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RESEARCH ARTICLE

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Varietal Difference in Seed Germination under Different Levels of Salt Stress in Rice

T. Shafeeqa[1], V. Ravichandran[1], A. Senthil[1], L. Arul[2], S. Radhamani[3]

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ABSTRACT

Background: Salinity, major abiotic stress affects many field crops including rice. Salinity account 20% of cultivated area and may cover 50% irrigated area by the end of 2050 due to climate change. Global food security ensured by developing high yielding rice varieties tolerant to different abiotic stress. Salinity affect both germination and other growth stages of rice.

Methods: Present study conducted in Tamil Nadu Agricultural University aim to assess the seed germination of rice under salinity stress using germination and growth parameters. In our study we included 11 rice genotypes with one tolerant check Pokkali and one susceptible check IR 64 recorded germination percentage, mean germination time, germination index, seedling vigour index, shoot length, root length, fresh weight, dry weight, root length to shoot length ratio and stress tolerance index.

Result: Two way ANOVA used for comparing means. Two component extracted through principle component analysis which explained a total of 83.54% variance. Component 1 explained 64.96% and component 2 explained 18.57%. Stress tolerance index recorded higher for TRY 3 under 150mM NaCl followed by TRY 2.

Key words: Principle component analysis, Rice, Salinity, Seedling vigour index.

INTRODUCTION

Half of the world population depend on rice for their staple food in their daily life, so it is very important to improve its production and productivity to attain food security for growing population (Dawe *et al*., 2010). One of the abiotic stress which affect rice growth is salinity. Which account 20% of cultivated area and 50% of the irrigated area (Devkota *et al*., 2015). In Indian soil, 2.1% land area affected by salinity and this was all total of 2.96 million ha saline soil and 3.77 million ha sodic soil. Saline soil or white alkali soil having EC>4, pH< 8.5 and ESP<15 and sodic soil possess EC<4 dsm^{-1}, ESP>15 and pH= 8.5 to 10. Hence, salt problem is very evident in arid and semi-arid region (Kumar and Sharma, 2020). Screening of various rice genotypes for their salt tolerance capacity at germination stage is necessary to improve direct seeded crop. At vegetative stage the efficient screening of rice plant under varying salinity level can be done through hydroponic screening (Revathi and Arumugam Pillai, 2015).

Many studies proved that increasing salinity reduces seed germination, shoot length, root length, fresh weight and dry weight (Munns and Tester, 2008). Salinity affect many physiological, biochemical and genetic factors and also it showed poor correlation in early seedling and reproductive stage. Reduction in the chlorophyll content showed in a study conducted in rice genotypes under salt stress at four different stages such as vegetative, tillering, panicle initiation and flowering stage by Djanaguiraman and Ramadass (2004). Rice is salt sensitive crop even 3 dsm^{-1} may cause reduction in growth, development and yield in rice plants (Lutts *et al*. 1995). Latest studies gave an evidence that rice showed higher sensitivity in germination and vegetative stage rather than seedling and reproductive stage (Bundo *et al*., 2022). Yield reduction was very evident in rice under 3 dsm^{-1} salt concentration which was around 12% however it reached 50% when the salinity become 6 dsm^{-1} (Linh *et al*., 2012). Studies conducted in natural saline soil having EC more than 5 to 8 and pH 7.8 to check the tolerant capacity of various rice genotypes under local environment (Rani *et al*., 2008). In present scenario genetic studies of tolerant varieties unveil the saline tolerant mechanism may help molecular breeding programme (Niu *et al*., 2022).

The main reason for poor seedling growth under salinity is the poor seed germination parameters (Hasanuzzaman *et al*., 2009). Hence the present study aim to understand

[1]Department of Crop Physiology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[2]Department of Plant Biotechnology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[3]Department of Agronomy, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

Corresponding Author: T. Shafeeqa, Department of Crop Physiology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
Email: shafeeqahamza@gmail.com

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the salt tolerance capacity of 11 rice genotypes at it germination stage for further salt tolerance studies and its mitigation researches.

