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Association and Genetic Variability Studies for Reproductive Stage Salinity Tolerance Traits in Rice (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author VPM conducted the work, performed data analysis, and wrote the manuscript. Authors VRRP and JB contributed to methodology and data curation. Author SC contributed to methodology. Author RD performed data analysis and developed software. Author VDRD contributed to writing and editing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Salinity is the second major abiotic stress which significantly affect the rice crop particularly at reproductive stage. Understanding the genetic variability and trait relationship in terms of direct and indirect effects is important to strength the breeding programme to develop salt tolerant rice varieties. Hence, a study was carried out in rice to evaluate 204 $F_{3:4}$ (MTU 1061 × MTU 1121) progenies along with two parents and checks during *kharif* 2021 and *rabi* 2021-22. In this study, highest positive direct effects were observed for the traits number of total grains/panicle (0.4536, 0.4499), panicle length (0.1015, 0.0994), productive tillers per plant (0.1008, 0.0974) and spikelet fertility (0.0913, 0.0953) at genotypic and phenotypic levels. High PCV and GCV was observed for spikelet fertility, number of filled grains/panicle, number of total grains/panicle and grain yield/plant. The traits panicle length (64.28%), flag leaf length (74.34%), plant height (80.34%), productive tillers per plant (94.94%), number of filled grains/panicle (99.66%) and number of total grains/panicle (99.99%) displayed high heritability values. Higher genetic advance over mean (GAM) was observed for spikelet fertility, number of filled grains/panicle, productive tillers per plant and number of total grains/panicle. High GAM and heritability were observed for the traits number of filled grains/ panicle, number of total grains/panicle, productive tillers per plant and spikelet fertility. Thus, these traits should be prioritized while selecting rice lines for increasing yield under salt stress conditions as they have moderate to high PCV, GCV, heritability and genetic advance over mean.

Keywords: Salinity; reproductive stage; variability; path analysis.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal crop which feeds more than half of the world’s population (Zheng et al*.* 2023). Chronic hunger affects more than 400 million people worldwide, including Africans, Asians and South Americans. This scenario demanded a 38% increase in food production within the next 30 years. Global acreage of 1125 mha include 20% of irrigated farmlands which were effected severely by salinity. Among the irrigated farmlands affected by salinity, India has more affected lands with 20 mha (Pruthi et al*.* 2022) followed by China (7 mha) and United States (5.2 mha). The problem of soil salinization is more severe in coastal areas as there is a continuous rise of sea level and the other natural phenomenon like flash floods, hurricanes and El Nino effects (Hossain, 2019) have further worsen the problem (Hairmansis et al*.* 2017). Satellite images have reported a continuous rise of sea level at the rate of about 0.34 cm/year which effect the water quality and soils nearby (Zheng et al*.* 2023). Rice is sensitive to salinity at seedling stage, become tolerant at vegetative stage and will be highly sensitive to salinity at reproductive stage which is expressed in terms of reduction in grain yield (Rasel et al*.* 2021 and Vani Praveena et al., 2023). At reproductive stage, salinity stress majorly effects biomass and grain yield components (Puram et al*.* 2018). Therefore, developing saline tolerant rice varieties through breeding can significantly improve coastal farming, thus enabling to address both the current and impending food problems.

Grain yield per plant is a complex quantitative trait which is controlled by many genes with minor effects and highly influenced by environment. Grain yield is related with agronomic traits which influence the yield (Hasan et al*.* 2020), (Biswas et al*.* 2006) and other quality traits (Chakrabarty et al*.* 2018). So, direct selection for yield may not be rewarding. Path coefficient analysis will split correlation coefficients into both direct and indirect effects which permits one to understand the casual relationship among different variables. In any crop improvement programmes selection of superior genotypes to achieve a particular trait improvement is based on the availability of exploitable variation. The knowledge on the magnitude of heritable component of this variation plays a vital role in guiding the breeder to select the superior parents for crop improvement which can be worked out by studying genetic variability parameters (Manohara and Singh 2015).

