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# **Evaluation of Rice Genotypes for Salinity Tolerance at Reproductive Stage Using Phenotypic and Molecular Markers**

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#### *Authors' contributions*

*This work was carried out in collaboration among all authors. Author AK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DKD and PKB managed the analyses of the study. Authors Vineeta Singh, Vikas Singh, PK and NAK managed the literature searches. All authors read and approved the final manuscript.*

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# **ABSTRACT**

Rice is the single largest source of food energy for more than half of the world's population. Salinity may be a serious environmental constraint to crop production. Salinity screening of twenty rice genotypes were performed at the reproductive stages, in the net house of department of PMB&GE. Phenotyping of the genotypes was done at EC 12dS/m at reproductive stage in net house. Most desirable genotypes days to 50% flowering were IR-68144-2B-2-2-3-1-120, CSR-13, FL-478, NDR-359, AYYAR and NUD-2, SAMBHA MANSURI and MTU-1010; for plant height IR-68144-2B-2-2-3- 1-127, NUD-3, NUD-2, NDRK-2008, IR-91171-66-3-2-1-3, SAMBHA MANSURI, TARAMON and MTU-1010; for panicle bearing tillers/plant FL-478, NDR-359 and SWARNA; for panicle length IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-29, FL-478, NDRK-2008 and IR-92953-49-1-3; for spikelets/panicle IR-91167-99-1-1-1-3, NDRK-2008, SWARNA , IR-92953-49-1-3, IR-91171-66-3-2- 1-3, IR-83668-35-2-2-2 and MTU-1010; for grains/panicle SWARNA, IR-92953-49-1-3, IR-91171-66-

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3-2-1-3, IR-83668-35-2-2-2, NDRK-2008 and MTU-1010 for spikelet fertility % NUD-3, IR-29, FL-478, NDRK-2008, SWARNA, IR-91171-66-3-2-1-3 and IR-83668-35-2-2-2; for test weight NUD-3, NDRK-2008, IR-29 and SWARNA for biological yield/plant AYYAR, TARAMON and NUD-3; for harvest index FL-478, IR-68144-2B-2-2-3-1-120, IR-91167-133-1-1-2-3 and NUD-2; for Na+ NUD-3 and FL-478; for K<sup>+</sup> IR-91167-133-1-1-2-3, NDR-359 and MTU-1010; for Na<sup>+</sup>/K<sup>+</sup> IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, NUD-3, FL-478, IR-64 and SAMBHA MANSURI; for grain yield/ plant (g) AYYAR and FL-478 were reported highly significant in mean performance for yield and its components traits. Analysis of variance, estimates of phenotypic and genotypic coefficient of variation, estimates of heritability and genetic advance in percent of mean were recorded for all the characters among 20 rice genotypes in saline condition. Molecular analysis with SSR markers differentiates the rice genotypes into tolerant and susceptible based on banding pattern. The tolerant rice genotypes were NUD 3, IR-68144-2B-2-2-3-166, IR68144-2B-2-2-3-1-120, IR68100- 2B-2-2-3-1-127, IR-1167-31-3-1-33 and IR-91171-66-3-2-1-3 and susceptible were NDR-359, Taramon, MTU-1010, Swarna and IR-64. The identified salt tolerant genotypes can be potential germplasm sources for future breeding programmes.

*Keywords: Rice; salinity; reproductive stage; phenotyping; genotyping; molecular markers.*

#### **1. INTRODUCTION**

Rice (*Oryza sativa* L.) may be a diploid (2n = 24) and self-fertilized monocot. As a food crop, it forms the staple food of quite three billion people accounting for about 50-80% of their daily calorie intake  $\begin{bmatrix} 1 \end{bmatrix}$ . It's the  $2^{nd}$  most significant crop within the planet after wheat, covering almost 90% of area across Asia alone. Asia is the largest producer of rice (90%) with a mean productivity 3.9 tonnes per hectare. China and India account for about 50% of the world's rice area and 56% of production [2]. Rice may be a most vital cereal crop in India and it contributes about 45% to the cereal production, 41% of the entire grain production and accounts for 20-25 per cent of the agricultural GDP. In India, rice occupies 43.90-million-hectare area with total production of 109 million ton with productivity of 2.59 ton/hectare. Global rice production was only 483.9 million tonnes in (2017-18). Rice is cultivated worldwide over a neighbourhood of about 153.51 million hectares with annual production of 650.19 million tonnes. India ranked first in area having 45.2 million hectares and second in production 104.32 million tonnes [3]. A group of environmental factors like drought, temperature, salinity, soil conditions etc., which varied across sites, seasons and years. Salinity could also be a significant environmental constraint to crop production in many parts of the world. It especially prevalent in irrigated agriculture and in marginal lands, related to poor drainage or high-water tables. Estimates for the extent of salinity damage vary from 25-50 percent of the world's irrigated land. A soil is often termed as saline if its EC is  $4 \text{ dsm}^{-1}$ (equivalent to approximately 40 mM NaCl) and pH exist between 7-8.5 with a pressure of roughly 0.2 MPa. Variety of morpho-physiological growth factors are suffering from NaCl stress [4]. The identification of major gene locus for salt tolerance near a microsatellite marker-are<br>often-employed by-plant breeders-to pick often employed by plant breeders to more efficiently and to raised understand salt tolerance, at vegetative and reproductive growth stages [5]. SSR or microsatellite markers are proved to be ideal for creating genetic maps [6,7] assisting selection [8] and studying genetic diversity in genotype. SSR markers are playing important role to spot gene for salt tolerance which will be helpful for plant breeders to develop new cultivars. The aim of this study was to screen rice genotypes under saline and non-saline conditions and to gauge microsatellite markers for the identification of salt tolerant genotypes at the reproductive stage.

