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Correlation studies in seedling and reproductive stage salinity tolerance in rice (*Oryza sativa* L.) under salinity

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Abstract
Salinity is a major abiotic stress limiting rice productivity worldwide. The present study had 234 $F_{2:3}$ population of a salt-tolerant donor Indra (MTU 1061) in the genetic background of Sri Druthi (MTU 1121) to elucidate the genetic basis of seedling and reproductive stage salinity tolerance. The $F_{2:3}$ population were evaluated under salt stress at seedling stage (EC 6 &12 dSm^{-1}) and reproductive stage (EC 6 & 12 dSm^{-1}). At seedling stage, salt injury score had positive significant correlation with shoot Na^+ concentration and Na/K ratio while it was negative & significant with K^+ concentration, shoot length, root length and shoot dry weight. This indicated that homeostasis between Na^+ and K^+, plays a key role in the seedling stage tolerance to salt stress. In reproductive stage grain yield per plant play pivotal role for salinity tolerance comparative to remaining parameters such as number of spikelets, filled spikelets and unfilled spikelets. Results showed positive significant correlation of grain yield with days to flowering, number of total grains and productive tillers while it was negative significant with panicle length. The results of this study confirmed that salinity tolerance at the seedling and reproductive stages are independent to each other.

Keywords: Rice, salinity tolerance, seedling stage, reproductive stage, $f_{2:3}$ population, correlation

Introduction
Enormous environmental changes have brought negative impact on food crops in the world. Among these environmental factors, salinity is known to inhibit plant growth and ultimately cause crop yield loss. Salinity affecting approximately 1 billion ha of land globally. This equals more than 6% of the world's total farming area and nearly 20% of the globally irrigated area (Kakar *et al*., 2019) [8]. As the most important staple food in Asia, a significant portion of rice crops are grown along the coastal areas where rice paddy fields suffer various degrees of salinity (Naveed *et al*., 2018) [15].

In general, salinity imposes both osmotic and ionic stresses on plant. The gradient between the soil and root cells due to high salt content reduces the absorption capacity resulting in water deficit in the plant system. At the same time, the altered proportion of Na^+/K^+ and high Cl^- concentration impedes the normal physiological activity of the plant. Further, high level of salinity can upset the nutrient balance in the plant or interfere with the uptake of some nutrients (Venkata Ramana Rao *et al*., 2018) [19].

Rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage, when 2-3 leaves appeared), and it gains tolerance during vegetative growth stage, becomes sensitive during pollination and fertilization, and then becomes increasingly more tolerant at maturity (Mondal *et al*., 2016) [13]. Salinity has in fact independent effects at these two critical stages, that is, tolerance at the seedling stage is not necessarily associated with tolerance at the reproductive stage and vice versa, suggesting that they are regulated by different processes and genes (Krishnamurthy *et al*., 2016) [9]. Hence, pyramiding of contributing traits at both stages is needed for developing resilient salt-tolerant cultivars (Mohammadi *et al*., 2014) [12].

The overall goal of the present study was to screen 234 $F_{2:3}$ population to develop tolerance to salt stress at the seedling and reproductive stage using salt tolerant rice cultivar Indra (MTU

1061). In this study identified tolerant $F_{2:3}$ lines will facilitate the development of adaptable salt tolerant varieties.

Materials and Methods

Parental Material Utilized for the Study: Indra (MTU 1061) salinity tolerant variety at both the seedling and reproductive stage was used as donor. Sri Druthi (MTU 1121) a high yielding, early maturing, leaf blast and BPH tolerant variety with low grain shattering but sensitive to salinity was used as recipient parent. Crossing was taken with Indra as male and Sri Druthi as female in Rabi 2017-18. In kharif 2018 F_1s were selfed to generate F_2 population. The evaluation of $F_{2:3}$ for seedling salinity tolerance was taken up during Rabi 2018-19 and Kharif 2019. Phenotyping of $F_{2:3}$ for reproductive salinity tolerance was taken up in Rabi 2019-20.

Phenotypic Evaluation of $F_{2:3}$ Seedlings under Salinity at Seedling Stage: A total of 234 $F_{2:3}$ population along with Indra and Sri Druthi were screened for salinity tolerance at seedling stage using hydroponics (Gregorio *et al*., 1997) [4]. All $F_{2:3}$ lines were germinated and were transferred to Yoshida *et al*. (1976) [20] nutrient solution. When the seedlings were at two leaf stage, salt stress of EC= 6dSm^{-1} was imposed by adding NaCl to nutrient solution. After eight days of initial salinization, the stress was increased to 12 dSm^{-1} (Fig.1). Scoring for SES was recorded as per IRRI 1997 [7]. The score for salt injury was noted at 16 days after initial stress. The score 1 indicated high tolerance while score 9 indicated high susceptibility. Root length and shoot length were measured after final scoring. After screening the shoots were oven dried at 60 °C for 3 days, and the dry weights were measured. For estimating the concentrations of Na^+ and K^+ in the shoot, plant sample was powdered and one gram was kept in 150 ml Erlenmeyer flask and digestion was done using diacid mixture (HNO_3 and $HClO_4$ in 9:4 ratio) at 50-55 °C heating block. The final Na^+ and K^+ concentrations in the shoots were estimated by flame photometer.