Therefore, with this backdrop, the present study was undertaken with the objectives to estimate the magnitude of genetic parameters and to study the direct and indirect effects on grain yield using path coefficient analysis.

2. MATERIALS AND METHODS

Pot culture experiment for reproductive stage salinity screening was carried out in greenhouse at RARS, Maruteru, with ambient temperature and light condition using 204 $F_{3:4}$ progeny obtained from the cross between MTU 1061 (saline tolerant) and MTU 1121 (saline sensitive).

The screening experiment was conducted during *kharif* 2022 in a complete randomized design with 2 replications. Seeds of 204 $F_{3:4}$ lines, both parents (MTU 1061 and MTU 1121) and checks (FL 478 and MTU 1010) were pre-germinated for 24 h by placing them in petri dishes containing distilled water just sufficient to soak the seeds. Six pre-germinated seeds were placed on the soil surface in each plastic pot filled with fertilized soil (50N, 25P and 25K mg kg^{-1}) and 7 pots were kept in each plastic tray filled with ordinary tap water. The water level in the plastic tray was kept level with the soil in the pots. Six plants were grown in each pot with proper labelling but were later thinned to three plants per pot.

All $F_{3:4}$ individuals, checks and both parents were grown under control conditions (EC @ 0 dS/m) until the flag leaf appeared. Salt stress was given at the same growth stage for all the population. Salt stress was imposed by dissolving NaCl in water to raise the electrical conductivity up to 6 dSm^{-1} at booting stage. At the time of maturity plant height, days to flowering, SPAD, flag leaf length, flag leaf width, productive tillers per plant were taken in each replication. At the time of harvesting, individual plants were harvested and data on panicle length, number of total grains/panicle, number of filled grains/panicle, spikelet fertility (%) and grain yield/plant were recorded.

2.1 Statistical Analysis

The analysis of variance (ANOVA) was worked out using OPSTAT software for 204 $F_{3:4}$ progenies along with parents and checks. Phenotypic and genotypic variances; phenotypic and genotypic coefficient of variation; heritability and genetic advance as per cent of mean were calculated using the formulas

Phenotypic variance ($\sigma^2 p$) = $\sigma^2 g + \sigma^2 e$
Genotypic variance ($\sigma^2 g$) = MSg - MSe/r.
GCV (%) = $\{(\sqrt{\sigma^2 g}) / x\} \times 100$
PCV (%) = $\{(\sqrt{\sigma^2 p}) / x\} \times 100$
Heritability (h^2) = $\sigma^2 g / \sigma^2 p$
Genetic advance (GA) = K. $\sigma^2 p$. h^2
Genetic advance percentage of mean = (GA/ x) × 100

Where σ^2 g = genotypic variance, σ^2 p = phenotypic variance, x = grand mean for the trait, MSg = genotype mean sum of square, MSe = error mean sum of square, r = No. of replications, GA = genetic advance, K = selection differential, δp = phenotypic standard deviation and h^2 = broad sense heritability, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation.

Path coefficient analysis was carried out using the method suggested by Wright, 1921 and illustrated by Dewey and Lu, 1959. The data obtained in the study was analysed using R studio to generate estimates on path coefficients and genetic variability parameters.

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Analysis of variance (ANOVA) revealed the existence of statistically significant differences among the lines for all the studied traits (Table 1) which indicated that the exploitable variability existed among the lines. Similar results were earlier reported by Nath et al. 2008, Bisawas et al. 2014, Puram et al. 2017, Sarker et al. 2017, Allel et al. 2019 and Chattopadhyay et al. 2021.

3.2 Path Coefficient Analysis

The estimates on direct and indirect effects obtained using path coefficient analysis was presented in the Table 2 (genotypic path coefficient analysis) and Table 3 (phenotypic path coefficient analysis). The association between the variables studied using correlation coefficients can be reliable only for limited number of variables because with the increase in number of traits the indirect relationship between them will become significant and complex. In this scenario path coefficient analysis helps to identify both direct and indirect causes influencing the relationship between the traits (Kulsum et al. 2022).