MATERIALS AND METHODS

Germination of rice seeds

Eleven rice genotypes were selected for present study including one check landrace Pokkali and one susceptible variety IR 64 remaining genotypes are CO 51, CO 53, CO54, TRY 2, TRY3, TRY4, ADT 53, ASD 16 and CO 43 which include both tolerant and susceptible genotypes. The study was conducted in Tamil Nadu Agricultural University, Department of Crop Physiology during 2022. We used petridishes for laboratory experiment and performed 4*11 factorial design. The treatments were control, 75 mM NaCl, 125 mM NaCl and 150 mM NaCl. Fifteen rice seeds in each petridishes with 3 replication kept under room temperature with normal light. Required amount of NaCl added in water and poured equal volume of salt solution in each petridishes.

Germination parameters measured

1. Germination percentage (GP) =

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100 \quad \text{.... eq (1)}$$

2. Germination index (GI) =

$$GI = \Sigma \frac{\text{Number of germinated seeds on day t}}{\text{Number of days}} \quad \text{....eq (2)}$$

Hakim *et al*., (2010)

3. Mean germination time (MGT) =

$$MGT = \frac{\Sigma \text{ Number of newly germinated seeds} \times \text{days of germination}}{\Sigma \text{ Number of germinated seeds}} \quad \text{.....eq (3)}$$

Alvarado *et al*., (1987)

4. Seedling vigour index (SVI) =

$$SVI = \text{Mean germination percentage} \times \text{Mean seedling length} \quad \text{...eq (4)}$$

(Mahender, 2015)

Growth parameters

Five uniformly grown rice seedling from each petridish were taken for the observation of shoot length, root length, fresh weight and dry weight. Length measured using rulers and expressed in cm on ninth days. Fresh and dry weight recorded in grams.

Salinity tolerance index

Stress tolerance index used to check tolerance level of rice seedlings

$$STI = \frac{Y_p \times Y_s}{Yp2} \quad \text{...eq (5)}$$

Y_p = Average seedling dry weight of a non-stressed condition.
Y_s = Average seedling dry weight at stressed condition.
Y_p= Average seedling dry weight of all genotypes at non stressed condition.

(Fernandez, 1992)

Statistical analysis

Statistical analysis were done by using SPSS software. Two way ANOVA used for comparing means. Principal component analysis was done to identify principle components. Pearson correlation checked to know the correlation between different parameters.

RESULTS AND DISCUSSION

Germination parameters

Increasing concentration of salinity significantly reduced the germination percentage of rice varieties (Table 1). In all the treatment (control, 75 mM NaCl, 125 mM NaCl and 150 mM NaCl) significantly higher and equal germination percentage recorded for 3 genotypes such as Pokkali, TRY3 and CO 53 and the values of these three genotypes are similar 100%, 99.67%, 99.33% and 99% respectively. Similarly IR 64 recorded significantly lower germination percentage in control, 75 mM NaCl, 125mM NaCl and 150mM NaCl the values are 100%, 80%, 80%, 70% respectively. Varietal difference of rice observed in germination percentage between 3 treatments (75mM NaCl, 125 mM NaCl,f 150 mM NaCl).Low germination percentage at higher concentration of salinity may be due to increase in osmotic stress by high salt concentration which may affect the imbibition of seeds it already proved by (Akbar and Ponnamperuma, 1982).

Germination index is the ratio of the summation of germination of seeds on day t to the total number of days (Fig 4). In all the treatments (Control, 75 mM NaCl,125 mM NaCl, 150 mM NaCl) significantly higher germination index recorded for Pokkali and the values are 68, 63.30, 60, 55 respectively and significantly lower germination index recorded for IR 64 and the values are 36,23.30, 17.50 and 14.90. Similar results were found in a study conducted by (Pushpam and Rangasamy, 2002) in rice. There are studies revealed that high yielding varieties showed higher germination index compared to local and coarse grain varieties and it was negatively correlated with salt concentration (Khan *et al*., 1997).