Phenotypic Evaluation of the Population under salt stress at Reproductive Stage: The 234 $F_{2:3}$ lines and the parents Indra (tolerant) and Sri Druthi (sensitive) were evaluated as suggested by Gregorio *et al.* (1997) [4]. Pre-germinated seeds were placed on the soil surface of plastic pot containing fertilized soil (50N, 25P and 25K mg kg^{-1}) and plastic pots were placed in tray filled with tap water. The water level in the plastic tray was maintained to the soil level in the pots. Six plants with proper labeling were placed in pot and thinned to three plants per pot in tillering stage. All $F_{2:3}$ individuals were maintained under control conditions (EC 0 dS/m) until the flag leaf appeared. Salt stress was imposed at the same growth stage to all $F_{2:3}$ population. NaCl was dissolved into the water in the tray so that electrical conductivity reaches 6 dSm^{-1} at booting stage and the salt stress was increased to 12 dSm^{-1} at blooming stage (Fig. 2). Data on plant height at maturity (cm), days to flowering, panicle length (cm), number of total grains per panicle, number of filled grains per panicle, spikelet fertility (%), grain yield per plant (g) and number of productive tillers per plant were recorded for each plant at appropriate stage.

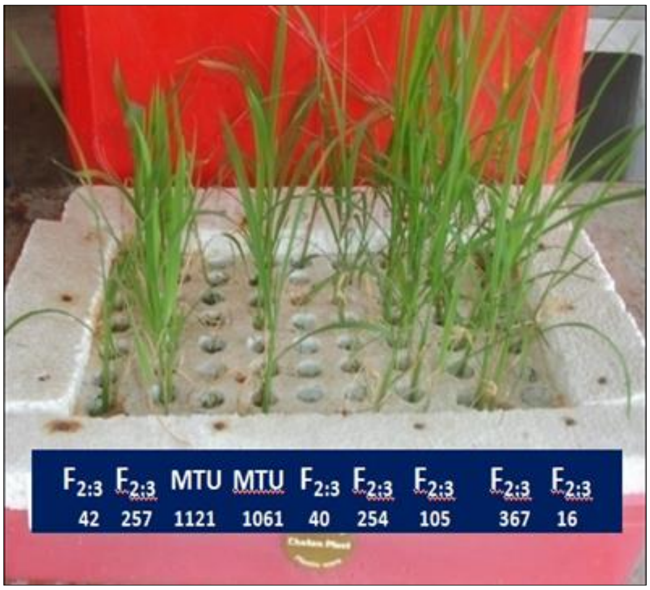

Fig 1: Final Scoring at 16 days after Salinization

Fig 2: Harvesting stage exposing to Salinization (EC-12dSm^{-1})

Results and Discussion

Correlation among different tolerance traits at Seedling Stage: The correlation coefficient matrix (Table 1) revealed salt injury score (SIS) had significant and positive association with shoot Na^+ concentration (0.576**), NaK ratio (0.860**) while it had negative significant correlation with K^+ concentration (-0.574**), shoot length (-0.670**), root length (-0.584**) and shoot dry weight (-0.598**). This results were in accordance with Venkata Ramana Rao *et al*. (2018) [19] who evaluated 112 ILs of Nona Bokra / Cheniere at the seedling stage. Positive and significant correlation was observed between shoot Na^+ concentration and shoot K^+ concentration (0.195**) and NaK ratio (0.634**) whereas it was negative and significant between shoot length (-0.425**), root length (-0.410**) and shoot dry weight (-0.309**). This results were in contrary with Batayeva *et al*. (2018) [2] who screened 27 accessions under salinity stress (EC @ 12 dSm^{-1}) at the seedling stage. The shoot K^+ concentration showed positive association with shoot length (0.437**), root length (0.321**), shoot dry weight (0.389**) and negative significant correlation with NaK ratio (-0.601**). The results were in confirmation with Venkata Ramana Rao *et al.* (2017 & 2018) [18, 19] and Naveed *et al*. (2018) [15]. The correlation between Na^+ and K^+ in shoot confirms that the tolerance to salt stress is governed by the homeostasis between Na^+ and K^+ rather than the concentration perse. The NaK ratio recorded negative and significant association with shoot length (-0.696**), root length (-0.586**), shoot dry weight (-0.546**). These results are in agreement with Teresa *et al.* (2015) [16] and Ahmadi *et al.* (2011) [1]. The association between shoot length and root length (0.704**), shoot length and shoot dry weight (0.657**) were positive and significant. Similar findings were reported by Venkata Ramana Rao *et al*. (2018) [19] and Bizimana *et al*. (2017) [3]. The root length was positively correlated with shoot dry weight (0.438**).