At genotypic level highest positive direct effects were revealed for the traits number of total grains/panicle (0.4536), panicle length (0.1015), productive tillers per plant (0.1008) and spikelet fertility (0.0913). Similarly, the traits number of total grains/panicle (0.4499), panicle length (0.0994), productive tillers per plant (0.0974) and spikelet fertility (0.0953) showed direct positive effects at phenotypic level. This explained the direct association of these traits with grain yield. So, direct selection will be effective for the traits number of total grains/panicle, panicle length, productive tillers per plant and spikelet fertility since these traits have manifested positive direct effects at both phenotypic and genotypic levels. Similar results were reported for filled grains per panicle (Panigrahi et al*.* 2018), (Sarker, 2020) and (Kulsum et al*.* 2022); panicle length (Abdul Fiyaz et al*.* 2011) and productive tillers (Kiruthikadevi et al*.* 2020), (Rajashekar et al*.* 2021). Highest positive indirect effects at genotypic and phenotypic levels were observed for the trait number of filled grains/panicle (0.3422, 0.3392) followed by plant height (0.0551, 0.0546) through number of total grains/panicle. These results showed the importance of indirect selection of the above traits to realize high grain yield under stress conditions. Indirect effects on grain yield through plant height, number of productive tillers, days to 50% flowering and plant height were reported (Afrin et al*.* 2017). Similarly, Sari et al*.* 2019 and Akshaya et al*.* 2020 in their study reported positive indirect effects of plant height on yield through flag leaf length.

The data obtained in the study also highlighted the residual effects at both genotypic and phenotypic levels with values of 0.883 and 0.887 respectively indicating only 12% contribution of component traits used in the study for grain yield, while rest of the 88% was contributed by factors not included in the study. This explains the importance of including the traits other than those used in the present study. On contrary to this, Kulsum et al*.* 2022 reported 86% contribution by 12 traits. This indicated that some traits which are due important in selection for increased grain weight under stress conditions need to be included in the study.

3.3 Genetic Variability

The estimates on genetic variability parameters were presented in the Table 4 and Fig. 1. In the present study the phenotypic coefficient of variation ranged between 4.41 (days to flowering) and 45.02 (number of filled grains/panicle). The genotypic coefficient of variation (GCV) ranged from 2.74 (days to flowering) to 44.94 (number of filled grains/panicle). Low PCV and GCV (4.41, 2.74) was observed for days to flowering while moderate PCV and GCV was observed for the traits plant height at maturity (11.49, 10.30) productive tillers per plant (15.71, 15.31), flag leaf length (14.74, 12.71) and SPAD (16.61, 12.44). Moderate GCV (14.81) and high PCV (26.29) was observed for flag leaf width whereas low GCV (9.48) and moderate PCV (11.83) was revealed for the panicle length. Narrow gap between the values of PCV and GCV was observed for all the studied traits except for flag leaf width and grain yield/plant which explained the predominant role of additive gene action. On the other hand highest coefficient of variations (PCV and GCV) was observed for spikelet fertility (28.13, 27.93), number of filled grains/panicle (45.02, 44.94) and number of total grains/panicle (42.65, 42.64) and grain yield/plant (44.36, 20.11) which can be inferred as least environmental influence and importance of additive gene action, thus provide the scope for selecting the genotypes for improving salt tolerance.

Low heritability was observed for grain yield/plant (20.54%) while moderate heritability was manifested by the traits flag leaf width (31.71%), days to flowering (38.46%) and SPAD (56.08%). High heritability was revealed for the traits panicle length (64.28%), flag leaf length (74.34%), plant height at maturity (80.34%), productive tillers per plant (94.94%), number of filled grains/panicle (99.66%) and number of total grains/panicle (99.99%). To achieve improvement of grain yield in advanced generations through selection estimates of heritability coupled with considerable amount of genetic advance is advisable. The efficiency of heritability and genetic advance were mutually related where the reliability of heritability estimates increase with genetic advance thus one can realize the genetic gain under selection. Hence along with heritability, genetic advance need to be worked out to appreciate the genetic gain achieved under selection which helps in planning breeding methods to achieve yield enhancement under stress conditions (Sarker, 2020).

