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Modified Standard Evaluation System: Is it Appropriate for Evaluation of Salinity Tolerance at the Reproductive Stage of Rice?

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Salinity is the most common and extensive soil problem in coastal rice production environments. Tolerance at the reproductive stage is the most crucial as it determines grain yield. An F_2 mapping population was developed from two rice genotypes contrasting in tolerance: NSIC Rc222 (sensitive) and BRRI dhan47 (tolerant). Salt stress injury scoring was performed to evaluate the performance of every F_2 individuals. In this experiment, no significant influence was established on salinity score on the productive and unproductive tillers per plant, number of filled, unfilled and total spikelets and yield. Correlation analysis between grain yield and Standard Evaluation System (SES) score was positive, which was unexpected as in general yield will be decreased with increasing salinity score. Therefore, SES scoring is not the ultimate indicator for concluding a variety either tolerant or sensitive.

(***Key words:*** *Salinity, Rice, Reproductive stage, Standard evaluation score*)

Rice is a diploid (2n = 2x = 24) glycophyte, of tropical origin, and is currently the model crop cereals (Jenkins *et al.,* 2008). It's relatively simple and completely sequenced genome and easy genetic manipulation, has made it a good model system for both investigations in genetics and functional genomics (Yu *et al.,* 2002 and Matsumoto, 2005). Nearly half of the world population depends on rice. About 90% of the rice in the world is grown in Asia and 85% is devoted for human consumption (IRRI, 1997). Although rice is an aquatic plant, it can be grown under a wide range of agro-climatic conditions ranging from favorable to various biotic and abiotic stresses, and even in upland conditions. Various abiotic stresses greatly affect rice yield and among the abiotic stresses, salinity is the second most prevalent soil problem in rice growing countries of the world and is considered as a serious threat to increased rice production worldwide (Gregorio, 1997). It has become increasingly important because more than 50% of the crop yield is negatively affected by abiotic stresses (Vij and Tyagi, 2007), with salinity causing a major loss (Chinnusamy *et al.,* 2005). More than 54 million ha of rice land in Asia are affected by salinity (Gregorio *et al.,* 2002). For this reason, millions of hectares in the humid regions of South and Southeast Asia are technically suited for rice production but are left uncultivated or are grown with very low yields because of salinity and problem soils. Salinity tolerance at the seedling and reproductive stages is only weakly associated (Mishra *et al.,* 1990; Moradi *et al.,* 2003); suggesting that tolerance at these two stages is regulated by different sets of genes (Hossain *et al.*, 2015). The reproductive stage is crucial as it ultimately determines grain yield. Conventional breeding was used to develop stress tolerance high yielding varieties of rice. But plant selection for salt tolerance in this method is not easy because of the large environmental effects and low heritability of salt tolerance (Gregorio and Senadhira, 1993; Gregorio *et al.,* 2002).

Standard Evaluation System (SES) scoring is one of the indicators of measuring salinity tolerance in seedling and reproductive stages of rice plant. But it may not be the ultimate stress indicator, rather yield is the ultimate indicator of rice. Therefore, the experiment was conducted to phenotype the population using various parameters attributing to reproductive stage salinity tolerance and to determine whether SES score is the eventual parameter for concluding a population either tolerant or sensitive to salinity at reproduction stage of rice.

MATERIALS AND METHODS

The experiment was conducted at the artificially salinized field of the International Rice Research Institute (IRRI) in 2013. A salt-tolerant Bangladeshi

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variety, BRRI dhan47 (IR63307-4B-4-3) and a salt-sensitive variety, NSIC Rc222 which was originated from the Philippines were used as parents to develop the mapping population. The cross was developed at IRRI's crossing program and F_1's were generated to produce the F_2 progenies. Among them, 92 F_2 progenies were identified randomly and used as mapping population. Field screening was conducted in artificially salinized concrete field, located in the IRRI experimental station; which was similar to field conditions minus the soil heterogeneity. The seeds of the F_2 plants including their parents were sown in the germination tray and after 21 days, seedlings were transplanted in the artificially salinized concrete field. The F_2 progenies and parental checks (NSIC Rc222 and BRRI dhan47) were planted in 3 m x 5 m plots with one seedling per hill with the spacing of 20 cm x 20 cm. The initial salinity (EC) of 6 to 8 dS m^{-1} was imposed during the vegetative stage and it was increased to EC 10 dS m^{-1} at panicle initiation stage. Salt stress of EC 10 dS m^{-1} was maintained until maturity. The soil was fertilized with the recommended NPK only (50N, 25P and 25K kg ha^{-1}). Other intercultural operations like weeding was done during the experiment and some marigold plants were grown around the block to protect the plants from insects as it works as a natural insect repellent. EC level was checked every alternate day with a portable EC meter for the maintenance of salinity level. Saline/tap water was added to maintain desired EC (6-10 dS m^{-1}) when necessary.

