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M. Vani Praveena

Ph.D. Scholar, Department of Genetics and Plant Breeding, Agricultural College, Bapatla, ANGRAU, Hyderabad, Telangana, India

P. Venkata Ramana Rao

Principal Scientist, Department of Plant Breeding, Regional Agricultural Research Station, Maruteru, Andhra Pradesh, India

Jyothi Badri

Senior Scientist, Department of Plant Breeding, ICAR-Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, New Delhi, India

Ch. Sreenivas

Principal Scientist, Department of Soil Science and Agricultural Chemistry, Regional Agricultural Research Station, Maruteru, Andhra Pradesh, India

D. Ramesh

Assistant Professor, Department of Agricultural College, Statistics and Computer Applications, Bapatla, Andhra Pradesh, India

Corresponding Author:

P. Venkata Ramana Rao

Principal Scientist, Department of Plant Breeding, Regional Agricultural Research Station, Maruteru, Andhra Pradesh, India

Studies on path coefficient analysis and variability parameters for seedling stage salinity tolerance in rice (*Oryza sativa* L.)

M. Vani Praveena, P. Venkata Ramana Rao, Jyothi Badri, Ch. Sreenivas and D. Ramesh

Abstract

An experiment was conducted during *rabi* 2021-22 and *kharif* 2022 using hydroponics system for screening 204 F_{3:4} population derived from the cross MTU 1061 × MTU 1121 in complete randomized block design with two replications to study path coefficient analysis and genetic variability parameters at Regional Agricultural Research Station, Maruteru. Highly significant differences among the screened genotypes were revealed by Analysis of variance. Highest positive direct effects were observed for shoot length (0.944), SIS (0.446), root length (0.191) and shoot potassium concentration (0.111) at genotypic level. Negative direct effects at genotypic level were revealed by shoot sodium concentration (Na⁺) and shoot sodium to potassium ratio (Na⁺/K⁺). Shoot K⁺ concentration (K⁺) and root length showed positive indirect effect. Phenotypic co-efficient of variation (PCV) ranged between 13.74 (shoot length) and 64.89 (Na⁺/K⁺) while the genotypic co-efficient of variation (GCV) ranged between the traits Na⁺/K⁺ (0.54) and K⁺ (30.06). High heritability was revealed for the traits K⁺ (95.61%), shoot length (76.23%), root length (66.91%) and Na⁺ (64.14%). High GAM was observed for shoot dry weight, SIS, Na⁺ and K⁺. High heritability and GAM was observed for K⁺ and Na⁺ and selection for these traits can increase shoot dry weight under salt stress condition.

Keywords: Rice, salinity, seedling stage, variability and path coefficient

1. Introduction

Rice is a major cereal crop cultivated nearly in 43.50 million ha, with a production of 130 mt and productivity of 2713 kg/ha. The majority of the world's rice comes from China, India, Bangladesh, Indonesia, and Vietnam. According to UN-based population and income predictions, the world's rice consumption has gradually scaled from 439 Mt in 2010 to 496 Mt in 2020 and will continue to rise to 555 Mt in 2035, representing a 26% increase globally over the next 25 years (Fahad *et al.*, 2019) [8]. However, due to climate change under irrigated conditions, rice yields were lowered by 4% in 2020 and are projected to decrease by another 7% in 2050 (Kumar, 2017) [16]. The various abiotic factors that contribute to climate change put diverse plant species under stress. Salinity is a second important abiotic stress after drought affecting differently at different growth stages. More than 6% of the world's land area, approximately accounting for 800 million hectares, has been devastated by salinity, including 45 Mha (20%) of irrigated land utilised for crop production, making salinity a global problem. Salinity is due to presence of excessive soluble cationic salts (Osman, 2018) [17] like sodium (Na⁺), calcium (Ca⁺), magnesium (Mg⁺) and anions like bicarbonates (H₂CO₃⁻), chlorides (Cl⁻) and sulphates (SO₄²⁻). Rice yield has been significantly reduced by about 30-50% in salt-affected areas (Kim *et al.*, 2023) [14]. At seedling stage, salt stress results in whitened leaf tips, chlorotic patches, stunting, reduction of tillering ability and finally death (Puram *et al.*, 2018) [19]. Understanding the scope and magnitude of genetic variability along with the association between different agro-morphological features and grain yield is important for crop improvement under saline conditions. Genetic parameters like coefficients of variation (PCV and GCV), heritability and genetic advance as percent of mean help us to understand the nature of gene action, thus helps in identification of genetic components of variances which facilitates the selection of a desirable breeding method (Bisne *et al.*, 2009) [1]. Additionally, path coefficient analysis splits up the correlation coefficients into direct effect and series of indirect effects, which allows the breeder to understand the contribution of each trait towards dependent trait and facilitate the selection of suitable traits for indirect selection.