Low genetic advance as percent of mean (GAM) was observed for days to flowering (2.82) whereas moderate GAM was observed for the traits *viz*., flag leaf width (13.52), panicle length (13.71), grain yield/plant (14.25), flag leaf length (15.23), SPAD (16.45) and plant height at maturity (17.52). High GAM was observed for number of filled grains/panicle (89.89), number of total grains/panicle (85.27), spikelet fertility (55.57) and productive tillers per plant (27.06).

In the present study high genetic advance as percent of mean and heritability was observed for number of filled grains/panicle, number of total grains/panicle, productive tillers per plant and spikelet fertility. This explained that these traits are highly under the influence of additive gene action and selection is effective for these traits. Similar results were reported earlier by Karthikeyan et al. 2010, Abdul Fiyaz et al. 2011, Sarker, 2020 and Pranaya et al. 2024. Moderate GAM and high heritability was observed for flag leaf length, plant height and panicle length which highlighted the importance of additive gene action in the inheritance of these traits and suitability of selecting these traits in the population for improving grain yield/plant. Low to moderate GAM and heritability was observed for days to flowering and grain yield/plant which explained the role of non-additive gene action in transmission of these traits from parents to offspring. These results were similar with the reports of Sarker, 2020, Talekar et al. 2022, Vanipraveena et al. 2022 and Abdul Fiyaz et al. 2011.

Table 1. Analysis of variance for reproductive stage salinity tolerance traits in rice

	Treatments	Error	Total
Degrees of freedom	207	208	415
Days to maturity	26.46**	11.76	
Plant height	101.46**	11.06	
Flag leaf length	30.16**	4.44	
Flag leaf width	0.54**	0.28	
SPAD	14.93**	4.20	
Panicle length	7.68**	1.67	
Number of total grains/panicle	11606.28**	0.79	
Number of filled grains per/panicle	3233.01**	5.67	
Spikelet fertility	413.05**	2.902	
Productive tillers per plant	0.78**	0.02	
Grain yield/plant	0.71**	0.47	

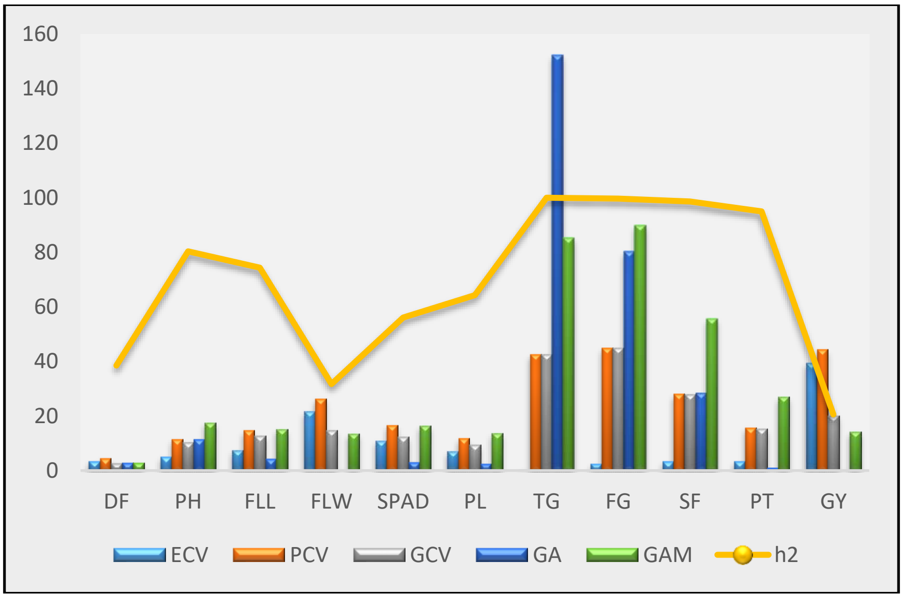

Fig. 1. the variabitlity parameters for reproductive stage salinity tolerance traits in rice (*Oryza sativa* L.)

