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Research Article

Assessment of seedling traits of rice landraces under different saline conditions

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Abstract

Salinity is one of the major abiotic stresses affecting rice growth and yield worldwide. In rice, the most critical stages which affect salinity at a greater level are germination, vegetative and reproductive stages.It is very important to know the genotypic variation among landraces under saline conditions at the seed germination stage to reduce the harmful effect of salinity. The present study conducted on Petri plate was mainly for assessing germination, relative water content and seedling parameters of eleven rice landraces with check landrace Pokali under three different salt concentrations (75mM, 125mM and 150mM). Two-way ANOVA gave the variations among the genotypes, treatments and their interactions. The present study showed that Mundan, Odiyan, Muttadan, Kallimadiyan and Vellimuthu had less percentage reduction in growth parameters at the germination stage. Odiyan and Mundan showed less percentage reduction in fresh weight (36.09%) and shoot length (25.61%) respectively, in relative water content (10.70% and 16.07%, respectively) at higher concentrations of salinity (150mM) compared to control. Pokali, Chembakam and Odiyan showed good germination parameters under three different saline treatments compared to other genotypes. Biplot analysis showed 65.4% variation between the treatments, whereas the variation between the genotypes was around 13.3%. Screening of landraces for salinity tolerance at the seed germination stage is the most reliable method to identify the salt tolerant line at the early seedling stage. The present study can be used for further screening programme at the vegetative stage for the identification of potential salt tolerant lines to improve breeding and gene introgression studies.

Keywords: Cluster heat map, PCA, Rice, Salt stress, Seed germination

INTRODUCTION

Rice, major cereal crop growing worldwide, have wider adaptability under different climatic conditions. Even though adverse climatic conditions do not completely arrest rice plants' growth, various abiotic and biotic stresses greatly limit its yield parameters. Salinity is one of the abiotic stress after drought, which reduces rice yield (Mohammed *et al*., 2007). Around 50% of the irrigated area and 20% of the total cultivated area affected by salinity (Devkota *et al*., 2015). Yield reduction is noticed even under 3 ds/m saline condition, which accounts for a yield loss of 10%, but yield reduction may reach up to 50% under moderate saline condition, that is 7ds/m (Umali, 1993). Salt accumulation in soil was increased by sea water inundation, indiscriminate use of fertilizers and deposition of salt through irrigation water. Among total rice growing areas 30% of the cultivated area was affected by salinity. Most of the rice genotypes were severely injured under high EC (8-10 ds/m) in submerged condition, especially seen in coastal areas (Mohamed *et al*, 2007). Salinity stress initiates other stresses like oxidative, ion toxicity, and osmotic stress (Ghosh *et al*., 2016). So the complexity of the stress also the reason for the complex salt tolerance mechanism in pants (Reddy *et al*.,2017).The negative impact of salinity is mainly seen in the seed germination and seedling growth stage, thereby reducing the plants' leaf area and photosynthetic capacity (Mansour and Salama, 2004). Critical stages of rice on salinity stress can be chosen to see the genetic variations of genotypes for salt tolerance (Lauchli and Epstein, 1990). Increasing the nature of soil salinity at present necessarily need to identify the genotypes which are tolerant to salinity. This can be manipulated to develop saline tolerant varieties with satisfying yield.

Germination (Pradheeban *et al*., 2014), vegetative, and reproductive stages (Anshori *et al*., 2018) are major phases of rice where the salt injury is more prominent. Compared to vegetative and reproductive stage seed germination stage relatively tolerant to salinity stress (Singh and Flowers,2010).To reduce cost of cultivation and operational simplicity, direct seeding is the major method followed in many Asian countries, so it is very important to work out the method for improving seed germination under saline condition (Fujino *et al*., 2004). Seed germination is a complex process, which affected by endogenous factors like Phyto-hormones and environmental factors like light, temperature and water (Weitbrecht *et al*., 2011). The antagonistic action of gibberellic acid(GA) and Abscisic acid (ABA) plays a major role in the regulation of seed germination (Shu *et al*., 2016). In a recent study explained that NaCl reduced seed germination is due to the decreased activity of bioactive gibberellins (GA1 and GA4), which ultimately leads to the inhibition of alpha-amylase activity, it is the key enzyme responsible for seed germination (Liu *et al*., 2018)

Difficulties in identifying salt tolerance mechanisms at the germination stage make the identification of saline tolerance in rice arduous. Salt tolerance mechanisms include ion exclusion and sequestration, Oxidative stress tolerance, K retention in cytosol and control of ions in the xylem (Chakraborty *et al*., 2016). Water uptake through imbibition is the first step in seed germination (Levitt, 1980) which can be affected by higher salt concentration in water and which leads to ion toxicity inside seed. Recent studies reveal that imbibition rate negatively correlated with seed germination in saline condition. Seed germination may improve by decreasing imbibition. Seed germination not only experienced by primary stress like ionic imbalance but also generate secondary stress like oxidative stress due to overproduction of reactive oxygen species (Zhu, 2001). Most of the time, screening for salt tolerance is limited to one or two parameters and scoring, which is a qualitative character, but the quantitative measure is necessary for salinity tolerance (Jahan *et al*., 2020). Hence at present scenario selection index methods are used for screening of salt tolerance using linear regression (Farid *et al.,*2021). The present study included seed germination assessment and root phenology of rice seedlings under different saline conditions to check the salt tolerance capacity of rice seedlings.