Statistical analysis of mean germination time (Table 1) revealed that in all the treatments significantly higher mean germination time recorded for IR 64 and the values are 2.81 days, 3.62 days, 4.73 days, 4.81 days in control, 75mM NaCl, 125 mM NaCl and 150 mM NaCl respectively. Similarly significantly lower mean germination time recorded for Pokkali and the values are 1.74 days, 1.83 days, 1.85 days and 2.10 days, similar results were found in (Shi *et al*., 2017). Speed of germination decreases with increasing concentration of salinity irrespective of genotypes highest speed of germination noticed in control and lowest recorded for 12 dsm^{-1} which reduced 70% of speed of germination with respect to non-saline condition (Hakim *et al*., 2010).

Shoot length and root length

Among genotypes significantly higher shoot length were measured for Pokkali in Control, 75 mM NaCl, 125 mM NaCl and 150 mM NaCl and the values are7.73 cm, 6.03 cm, 4.27 cm and 2.37 cm respectively and significantly lower shoot length recorded for IR 64 and the values are 1.67 cm, 0.57 cm, 0.40 cm and 0.30 cm. Second highest shoot length recorded for CO 53 in control and 75 mM NaCl, the values are 5.13 cm and 4.37 cm however it was CO 51 in 125 mM NaCl and 150 mM NaCl treatments and the values are 2.47 cm and 2.27 cm (Fig 2).

Significantly higher root length recorded for Pokkali followed by TRY2 and the values are 8.17 cm,7 cm,5.23 cm, 3.80 cm and 5.53 cm, 3.73 cm, 3.07 cm, 2.63 cm in control, 75mM NaCl, 125 mM NaCl and 150 mM NaCl

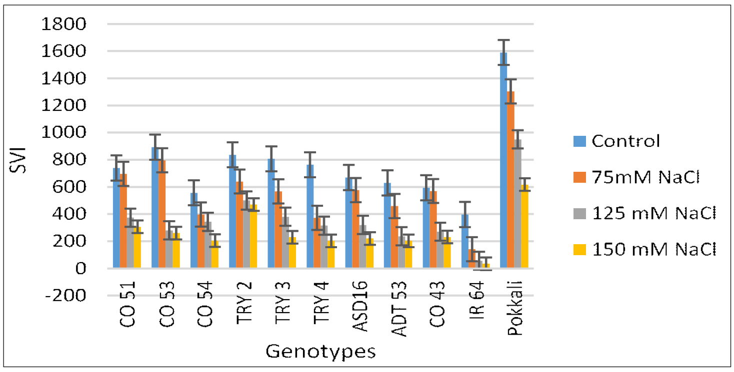

Fig 1: Effect of salinity on seedling vigour index (SVI) of rice genotypes.

Table 1: Effect of salinity on germination percentage (%) and Mean germination time (days) of rice varieties.

	Varieties	Control	75 mM NaCl	125 mM NaCl	150 mM NaCl	Mean
GP (%)	CO 51	100.00	99.00	98.33	97.67	98.75
	CO 53	100.00	99.67	99.33	99.00	99.50
	CO 54	100.00	98.33	97.67	93.67	97.42
	TRY 2	100.00	99.33	99.00	98.67	99.25
	TRY 3	100.00	99.67	99.33	99.00	99.50
	TRY 4	100.00	90.00	80.00	70.00	85.00
	ASD16	100.00	99.33	99.00	98.67	99.25
	ADT 53	100.00	98.67	98.33	98.00	98.75
	CO 43	100.00	99.67	99.33	92.33	97.83
	IR 64	100.00	80.00	80.00	70.00	82.50
	Pokkali	100.00	99.67	99.33	99.00	99.50
	Mean	100.00	96.67	95.42	92.36	96.11
	CD	Genotypes		Treatments	G×T	
	P≤0.05	4.01		2.42	2.96	
MGT (days)	CO 51	1.92	2.05	2.12	2.15	2.06
	CO 53	1.82	2.15	2.24	2.28	2.12
	CO 54	2.24	3.34	3.44	3.59	3.15
	TRY 2	1.81	2.85	2.93	3.08	2.67
	TRY 3	1.92	2.83	2.93	2.95	2.66
	TRY 4	2.12	3.29	3.80	4.17	3.35
	ASD16	1.92	2.83	2.95	2.96	2.66
	ADT 53	1.85	2.93	2.93	2.95	2.67
	CO 43	1.82	1.91	2.91	4.07	2.68
	IR 64	2.81	3.62	4.73	4.81	3.99
	Pokkali	1.74	1.83	1.85	2.10	1.88
	Mean	2.00	2.69	2.98	3.19	2.72
	CD	Genotypes		Treatments	G×T	
	P≤0.05	0.133		0.080	0.098	