Table 1: Pearson Correlation of morph physiological traits in $F_{2:3}$ lines under salt stress (EC = 12 dSm^{-1}) at seedling stage

	SIS	Na^+	K^+	NaK	SHL	RTL	DWT
SIS	1						
Na^+	0.576**	1					
K^+	-0.574**	0.195**	1				
NaK	0.860**	0.634**	-0.601**	1			
SHL	-0.670**	-0.425**	0.437**	-0.696**	1		
RTL	-0.584**	-0.410**	0.321**	-0.586**	0.704**	1	
DWT	-0.598**	-0.309**	0.389**	-0.546**	0.657**	0.438**	1

SIS, salt injury score; Na^+, shoot sodium concentration; K^+, shoot potassium concentration; NaK, ratio of the shoot sodium and shoot potassium concentration; SHL, shoot length; RTL, root length; DWT, shoot dry weight.
** Significant at 0.05 (two tail)

Correlation among different traits at Reproductive stage: The Pearson correlation coefficients (Table 2) revealed that the plant height showed negative correlation with panicle length (-0.070) and productive tillers per plant (-0.022). Days to flowering had positive significant association with number of filled grains per panicle (0.414**), number of total grains per panicle (0.600**), spikelet fertility (0.486**), grain yield per plant (0.412**) and productive tillers per plant (0.593**) while it was negative significant with panicle length (-0.582**). Similarly Tiwari *et al*. (2016) [17] also reported the findings which are in accordance with present study. Panicle length recorded negative and significant association with number of total grains per panicle (-0.442**), spikelet fertility (-0.222**), grain yield per plant (-0.434**) and productive tillers per plant (-0.511**). These results were in contrary with the findings of Hakim *et al*. (2013) [5] who screened five rice varieties under different salinity levels at reproductive stage. The number of filled grains $panicle^{-1}$ had significant and positive correlation with number of total grains per panicle (0.325**), spikelet fertility (0.412**) and productive tillers per plant (0.306**). The results were in confirmation with Hakim *et al.* (2013) [5], Hossen *et al.* (2017) [6] and Mondal *et al.* (2019) [14]. The number of total grains per panicle was positively correlation with spikelet fertility (0.622**), grain yield per plant (0.777**) and productive tillers per plant (0.832**). The findings are in conformity with the reports of Mohammadi *et al*. (2013) [11]. The spikelet fertility is positive and significantly associated with productive tillers $plant^{-1}$ (0.478**). Similar findings were reported by Mondal *et al.* (2019) [14] who evaluated 92 F_2 progenies of NSIC Rc222 / BRRI dhan 47 at reproductive stage under different salinity stress (EC @ 6, 8 &10 dSm^{-1}). The grain yield per plant had positive significant association with productive tillers per plant (0.692**). These results are in conformity with the findings of Mondal *et al.* (2019) [14], Krishnamurthy *et al.* (2016) [9] and contrary with Mansuri *et al*. (2012) [10].

Table 2: Pearson Correlation matrix of yield components of $F_{2:3}$ population under salt stress (EC @ 12 dSm^{-1}) at the reproductive stage

	Plant height	Days to flowering	Panicle length	Number of filled grains $panicle^{-1}$	Number of total grains $panicle^{-1}$	Spikelet fertility (%)	Grain yield/plant (g)	Productive tillers $plant^{-1}$
PHT	1							
DFL	0.036	1						
PNL	-0.070	-0.582**	1					
NFG/Pn	-0.009	0.414**	-0.070	1				
NTG/Pn	0.030	0.600**	-0.442**	0.325**	1			
SPF (%)	0.002	0.486**	-0.222**	0.412**	0.622**	1		
GY/Pl (g)	0.054	0.412**	-0.434**	0.094	0.777**	0.032	1	
PT/Pl	-0.022	0.593**	-0.511**	0.306**	0.832**	0.478**	0.692**	1

PHT, plant height; DFL, days to flowering; PNL, panicle length; NFG/Pn, number of filled grains $panicle^{-1}$, NTG/Pn, number of total grains $panicle^{-1}$, SPF, spikelet fertility, GY/Pl, grain yield/plant; PT/Pl, Productive tillers $plant^{-1}$.
**Correlation is significant at the 0.01 level (2-tailed).

Conclusion

We observed that at seedling stage, the $F_{2:3}$ lines had high Na^+ and high K^+ concentration in the shoot suggesting that homeostasis between Na^+ and K^+ plays a key role in the seedling stage tolerance to salt stress. In reproductive stage grain yield per plant play pivotal role for salinity tolerance comparative to remaining parameters such as number of spikelets, filled spikelets and unfilled spikelets. It can be concluded from the study that tolerance at the seedling stage is not necessarily associated with tolerance at the reproductive stage and vice versa.

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