#### **2. MATERIALS AND METHODS**

#### **2.1 Plant Materials**

Total twenty rice genotypes were used in this study, which were IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-28, FL-478, NUD-2, CSR-13, AYYAR, NDRK-2008, IR-64, SWARNA, IR-92953-49-1-3, IR-91171-66-3- 2-1-3, IR-83668-35-2-2-2, SAMBHA MANSURI, TARAMON and MTU-1010. Two markers viz. RM 10772 and RM 10745 were used to evaluate twenty rice genotypes for salt tolerance.

**Screening of rice genotypes at the reproductive stage:** The genotypes were evaluated for their tolerance to salinity under net house. The experimental design was completely randomized block design with three replications.

Primer	motif	(Mb)	(bp)	Repeat Physical Amplicon Amplicon size No. of No. of No. of PP position size range difference (bp) alleles		uniaue alleles	shared alleles		<b>PIC</b>
RM 10772	$(CTT)_{16}$ 12.2		362–490	128				50.00	0.635
RM 10745	(TATG)。11.7		183–200	017				0.00	0.648
DD: Dolumornhiam noroont:									

**Table 1. Analysis of 2 SSR primer used for the amplification of genomic DNA extracted from 20 rice varieties**

*PP: Polymorphism percent;*

*PIC: polymorphism information content*

Two setups were maintained: Normal and salinized. Pregerminated seeds of rice genotypes were sown in earthen pots. After 2 weeks, seedlings were thinned and the water level was raised to about 1 cm. The pots were salinized at EC 12 dSm<sup>-1</sup> after three weeks of sowing and EC was monitored every week. Data were recorded for plant height (cm), days to flowering, panicle bearing tillers/plants, spikelets/panicle, panicle length, grain/panicle, spikelet fertility (%), test weight (g), biological yield (g), harvest index (%), grain yield (g), and  $Na^{+}/k^{+}$  and the data were compiled by taking mean values over randomly selected plant from three replications. Data was subjected to statistical analysis. The variance was estimated as per procedure as suggested by Panse and Sukhatme [9], PCV and GCV were calculated by the formula given by Burton [10], heritability in broad sense  $(h^2)$  by Burton and De Vane [11] and genetic advance i.e. the expected genetic gain were calculated by using the procedure given by Johnson et al. [12].

**Genotyping of salinity tolerant rice genotypes:** Modified CTAB method was used for DNA extraction for 25-day old seedling [13]. Two primers were used for this study, (Table 1). Each PCR reaction carried out with 20.0 μl reactions containing 2.0 µl 10x buffer, 2.0 µl dNTPs, 0.5 μl forward primer, 0.5 μl reverse primer,  $0.5$  μl taq polymerase, 13.5 μl ddH<sub>2</sub>O and 1.0 μl of each template DNA samples. PCR profile was maintained as initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at  $94^{\circ}$  C for 1 min, annealing at 55°C for 1 min and polymerization at 72°C for 2 min and final extension by 7 min at 72°C. Then electrophoresis in 2.5% agarose gel was done after polymorphism in the PCR products and stained in ethidium bromide. Banding patterns were visualized with ultraviolet gel documentation system. Susceptible and tolerant genotypes was identified on the basis of banding patterns obtained from gel documentation system.