respectively (Fig 3). Significantly lower root length recorded for IR 64 in all the treatments and the values are 2.30 cm, 1.20 cm, 0.30 cm and 0.20 cm. Salinity reduced seedling growth as the reduction in shoot and root length compared to control in rice so that increasing salinity showed a decreasing trend Vibhuti *et al*., (2015) and significantly higher root length recorded in control condition compared to higher salt stress condition (12 dsm^{-1}) in 10 days old genotypes. Root length to shoot length ratio recorded were significantly different between genotypes, treatment and their interaction (Fig 5). Under control significantly higher root length to shoot length ratio recorded for TRY4, the values are 2.58, 2.63, 2.57 and 2.77.Significantly lower root length to shoot length ratio recorded for ADT 53 and the values are 0.40, 0.50, 0.23 and 0.15.

Fresh weight and dry weight

Fresh weight also have decreasing trend with increasing concentration of salinity, which significantly different from

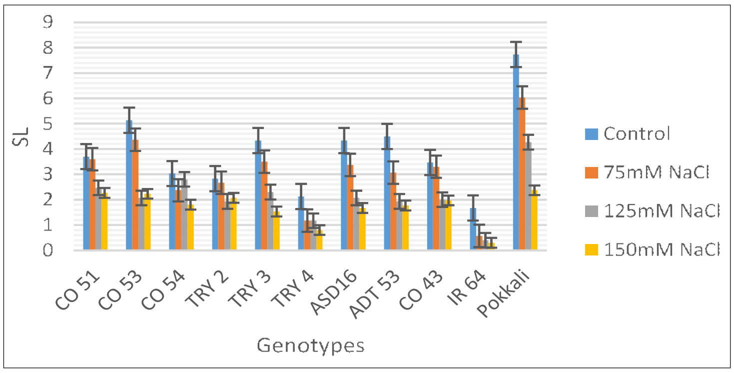

Fig 2: Effect of salinity on shoot length (SL) of rice genotypes.

Table 2: Effect of salinity on fresh weight (g) and dry weight (g) of rice varieties.

	Varieties	Control	75 mM NaCl	125 mM NaCl	150mM NaCl	Mean
FW (g)	CO 51	0.14	0.13	0.07	0.07	0.10
	CO 53	0.13	0.13	0.10	0.07	0.11
	CO 54	0.10	0.09	0.06	0.05	0.07
	TRY 2	0.17	0.14	0.09	0.07	0.12
	TRY 3	0.14	0.12	0.11	0.09	0.12
	TRY 4	0.12	0.10	0.06	0.05	0.08
	ASD16	0.14	0.13	0.09	0.08	0.11
	ADT 53	0.09	0.08	0.06	0.05	0.07
	CO 43	0.14	0.13	0.07	0.06	0.10
	IR 64	0.08	0.07	0.05	0.04	0.06
	Pokkali	0.16	0.13	0.10	0.06	0.11
	Mean	0.13	0.11	0.08	0.06	0.10
	CD	Genotypes		Treatments	G×T	
	P≤0.05	0.003		0.002	0.002	
DW (g)	CO 51	0.029	0.026	0.014	0.012	0.020
	CO 53	0.026	0.025	0.019	0.014	0.021
	CO 54	0.021	0.017	0.011	0.010	0.015
	TRY 2	0.034	0.027	0.019	0.013	0.023
	TRY 3	0.027	0.023	0.021	0.018	0.022
	TRY 4	0.023	0.021	0.013	0.010	0.017
	ASD16	0.027	0.024	0.017	0.014	0.020
	ADT 53	0.018	0.017	0.012	0.011	0.014
	CO 43	0.027	0.026	0.014	0.012	0.020
	IR 64	0.017	0.014	0.011	0.009	0.013
	Pokkali	0.032	0.026	0.019	0.011	0.022
	Mean	0.025	0.022	0.016	0.012	0.019
	CD	Genotypes		Treatments	G×T	
	P≤0.05	0.007		0.004	0.005	

Table 3: Total variance explained through PCA.

