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Annals of Biological Research, 2016, 7 (5):46-54
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Early seedling stage salt tolerance evaluation of genetically diverse rice genotypes

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ABSTRACT

The present study carried out a comparative evaluation of 38 rice genotypes (including 17 landraces) for salinity tolerance at germination and early seedling stage salt tolerance of genotype is stage specific. Hence to understand the significance of the contribution of different salt tolerance mechanisms among genotypes, its primary requisite to reveal its tolerance level at an early seedling stage before. A refined scoring system for salt tolerance evaluation at the early seedling stage was designed to reveal genotypes salt tolerance in a given experimental conditions. The extent of genetic similarity was assessed by AFLP among 21 of them. Comparative analysis in similar growth conditions, stage and time-points provided a better understanding of theinvolvement of stress effect mitigating genes among diverse rice genotypes. This study evaluated salinity tolerance of several unexplored genotypes/land races to enable effective use of these in particular soil conditions and can be utilized to improve tolerance of sensitive but agronomically important landraces.

Keywords: Early seedling stage, *Oryza sativa*; salt tolerance grade (STG).

INTRODUCTION

Abiotic stress is detrimental to all plants and a major cause of reduced crop productivity. Among them, salinity is an important agricultural problem as it affects more than 6% of the total and ~20% of irrigated land globally. To make matter worse salinity affected area is gradually increasing due to natural factors and human activities (Tuteja 2007). Hence, development of tolerant varieties using classical and biotechnological approaches is highly desirable and is an active area of research (Negrão et al. 2011). However, being polygenic trait salinity tolerance is a highly complex phenomenon and a thorough understanding of various mechanisms contributing to it is required to realize the above-mentioned goals (Sreenivasulu et al. 2007). Salinity tolerance is widely varied with plant species and genotypes within a species. Rice (*Oryza sativa* L.), a globally important crop, is relatively more sensitive to salinity than other cereal crops such as barley and wheat (Munns and Tester 2008). Variability in salinity tolerance has been reported in rice at different stages of growth, with germination and active tillering stages being more tolerant than panicle initiation, fertilization and early seedling stages (Walia et al. 2005 and references therein).The response to salt stress is highly complex and involves diverse mechanisms aimed at minimizing the salinity-induced cellular damage. Since last two decades, various mechanisms and characterization of genes involved in these mechanisms have been revealed. For best utilization of this information in germplasm improvement, one should know exactly which mechanisms are predominant and or lacking in a given genotype. Therefore,choice of germplasm in screening and further experimental analysis is most crucial for the success of studies. Also, for effective utilization of agronomically important genotypes in particular soil region, it is primary requisite to know their salt tolerance level. The present study includes geographically distinctive population landraces, which are endowed with tremendous genetic variability and represents a unique source of useful traits for rice genetic improvement.

MATERIALS AND METHODS

Plant material

A total of 38 rice (*Oryza sativa* L.) genotypes including 12 obtained from International Rice Research Institute (IRRI, Philippines) and 26 from University of Pune (Pune, India) were used in the present study (Table 2). The genotypes included both known salt tolerant and sensitive genotypes as well as seventeen landraces with no information on their salt tolerance. All the genotypes were evaluated for the effect of salt stress on germination and at early seedling stage (6-day old seedlings). Subsequently, 21 genotypes were used for genetic diversity analysis (Table 2).

Conditions for germination, growth of seedlings, salinity treatment, and analysis

Seeds were surface sterilized with 0.1% $HgCl_2$ (Hi-media, India), imbibed for 24 h in distilled water (D/W), and used for germination and early seedling stage analysis.

Seed germination analysis: For each genotype 20-30 seeds were kept for germination in the dark on a sterile moist blotting paper in a glass petri plate and the data was recorded up to seven days. Control seeds were germinated on blotting paper moistened with Hoagland's basal nutrient media (Hi-media, India) whereas, the nutrient media supplemented with sodium chloride (0-200 mM NaCl) was used for salt stress treatment. Germination of seeds was recorded as per Liu et al. (2007), where seeds were considered to have germinated when the emerging radical was about the half-length of seed. Germination percentage was scored till 7^{th} day and was used for calculating mean germination time (MGT) as per Ellis and Roberts (Ellis and Roberts 1980). The analysis was repeated three times.

