13
IR 29	31.06	22.70	9.33	21.03	19.36	16.10	7.10	14.19	11.70	6.60	2.23	6.84	60.43	40.99	31.41	44.28
Mean	32.44	26.79	21.33	26.85	20.36	17.24	14.23	17.28	12.09	9.54	7.11	9.58	59.19	55.11	47.83	54.04
Genotype	1.27***				0.798***				0.478***				2..44***			
Salinity	0.987***				0.618***				0.37***				1.90***			
Genotype × Salinity	2.20***				1.38***				0.828***				4.24***			
CV	5.0				4.8				5.2				4.8			

*** Significant at $P < 0.001$ %. + Dry weight of 10 seedlings

Table 6 Sodium potassium ratio in leaves, shoot and root at 14 days after salinization

Genotype	$Na^{+}:K^{+}$ ratio																			
	4th leaf				5th leaf				6th leaf				Shoot				Root			
	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean
FL 478	0.23	0.69	1.03	0.65	0.18	0.24	0.84	0.42	0.16	0.17	0.50	0.28	0.09	0.24	1.12	0.48	0.39	1.21	2.02	1.21
IR 74802	0.20	0.57	1.18	0.65	0.18	0.32	0.59	0.36	0.16	0.26	0.61	0.34	0.11	0.26	1.39	0.59	0.40	1.55	1.90	1.28
IR 73104	0.24	0.61	0.94	0.60	0.20	0.37	0.85	0.47	0.18	0.30	0.58	0.35	0.17	0.23	1.18	0.53	0.33	1.38	1.96	1.22
IR 72593	0.22	0.60	0.90	0.57	0.19	0.43	0.60	0.41	0.16	0.33	0.47	0.32	0.16	0.18	0.50	0.28	0.37	1.73	1.55	1.22
IR 29	0.20	1.40	2.52	1.37	0.19	1.25	1.99	1.14	0.15	1.13	1.60	0.96	0.20	0.95	1.47	0.87	0.32	2.62	4.66	2.53
Mean	0.22	0.78	1.31	0.77	0.19	0.52	0.97	0.56	0.16	0.44	0.75	0.45	0.15	0.37	1.13	0.55	0.36	1.70	2.42	1.492
Genotype	0.030***				0.026***				0.018***				0.034***				0.06***			
Salinity	0.23****				0.20***				0.142***				0.26***				0.49***			
Genotype × Salinity	0.052***				0.045***				0.031***				0.05***				0.01***			
CV	4.1				4.8				4.3				6.5				4.5			

*** Significant at $P < 0.001$

lower in older leaves and then increased with decreasing leaf age but with relatively slower increase under salt stress. Across the genotypes, Na^+/ K^+ ratio was much lower in younger leaves than in older leaves. Na^+/ K^+ ratio showed a lower rate of reduction in leaves number 4 and 5 and sharp decline in leaf 6.

Under normal conditions, salt tolerant lines FL 478, IR 74802, IR 73104 and IR 72593 seem to accumulate significantly higher potassium in their roots compared to susceptible genotype IR 29. In general, salinity reduced K^+ concentration and increased Na^+ concentration in roots. Concentration of K^+ in the shoot of IR 29 was lower than that of other genotypes under normal conditions. Potassium concentration in the shoot of all genotypes decreased substantially under salt stress with much higher reduction in K^+ in the susceptible cultivar IR 29 than in other genotypes. Unlike in other plant parts concentration of K^+ in leaves was not much affected by salt stress especially in 5th and 6th leaves. Rice seems to accumulate much higher sodium in roots, shoots and lowest in leaves.