Data on yield, yield components and important agronomic parameters were collected from reproductive stage screening. Plant height, number of productive and unproductive tillers, fresh weight of plants, number of filled and unfilled spikelet and grain yield were collected at maturity. Plant height was measured from soil surface to the tip of the tallest panicle (awns excluded) at the time of harvest. Salinity scoring was done to identify the tolerant and sensitive plants based on visual symptoms by using Modified Standard Evaluation System (Table 1) of IRRI (IRRI, 2013).

RESULTS AND DISCUSSIONS

Phenotypic experiment was conducted in order to determine the response of the 92 F_2 individuals of NSIC Rc222 and BRRI dhan47 to document the effect of salinity stress of EC 10 dS m^{-1} at reproductive stage of rice under artificially salinized field condition. The phenotypes of the NSIC Rc222 (sensitive parent) and BRRI dhan47 (tolerant parent) and their F_2 progenies salinized with EC 10 dS m^{-1} showed significantly different responses at reproductive stage. The effects of 10 dS m^{-1} salinity on different agronomic parameters are discussed below:

Plant height

Plant height of the sensitive (NSIC Rc222) and tolerant (BRRI dhan47) parents was 74 cm and 98 cm, respectively, and that of their progenies varied from 66 to 106 cm. Some F_2 progenies had higher plant height than the tolerant parent under saline stress. The distribution is negatively skewed (-0.712), so the population is highly affected by salinity stress, i.e. salinity caused significant reduction in the plant height of the F_2 population (Mondal, 2014). Out of 92 F_2 progenies, 25 individuals had plant height between 81-90 cm. The plant height of two individuals (F_2) was less than that of the sensitive parent and 25 individuals were greater than the tolerant parent. Hence, 2.17% of the total population had plant height less than the sensitive parent (NSIC Rc222), while 27.1% of the progenies were taller than the tolerant parent (BRRI dhan47), suggesting transgressive segregation in both directions for this trait. The salinity score of sensitive and tolerant parents was 3 and 9, respectively; while that of the 92 F_2 individuals varied from 4 to 9. Among the individuals, 50% was highly sensitive to salinity (score 9). Average plant height of 92 F_2 progenies decrease with the increased level of salinity (Fig. 1) and can be expressed by the equation:

$$Y = 103.14 - 1.53\ (X) \qquad R^2 = 0.905$$

where Y = Plant height (cm)
X = Salinity score (eg. 1, 3, 5…., 9)

Table 1. *Modified Standard Evaluation System (SES) for scoring of visual salt injury at seedling and reproductive stages in rice (IRRI 2013)*

Score	Observation	Rating
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leaves are rolled; few elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Sensitive
9	Almost all plants dead or dying	Highly sensitive

Tillers per plant

Total tillers per plant of the progenies decreases with increasing salinity score (Fig. 2). But there is no significant influence on salinity score on the productive and unproductive tillers per plant. These two traits did not show any trend with the salinity score.