DF =Days to flowering, PH=Plant height at maturity, FLL=Flag leaf length, FLW=Flag leaf width, SPAD=Chlorophyll reading, Panicle length=PL, TG=Total number of grains per panicle, FG =Number of filled grains per panicle, SF=Spikelet fertility, PT=Productive tillers per plant, GY= grain yield per plant, ECV= Environment coefficient of variation, PCV = Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation, h^2 BS=heritability broad sense, GA=Genetic advance, GAM= Genetic advance as per cent of mean

Table 2. Genotypic path coefficients for salinity related traits of $F_{3:4}$ population under salt stress (EC @ 12 dSm^{-1}) at reproductive stage in rice

Traits	DF	PH	FLL	FLW	SPAD	PL	TG	FG	SF	PT	r value for Grain yield/plant
DF	-0.0018	-0.0001	0.0037	-0.0004	0.0009	0.0138	-0.0370	0.0059	0.0051	0.0001	-0.0100
PH	0.0000	0.0051	0.0028	0.0003	0.0007	0.0173	0.0551	-0.0140	0.0027	0.0143	0.0840
FLL	-0.0001	0.0003	0.0385	0.0007	0.0016	0.0119	-0.0606	0.0203	-0.0016	-0.0103	0.0007
FLW	0.0001	0.0001	0.0025	0.0105	0.0002	0.0064	-0.0295	0.0249	-0.0180	0.0035	0.0008
SPAD	-0.0001	0.0002	0.0032	0.0001	0.0201	0.0111	0.0182	0.0047	-0.0099	0.0074	0.0540
PL	-0.0002	0.0009	0.0045	0.0007	0.0022	0.1015	-0.0030	0.0061	-0.0018	-0.0050	0.1000
TG	0.0002	0.0006	-0.0051	-0.0007	0.0008	-0.0008	0.4536	-0.1170	-0.0184	-0.0104	0.3000
FG	0.0001	0.0005	-0.0050	-0.0017	-0.0006	-0.0040	0.3422	-0.1550	0.0412	-0.0127	0.2000
SF	-0.0001	0.0002	-0.0007	-0.0021	-0.0022	-0.0020	-0.0910	-0.0700	0.0913	-0.0030	-0.0800
PT	0.0001	0.0007	-0.0039	0.0004	0.0015	-0.0050	-0.0470	0.0196	-0.0027	0.1008	0.0600
Residual effect: 0.883											

DF =Days to flowering, PH=Plant height at maturity, FLL=Flag leaf length, FLW=Flag leaf width, SPAD=Chlorophyll reading, PL= Panicle length, TG=Total number of grains per panicle, FG =Number of filled grains per panicle, SF=Spikelet fertility, PT=Productive tillers, GY = grain yield per plant

Table 3. Phenotypic path coefficients for salinity related traits of $F_{3:4}$ population under salt stress (EC @ 12 dSm^{-1}) at reproductive stage in rice