Early seedling stage analysis: Rice genotypes were grown hydroponically and screened for salt tolerance at an early seedling stage in a plant growth chamber (MLR-351H, Sanyo, Japan) under 13 h / 11 h light / dark cycle. Seeds were germinated as explained above, 2-day old seedlings were transferred to the 96-well PCR plates cut from the bottom and floated in a plastic tray containing Hoagland's media which was replaced every 48 h. Following growth conditions were used in the growth chamber: light intensity: 150 µmol m^{-2} s^{-1}, temperature: 28 ± 1°C (in light period) and 26 ± 1°C (in dark period), relative humidity: 65 ± 2%. For salt stress treatment, 6-day old seedlings were transferred to Hoagland's media supplemented with or without 150 mM NaCl.

Salinity tolerance evaluation at early seedling stage

Screening for salinity tolerance at seedling stage was carried out in hydroponics systems. A completely randomized design (CRD) with three replicates (at least 15 plants per replicate) was performed for the salinity tolerance evaluation and salinity tolerance grading. The plant growth condition was followed as given above. After 4 DIS, shoot length and visual salinity injury of individual seedling was recorded. Salt tolerance grade of rice seedlings at theearly seedling stage was calculated by introducing a fine scoring system based on SES method of IRRI (Gregorio GB et al, 1997)and the details are given in Table 1.

Table 1: Details of calculation of visual salt injury (VSI) penalty in rice seedlings at the early seedling stage.

I. For each of the 38 rice genotypes, at least 15 seedlings were used for analysis. Salt-induced damage observed in the percentage of rice seedlings was recorded in primary leaf [A], other leaves [B] and shoot [C]. In addition reduction in seedling growth [D] was also included calculations. The analysis was repeated three times.
II. The penalty was given on the basis of severity of VSI (1: part of tissue affected or 2: whole tissue affected) to primary leaf (1), and other leaves (2). In the case of shoot (3) penalty given was either 1 (contracted) or 2 (pale yellow/discoloration).
III. The percentage of seedlings affected was also included along with the penalties given above for the calculation.
IV. The penalty for each type ([A], [B], [C] and [D]) of salt-induced damage to rice seedlings was added up.
V. Total penalty score class: ≤ 250 (Salt tolerant); 251-400 (Salt moderate tolerant); ≥ 401 (Salt-sensitive); T: Salt tolerant, MT: Salt moderate tolerant, S: Sensitive

Visual salt injury (VSI) symptoms observed / Number of seedlings affected (in %)	[A] VSI (contracted and discoloration) on primary leaf (1)		[B] VSI (contracted and discoloration) on other leaves (2)		[C] VSI on shoot (3)		[D] % Reduction in growth rate (in)
	Leaf tip (1)	Whole leaf (2)	Leaf Tip (1)	Whole leaf (2)	Contracted (1)	Pale yellow (2)	
< 30% (10)	10 x 1 x 1	10 x 1 x 2	10 x 2 x 1	10 x 2 x 2	10 x 3 x 1	10 x 3 x 2	< 30% (10)
30% - 60% (20)	20 x 1 x 1	20 x 1 x 2	20 x 2 x 1	20 x 2 x 2	20 x 3 x 1	20 x 3 x 2	30 – 60% (20)
60% - 80% (30)	30 x 1 x 1	30 x 1 x 2	30 x 2 x 1	30 x 2 x 2	30 x 3 x 1	30 x 3 x 2	60 – 80% (30)
> 80% (40)	40 x 1 x 1	40 x 1 x 2	40 x 2 x 1	40 x 2 x 2	40 x 3 x 1	40 x 3 x 2	> 80% (40)

Assignment of salt tolerance grade

Based on genetic relatedness and early seedling stage salinity tolerance 11 rice genotypes were selected for salt tolerance grade assessment. While assigning salinity tolerance grade visual salt injury as well as percent growth rate retardation after 2 and 4 DIS was taken into consideration. Penalty scoring system was similar as followed for salinity tolerance evaluation. The range of total penalty scores [A+B+C+D] after 2 DIS was found to be in the 100-350 and after 4 DIS: 160-520. The range of penalty score was classified into 1 (lowest) – 10 (highest) grades with each class of 30 for 2 DIS and 50 for 4 DIS. Average of 2 DIS grade and 4 DIS grade was assigned as genotypes salinity tolerance grade at early seedling stage (Table 2).