MATERIALS AND METHODS

Germination experiment in petriplate

Eleven rice landraces were included and surface sterilized for germination. The landraces are Pokali, Mundan, Odiyan, Upputhuran, Marathondi, Vellimuthu, Chembakam, Cheriviripp u, Kuttadan, Muttadan and Kallimadiyan. These landraces were from Kerala (Pokali, Upputhuran, Marathondi, Vellimithu, Marathondi, Chembakam and Muttadan) and Tamilnadu (Mundan, Odiyan, Kuttadan and Cheriviripp u) to check the saline tolerance level of rice under different concentrations of NaCl salt. This experiment was performed in Petriplate with factorial, completely randomized design in 4*10 factorial. The treatments were: control, 75mM NaCl, 125mM NaCl and 150mM NaCl. Thirty rice seeds are surface sterilized and placed in petriplate containing filter paper with 3 replications, watered with 3 different saline solutions. Filter papers were changed regularly to prevent salt accumulation and maintain proper EC. It was monitored 10 days to the completion of germination. Uniformly grown seedlings were selected from each treatment for morphological observation

Assessment of root phenotypes

Roots were separated from the shoot and washed thoroughly, and cleaned roots were floated in the thin film of water in a Plexiglas tray. To reduce the overlap of roots, it set apart using a needle or paintbrush. Scanning of roots was done in a dual scan optical scanner. Root morphology was measured using WinRHIZO optical scanner (Version 5) software with 400 dpi resolution and colour scale. The root area was measured using this instrument. The highest Root length and shoot length were measured manually using rulers.

Assessment of seed germination

Germination percentage

It is the ratio of total germinated seeds to the total seed sown, it expressed in percentage

Germination index

It is the sum of the ratio of the number of seeds germinated on day t, to the number of days (Hakim *et al*., 2010)

Mean germination time

It is the sum of the ratio of newly germinated seeds at time T to the number of newly germinated seeds (Alvarado *et al*., 1987)

Seedling vigor index

It is the product of mean germination percentage and mean seedling length (Mahender *et al*., 2015).

Stress tolerance index

Salt tolerance index can be used as the salinity tolerance capacity of rice plants (Fischer and Maurer, 1978), Fernandez, 1992)

$$STI= (Yp \times Ys)/(Yp)^2 \quad ......(Eq.1)$$

Y_p =Average seedling dry weight of non-stressed plants

Y_s = Average seedling dry weight of stressed plants

Relative water content

It was measured by the method suggested by Barrs and weatherly (1962) and it expressed in percentage

$$RWC= (Fresh\ weight-Dry\ weight)/Turgid\ weight - Dry\ weight) \times 100 \quad ....(Eq.2)$$

Statistical analysis

Principal component analysis and cluster heat map analysis were assessed by using R statistical software and two way Anova was carried out using spss soft ware.

RESULTS

Main and interaction effect of genotypes and salinity

All measured parameters were significantly different ($P< 0.05$) between treatment, genotypes and their interaction (Table 1). Germination percentage (GP) was affected in most of the genotypes under the higher concentration of salinity. However, germination index (GI), seedling shoot length and root length, seedling fresh and dry weight, seedling vigor index (SVI) and relative water content were reduced in all genotypes over increasing concentration of salinity except mean germination time (MGT), which was inversely proportional to salinity (Fig. 1). Principal component analysis biplot graph showed variation of 13.3% between the genotypes. However between treatments, the variation was 65.4%, shown in dimension 2 and 1, respectively.

Assessment of germination

GP, GI, GVI, MGT calculated to assess the germination of rice landraces showed that all parameters were significantly different ($P< 0.05$) between treatment, genotypes and their interaction (Table.2). Salinity check landrace, Pokali showed 100% germination in all treatments but it showed reduced Germination index under the higher concentration of NaCl, indicating ha Pokali was also affected by the higher concentration of salinity (150mM). Among other landraces Kallimadiyan, Muttadan and Odiyan showed a comparatively higher

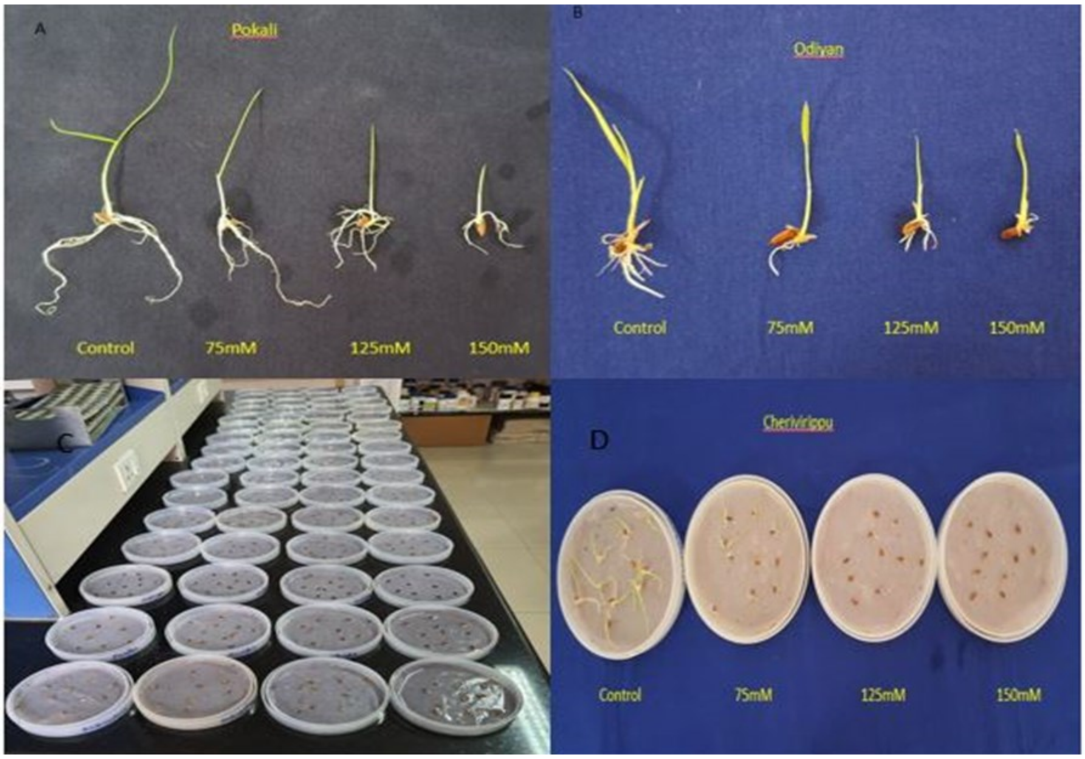

Fig. 1. *A and B; Seedling growth of Pokali and Odiyan Under different saline treatment C and D; Seed germination study conducted in petriplates*