Traits	DF	PH	FLL	FLW	SPAD	PL	TG	FG	SF	PT	r value for Grain yield/plant
DF	-0.00220	-0.00005	0.00370	-0.00040	0.00100	0.01330	-0.0369	0.00600	0.00510	-0.00020	-0.0110
PH	0.00002	0.00540	0.00290	0.00030	0.00070	0.01690	0.0546	-0.01430	0.00280	0.01340	0.0820
FLL	-0.00020	0.00040	0.03930	0.00070	0.00170	0.01170	-0.0594	0.02020	-0.00160	-0.00960	0.0020
FLW	0.00009	0.00010	0.00260	0.01010	0.00020	0.00620	-0.0293	0.02510	-0.01880	0.00410	0.0003
SPAD	-0.00010	0.00020	0.00320	0.00010	0.02090	0.01070	0.0179	0.00480	-0.01020	0.00690	0.0540
PL	-0.00030	0.00090	0.00460	0.00060	0.00230	0.09940	-0.0035	0.00610	-0.00180	-0.00470	0.1000
TG	0.00020	0.00070	-0.00510	-0.00060	0.00080	-0.00070	0.4499	-0.11840	-0.01910	-0.00980	0.2900
FG	0.00008	0.00050	-0.00500	-0.00160	-0.00060	-0.00390	0.3392	-0.15710	0.04300	-0.01190	0.2020
SF	-0.00010	0.00020	-0.00070	-0.00200	-0.00220	-0.00190	-0.0906	-0.07090	0.09530	-0.00280	-0.0760
PT	0.00010	0.00070	-0.00380	0.00040	0.00150	-0.00470	-0.0453	0.01920	-0.00270	0.09740	0.0620
Residual effect: 0.887											

DF =Days to flowering, PH=Plant height at maturity, FLL=Flag leaf length, FLW=Flag leaf width, SPAD=Chlorophyll reading, PL= Panicle length, TG=Total number of grains per panicle, FG =Number of filled grains per panicle, SF=Spikelet fertility, PT=Productive tillers per plant, GY = grain yield per plant

Table 4. Genetic variability parameters for salinity related traits in rice (*Oryza sativa* L.) at reproductive stage

	Gen var	Phen var	ECV	PCV	GCV	h^2BS	GA	GAM
DF	7.35	19.11	3.46	4.41	2.74	38.46	2.79	2.82
PH	45.20	56.26	5.09	11.49	10.30	80.34	11.44	17.52
FLL	12.86	17.30	7.47	14.74	12.71	74.34	4.30	15.23
FLW	0.13	0.41	21.73	26.29	14.81	31.71	0.33	13.52
SPAD	5.36	9.57	11.01	16.61	12.44	56.08	3.06	16.45
PL	3.01	4.68	7.07	11.83	9.48	64.28	2.51	13.71
TG	5802.74	5803.54	0.50	42.65	42.64	99.99	152.32	85.27
FG	1613.78	1619.23	2.61	45.02	44.94	99.66	80.35	89.89
SF	205.06	207.99	3.34	28.13	27.93	98.59	28.49	55.57
PT	0.38	0.40	3.54	15.71	15.31	94.94	1.08	27.06
GY	0.12	0.59	39.54	44.36	20.11	20.54	0.25	14.25

DF =Days to flowering, PH=Plant height at maturity, FLL=Flag leaf length, FLW=Flag leaf width, SPAD=Chlorophyll reading, Panicle length=PL, TG=Total number of grains per panicle, FG =Number of filled grains per panicle, SF=Spikelet fertility, PT= Productive tillers per plant, GY = grain yield per plant, Gen var= genotypic variance, Phen var=Phenotypic variance, ECV=Environment coefficient of variation, PCV=Phenotypic coefficient of variation, GCV=Genotypic coefficient of variation, h^2 BS=heritability broad sense, GA=Genetic advance, GAM= Genetic advance as per cent of mean

Based on the above studied genetic parameters *i.e.*, PCV, GCV, heritability (h^2 BS) and GAM the traits namely number of filled grains/panicle, number of total grains/panicle, productive tillers per plant and spikelet fertility showed high heritability and GAM with least difference observed between PCV and GCV. Hence, selection will be effective for these traits to enhance grain yield/plant in rice under salinity stress conditions.

4. CONCLUSION

In the present study number of total grains/panicle, panicle length, productive tillers per plant and spikelet fertility showed high positive direct effects at genotypic and phenotypic levels. However high heritability coupled with GAM with narrow gap between PCV and GCV was observed for number of total grains/panicle, productive tillers per plant and spikelet fertility. Thus selection for these traits at reproductive stage will be effective for increasing grain yield under stress.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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