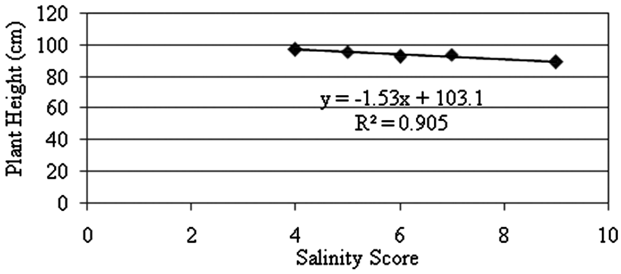

***Fig. 1.** Effect of salinity on plant height of 92 F_2 progenies of BRRI dhan47 and NSIC Rc222*

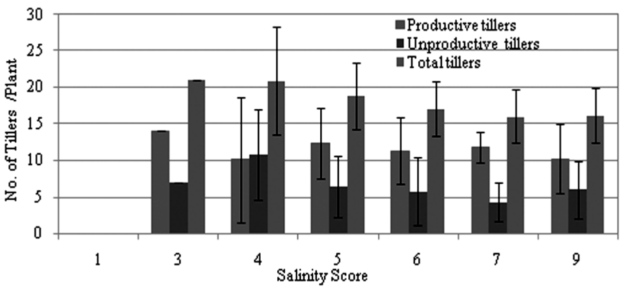

***Fig. 2.** Variations in productive, unproductive and total tillers per plant under different salinity scores in the reproductive stage (vertical and capped bar indicated standard deviation)*

The number of productive tillers for NSIC Rc222 and BRRI dhan47 was 10 and 14 per plant, respectively and their F_2 population ranged from 0 to 24 per plant, which followed the normal distribution pattern (Mondal, 2014). Out of 92 F_2 progenies, 32.6% of the population had less number of productive tillers per plant than the sensitive parent, while 21.7% of the progenies had more productive tillers per plant than tolerant parent, suggesting transgressive segregation in both directions for this trait.

The number of unproductive tillers for NSIC Rc222 and BRRI dhan47 was 9 and 7 per plant, respectively and their F_2 population varied from 0 to 19 per plant, which followed positively skewed distribution (Mondal, 2014). Among the 92 F_2 progenies, 53.2% of the total population had less number of unproductive tillers per plant than the tolerant parent and 22.8% had more number of unproductive tillers per plant than the sensitive parent, suggesting transgressive segregation in both directions for this trait.

The number of total tillers for sensitive and tolerant parent was 19 and 21 per plant, respectively and that of their progenies varied from 7 to 34, which showed positively skewed distribution (Mondal, 2014). Of the 92 progenies, about 67% of the 92 F_2 population had less number of total tillers per plant than sensitive parent and 18% had higher tillers than the tolerant parent, suggesting transgressive segregation in both directions for this trait.

Spikelets per plant

Total spikelets per plant of the 92 F_2 progenies varied from 40 to 2152, average of which ranged within 900.1 to 1026.2 per plant across all the salinity levels (Fig. 3). Similarly, the number of filled (175.3-252.5) and unfilled (684.4-773.7) spikelets varied across the salinity level.

In case of percent filled spikelet, the tolerant (score 3) and sensitive (score 9) parents showed normal trend but no significant changes were observed among the F_2 progenies (Fig. 4). The percentages of filled

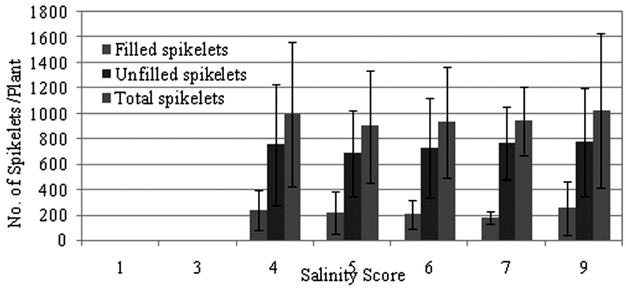

***Fig. 3.** Variations in filled, unfilled and total spikelets per plant under different salinity scores in the reproductive stage (vertical and capped bar indicated standard deviation)*

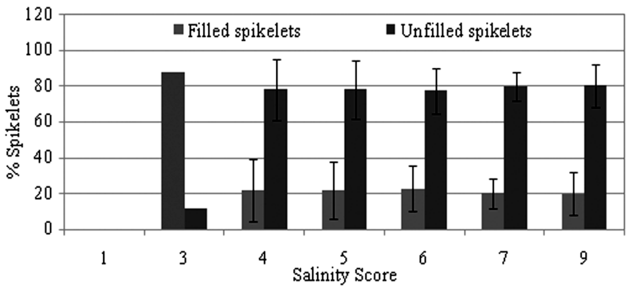

***Fig. 4.** Variations in the percentages of filled and unfilled spikelets under different salinity scores in the reproductive stage (vertical and capped bar indicated standard deviation)*