DNA isolation and AFLP analysis: Genomic DNA was isolated from shoot tissue of the rice seedling as per the protocol of Nalini et al. (Nalini et al. 2004). AFLP analysis was performed as described in Vos et al. (1995) with minor modifications. Genomic DNA was double-digested with restriction enzymes *Pst*I and *Mse*I (New England Biolabs, USA). After heat inactivation of restriction enzymes, the adapters specific for *Pst*I (*Pst*I-a: 5′-CTCGTAGACTGCGTACATGCA-3′, *Pst*I-b: 5′-TGTACGCAGTCTAC-3′) and *Mse*I (*Mse*I-a: 5′-GACGATGAGTCCTGAG-3′, *Mse*I-b: 5′- TACTCAGGACTCAT-3′) were ligated to the restricted fragments using DNA ligation kit LIG-1 (Sigma-Aldrich, USA) as per the recommended protocol. The restriction-ligation mixture was diluted 5 times used as a template for pre-selective amplification using adapter specific primers for *Pst*I (P: 5′-GACTGCGTACATGCAGA-3′), and *Mse*I (M: 5′-GATGAGT-CCTGAGTAAC-3′). Pre-selective PCR product was diluted 50 times and used as a template for selective amplification using 20 *Pst*I and *Mse*I adapter primers combinations with 3-base extensions at 3′- end(P_{NNN}+M_{NNN}) (Table 4). PCR products were resolved on 5 % denaturing polyacrylamide gel and DNA fragments were visualized by silver staining. The AFLP gels were scored for presence (1) and absence (0) of DNA fragments and data from 20 primer combinations was used for analysis of genetic diversity by aun-weighted pair- group method with arithmetic average (UPGMA) of TREECON program version 1.3b (Van de Peer and De Wachter 1993).

RESULTS AND DISCUSSION

Effect of salinity on germination

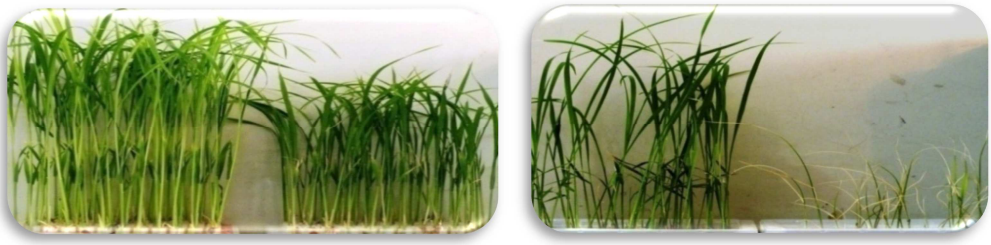
Salinity affected the seed germination to a different extent among the rice genotypes analyzed (0 - 60 % reduction, Table 2). Analysis of DMGT values revealed fine differences in the effect of salinity on germination. Among the genotypes, CSR06220, Delhi rice, RDN local, Pusa Basmati, Pusa Sugandha5 and Gopal Bhog showed minimum DMGT whereas IR29 showed the maximum (Table 2).

Effect of salinity on growth at early seedling stage

Rice genotypes showed 35-82% growth retardation under salinity at early seedling stage (Table 2). Analysis of variance (ANOVA) showed significant differences in retardation in seedling growth under salinity among tolerant, moderate tolerant and salt sensitive rice genotypes (P= ≤0.05) (Table 3).