Therefore, an effort has been taken up to estimate path coefficients and genetic variability parameters in rice to develop an efficient selection criterion for seedling stage salinity tolerance.

2. Materials and Methods

2.1 Material

Crossing was taken up between MTU 1121 (female parent), a high yielding, salinity sensitive and MTU 1061 (as donor parent), a high yielding, salinity tolerant rice variety during *rabi* 2017-18 (Durga *et al.*, 2021) [6]. F₁S were evaluated and selfed to generate F₂ and F₃ populations and phenotyping of 204 F_{3:4} families for seedling stage salinity tolerance was conducted during *rabi* 2021-22 and *kharif* 2022 using hydroponics following the protocol of Gregorio *et al.* (1997) [10] with some modifications.

2.2 Method

A total of 204 lines from F_{3:4} population, two parents *i.e.*, MTU 1061 and MTU 1121 along with tolerant check FL478 and susceptible check MTU 1010 were screened for salinity tolerance at seedling stage in greenhouse following the standard protocol of IRRI with some modifications (Gregorio *et al.*, 1997) [10]. The screening experiment was conducted in a complete randomized design with 2 replications. Sterilized seeds of 204 F_{3:4} lines, parents and checks were placed in petri dishes with moistened filter papers and incubated at 30 °C for 48 hr. One pre-germinated seed per hole was placed on the Styrofoam seedling float. Initially, the seedlings were grown in normal water for two days, followed by Yoshida *et al.* (1976) [28] nutrient solution for two to three days and at two leaf stage, they were subjected to initial salinity stress of EC=6 dSm⁻¹ for seven days and final salinization at an EC of 12 dSm⁻¹ for seven days by adding NaCl to nutrient solution and the pH was adjusted to 5.0 using NaOH and HCl.

The final score was recorded at 14 days after initial salinization. The plants were separated carefully and data on root length and shoot length were recorded. After screening

experiment, the shoots of the lines were oven dried at 60 °C for 3 days, and the dry weights were recorded. For measuring the concentrations of Na⁺ and K⁺ in the shoot, one gram of powdered plant sample was taken in 150 ml Erlenmeyer flask and digested with diacid mixture (HNO₃ and HClO₄ in 9:4 ratio) at 50-55 °C heating block for 3 h. The total amount of Na⁺ and K⁺ was measured using flame photometer.

2.3 Statistical analysis

Analysis of variance was performed for the mean data generated from 204 F_{3:4} population using OPSTAT software and the total variation was partitioned into 'within treatment' and 'between treatment variations'. To know the direct and indirect effects of the salinity tolerance components, path coefficient analysis was carried out using the simple correlation coefficient as suggested by Dewey and Lu (1959) [5]. PCV (Phenotypic coefficient of variation) and GCV (Genotypic coefficient of variation) were calculated according to Burton 1952 [2]. Heritability in broad sense and genetic advance were calculated using formulae given by Johnson *et al.*, 1955 [13]. The data was analysed in R software version 4.1.2 to draw valid conclusions.