	Component	
	1	2
GP	.653	-.587
GI	.900	-.181
MGT	-.886	.259
SL	.888	-.137
RL	.807	.514
RL/SL	.021	.924
FW	.884	.147
DW	.860	.170
SVI	.931	.201
Variance (%)	64.96	18.57

genotypes, treatments and their interaction (Table 2). Under higher concentration of salinity significantly lower fresh weight recorded for IR 64 and the values are0.08 g, 0.07 g, 0.05 g and0.04 g. significantly higher fresh weight recorded for TRY2 in control and 75mM NaCl, the values are 0.17 g and 0.14 g. In 125 mM NaCl and 150 mM NaCl it was TRY 3 and the values are 0.11 g and 0.09 g.

Significantly higher dry weight recorded for TRY2 In control and 75 mM NaCl, the values are 0.034 g, 0.027 g however in 125 mM NaCl and 150mM NaCl treatment significantly higher dry weight recorded for TRY3, the values are 0.021 g and 0.018 g similarly significantly lower fresh weight in all the treatment recorded for IR 64, the values are 0.017 g, 0.014 g, 0.011 g and 0.009g (Table 2). Fresh

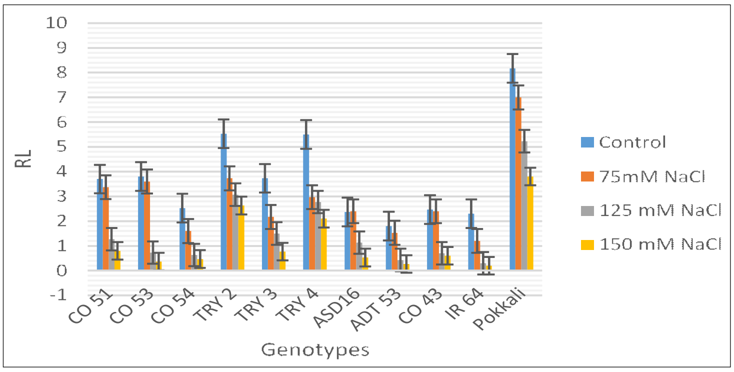

Fig 3: Effect of salinity on root length (RL) of rice genotypes.

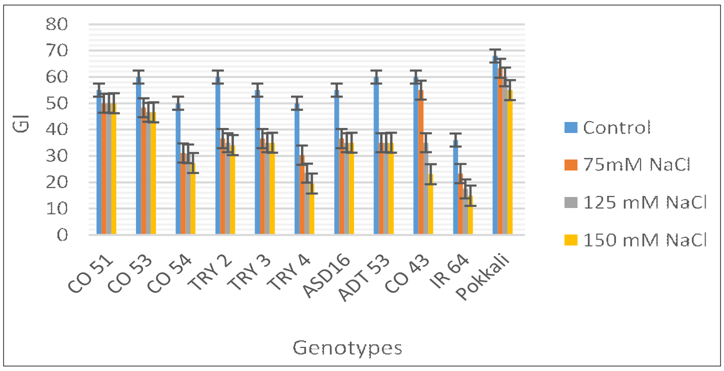

Fig 4: Effect of salinity on germination index (GI) of rice genotypes.

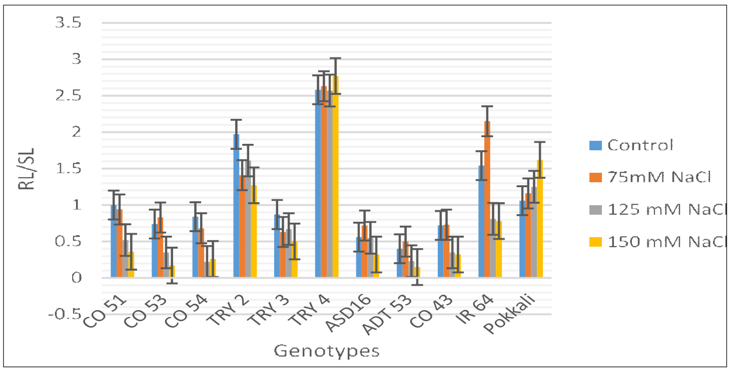

Fig 5: Effect of salinity on RL/SL of rice genotypes.