Figure 1: Rice genotypes grown with or without 150 mM NaCl for four days

NSICRc106 (Salt Tolerant) IR29 (Salt Sensitive)



Control **Stress** **Control** **Stress**

Table 2: Details of effect of salinity on germination, growth and visual salt injury (VSI) symptoms observed in seedlings of rice genotypes at the early seedling stage

No	Genotype	Salt Tolerance of Genotype	Average % Germination[b]	DMGT[c] (±SE)	Day In Stress	% Seedlings with VSI and penalty score[d]			% Growth Retardation ± SE,(Penalty score)	Total penalty Score	Salt Tolerance at Early Seedling Stage[e]	Salt Tolerance Grade (STG)[f]
						Primary leaves	Other leaves	Seedlings				
1	NSICRc106*	T	100	0.80 (±0.1)	2 DIS	>80% (40)	<30% (20)	<30% (30)	30 ± 2.5 (10)	100	T	2
					4 DIS	>80% (80)	30-60% (40)	<30% (30)	35 ± 4.0 (20)	170		
2	Cherivirappu*	T	100	1.20 (±0.13)	2 DIS	>80% (40)	<30% (20)	<30% (30)	32 ± 6.0 (20)	110	T	3
					4 DIS	>80% (80)	30-60% (40)	30-60% (60)	35± 5.0 (20)	200		
3	Delhi rice*	NA	100	0.10	2 DIS	>80% (40)	<30% (20)	<30% (20)	40 ± 2.5 (10)	110	T	3.5
					4 DIS	>80% (80)	>80% (80)	30-60% (60)	36 ± 5.0 (20)	240		
4	Panvel03*	T	100	0.25 (±0.05)	2 DIS	>80% (80)	<30% (20)	<30% (30)	35 ± 2.5 (20)	150	MT	5
					4 DIS	>80% (80)	>80% (160)	30-60% (60)	38 ± 6.0 (20)	320		
5	PSBRc50*	T	100	0.40 (±0.05)	2 DIS	>80% (80)	60-80% (60)	<30% (30)	45 ± 4.0 (20)	150	MT	5.5
					4 DIS	>80% (80)	>80% (160)	30-60% (60)	42 ± 5.0 (20)	320		
6	PSBRc84*	T	100	1.00 (±0.1)	2 DIS	>80% (80)	60-80% (60)	30-60% (120)	32± 10.0 (20)	280	MT	7
					4 DIS	>80% (80)	60-80% (120)	60-80% (120)	55 ± 6.0 (20)	340		
7	Nonabokra*	T	100	1.50 (±0.1)	2 DIS	>80% (80)	30-60% (80)	30-60% (60)	66 ± 5.0 (30)	250	MT	7
					4 DIS	>80% (80)	60-80% (120)	60-80% (180)	52 ± 6.0 (20)	400		
8	Karjat03*	S	100	1.40 (±0.1)	2 DIS	>80% (80)	60-80% (60)	30-60% (90)	45 ± 5.0 (20)	250	S	7.5
					4 DIS	>80% (80)	>80% (160)	60-80% (180)	70 ± 5.0 (30)	450		
9	Gham*	NA	100	1.41 (±0.1)	2 DIS	>80% (40)	>80% (80)	60-80% (90)	65 ± 2.5 (30)	240	S	8
					4 DIS	>80% (80)	>80% (160)	>80% (240)	75 ± 10.0 (30)	510		
10	RDN local*	NA	100	0.10	2 DIS	>80% (80)	>80% (160)	30-60% (60)	40 ± 2.5 (20)	320	S	9
					4 DIS	>80% (80)	>80% (160)	>80% (240)	82 ± 5.0 (40)	520		
11	IR29*	S	40	2.04 (±0.22)	2 DIS	>80% (80)	>80% (160)	60-80% (90)	45 ± 4.0 (20)	350	S	9.5
					4 DIS	>80% (80)	>80% (160)	>80% (240)	65 ± 2.5 (30)	510		
12	PSBRc48*	T	100	0.70 (±0.05)	4 DIS	>80% (80)	60-80% (120)	30-60% (60)	45 ± 5.0 (20)	280	MT	-
13	PSBRc86*	T	100	1.30 (±0.1)	4 DIS	>80% (80)	60-80% (120)	60-80% (90)	40 ± 5.0 (20)	310	MT	-
14	PSBRc88*	T	100	0.50 (±0.1)	4 DIS	>80% (80)	60-80% (120)	60-80% (90)	40 ± 6.0 (20)	310	MT	-
15	CSR06220*	NA	100	0.06	4 DIS	>80% (80)	60-80% (120)	60-80% (90)	50 ± 5.0 (20)	310	MT	-