The ratio of Na^+/K^+ in roots could not differ much among tolerant genotypes under salt stress condition, but was significantly higher in roots, shoots and leaves of susceptible genotype IR 29. The genotype IR 72593 recorded lower shoot, root, and leaves Na^+/k^+ ratio at 120 mM. The variation in sodium and potassium concentration and Na^+/K^+ ratio in individual leaves of each of rice genotypes showed greater reduction in lower leaves 4 than 6th leaf. Significant difference in cultivars between normal, 60mM and 120 mM were observed when treatment was prolonged to 14 days. Salinity resulted in substantial reduction in biomass. Genotypic difference in the extent of salinity induced reduction in root, shoot and total seedling biomass were also apparent with the greatest reduction experienced by the intolerant cultivar, IR 29. On average, root-shoot ratio increased by about 28.62 % due to salt stress and with the significant variation among cultivars in response to salinity. The IR 29 showed the lowest increase in root-shoot ratio at 120 mM (31.41%). The vast decrease in root-shoot ratio of IR 29 under salinity was mostly because of reduction in shoot dry matter. Root, shoot and total dry matter significantly decreased with an average of 9.58%, 17.28% and 26.85% respectively. The susceptible genotype showing greater reduction in total biomass due to salt stress than tolerant cultivars. Thus reduction in total biomass seems to be a better indicator of salinity tolerance.

The modified standard evaluation score in rating the visual symptoms of salt toxicity established at IRRI was used to discriminate the susceptible, tolerant and moderately tolerant genotypes (Gregario *et al.* 1997). Among the selected genotypes and hybrids tested in the first experiment, genotypes, viz. IR 74802, IR 72593, IR 73104 and FL 478 exhibited higher tolerance at 60 and 120 mM salt concentration in terms of SES. From the SES data of the second experiment, the salt tolerance of the above mentioned genotypes was confirmed while with the genotype IR 29 was found to be susceptible. Gregario *et al*. (1997) confirmed the reliability of SES for salt tolerance by comparing it with grain yield. Like wise they established a high positive correlation between SES and Na^+/K^+ ratio.

The regression analysis between Na^+/K^+ content is ($r^2 = 0.22$) and for seedling vigor and Na^+/K^+ ratio ($r^2 = 0.3$) is weakly related, this may be due to seedling tolerance may not be positively correlated to reproductive stage tolerance, hence accumulation of salts are profound at the later stage of crop growth. This study infers that the seedling stage tolerance is only inference, but not the tolerance of whole crop stage. This seedling and vegetative stage tolerance is weakly associated in most of the genotypes, however some genotypes have the mechanism to respond to tolerance in all the stage of crop growth. Based on the Na^+ and K^+ content in leaves and shoot samples, the genotypes, viz. IR 74802, IR 73104, IR 72593 and FL 478 were found to contain low Na^+/K^+ ratio. Roy *et al.* (2005) stated that shoot Na^+/K^+ ratio is considered as a reliable parameter to evaluate salt tolerant genotypes in rice. The typical mechanism of salt tolerance in rice is the Na^+ exclusion or reduced uptake of Na^+ and increased absorption of potassium to maintain a good Na^+/K^+ balance in the shoot.Bal *et al.* (1986) reported that salt tolerant rice varieties accumulate lesser Na^+ and higher K^+ than susceptible varieties.

Lutts *et al.* (1996) found that the resistant cultivars accumulated less Na, Cl, Zn and proline and more K in roots and shoots than salt sensitive cultivar. Accumulation of Na and decrease in K content in shoots were restricted to the oldest leaves in salt resistant cultivars. Studies on salt tolerance in Korean rice cultivars by Cho Dong Ha (1996) revealed that the extent of 'K' exclusion by 'Na' in the roots might be related to salt tolerance. Asch *et al.* (1997) selected five lines I Kang Pao, IR 64, IR 4630-22-2, CSR 10 and Aiwu to be salt tolerant based on the traits grain yield decline, spikelet sterility and sodium and potassium distribution in top three leaves, stems, stem base and roots. They reported that potassium/ sodium ratio in the three leaves from the top was high in these genotypes and this could be used as a reliable index for salt tolerance. Low Na^+/K^+ ratio of ion uptake is positively correlated with a high level of salt tolerance and can be taken into consideration as a desired characteristic while screening rice lines (Mishra *et al.* 1997).