3. Results and discussion

3.1 Analysis of variance

The analysis of variance for seven salinity related traits studied at seedling stage of 204 F_{3:4} mapping population along with parents and checks was presented in Table 1. It was evident from the analysis of variance that the genotypic differences were highly significant for all the traits under study. A wide range of variation among the genotypes indicated the existence of variability. The availability of accountable variability in the generated experimental material signifies the improvement of different quantitative and qualitative traits of rice through selection (Talekar *et al.*, 2022) [26]. Similar results were reported by Krishnamurthy *et al.*, 2014 [15], Tiwari *et al.*, 2016 [27], De Leon *et al.*, 2017 [4] and Puram *et al.*, 2017 [18].

Table 1: Analysis of variance for seedling stage salinity tolerance traits in rice (*Oryza sativa* L.)

Sl. No.	Sources of variation	df	Mean sum of squares						
			SIS	RL	SL	Na ⁺	K ⁺	Na ⁺ /K ⁺	SDW
1	Treatments	207	7.44**	5.75**	18.46**	405.09**	612.43**	0.94**	0.27**
2	Error	208	3.34	1.14	2.49	88.45	14.86	0.59	0.15
3	Total	415							

SIS= Salt injury score, RL=Root length, SL=Shoot length, Na⁺=Shoot sodium concentration, K⁺= Shoot potassium concentration, Na⁺/K⁺=Ratio of shoot sodium to potassium concentration, SDW=Shoot dry weight

3.2 Path coefficient

The values of correlation coefficients are seldom affected by the third factor. So, the true association between the variables cannot be realized by studying correlation coefficients alone. Hence, path analysis which estimate the cause and effect relationship need to be studied. It establishes an actual association between independent and dependent variable (Chavan *et al.*, 2020) [3].

The estimates of path coefficient analysis in this study were presented in Table 2 and Table 3. Highest positive direct effects were observed for shoot length (0.944) followed by the traits SIS (0.446), root length (0.191) and shoot potassium concentration (0.111) at genotypic level (Table 2). Similarly, highest positive direct effect at phenotypic level (Table 3) was observed for shoot length (0.509). This indicated that under

salt stress conditions, the above mentioned traits were associated directly with shoot dry weight and less effected by environment as these traits manifested positive direct effects at phenotypic and genotypic levels. However, only shoot length manifested highest positive correlation with shoot dry weight along with positive direct effect at both phenotypic and genotypic levels which explains that direct selection of lines with higher shoot length would be beneficial to increase shoot dry weight under salt stress condition. Negative direct effects at genotypic level (Table 2) were revealed by Na⁺ (-0.046) and Na⁺/K⁺ (-0.390) explaining an increment in Na⁺ and Na⁺/K⁺ will reduce the shoot dry weight. Shoot K⁺ concentration (0.282) and root length (0.126) showed positive indirect effect on shoot dry weight through Na⁺/K⁺ (ratio of shoot sodium to potassium concentration) under stress at

genotypic level (Table 2). So, indirect selection for these traits under stress conditions will be an effective strategy. It can be inferred that selection for the genotypes with longer roots can penetrate deeper into the soil to avoid detrimental effects of toxic sodium ions by reduced uptake of Na⁺ or the genotypes with higher uptake of potassium will result in low Na⁺/K⁺ which allow the plant to perform all the physiological processes normally, and reduce the effect of salt stress thus reducing the yield loss at later stages of crop growth by enhancing seedling stage salinity tolerance.

The data also reported residual effects of 0.06 and 0.73 at genotypic and phenotypic levels which explains contribution of 94% variability by the characters included in the study at genotypic level. These results were in conformation with the findings of Krishnamurthy *et al.*, 2014 [15], Rahman *et al.*, 2016 [20], Rasel *et al.*, 2018 [22] and Rahman *et al.*, 2019 [21]. SES showed negative correlation with shoot dry weight and highly positive direct effect which highlighted the importance of using restricted simultaneous selection model (Singh and Kakar 1977; Rashid *et al.*, 2010) [25, 23].