16	Damodar*	MT	100	0.20 (±0.05)	4 DIS	>80% (80)	60-80% (120)	60-80% (90)	42 ± 4.0 (20)	310	MT	-
17	Getu*	MT	100	0.60 (±0.05)	4 DIS	>80% (80)	60-80% (120)	60-80% (90)	45 ± 6.0 (20)	310	MT	-
18	Kalanamk*	NA	100	1.20 (±0.05)	4 DIS	>80% (80)	30-60% (80)	30-60% (90)	38 ± 4.0 (20)	270	MT	-
19	Chimansal*	NA	95	0.20 (±0.1)	4 DIS	>80% (80)	30-60% (80)	60-80% (90)	55 ± 5.0 (20)	270	MT	-
20	Gandhsale*	NA	77	1.47 (±0.1)	4 DIS	>80% (80)	60-80% (120)	60-80% (180)	60 ± 7.0 (30)	410	S	-
21	Ghansal*	NA	53	1.53 (±0.1)	4 DIS	>80% (80)	>80% (160)	>80% (240)	65 ± 4.0 (30)	510	S	-
22	Pandhara dodki*	NA	90	1.64 (±0.2)	4 DIS	>80% (80)	60-80% (120)	>80% (240)	65 ± 10.0 (30)	470	S	-
23	Champakali	NA	95	0.40 (±0.2)	4 DIS	>80% (80)	60-80% (120)	>80% (240)	65 ±2.0 (30)	510	S	-
24	Gari Kolpi	NA	100	0.20 (±0.1)	4 DIS	>80% (40)	30-60% (40)	>30% (60)	45 ± 5.0 (20)	160	T	-
25	Pakistan Basmati	NA	100	0.20 (±0.1)	4 DIS	>80% (80)	30-60% (80)	60-80% (90)	48 ± 5.0 (20)	270	MT	-
26	Jeera Sona	NA	95	0.68 (±0.2)	4 DIS	>80% (80)	30-60% (80)	60-80% (90)	35 ± 5.0 (20)	270	MT	-
27	Geerige Sanna	NA	100	0.42 (±0.2)	4 DIS	>80% (80)	30-60% (80)	>80% (120)	45 ± 6.0 (20)	300	MT	-
28	Kolamb	NA	95	0.20 (±0.2)	4 DIS	>80% (80)	60-80% (120)	>80% (240)	58 ± 4.0 (20)	460	S	-
29	Super Basmati	NA	95	0.40 (±0.1)	4 DIS	>80% (80)	60-80% (120)	60-80% (180)	65 ± 3.0 (30)	410	S	-
30	Pusa Basmati	S	100	0.10	4 DIS	>80% (80)	60-80% (120)	60-80% (180)	65 ± 8.0 (30)	410	S	-
31	Pusa Basmati1	NA	95	0.33 (±0.1)	4 DIS	>80% (80)	30-60% (80)	30-60% (120)	45 ± 4.0 (20)	300	MT	-
32	Pusa Sugandha	NA	100	0.10	4 DIS	>80% (40)	30-60% (40)	30-60% (60)	38 ± 3.0 (20)	160	MT	-
33	Pusa Sugandha5	NA	95	0.22 (±0.2)	4 DIS	>80% (80)	30-60% (40)	60-80% (90)	45 ± 5.0 (20)	230	MT	-
34	Dusara	NA	77	1.67 (±0.05)	4 DIS	>80% (80)	30-60% (80)	60-80% (90)	45 ± 5.0 (20)	270	MT	-
35	Gopalbhog	S	100	0.10	4 DIS	>80% (80)	>80% (160)	>80% (240)	65 ± 4.0 (30)	510	S	-
36	Swarna	S	95	0.30 (±0.04)	4 DIS	>80% (80)	60-80% (120)	30-60% (120)	46 ± 8.0 (20)	340	MT	-
37	Girga	NA	92	0.50 (±0.05)	4 DIS	>80% (80)	30-60% (40)	30-60% (120)	55 ± 3.0 (20)	260	MT	-
38	HaliKolpi	NA	100	0.20 (±0.1)	4 DIS	>80% (40)	30-60% (40)	30% (60)	38 ± 3.0 (20)	160	T	-