# **3. RESULTS AND DISCUSSION**

**Screening of rice genotypes for salt tolerance at the reproductive stage:** Under salt stress 20 rice genotypes showed wider variation for yield and yield contributing characters. Analysis of variance involving 20 genotypes was done for 14 characters under saline conditions. The mean sum of squares due to replication, treatment and error under saline conditions are present in (Table 2). On the basis of mean performance of genotypes, under saline conditions the most desirable genotypes for days to 50% flowering were IR-68144-2B-2-2-3-1-120, CSR-13, FL-478, NDR-359, AYYAR and NUD-2, SAMBHA MANSURI and MTU-1010; for plant height IR-68144-2B-2-2-3-1-127, NUD-3, NUD-2, NDRK-2008, IR-91171-66-3-2-1-3, SAMBHA MANSURI, TARAMON and MTU-1010; for panicle bearing tillers/plant FL-478, NDR-359 and SWARNA; for panicle length IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-29, FL-478, NDRK-2008 and IR-92953-49-1-3; for spikelets/panicle IR-91167-99-1-1-1-3, NDRK-2008, SWARNA , IR-92953-49-1-3, IR-91171-66-3-2-1-3, IR-83668- 35-2-2-2 and MTU-1010; for grains/panicle SWARNA, IR-92953-49-1-3, IR-91171-66-3-2-1- 3, IR-83668-35-2-2-2, NDRK-2008 and MTU-1010 for spikelet fertility % NUD-3, IR-29, FL-478, NDRK-2008, SWARNA, IR-91171-66-3-2-1- 3 and IR-83668-35-2-2-2; for test weight NUD-3, NDRK-2008, IR-29 and SWARNA for biological yield/plant AYYAR, TARAMON and NUD-3; for harvest index FL-478, IR-68144-2B-2-2-3-1-120, IR-91167-133-1-1-2-3 and NUD-2; for Na+ NUD-3 and FL-478; for K<sup>+</sup> IR-91167-133-1-1-2-3, NDR-359 and MTU-1010; for Na<sup>+</sup>/K<sup>+</sup> IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, NUD-3, FL-478, IR-64 and SAMBHA MANSURI; for grain yield/ plant (g) AYYAR and FL-478 were reported highly significant in mean performance for yield and its components traits (Table 4). Tolerant genotypes had lower reduction than the<br>susceptible genotypes. This result was susceptible genotypes. This result consistent with the result observed by Gyawali [14] recorded highest plant height was in the

variety NR11289-B-16-3 but the variety LPNBR-1628 was the shortest in plant height. LPNBR 1628 produced the highest 1000 grains weight on the other hand the variety Khumal-4, produced the lowest 1000 grains weight. The highest yield per  $m<sup>2</sup>$  was produced by the LPNBR 1628, whereas, the lowest yield per  $m<sup>2</sup>$ was produced by the variety NR11130-B-B-B-8- 3. Warkad et al. [15] evaluated 80 rice genotypes for yield and quality. Whereas Under saline condition the panicle bearing tillers per plant (32.34 %) showed highest phenotypic coefficient of variation followed by grains per panicle (27.53%), spikletes per panicle (25.64%), K+ (23.36%), grain yield per plant (g) (16.76%), Na+ /K+ (15.74%), biological yield per plant (g) (15.73%). In saline condition the genotypes had less vigorous growth whereas in non-saline condition they had been showed vigorous growth (Pic. 1). Similar results were also reported by Idris et al. [16], Yadav et al. [17] and Sandhya et al. [18]. (Table 3). among the all traits, panicle bearing tillers per plant (32.34 and 29.53) exhibited high estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), high values of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for these traits suggested the possibility of yield improvement through selection of these traits. Close relationship between GCV and PCV was found altogether the characters and PCV values were slightly greater than GCV, revealing little or no influence of environment for expression. Whereas in saline condition highest broad sense heritability was recorded in the case of plant height (98.97) followed by days to 50% flowering (98.03) grains per panicle (97.96),  $K^+$  (97.87) and spikelet fertility (%) (94.77) and maximum genetic advances was recorded in grains per panicle (55.55) followed by panicle bearing tillers per plant (55.53) in saline condition (Table 3). In general heritability along with genetic advance are often useful in selection programmes. High heritability with high genetic advance as percent of mean indicates that these characters are largely controlled by additive gene action, which indicates that improvement in these characters is possible through mass selection and progeny selection.

**Genotyping evaluation of rice genotypes using SSR markers:** Molecular analysis with two SSR markers differentiate the rice genotypes into tolerant and susceptible based on banding pattern. The tolerant rice genotypes were NUD 3, IR68144-2B-2-2-3-1-120, IR-681002B-2-2-3-1- 127, NDRK-2008 and IR-91171-66-3-2-1-3 and susceptible were NDR-359, Swarna, Taramon, Ayyar and IR-64. These two primers (RM10772 and RM10745) showed polymorphisms in rice genotypes under study and discriminated tolerant genotypes from susceptible (Figs. 1, 2). Those markers showed as highly polymorphic in IR29 x Pokkali for tagging salt tolerant genes as reported by Islam, 2004 and Niones, 2004. Chakravarthi and Naravaneni [20] also reported that SSR primers had distinct polymorphism in rice while they studied 30 SSR primers on 15 rice genotypes. Yadav et al. [17] also reported different banding pattern on 30 rice genotypes with three SSR primers.