Table 1. ANOVA table (sum of squares) showing different parameters of rice landraces under saline treatments

Trait	**Source of variations**			
	T	**G**	**TXG**	**Error**
df	**3**	**10**	**30**	**88**
GP	1777.705**	668.5227**	104.1045**	0.795455
GI	627.604**	153.039**	10.679**	0.001
SL	80.13828**	10.08014**	2.095672**	0.044545
RL	131.1314**	14.45185**	2.433303**	0.022273
MGT	3.491**	0.936**	0.079**	0.001
SVI	5248453**	374553.8**	45968.86**	1.061
RWC	2144.628**	220.0509**	46.16305**	2.450569
DW	0.002**	0.00007431**	0.0000444**	0.000002008
FW	0.052**	0.002**	0.001**	0.000002295
RA	1047.898**	170.075**	19.761**	0.2
R/L	0.11208**	0.45773**	0.193275**	0.005875

GP-Germination percentage, GI-Germination index, SL-Shoot length, RL-Root length, MGT- Mean germination time, SVI-Seedling vigour index, RWC-relative water content, DW- dry weight, FW- fresh weight, RA- Root area, R/L- Root length to shoot length ratio

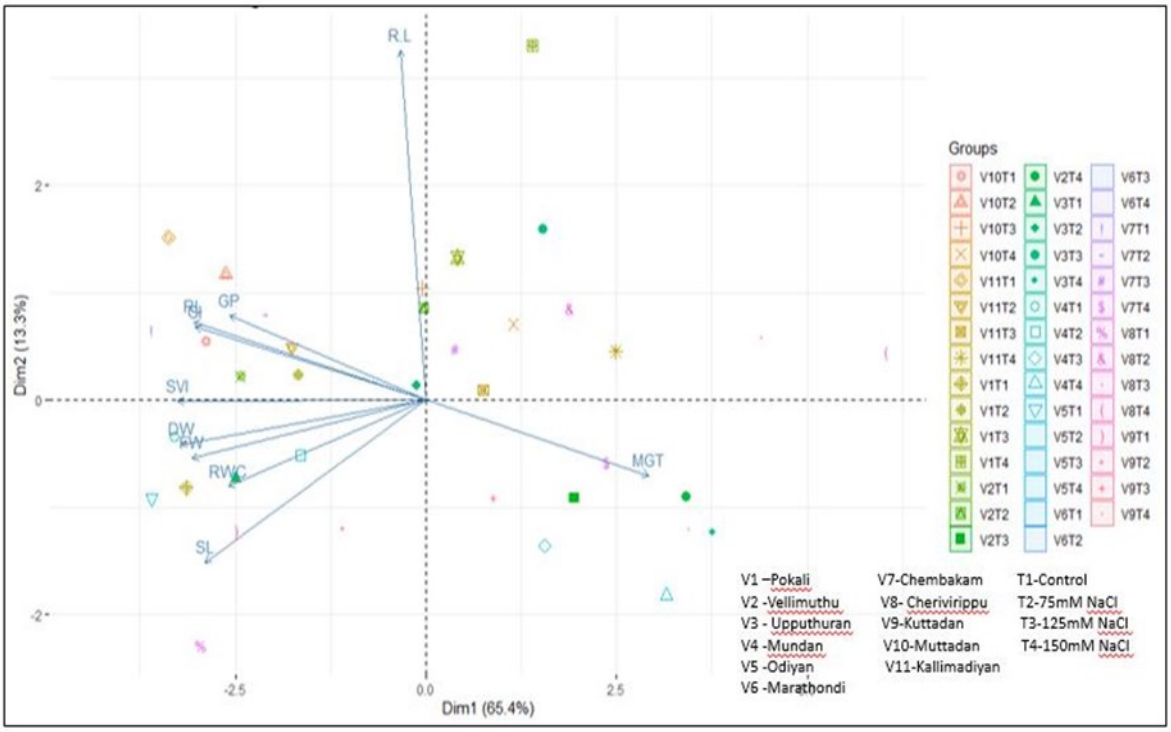

Fig. 2. *Biplot diagram of principal component analysis of 11 landraces in 4 different treatments. Growth and germination parameters projected in the diagram. GP-Germination percentage, GI-Germination index, SL-Shoot length, RL-Root length, MGT- Mean germination time, SVI-Seedling vigour index, RWC-relative water content, DW- dry weight, FW- fresh weight, RA- Root area, R/L- Root length to shoot length ratio*

germination percentage than other landraces in all treatments. Germination percentage was very much affected in Cherivirippu in all concentrations of salinity. At higher concentrations of salinity (150mM), Upputhuran (34%), Vellimuthu (29%) and Mundan (29%) showed a higher reduction percentage of germination than control. Germination index indicated how fast the seeds get to germinate, which were noted to be higher for Kallimadiyan, Muttadan and Marathondi in control. In all treatments, Pokali followed by Chembakam resulted in less percentage reduction of germination index from the control. Seedling vigour index showed higher for Odiyan and Marathondi in control, irrespective of treatments. Due to salinity treatments, the seedling vigour index was reduced in all genotypes by increasing salinity concentration. Genotype Cherivirippu showed a higher reduction percentage when compared to control in all treatments, which were 48.97%, 76%, 86.93% in 75mM, 125mM, and 150mM treatments, respectively. Pokali improved seedling vigour index by around