'' Indicates the 22 rice genotypes included for analysis of genetic diversity by AFLP.*
'a' Known salt tolerance of genotype (T: tolerant, MT: moderately tolerant, S: sensitive, NA: no information available)
'b' Average germination as per Liu et al. (2007)
'c' DMGT (Delayed Mean Germination Time) as per Kaya et.al. (2009).
'd' Provides details of apercent of seedlings affected by visual salt injury (VSI) at different parts (primary leaves, other leaves, whole seedlings) along with the assigned penalty score (in parenthesis). 'e' indicates the salt tolerance of rice genotypes and land races at early seedling stage
'f' indicates the salt tolerance grade (STG) values of 11 genotypes subsequently used for detailed analysis of proline accumulation under salinity

Table 3: Result of ANOVA analysis – shoot length growth retardation under stress and genotypes salt tolerance at early seedling stage

Class	Mean	Variance	Number of samples
Tolerant genotypes	37.8	17.7	5
Moderately tolerant genotypes	42.1	62.5	21
Sensitive genotype	61.3	152.4	12

Fisher (F) value: 19.66; Probability: 1.9E-6, at the probability level 0.05 the means are found to be significantly different

Differential effect of salinity on germination and early seedling stage

In the present study, salinity tolerance of 38 rice genotypes (including several landraces) was evaluated at an early seedling stage in terms of salt tolerance grade (STG) (Table 2).

In the majority of the rice genotypes, germination stage was more tolerant to salinity as also observed in previous reports (Negrão et al. 2013 and references therein). However, exceptions were observed in certain moderately tolerant (at early seedling stage) genotypes that showed higher sensitivity at germination stage. Similarly, in some sensitive genotypes germination remained unaffected under salt stress (Table 2). Observed salt tolerance in rice at seedling stage is generally reported to be in agreement with the tolerance of the genotypes (Islam et al. 2012). Our study shows that tolerance at the early seedling stage is also in agreement with a salt tolerance of rice genotypes observed at seedling stage (Islam et al. 2012). Hence, screening at the early seedling stage can be effectively employed for screening of rice genotypes. In present study multiple rice genotypes including several agronomically important and unexplored landraces/localselections (with no information on salt tolerance) were analyzed for salt toleranceat germination and early seedling stage. Some of the landraces analyzed includescented non-basmati type genotypes known for good aroma (Champakali, Gham,Ghansal, Kalanamak, GopalBhog etc.) and grain shape characteristics (Delhi rice, GariKolpi, HaliKolpi) (Mathure et al. 2010, Mathure et al. 2011). Thisinformation will also be useful for effective utilization of such landraces/localselections with important traits in particular soil conditions.In response to salt stress wide degree of biochemical and physiological changes has been reported (Munns and Tester 2008). To associate the significance of thecontributionto change in biochemical level and physiological modulations in enhancing salt tolerance, it is primary requisite to reveal genotypes salt tolerance in a given experimental condition. Hence, arefined method for salt tolerance evaluation at early seedling has been followed. We have seen that in rice genotypes degree of salt stress effect differs stage to stage and also genotype to genotype. While seedling growth in salt condition, we observed that, though some genotypes were able to tolerate or showed relatively less visual salt injury symptom in early stress period after 4 DIS there visual salt injury score / salt tolerance level was similar to genotypes with relatively low salt tolerance. Hence to avoid such ambiguity and to see theprobable correlation between there salt tolerance and biochemical-molecular level response to salt (future studies), VSI symptom at 2^{nd} day was included along with 4^{th}-day time-points for refined STG scoring among genotypes with similar salinity tolerance. A positive correlation (R=0.89) between STG of genotypes and percent shoot growth retardation indicates that fast seedling growth rate is also an important factor for salt tolerance in rice, as also observed in a previous report (Anil et al. 2005).

Genetic relatedness among rice genotypes with varying salt tolerance

Of the total 38 rice genotypes, 21 were further analyzed for genetic diversity by AFLP (Table 2). Twenty primer combinations were used for the AFLP analysis. Out of a total of 461 scorable bands, 265 were polymorphic. The polymorphism percentage was 57.84%.