<b>Characters</b>	d.f.	Sources of variation				
	<b>Replication</b>	<b>Treatments</b>	Error			
	$\mathbf{2}$	19	38			
Days to 50% flowering	0.43	185.31**	1.23			
Plant height (cm)	0.42	130.88**	0.45			
Panicle bearing tillers/plant	0.62	$7.42**$	0.46			
Panicle length (cm)	2.31	28.28**	1.66			
Spikelets/panicle	10.51	2832.35**	25.20			
Grains/panicle	4.48	2284.54**	15.75			
Spikelet fertility (%)	1.37	35.60**	0.64			
Test weight (g)	0.23	$16.58**$	0.44			
Biological yield/plant (g)	7.08	63.74**	2.88			
Harvest index (%)	2.59	52.46**	2.13			
$Na+$	0.01	$0.76**$	0.07			
$K^*$	0.21	42.55**	0.30			
$Na+/K+$	0.0000	$0.0023**$	0.0001			
Grain yield/plant (g)	1.19	$9.15***$	0.46			

**Table 2. Analysis of variance for randomized block design for 14 characters of rice under saline condition**

*\*, \*\* significant at 5 and 1% probability levels, respectively*

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 $IR-29$ 



# **Picture 1. Response of rice genotype under control and saline condition at reproductive stage**

 $FL-478$ 

**Table 3. Estimates of general mean, phenotypic coefficient of variability (PCV), genotypic coefficient of variability (GCV), heritability in broad sense (h<sup>2</sup> b) and genetic advance in per cent of mean for 14 characters in rice in saline condition**





# **Table 4. Mean performance of different rice genotypes under saline condition**

*\*\*- Highly significant value; \*- Significant value*



**Fig. 1. Amplification of 20 rice genotypes with SSR primer RM 10772**





#### **4. CONCLUSION**

The present investigation included 20 genotypes of rice was carried out in order to study the nature and amount of variability, heritability and genetic advance for 14 quantitative characters. Most desirable genotypes days to 50% flowering were IR-68144-2B-2-2-3-1-120, CSR-13, FL-478, NDR-359, AYYAR and NUD-2, SAMBHA MANSURI and MTU-1010; for plant height IR-68144-2B-2-2-3-1-127, NUD-3, NUD-2, NDRK-2008, IR-91171-66-3-2-1-3, SAMBHA MANSURI, TARAMON and MTU-1010; for panicle bearing tillers/plant FL-478, NDR-359 and SWARNA; for panicle length IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-29, FL-478, NDRK-2008 and IR-92953-49-1-3; for spikelets/panicle IR-91167-99-1-1-1-3, NDRK-2008, SWARNA , IR-

92953-49-1-3, IR-91171-66-3-2-1-3, IR-83668- 35-2-2-2 and MTU-1010; for grains/panicle SWARNA, IR-92953-49-1-3, IR-91171-66-3-2-1- 3, IR-83668-35-2-2-2, NDRK-2008 and MTU-1010 for spikelet fertility % NUD-3, IR-29, FL-478, NDRK-2008, SWARNA, IR-91171-66-3-2-1- 3 and IR-83668-35-2-2-2; for test weight NUD-3, NDRK-2008, IR-29 and SWARNA for biological yield/plant AYYAR, TARAMON and NUD-3; for harvest index FL-478, IR-68144-2B-2-2-3-1-120, IR-91167-133-1-1-2-3 and NUD-2; for Na+ NUD-3 and FL-478; for  $K^+$  IR-91167-133-1-1-2-3. NDR-359 and MTU-1010; for Na<sup>+</sup>/K<sup>+</sup> IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, NUD-3, FL-478, IR-64 and SAMBHA MANSURI; for grain yield/ plant (g) AYYAR and FL-478 were reported highly significant in mean performance for yield and its components traits. Analysis of variance indicated presence of high degree of variability for all the characters among 20 rice genotypes. The high estimates of phenotypic and genotypic coefficient of variation were recorded for panicle bearing tillers/plant. High estimates of heritability are found in plant height and genetic advance in percent of mean were recorded for grains per panicle in both conditions. SSR markers differentiate the rice genotypes into tolerant and susceptible based on banding pattern, the tolerant rice genotypes were NUD 3, R68144-2B-2-2-3-1-120, IR68100-2B-2-2-3-1- 127, IR-1167-31-3-1-33 and IR-91171-66-3-2-1-3 and susceptible were NDR-359, Taramon, MTU-1010, Swarna and IR-64. The quantitative and molecular screening is the best way to find out the tolerant rice varieties salinity tolerance at reproductive stage. The identified salt tolerant genotypes can be potential germplasm sources for future breeding programmes.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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