Table 2. Germination parameters of eleven rice landraces under four treatments

	Genotypes	**Control**	**75mM NaCl**	**% Change**	**125mM NaCl**	**% Change**	**150mM NaCl**	**% Change**
GP (%)	Pokali	100	100	0.00	100	0.00	100	0.00
	Vellimuthu	93	86	-7.53	80	-13.98	66f	-29.03
	Upputhuran	100	93	-7.00	73	-27.00	66f	-34.00
	Mundan	93	93	0.00	93b	0.00	66f	-29.03
	Odiyan	100	86	-14.00	86c	-14.00	86c	-14.00
	Marathondi	100	86	-14.00	80d	-20.00	80d	-20.00
	Chembakam	93	86	-7.53	80d	-13.98	80d	-13.98
	Cheriviripp u	86	73	-15.12	66f	-23.26	66f	-23.26
	Kuttadan	93	86	-7.53	80d	-13.98	73e	-21.51
	Muttadan	93	93	0.00	93b	0.00	86c	-7.53
	Kallimadiyan	100	100	0.00	93b	-7.00	93b	-7.00
CD	G		T		GXT			
(0.05%)	0.717		0.432		1.434			
GI	Pokali	20.49	18.99	-7.32	18.00	-12.15	16.50	-19.47
	Vellimuthu	18.99	13.98	-26.38	12.32	-35.12	10.50	-44.71
	Upputhuran	18.00	15.33	-14.83	13.38	-25.67	9.66	-46.33
	Mundan	21.00	17.31	-17.57	13.14	-37.43	9.06	-56.86
	Odiyan	25.98	17.04	-34.41	14.16	-45.50	11.16	-57.04
	Marathondi	27.99	15.99	-42.87	14.82	-47.05	11.64	-58.41
	Chembakam	21.99	20.64	-6.14	18.66	-15.14	14.31	-34.92
	Cheriviripp u	16.71	10.32	-38.24	9.00	-46.14	6.99	-58.17
	Kuttadan	21.00	18.99	-9.57	13.98	-33.43	9.81	-53.29
	Muttadan	27.00	24.00	-11.11	18.33	-32.11	16.98	-37.11
	Kallimadiyan	27.99	25.98	-7.18	21.00	-24.97	18.00	-35.69
CD	G		T		GXT			
(0.05%)	0.025		0.015		0.049			
SVI	Pokali	1016	1343	32.19	1060	-4.33	716	-29.53
	Vellimuthu	1434	1210	-15.62	860	-40.03	588	-59.00
	Upputhuran	1656	1368	-17.39	906	-45.29	533	-67.81
	Mundan	1652	1452	-12.11	1098	-33.54	713	-56.84
	Odiyan	1753	1331	-24.07	855	-51.23	751	-57.16
	Marathondi	1745	1369	-21.55	989	-43.32	914	-47.62
	Chembakam	1683	1418	-15.75	1029	-38.86	770	-54.25
	Cheriviripp u	1354	691	-48.97	325	-76.00	177	-86.93
	Kuttadan	1368	1161	-15.13	898	-34.36	711	-48.03
	Muttadan	1309	1633	-24.75	1104	-15.66	892	-31.86
	Kallimadiyan	1596	1279	-19.86	852	-46.62	543	-65.98
CD	G		T		GXT			
(0.05%)	0.684		0.412		1.368			
MGT (day)	Pokali	1.70	1.70	0.00	1.80	5.88	2.10	23.53
	Vellimuthu	1.64	2.06	25.79	2.25	37.20	2.30	40.24
	Upputhuran	1.80	2.14	18.89	2.27	26.11	2.30	27.78
	Mundan	1.50	1.86	24.00	2.36	57.33	2.40	60.00
	Odiyan	1.27	1.92	51.18	2.23	75.59	2.46	93.70
	marathondi	1.13	1.92	69.91	2.00	76.99	2.25	99.12
	Chembakam	1.43	1.46	2.10	1.50	4.90	1.92	34.06
	Cheriviripp u	2.00	2.18	9.00	2.30	15.00	2.90	45.00
	Kuttadan	1.64	1.69	3.05	2.00	21.95	2.36	43.90
	Muttadan	1.07	1.29	20.90	1.79	67.76	1.85	73.38
	Kallimadiyan	1.13	1.27	12.39	1.50	32.74	1.86	64.60
CD	G		T		GXT			
(0.05%)	0.026		0.016		0.053			

GP-Germination percentage (%); GI-Germination index; MGT- Mean germination time (Day); SVI-Seedling vigour index