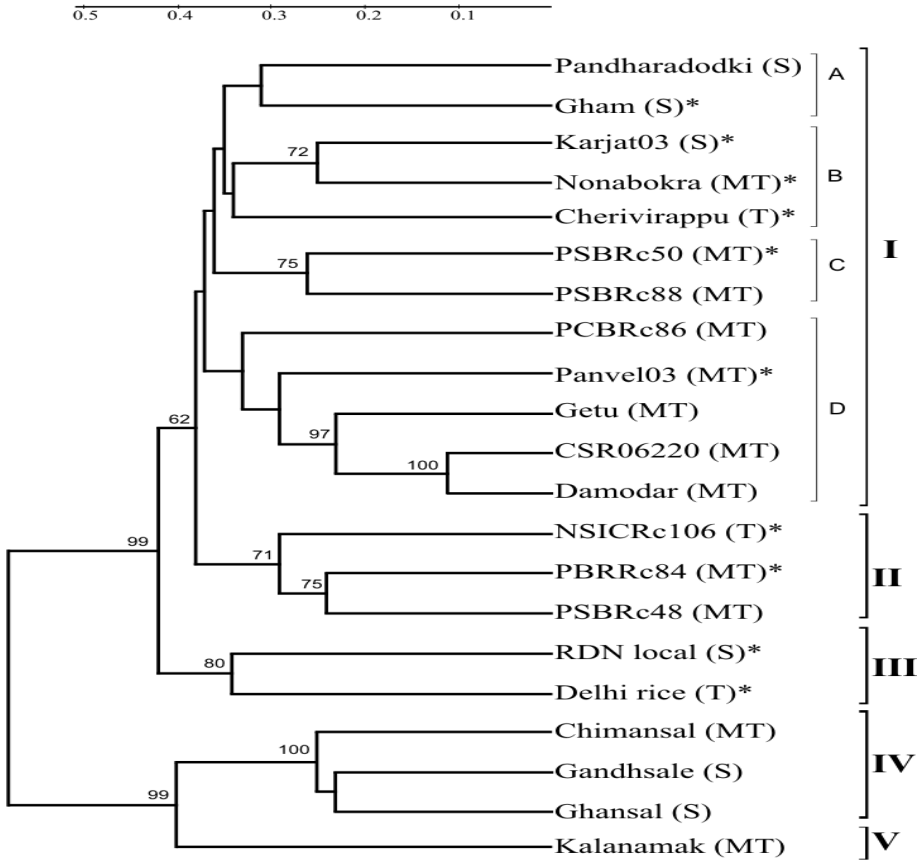
Table 4: List of AFLP primers and number of PCR amplified bands generated across 21 rice accessions

Sr.No.	Primer combinations (PstI/MseI) and sequence of the last three bases	Total No of scorable bands	No of Polymorphic bands	% Polymorphic bands
1	ACG/CAC	19	12	63
2	ACT/CTG	11	5	45
3	AGT/CAC	18	6	33
4	AGT/CTG	16	7	43
5	AGG/CAC	23	13	57
6	AGG/CAG	13	9	69
7	ACA/CTC	21	12	57
8	ACT/CTA	24	13	72
9	AGT/CAG	11	6	54
10	AAC/CTA	25	17	68
11	ACG/CTA	30	18	60
12	ACG/CAG	25	9	36
13	AAC/CAC	32	18	56
14	ACA/CAC	34	24	71
15	AGT/CTA	10	5	50
16	ACC/CAC	22	13	59
17	ACA/CTG	30	22	73
18	AGT/CTC	38	25	66
19	AAC/CTT	35	15	43
20	AGT/CAA	30	16	53
Total		**461**	**265**	**57.84%**