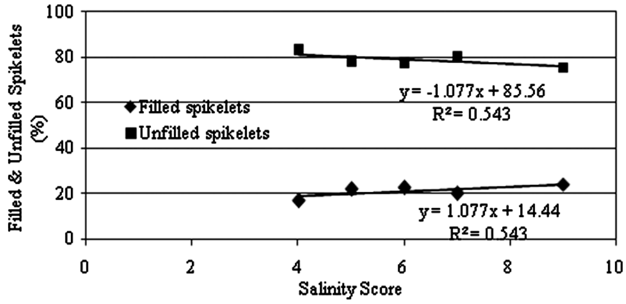

***Fig. 5.** Relations between percent filled and unfilled spikelets with different salinity scores in the reproductive stage*

spikelet in the sensitive and tolerant parent were 8.2% and 88.0%, respectively while the filled spikelets of the F_2 population ranged from to 0 to 88.0%. Eighteen individuals showed less percentage of filled spikelet than the sensitive parent, but none of the progenies exhibited higher percentage than the tolerant parent. But still, the population was positively skewed and very near to normal distribution because 54.25% of the total population had 20-60 percent filled spikelets, suggested transgressive segregation in one direction only (Mondal, 2014).

A significant range (0-100%) was observed in the F_2 individuals for percent unfilled spikelet. Out of 92 F_2 progenies, 52 individuals showed 72% to 96% unfilled spikelet. The distribution is negatively skewed (Mondal, 2014). Four individuals had less percentages of unfilled spikelet than the tolerant parent, while 14 progenies showed higher percentages than the sensitive parent suggesting transgressive segregation in both directions.

The study reveled that there is no relation between the total spikets per plant across the salinity level of the 92 F_2 progenies (Fig. 3). Rather percent filled spikelet showed increasing trend and unfilled spikelet showed declined trend with the increasing level of salinity score (Figs. 4 and 5). Therefore, modified standard evaluation systems (SES) scoring is not an ultimate indicator of concluding salinity stress at reproductive stage of rice.

Yield per plant

Average yield of the 92 F_2 individuals and their parents across the salinity level is shown in Fig. 6. The yields of susceptible (NSIC Rc222) and tolerant (BRRI dhan47) parents were 6.2 g plant^{-1} and 14.5 g plant^{-1}, respectively while that of the F_2 population ranged from 0 to 14.4 g plant^{-1}. The distribution is positively skewed (Mondal, 2014). Out of 92 F_2 progenies, 82 individuals had yield in the range of 0 to 8 g plant^{-1}. The yield of 66 individuals was less than the value of the sensitive parent and no individual had higher yield than the tolerant parent. In this study, 71.7% of the total population exhibited lower yield than sensitive parent (6.2 g plant^{-1}) suggesting transgressive segregation in one direction for this trait (Mondal, 2014). Generally, rice yield will decrease with increasing salinity score, but the average yield of the progenies were observed around 4 g plant^{-1}across the salinity score of 4 to 9. Therefore, the modified SES salinity scoring may not always be used as a stress indicator at reproductive stage of rice.

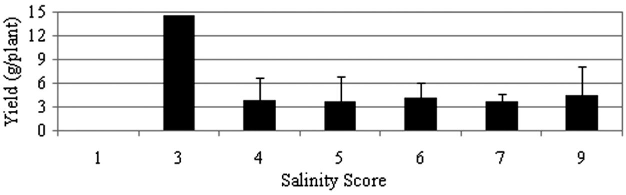

Fig. 6. *Variations in yield of rice under different salinity scores in the reproductive stage (vertical and capped bar indicated standard deviation)*