Table 3. Growth parameters of eleven rice landraces under four treatments

	Genotypes	**Control**	**75mM NaCl**	**% Change**	**125mM NaCl**	**% Change**	**150M NaCl**	**% Change**
	Pokali	0.198	0.12	-39.39	0.101	-48.99	0.088	-55.56
	Vellimuthu	0.146	0.107	-26.71	0.086	-41.10	0.062	-57.53
	Upputhuran	0.123	0.084	-31.71	0.065	-47.15	0.066	-46.34
	Mundan	0.162	0.136	-16.05	0.074	-54.32	0.066	-59.26
	Odiyan	0.133	0.127	-4.51	0.106	-20.30	0.085	-36.09
FW(g)	Marathondi	0.184	0.128	-30.43	0.102	-44.57	0.078	-57.61
	Chembakam	0.163	0.128	-21.47	0.098	-39.88	0.056	-65.64
	Cheriviripppu	0.234	0.093	-60.26	0.056	-76.07	0.051	-78.21
	Kuttadan	0.147	0.118	-19.73	0.095	-35.37	0.058	-60.54
	Muttadan	0.155	0.121	-21.94	0.107	-30.97	0.091	-41.29
	Kallimadiyan	0.133	0.113	-15.04	0.089	-33.08	0.067	-49.62
CD (0.05%)	G 0.001		T 0.001		GXT 0.002			
	Pokali	0.036	0.024	-33.33	0.019	-47.22	0.016	-55.56
	Vellimuthu	0.029	0.021	-27.59	0.016	-44.83	0.01	-65.52
	Upputhuran	0.025	0.016	-36.00	0.011	-56.00	0.011	-56.00
	Mundan	0.032	0.027	-15.63	0.013	-59.38	0.011	-65.63
	Odiyan	0.026	0.026	0.00	0.021	-19.23	0.016	-38.46
DW(g)	Marathondi	0.035	0.026	-25.71	0.02	-42.86	0.014	-60.00
	Chembakam	0.032	0.026	-18.75	0.019	-40.63	0.008	-75.00
	Cheriviripppu	0.042	0.017	-59.52	0.008	-80.95	0.007	-83.33
	Kuttadan	0.029	0.024	-17.24	0.018	-37.93	0.008	-72.41
	Muttadan	0.031	0.024	-22.58	0.021	-32.26	0.017	-45.16
	Kallimadiyan	0.026	0.023	-11.54	0.017	-34.62	0.011	-57.69
CD (0.05%)	G 0.001		T 0.001		GXT 0.002			
	Pokali	8.43	7.40	-12.22	6.33	-24.87	4.80	-43.06
	Vellimuthu	8.73	8.30	-4.96	5.33	-38.93	4.30	-50.76
	Upputhuran	8.50	8.03	-5.49	7.50	-11.76	3.60	-57.65
	Mundan	9.50	8.10	-14.74	5.17	-45.61	4.60	-51.58
	Odiyan	8.27	8.00	-3.23	4.40	-46.77	3.70	-55.24
RL(cm)	Marathondi	9.17	8.47	-7.64	7.57	-17.45	7.10	-22.55
	Chembakam	10.57	9.60	-9.15	7.30	-30.91	4.50	-57.41
	Cheriviripppu	7.27	5.77	-20.64	2.67	-63.30	1.50	-79.36
	Kuttadan	7.10	6.40	-9.86	5.17	-27.23	4.30	-39.44
	Muttadan	7.69	10.16	32.12	6.79	-11.70	5.69	-26.01
	Kallimadiyan	9.46	6.63	-29.92	4.59	-51.48	2.90	-69.34
CD (0.05%)	G 0.122		T 0.074		GXT 0.244			

Contd……

Table 3. Contd….

SL(cm)	Pokali	7.73	6.03	-21.99	4.26	-44.89	2.36	-69.47
	Vellimuthu	6.30	5.66	-10.16	5.43	-13.81	4.53	-28.10
	Upputhuran	8.06	6.63	-17.74	4.86	-39.70	4.40	-45.41
	Mundan	8.20	7.46	-9.02	6.60	-19.51	6.10	-25.61
	Odiyan	9.26	7.36	-20.52	5.46	-41.04	4.96	-46.44
	Marathondi	8.30	7.33	-11.69	4.80	-42.17	4.33	-47.83
	Chembakam	7.46	6.76	-9.38	5.56	-25.47	5.13	-31.23
	Cheriviruppu	8.36	3.66	-56.22	2.23	-73.33	1.16	-86.12
	Kuttadan	7.56	7.00	-7.41	6.06	-19.84	5.40	-28.57
	Muttadan	6.33	7.33	15.80	5.03	-20.54	4.60	-27.33
	Kallimadiyan	6.50	6.10	-6.15	4.53	-30.31	2.93	-54.92
CD	G		T		GXT			
(0.05%)	0.173		0.104		0.345			
R/L	Pokali	1.09	1.23	12.84	1.55	42.20	2.03	86.24
	Vellimuthu	1.32	1.47	11.36	0.98	25.76	0.95	-28.03
	Upputhuran	1.06	1.21	14.15	1.54	45.28	0.82	-22.64
	Mundan	1.16	1.09	-6.03	0.79	-31.90	0.75	-35.34
	Odiyan	0.89	1.09	22.47	0.81	-8.99	0.74	-16.85
	Marathondi	1.10	1.15	4.55	1.58	43.64	1.64	49.09
	Chembakam	1.42	1.42	0.00	1.31	-7.75	0.88	-38.03
	Cheriviruppu	0.87	1.57	80.46	1.20	37.93	1.28	47.13
	Kuttadan	0.94	0.91	-3.19	0.85	-9.57	0.80	-14.89
	Muttadan	1.22	1.39	13.93	1.35	-10.66	1.24	1.64
	Kallimadiyan	1.45	1.09	-24.83	1.01	-30.34	0.99	-31.72
CD	G		T		GXT			
(0.05%)	0.068		0.038		0.125			
RA (Cm^2)	Pokali	18.78	6.18	67.09	5.41	71.19	1.60	91.48
	Vellimuthu	13.54	11.05	18.39	6.56	51.55	4.24	68.69
	Upputhuran	15.27	13.05	14.54	5.95	61.03	4.98	67.39
	Mundan	26.13	17.77	31.99	11.37	56.49	9.70	62.88
	Odiyan	27.97	21.50	23.13	9.29	66.79	4.25	84.81
	Marathondi	12.52	9.85	21.33	6.86	45.21	2.86	77.16
	Chembakam	10.38	7.37	29.00	3.77	63.68	1.99	80.83
	Cheriviruppu	12.92	7.16	44.58	1.51	88.31	0.79	93.89
	Kuttadan	18.99	9.90	47.87	6.16	67.56	3.21	83.10
	Muttadan	10.54	6.89	34.63	3.96	62.43	3.51	66.70
	Kallimadiyan	11.88	5.35	54.97	3.77	68.27	1.32	88.89
CD	G		T		GXT			
(0.05%)	0.357		0.215		0.714			