Table 2: Estimation of genotypic path coefficients for seedling stage salinity related traits in rice (*Oryza sativa* L.)

	SIS	RL	SL	Na ⁺	K ⁺	Na ⁺ /K ⁺	r for SDW
SIS	0.446	-0.075	-0.048	-0.008	-0.077	-0.416	-0.17
RL	-0.177	0.191	0.036	-0.006	0.042	0.126	0.21
SL	-0.023	0.007	0.944	0.009	-0.007	0.027	0.95
Na ⁺	0.075	0.023	-0.183	-0.046	0.051	-0.104	-0.18
K ⁺	-0.312	0.072	-0.060	-0.021	0.111	0.282	0.07
Na ⁺ /K ⁺	0.477	-0.062	-0.066	-0.012	-0.080	-0.390	-0.13
Residual effect: 0.06							

SIS= Salt injury score, RL=Root length, SL=Shoot length, Na⁺=Shoot sodium concentration, K⁺= Shoot potassium concentration, Na⁺/K⁺=Ratio of shoot sodium to potassium concentration, SDW=Shoot dry weight

Table 3: Estimation of phenotypic path coefficients for seedling stage salinity related traits in rice (*Oryza sativa* L.)

	SIS	RL	SL	Na ⁺	K ⁺	Na ⁺ /K ⁺	r for SDW
SIS	-0.066	-0.012	-0.007	-0.0007	-0.017	0.009	-0.09
RL	0.016	0.051	0.005	-0.0007	0.012	-0.004	0.07
SL	0.0009	0.0005	0.509	0.001	-0.002	-0.0008	0.5
Na ⁺	-0.006	0.0043	-0.078	-0.007	0.016	0.005	-0.06
K ⁺	0.030	0.015	-0.030	-0.003	0.037	-0.009	0.04
Na ⁺ /K ⁺	-0.032	-0.010	-0.022	-0.002	-0.019	0.019	-0.06
Residual effect: 0.73							

SIS= Salt injury score, RL=Root length, SL=Shoot length, Na⁺=Shoot sodium concentration, K⁺= Shoot potassium concentration, Na⁺/K⁺=Ratio of shoot sodium to potassium concentration, SDW=Shoot dry weight

3.3 Variability

While choosing superior genotypes for crop improvement programmes, variability is crucial since it enables us to determine if the exploitable variation is heritable or non-heritable. However, it is important to determine what extent of this variance is due to heritable component which plays a major role in choosing better parents for crop development. The estimates on different studied genetic parameters obtained in the present study were presented in Table 4 and Figure 1. Phenotypic co-efficient of variation (PCV) ranged between 13.74 (shoot length) and 64.89 (Na⁺/K⁺) while the genotypic co-efficient of variation (GCV) ranged between the traits Na⁺/K⁺ (0.54) and K⁺ (30.06). Higher PCV and low GCV values were observed for the traits shoot dry weight (44.49, 0.55) and SIS (42.52, 3.80), whereas high PCV and GCV values were reported for the traits K⁺ (59.87, 30.06) and Na⁺ (47.55, 26.45). Moderate GCV and PCV was observed for root length (10.70, 14.19) while shoot length (20.56, 13.74) manifested high GCV and moderate PCV. Highest coefficient variations (PCV and GCV) were observed in the present study with narrow gap between the studied traits which indicated the negligible effect of environment on the phenotypic expression of these traits. Low heritability was observed for Na⁺/K⁺ (17.48%) and shoot dry weight (28.57%); moderate heritability was reported for SIS (43.90); high heritability was revealed for the traits K⁺ (95.61%), shoot