Shannon *et al.* (1998) observed that leaf tissues of plants grown at 16 dS/m had five times increase in Na^+ and three times increase in Cl^- as compared to those of controls. Leaf concentration of K^+ was decreased by about 40% by salinity, but tissue levels of Ca^{2+} and Mg^{2+} were unaffected. Relative salt tolerance differences were found to be negligible among cultivars leading to the conclusion that genetic differences are limited. Highest K^+/Na^+ ratio noticed in the lower leaves (4th leaf) and it decreases towards younger 'Y' leaf, this leads to cessation of growth in lower leaves due to more accumulation of salts. This is quite visible in sensitive cultivars. Reduction in K/Na ratio was observed with increasing soil salinity. Ansari *et al.* (2003) have reported that sodium uptake increased and potassium decreased with increasing salinity. In a study performed using 2 week old seedlings of Shua-92 and IR 8C, the Na/K ratio was found to be increased with time in IR 8, whereas the ratio reduces in more tolerant cultivar Shua-92.For many salt sensitive plants, a major part of the growth inhibition is caused by excess Na^+. High sodium disturbs potassium (K) nutrition and when accumulated in cytoplasm, inhibits many enzymes (Jaleel *et al.* 2007). The results of the second experiment also revealed that the salt tolerant genotypes FL 478, IR 74802, IR 73104 and IR 72593 exhibited a significant reduction in accumulation of sodium in the leaves even at higher level of salinity. However, salt sensitive genotype IR 29 had accumulated high sodium in the leaves under lower level of salinity itself. The rice plants have K^+ absorption capacity to balance the Na^+ in the cell (Gregario and Senadhira 1993). Therefore, the genotypes with low Na^+/K^+ ratio have an ability to adjust the sodium content in parts of the plants to prevent toxicity of ions. The cultivars which have the ability of Na^+/K^+ balance (low Na and K ratio) is classified as salt tolerant genotypes. Din *et al.* (2008) reported an inverse relationship between shoot sodium content and salt tolerance in rice. A significant negative correlation between dry matter production and Na^+/K^+ concentration indicates the reduction of source activity, which may impart the accumulation of photo-assimilates within the grain (Ashraf and O'Leary 1996 and Poustini and Siosemardha 2004). Hence, it can be concluded that under salt stress condition, the plants with low Na^+/K^+ ratio and low SES (IR 73104, IR 74802 and IR 72593) are salt tolerant.

Lower Na^+/K^+ ratio is related to salt tolerance in crop plants (Mishra *et al.* 2000). High salt (NaCl) uptake competes with the uptake of other nutrient ions, especially K^+ leading to K^+ deficiency (Parida and Das 2005). Increased concentration of NaCl induces increase in Na^+ and Cl^- and decrease in Ca^{2+}, K^+, and Mg^{2+} levels in a number of plants (Khan *et al.* 2000). There is a positive relationship between Na^+ and Cl^- and a negative relationship between Na^+ and K^+ concentration in roots and leaves (Parida and Das 2005). Under salt stress, plants maintain high concentrations of K^+ and low concentrations of Na^+ in the cytosol. Maintenance of relatively lower Na^+/K^+ ratio in the shoot in the presence of salinity stress coupled with its ability to grow fast despite NaCl stress is a characteristic stress adaptation seen in Pokkali (Walia *et al.* 2005). An increase in Na^+/K^+ ratio was evident in the susceptible genotype (IR 29) at higher salt levels (Singh *et al.* 2007).

Dry matter production and partitioning between different plant organs was substantially altered by salt stress. IR 72593 had higher dry matter accumulation, followed by IR 73104. However, IR 29 showed a poor performance and reduced dry matter under increased salt stress concentration. The greater reduction in shoot biomass under salt stress, suggested that growth of rice roots is less sensitive to salinity than shoot growth. Munns (2006) reported that immediate response of salinity results in increase in root-shoot ratio with salt stress.

SUMMARY

The tolerant genotypes have compensating mechanism of Na^+ exclusion and K^+ absorption which buffers to excessive salt stress, whereas susceptible genotypes respond quickly to salinity and did not recover after a week of salinity leading to complete death of whole plants. The reduced level of CO_2 assimilation, stomatal closure, transpiration rate, exclusion of Na^+ ion and K^+ absorption and increased level of antioxidant enzymes serve as a potential indicator in identification of tolerant genotypes at early seedling stage. Therefore, screening of genotypes at seedling stage by using these criteria would be quite effective and time saving method.

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