SL-Shoot length (cm); RL-Root length(cm); DW- dry weight (g); FW- fresh weight(g); RA- Root area(Cm^2); R/L- Root length to shoot length ratio

32.19% in 75mM NaCl treatment, but seedling vigour index (SVI) reduced other treatments like 125mM (4.33%) and 150mM (29.53%) when compared to control. Among all genotypes, the seedling vigour index reduction percentage was less for Pokali in all treatments, followed by Mundan. Mean germination time increased in all genotypes for all three NaCl treatments when compared to control. Among all treatments of 75mM, 125mM and 150mM NaCl, the mean germination time increment was noted less for Pokali and Chembakam; however, the mean germination time increased much in landrace Marathondi compared to control.

Morphological assessment

Shoot length and root length of the investigated landraces were negatively affected by the salinity treatments (75mM, 125mM, and 150mM) (Table 3). There were genotypic variations in the root and shoot length. The reduction of the shoot length from the control was noticed higher for the landrace Cheriviruppu followed by the Pokali in all treatments. Reduction percentage of shoot and root length increased by increase in the concentration of salinity in the same genotype. Kuttadan, Mundan Vellimuthu showed a comparatively less reduction percentage of shoot length from control when compared to all other landraces in all the treatments. In the case of root length, landrace Cherivirippu showed a higher reduction percentage than control, especially in the higher concentration salinity of 125mM and 150mM, which was 63.30% and 79.36%, respectively. Muttadan showed improved root length in 75mM NaCl which was about 32.12%, comparatively less reduction percentage was noted as 11.70% and 26.01% in 125 and 150mM NaCl, respectively, when compared to control. Ratio of root length to shoot length increased in most of the genotypes at 75mM NaCl. However, similar trend was not followed in other treatments because increased salinity also affected root growth. Pokali showed an increased root-to-shoot ratio in all treatments compared to control. Kallimadiyan showed a consistent reduction of the root-to-shoot length ratio in all treatments. Percentage reduction of dry weight was higher for Cherivirippu in all treatments compared to the control, whereas the reduction percentage was less for Odiyan from the control. Root surface area measured through Win rhizo root scanner. Among all genotypes evaluated, the root area was observed more for Mundan and Odiyan in control than a gradual reduction in the root area noticed due to treatment. At higher concentrations of salinity, that is 125mM and 150mM NaCl, the reduction percentage of root area over increased salinity was 88.31% and 93.89%, respectively, for Cherivirippu. The least reduction of root area was noticed in landrace Mundan at 150mM NaCl.

Salinity tolerance

Salinity tolerance level measured in terms of salt tolerance index (STI) was reduced by the increase in the salinity concentration in all genotypes (Fig. 3). Among genotypes, Marathondi and check Pokali showed a comparatively higher stress tolerance index than other genotypes. Lowest STI recorded for landrace Upputhuran (0.414) at 75mM salinity. However, at higher salinity concentrations (125mM and 150mM), Cherivirippu showed the lowest stress tolerance index, which was 0.22 and 0.32, respectively.

Relative water content

Results of relative water content (RWC) showed that all genotypes gradually reduced the relative water content by increasing the concentration of the salinity (Table 4). At 75mM, the reduction percentage of RWC was noted higher for the genotype Vellimuthu (17.01%), whereas Pokali showed less reduction percentage (1.41%) compared to control. At 125mM NaCl, Cherivirippu (25.26%) followed by Upputhuran (23.35%) showed a higher reduction percentage, whereas Odiyan showed less reduction percentage (5.08%) in relative water content. At a higher concentration of salinity (150mM NaCl), the reduction percentage of RWC was noted to higher for Kuttadan (31.93%) followed by Marathondi (30.30%) least reduction percentage was noted for Odiyan (10.70%)

Grouping of rice genotypes based on cluster heat map analysis

Dendrogram was used to identify the similarity and dissimilarities between current populations. The cluster heat map and cluster gram attach two dendrograms in the same dimension. Based on the chart's colour intensity, the similarity and dissimilarity between the genotypes can be identified. There were two major clusters formed between the parameters measured: GP, GI, RWC, DW, FW, DW, SL, RL and SVI clustered in one group and mean germination time, root length to shoot length ratio clustered in another group. In the treatment side also, two major clusters were formed. All the con-

Table 4. Relative water content (RWC) (%) of eleven rice landraces under four treatments

Genotypes	Control	75mM NaCl	% Change	125mM NaCl	% Change	150M NaCl	% Change
Pokali	89.34	88.08	-1.41	78.73	-11.88	75.45	-15.54
Vellimuthu	91.45	75.90	-17.01	73.68	-19.43	71.60	-21.71
Upputhuran	90.46	77.28	-14.58	69.34	-23.35	66.41	-26.59
Mundan	82.49	78.11	-5.31	71.18	-13.71	69.05	-16.30
Odiyan	84.26	80.07	-4.97	79.98	-5.08	75.24	-10.70
Marathondi	77.43	69.27	-10.54	65.78	-15.04	53.96	-30.30
Chembakam	84.89	81.23	-4.30	73.15	-13.83	69.72	-17.87
Cherivirippu	85.66	84.57	-1.27	64.02	-25.26	61.99	-27.63
Kuttadan	92.68	87.62	-5.46	71.66	-22.68	63.09	-31.93
Muttadan	79.83	73.70	-7.68	70.05	-12.25	67.00	-16.07
Kallimadiyan	82.35	75.64	-8.15	72.74	-11.67	66.29	-19.50
CD (0.05%)	G 1.274		T 0.768		GXT 2.548		