Correlation analysis of yield and agronomic components

Correlation coefficient analysis was performed in order to determine relationship between grain yield and yield components under salt stress condition. Positive and significant correlations were observed with grain yield to the number of filled spikelets (0.979), percent filled spikelet (0.731), total spikelets (0.846), number of productive tillers (0.669), percent productive tiller (0.589), and total tillers (0.336) (Table 2). It was observed that the number of unfilled spikelets was positive and significantly correlated with grain yield (0.697), which was unexpected. The possible reason behind such response may be the tendency of producing excess sterile spikelets by the tolerant genotype (BRRI dhan47). On the other hand, percent unproductive filled spikelet, percent unproductive tiller, number of unproductive tillers showed negative and significant correlation with grain yield (Table 2). The correlation between grain yield and SES score was positive but not significant. This may prove that only SES score may not always be reliable parameter to measure salinity tolerance in rice. Therefore, yield is the ultimate stress indicator as well as some important yield components like number and percentages of filled spikelets, total spikelets, number and percentage of productive tillers and total tillers per plant.

CONCLUSIONS AND RECOMMENDATION

The phenotypes of the sensitive and tolerant parents and their F_2 progenies salinized with EC 10 dS m^{-1} showed significantly different responses at reproductive stage as every genotype is genetically different in the F_2 mapping population. The agronomic and yield traits evaluated were plant height, number of productive tillers, number of unproductive tillers, total tillers, number of filled spikelets, number of unfilled spikelets, total spikelets, percent filler spikelet, percent unfilled spikelet and yield.

Salinity stress injury scoring (SES) was performed to evaluate the performance of every F_2 individuals, but not considered as a reliable parameter to conclude the

Table 2.*Correlation coefficients for grain yield under stress and yield components using the 92 individuals (F_2) of NSIC Rc222/BRRI dhan47 under salt stress of 10 dS m^{-1}.*

	DAM	PLH	PT	UPT	TT	PPT	PUPT	FW	FS	UFS	TS	PFS	PUFS	YLD
Salscr	0.123	-0.386**	-0.16	-0.193	-0.318**	0.048	-0.048	-0.638**	0.132	0.136	0.145	-0.016	0.157	0.109
DAM		-0.004	-0.216*	0.196	-0.041	-0.232*	0.232*	0.083	-0.121	-0.166	-0.164	-0.155	0.13	-0.126
PLH			0.460**	-0.123	0.388**	0.325**	-0.325**	0.584**	0.396**	0.434**	0.455**	0.288**	-0.066	0.420**
PT				-0.517**	0.611**	0.809**	-0.809**	0.380**	0.662**	0.751**	0.782**	0.519**	0.015	0.669**
UPT					0.357**	-0.895**	0.895**	0.221*	-0.445**	-0.457**	-0.489**	-0.445**	-0.188	-0.425**
TT						0.048	-0.048	0.650**	0.316**	0.406**	0.408**	0.166	-0.072	0.336**
PPT							-1	0.026	0.600**	0.672**	0.701**	0.557**	0.155	0.589**
PUPT								-0.026	-0.600**	-0.672**	-0.701**	-0.557**	-0.155	-0.589**
FW									0.146	0.197	0.196	0.053	-0.026	0.197
FS										0.670**	0.832**	0.779**	-0.252*	0.979**
UFS											0.969**	0.298**	0.173	0.697**
TS												0.481**	0.045	0.846**
PFS													-0.342**	0.731**
PUFS														-0.285**
YLD														

* Significant at $P < 0.05$
** Significant at $P < 0.01$

Salscr = Salinity Score
DAM = Damage (%)
PLH = Plant height
PT = Productive tillers (no)
UPT = Unproductive tillers (no)
TT = Total tiller (no)
PPT = Percent productive tiller
PUPT = Percent unproductive tiller
FW = Fresh weight
FS = Filled spikelet (no)
UFS = Unfilled spikelet (no)
TS = Total spikelets
PFS = Percent filled spikelet
PUFS = Percent unfilled spikelet
YLD = Yield

tolerance level of population. In this study, no significant influence was established on salinity score on the productive and unproductive tillers per plant. They did not maintain any trend with the salinity stress injury score. Besides, number of filled, unfilled and total spikelets also followed the same trend as that of productive and unproductive tillers. Although yield is the ultimate parameter for reproductive stage, but after comparing with the score it did not exhibit any direct correlation. The study suggests that current injury score (SES) is not the stress indicator at reproductive stage of rice. Therefore, new SES scoring should be developed for salinity screening at reproductive stage of rice.

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