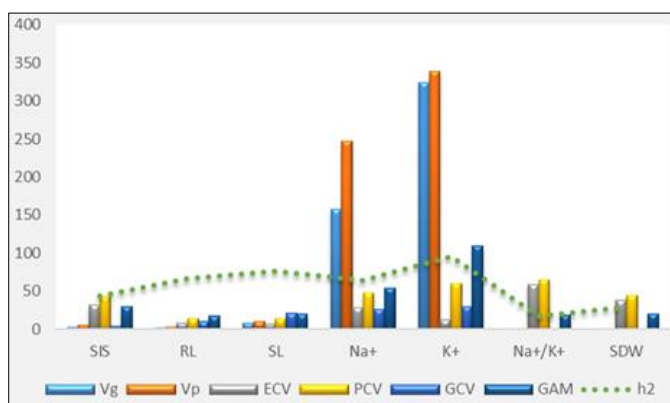
length (76.23%), root length (66.91%) and Na⁺ (64.14%). High GAM was observed for shoot dry weight (20.53), SIS (29.52), Na⁺ (53.90) and K⁺ (108.80) while moderate GAM was reported for the traits root length (17.29), Na⁺/K⁺ (18.25) and shoot length (19.81).

Moderate heritability and high genetic advance as percent mean (GAM) was observed for SIS which shows the predominance of additive gene action. Moderate GAM and high heritability was observed for shoot length and root length where trait improvement can be achieved by intermating superior genotypes of segregating population (Samadia *et al.*, 2005) [24]. High heritability and GAM was observed for K⁺ and Na⁺. Hence, direct selection is desired due to predominance of additive gene action. Low heritability was observed for shoot dry weight and Na⁺/K⁺ which showed the influence of environment on phenotypic expression of these traits. These observations were in confirmation with the earlier reports of Hosseini *et al.*, 2012 [11], Gana *et al.*, 2013 [9], Eti *et al.*, 2018, Rasel *et al.*, 2018 [22] and Ikhajagbe *et al.*, 2020. High PCV, GCV, GAM and heritability were observed for Na⁺ and K⁺. High heritability and GAM explains most of the heritable variation is additive in nature which helps in fixation of the traits and selection might be effective. Based on the above discussion in the present study selection is effective for low shoot Na⁺ and high K⁺ concentration to develop highly yielding varieties under salt tolerance.

Table 4: Genetic variability parameters for seedling stage salinity tolerance traits in rice (*Oryza sativa* L.)

	ECV	PCV	GCV	h ²	GA	GAM
SIS	31.84	42.52	3.80	43.90	1.69	29.52
RL	8.16	14.19	10.70	66.91	2.26	17.29
SL	6.70	13.74	20.56	76.23	4.67	19.81
Na ⁺	28.47	47.55	26.45	64.14	17.80	53.90
K ⁺	12.54	59.87	30.06	95.61	33.45	108.80
Na ⁺ /K ⁺	58.95	64.89	0.54	17.48	0.24	18.25
SDW	37.60	44.49	0.55	28.57	0.21	20.53

SIS= Salt injury score, RL=Root length, SL=Shoot length, Na⁺=Shoot sodium concentration, K⁺= Shoot potassium concentration, Na⁺/K⁺=Ratio of shoot sodium to potassium concentration, SDW=Shoot dry weight



SIS= Salt injury score, RL=Root length, SL=Shoot length, Na⁺=Shoot sodium concentration, K⁺= Shoot potassium concentration, Na⁺/K⁺=Ratio of shoot sodium to potassium concentration, SDW=Shoot dry weight

Fig 1: Graphs showing different genetic variability parameters for seedling stage salinity tolerance in rice (*Oryza sativa* L.)

4. Conclusion

In the present investigation although shoot length have highest positive direct effects at genotypic and phenotypic levels the traits shoot sodium and potassium concentrations have high heritability and genetic advance as percent of mean along with shoot length having high heritability and moderate GAM. Hence, selection for these traits can increase shoot dry weight under salt stress condition.

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