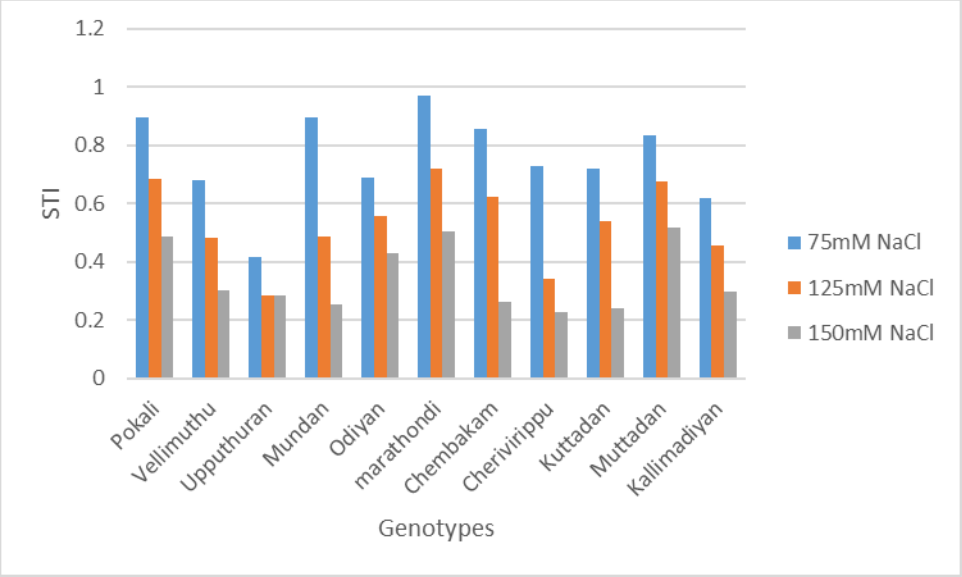

Fig. 3. *Salt tolerance index (STI) of all 11 landraces under 4 different treatments*

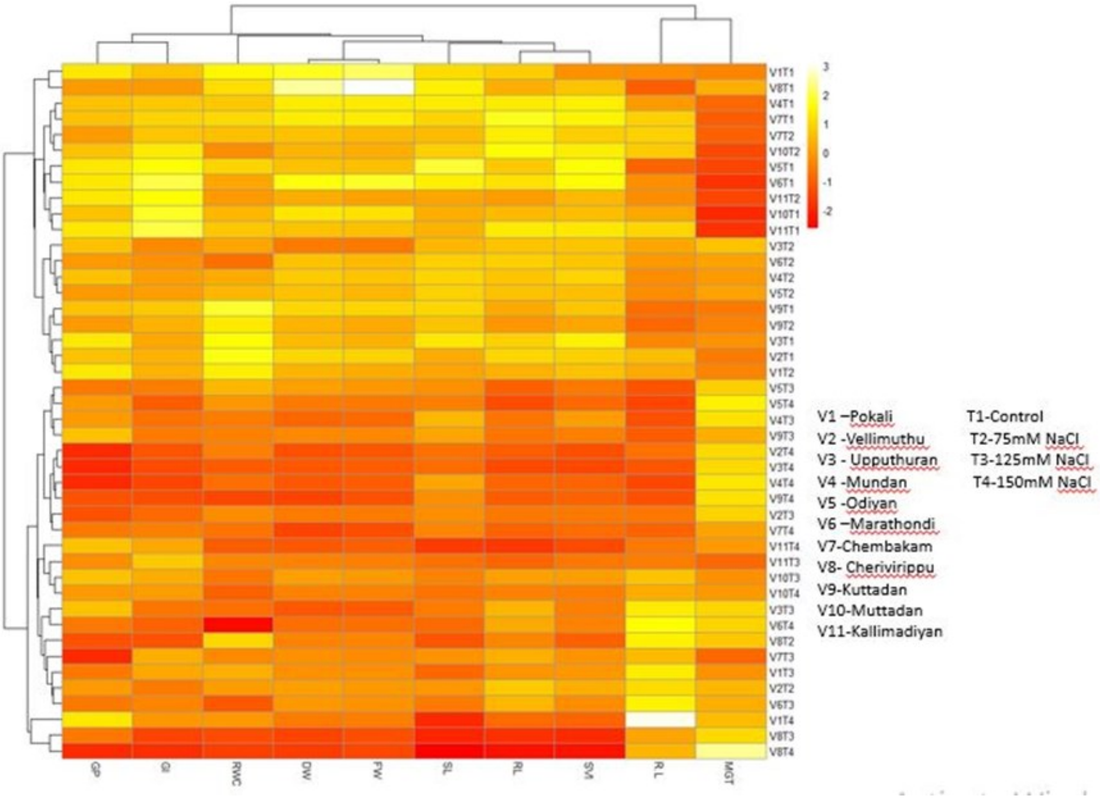

Fig. 4. *Cluster heat map based on measured traits among four treatments*

trol plants and part of 75mM NaCl treated plants were clustered in first group and the remaining plants in 75mM NaCl, all the plants treated with 125mM NaCl and 150mM NaCl clustered in the second group (Fig. 4).

DISCUSSION

The present study identified the salt-tolerant, sensitive and moderately tolerant categorization of rice landraces under saline conditions at the germination stage. Screening of landraces for their genetic variability under salinity stress is very important to improve the research on salt stress. A potential salt tolerant rice landrace Pokali was selected as a check to compare the other landraces. Seedling screening in the laboratory is an easy and viable method to identify the sensitivity of rice seedlings to salt stress. As a preliminary work, this can be utilized to identify the salt-tolerant genotypes that can be used for advanced salt tolerance studies. This study included 11 rice landraces to check their salinity tolerance capacity at the germination stage. Among the three salt treatments, 75mM saline did not much affect the seed germination of rice landraces, but 125 mM was considered the optimum salt concentration for salt tolerance studies in rice. The higher salt concentration used in this study was 150mM, becoming more concentrated to survive any rice genotypes. The results of this experiment showed that germination and morphological characteristics varied among the genotypes, treatments and their interaction.