The cluster analysis obtained with the UPGA revealed five main clusters (Figure 3). With some exceptions, genotypes with similar early seedling stage salt tolerance (visual salt injury based penalty score) were grouped in aclosed cluster. The first, cluster A, included seven accessions and could be divided into two sub-clusters. Sub cluster A1 included Cherivirappu [salt tolerant (200_{4DIS})], Nonabokra [salt moderately tolerant (400_{4DIS})] and Karjat03 (450_{4DIS}), Pandharadodki (470_{4DIS}) and Gham (510_{4DIS}) all salt sensitive genotypes. Cherivirappu and Nonabokra cultivar are non-scented varieties whereas Karjat03, Pandharadodki and Gham is scented non-basmati genotypes. Sub cluster A2 included salt moderately tolerant genotypes PSBRc50 (320_{4DIS}) and PSBRc88 (310_{4DIS}) with a common parent IR4630-22-2-5-1-3 (moderately salt tolerant donor line) (give areference). Cluster B included PSBRc86 (310_{4DIS}), Panvel03 (320_{4DIS}), Damodar (310_{4DIS}), CSR06220 (310_{4DIS}) and Getu (270_{4DIS}) which belong to moderately salt tolerant genotypes. Cluster C included another group of moderately salt tolerant genotypes viz.; PSBRc84 (340_{4DIS}), PSBRc48 (280_{4DIS}) and salt tolerant genotype NSICRc106 (170_{4DIS}). Cluster D included only two scented rice genotypes with contrasting early seedling stage salt tolerance viz.; RDN local (520_{4DIS}) and Delhi rice (240_{4DIS}). Cluster E includes 4 scented genotypes viz.; Kalanamak3131 (270_{4DIS}), Chimansal (270_{4DIS}), Ghansal (400_{4DIS}) and Gandhsale (510_{4DIS}). This cluster includes salt sensitive genotypes except Kalanamak3131and Chimansal which was found to be salt moderately tolerant genotype.

The AFLP analysis evaluated the extent of genetic diversity among rice genotypes and land races. Certain salt tolerant genotypes/landraces were identified which could be effectively used to improve the salt tolerance of agronomically important but salt sensitive genotypes/landraces. Intraspecies variability in salt tolerance does exist in rice and should be utilized to improve salinity tolerance of cultivars (Walia et al. 2005; Negrão et al. 2013). Evaluation of such variability among the unexplored landraces would enable their effective utilization, and may also lead to new *loci* governing salt tolerance (Walia et al. 2005). Results show that landraces Delhi rice, Gari Kolpi and Hari Kolpi were least affected by salinity and may serve as potential genotypes for use in the rice breeding programs. Furthermore, evaluation of salinity tolerance of several landraces including scented, non-basmati type (Champakali, Gham, Ghansal, Kalanamak, Gopal Bhog) and non-scented (Gari Kolpi, Hali Kolpi) landraces with good grain characteristics (Mathure et al. 2011) would be informative to select agronomically important landraces with similar traits for particular soil conditions. The moderately salt tolerant landraces (Chimansal, Jeera Sona, Pakistan Basmati, Geerige Sanna and Girga) would be more useful than the sensitive landraces. Salt tolerance is known to be a multigenic trait which means the presence of different combinations of genes/pathways in the genotypes that contribute to salt tolerance. And to reveal aprobable best combination of genes/pathways for enhancing salt tolerance, ideal genotypes are genotypes with similar salt tolerance but genetically diverse and vice versa (Table 4 and Figure 2). Genetically diverse salt tolerant genotypes (NSICRc106, Cherivirappu, and Delhi rice) may also serve as good material to study a different combination of molecular mechanisms involved in salinity tolerance.

Figure 2: Clustering analysis using the Unweighted Pair-Group Method with arithmetic Averages (UPGMA) for 21 rice genotypes



CONCLUSION

The present analysis of multiple rice genotypes identified some unexplored landraces, which can serve as new sources of salt tolerance, or can be utilized in breeding programs for improving tolerance of agronomically important genotypes. The information can also be utilized for efficient utilization of landraces in particular soil conditions. A refined scoring system for salt tolerance evaluation at theearly seedling stage is asimple and reproducible method which can be followed before assigning the significance of genes and or mechanism in enhancing salt tolerance in a given genotype at the early seedling stage.

Sources of funding
Department of Atomic Energy, Government of India

Acknowledgements
The authors are thankful to International Rice Research Institute IRRI, Philippines and University of Pune, Maharashtra India for providing the seed material for this study. The authors are also thankful to Dr. Ajay Saini and Mr. Ravi Sanyal for their support.

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