Table 4: Pearson correlation matrix.

	Correlations								
	GP	GI	MGT	SL	RL	RL/SL	FW	DW	SVI
GP	1								
GI	.647**	1							
MGT	-.765**	-.945**	1						
SL	.541**	.789**	.733**	1					
RL	.247**	.636**	-.562**	.683**	1				
RL/SL	-.401**	-0.091	0.108	-.209*	.477**	1			
FW	.488**	.678**	-.691**	.698**	.697**	0.111	1		
DW	.443**	.651**	-.657**	.677**	.680**	0.122	.979**	1	
SVI	.453**	.784**	-.717**	.907**	.925**	0.15	.769**	.746**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

GP-Germination percentage, GI- Germination index, MGT-Mean germination time.

SL- Shoot length, RL- Root length, FW- Fresh weight, DW- Dry weight, SVI- Seedling vigour index.

and dry weight reduced by increasing concentration of salinity in rice compared to control, greater reduction noticed in 150mM NaCl treatments (Jamil *et al*.,2012).

Seedling vigour index

Seedling vigour index recorded were significantly different between genotypes, treatment and their interaction (Fig 1). Significantly higher seedling vigour index recorded for Pokkali in all the treatment, the values are 1590, 1303, 950, 616.67 and significantly lower seedling vigour index recorded for IR 64, the values are 396.67, 141.33, 56.00, 35.00. Plant vigor is the major check for salt tolerance (Platten *et al*., 2013) which help to reduce toxic effect of salinity (Allbed and Kumar, 2013) fast growing genotypes showed comparatively lower sodium accumulation than slower growing genotypes. Plant vigour considered as avoidance mechanism rather than the tolerance mechanism (Yeo *et al*., 1990).

Assessment of salt tolerance using principal component

Principle component analysis did to identify the principle component of growth and germination parameters for identifying salt tolerance capacity of rice genotypes at germination stage. Principle component analysis revealed that there are 2 component extracted, which explained a total of 83.54% variance (Table 3) in that, component 1 explained 64.96% and component 2 explained 18.57%. First principal component showed higher values for all germination and morphological parameters and lesser loading recorded for RL/SL, second principal component recorded higher value for RL/SL, GP and RL and lesser loading recorded for remaining morphological and germination parameters. Pearson correlation revealed that GP correlated with GI, MGT, SL, RL, RL/SL, FW, DW and SVI. GI correlated with MGT, SL, RL, FW, DW and SVI. MGT correlated with SL, RL, FW, DW and SVI. SL correlated with RL, RL/SL, FW, DW and SVI. RL correlated with RL/SL, FW, DW and SVI. FW correlated with DW and SVI. DW correlated with SVI (Table 4).

CONCLUSION

Genetic variability of rice at germination stage is very important to identify tolerant genotypes. It is very difficult to understand seed germination under various conditions due to its genetic complexity. Germination stage is the most crucial stage under salinity which affect seedling growth at early vegetative stage. Among all the saline treatments studied in the present study *viz*.,control, 75 mM NaCl, 125 mM NaCl and 150 mM NaCl, Germination percentage, germination index and seedling vigour index were higher for Pokkali and it is lower for IR 64 however mean germination time higher for IR 64 lower for Pokkali. Similarly all growth parameters also higher for Pokkali and lower for IR 64 compared to other rice genotypes under saline treatment. Pearson correlation analysis revealed germination percentage and mean germination time showed significant negative correlation, seedling vigour index and shoot length showed significant positive correlation whereas seedling vigour index and mean germination time are negatively correlated. Principle component analysis explained 83.5% of total variance by two components. In that first component explained 64.9% and second component explained 18.5% of variance separately. Seedling vigour index, germination index, shoot length, fresh weight, root length and dry weight are the major parameters which exerts salt tolerance in rice at germination stage.

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Conflict of interest: None.

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