The present study revealed that germination percentage, germination index and seedling vigour index were reduced by increasing salt concentration but mean germination time increased according to salt stress increment. Pokali showed 100% germination in all treatments; no other genotypes showed 100% germination in all treatments. Even though Pokali germinated completely, its mean germination time increased over increasing salinity concentration. It is already known that salinity negatively correlates with germination percentage, germination index and seedling vigour index (Rehman *et al*., 2000). Low concentrations of salinity induce seed dormancy, but higher concentrations of salinity affected seed germination due to ion toxicity and high osmotic potential (Khan and Weber, 2008). Similar observations were observed in a study that seed germination energy and germination capacity of rice were decreased due to the accumulation of salt on the cell membrane, which may affect the imbibition process and lead to membrane permeability and leakage (Li, 2021).

In another study germination percentage of rice reduced under saline conditions was due to the reduction in GA activity that can be rescued by the external application of gibberellin at the seedling stage (Liu *et al*., 2018). Abbas *et al*. (2012) study on rice under different salinity resulted in salinity reducing the germination percentage, shoot and root length and dry weight of the rice plant. Due to salt stress, internal enzymes such as hydrolyses and proteases present in the seed will not be released properly to solubilize starch and protein that may lead to the delay in the germination process and stunted growth of shoot and root, reduced shoot and root fresh weight and poor seedling vigor index (Ruan and Xue, 2002; Guo *et al*., 2006). Zhang *et al*. (2021) evaluated the rice germplasm under salt stress at the germination stage, resulting for the development of an equation to calculate D value using the parameters like salt tolerance index and seedling vigour index. This equation can be used to identify the salt tolerance capacity of rice. Also, they identified seedling vigor index as the reliable parameter which can be used to assess salinity tolerance in rice. Khan *et al*. (1997) revealed germination index, seedling height, increase in germination speed, and decrease in dry matter accumulation in rice plants grown under salt stress. Mean germination time increased in rice genotypes under salinity which is shown in a study conducted by Momayezi *et al*. (2009.)

The present study recorded a slight decrease in the fresh weight of the root and shoot, which is in line with the study conducted by Chunthaburee *et al*. (2015) in rice. The result presented in Table 3 revealed that landraces Cherivirippu were very much affected by salinity in terms of fresh and dry weight, indicating that it is very sensitive to salt stress at a younger age. In another study, seed germination, radicle and plumule length were reduced due to salinity at the seed germination stage, especially delayed germination and the reduction in chlorophyll content (Tadesse *et al*., 2016). Root to shoot length ratio decreased under salinity in most of the genotypes under higher concentration of salinity. This is in line with the study by Zafar et al. (2015), which showed that root-to-shoot length reduced most of the genotypes except Basmathi -385. Even though shoot length was reduced under saline conditions, the root to shoot length ratio was also reduced due to the reduction of root length in parallel to shoot length.

Higher salt tolerance index (STI) indicated the genotype with higher stress tolerance and yield potential (Fernandez, 1992). In present study stress tolerance index showed higher for Marathondi followed by Pokali in all treatment (75mM, 125mM, 150 mM). Hence, the stress tolerance index decreased by an increase in the concentration of salinity, which was also noticed in a study conducted by Hosseini *et al*. (2012) on rice.

Like drought stress, salinity stress studies also require measuring relative water content to check the water maintenance capacity of the rice plant under salt stress, because salinity stress is considered physiological stress. In this study, reduction percentage of RWC at 75mM NaCl from control shown, Vellimuthu showed higher reduction percentage followed by Upputhuran however at higher concentration of salinity that is 125mM and 150mM higher reduction percentage of salinity was recorded for genotypes Cherivirippu and Kuttadan respectively. Percentage reduction of water content was less for the genotype Cherivirippu at 75mM; however, at 125mM and 150mM reduction percentage of water content was less for Odiyan. These results indicated Odiyan maintained water status in the plant even under a higher concentration of salinity.

A study conducted by Polash *et al*. (2018) revealed that salinity imbalance in plant osmotic character so that water uptake was negatively affected in root. To check the lethality of rice plants to salinity tested by Djaniguiraman *et al*. (2006) revealed that RWC reduction percentage reduced those plants pre-treated with low salinity treatment before severe saline condition. Reduced RWC may lead to impairment of metabolite transfer to the growing area; thus, dry matter accumulation gets reduced (Waisel, 1972).

From all the parameters studied, seedling growth and germination assessment can be done as the preliminary work for salinity screening. Still, there is a possibility to germinate seeds and transplant the seedlings, so there must be a validation screening at the vegetative stage. The check landrace Pokali responded positively to germination assessment parameters followed by, Chembakam, Odiyan, and Mundan, but there are variations among genotypes in terms of growth parameters. A higher salinity concentration affected Cherivirippu and Upputhuran as its salt tolerance index was low under125 and 150mM NaCl.

Conclusion

Screening of rice genotypes for saline tolerance is essential to identify genetic variability in rice under saline conditions. Initial screening using seedling traits is a good criterion for assessing rice genotypes' germination capacity under saline conditions. The present study revealed the germination and seedling growth parameters of 11 landraces including check landrace Pokali. It was concluded that all parameters were significant between treatment, genotypes and their interaction at germination to seedling stages. Salt tolerance index and relative water content also gave an account of the salt tolerance capacity of the rice seedlings. Manipulating genetic variability to develop salt-tolerant genotypes through breeding or biotechnological measures is much needed in the present situation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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