
Identification of best segregating family of NSIC Rc222/jumbo jet under salt stress at reproductive stage for use as a mapping population

Ordonio, J. L.^{1*}, Vergara, G. V.² and Gregorio, G. B.³

¹Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines; ²Plant Breeding Division, International Rice Research Institute, Los Baños, Laguna, Philippines; ³University of the Philippines Los Baños, College, Laguna, Philippines.

Ordonio, J. L., Vergara, G. V. and Gregorio, G. B. (2018). Identification of best segregating family of NSIC Rc222/jumbo jet under salt stress at reproductive stage for use as a mapping population. *International Journal of Agricultural Technology* 14(7): 1561-1574.

Abstract From the cross between the salt-tolerant rice landrace Jumbo Jet and the high yielding salt-sensitive variety NSIC Rc222, ten BC₁F₂ families were grown and evaluated for reproductive stage salt tolerance at International Rice Research Institute. Salt was applied to achieve an electrical conductivity of 6 dS/m during panicle initiation stage (60 days after sowing). Results of the salinity evaluation score showed wide variation in phenotypes among the ten BC₁F₂ families ranging from 3 to 8. The most saline tolerant families were T109284 (BC₁F₂-JJ10-P2) and T109285 (BC₁F₂-JJ10-P3) with salinity evaluation scores of 4 and 3, respectively, while the most sensitive to salt stress were T109286 (BC₁F₂-JJ10-P2), T109288 (BC₁F₂-JJ10-P2) and T109289 (BC₁F₂-JJ10-P2) scoring 8. Clear segregation pattern was observed in four families (T109282 (BC₁F₂-JJ04-P1), T109287 (BC₁F₂-JJ15-P3), T109290 (BC₁F₂-JJ29-P1) and T109291 (BC₁F₂-JJ29-P2)), showing susceptible and tolerant plants within the family. However, segregation pattern was most pronounced in T109290 (BC₁F₂-JJ29-P1), making it the best segregating family suited for use as a mapping population. The following traits were also evaluated at the reproductive stage: days to flowering, plant height, number of panicles, number of fertile spikelets, number of sterile spikelets, spikelet fertility, 100-grain weight and grain yield. The analysis of variance revealed significant differences between parents and among ten BC₁F₂ families for almost all traits except for the number of panicles and grain yield. Results also showed that among the eight parameters evaluated, grain yield was the most severely affected, and the reduction of number of fertile spikelets and spikelet fertility are the primary causes of yield loss. Based on correlation analysis, number of fertile spikelets and spikelet fertility have strong positive correlation with grain yield, hence, these traits are the most important traits contributing to grain yield of rice.

Keywords: Jumbo Jet, Mapping population, Rice, Salinity

Introduction

Development of high-yielding salt-tolerant rice varieties is being done to expand agriculture into the regions affected by salinity. Breeders' approach to

*Corresponding Author: Ordonio, J. L.; Email: jlordonio@clsu.edu.ph

this in the past is through conventional methods where significant progress has been made. However, such progress is not satisfactory in view of the current demand to increase crop productivity in salt-affected regions (Flowers, 2004). Conventional breeding has many limitations because salt tolerance is a complex trait controlled by many genes which is determined by a number of physiological and biochemical traits (Ashraf, 2009). The influence of environmental factors adds to the complexity which makes it difficult to select salt-tolerant cultivars. Furthermore, since salt tolerance varies depending on the growth stage, the reliability and efficiency of conventional breeding programs in selecting for salt-tolerant genotypes is often compromised.

The use of molecular markers that are tightly linked to traits related to salinity tolerance can speed up the development of salt-tolerant rice varieties. This can be done through the process of detecting tight linkage of a molecular marker to a gene of interest known as QTL mapping (Collard *et al.*, 2005). The identified QTL associated with the symptoms of salinity could improve the selection of salt-tolerant plants. Plants with the desirable traits can be selected in a segregating population in early generation based on their genotype rather than their phenotypes. The possibility of developing a variety in conventional breeding is more than 10 years while with the use of molecular markers, breeding period could be shortened.

Prior to QTL mapping or constructing linkage map, creation of an appropriate mapping population should be done. Most commonly, mapping population is derived from the crossing of two genetically divergent parents that show marked differences (polymorphism) for one or more traits of interest. However, parents should not be too genetically distant that they will not be able to cross or produce sterile progenies (Mohanrao and Deepak, 2015). Usually, a mapping population consists of 100 to 300 segregating lines or individuals, each of which is evaluated both for phenotypic traits and for molecular markers. However, larger population size is recommended when resources are available (Beavis, 1998).

There are several types of mapping populations used in QTL mapping and are usually obtained from controlled crosses (Mohanrao and Deepak, 2015). F₂ and Backcross (BC) populations are the most commonly used in molecular mapping studies. They are simple, easy to construct and require only a short period of time to be produced. An F₂ population is developed either by selfing or intermating the F₁ individuals while the BC population is developed by crossing an F₁ back to one of its parents. The heterozygous genetic makeup of these two populations makes them a good population for mapping quantitative traits.

Once the mapping population has been decided and selected, phenotyping of individual plants or measurement of important quantitative traits commences. The accuracy and precision of phenotyping determines how realistic the QTL mapping results are (Rahaman *et al.*, 2015). Also, each individual of the mapping population are genotyped using genetic markers. The next step is to perform statistical analysis that detects an association between the markers and the phenotype to identify the loci that are affecting the traits of interest (Collard *et al.*, 2005). It ranges from simple techniques, such as analysis of variance (ANOVA), to models that include multiple markers and interactions (Doerge, 2002).

Objectives: To evaluate the ten families for salinity tolerance at reproductive stage, characterize the agronomic traits and select mapping population.

Materials and methods

Plant Materials

A local landrace (Jumbo Jet) that shows high tolerance to salinity during reproductive stage and a salt-sensitive high yielding variety (NSIC Rc222) from the Philippines were used in the experiment as parents to produce an F₁ (Fig. 1). The NSIC Rc222 was used as female parent while Jumbo Jet serves as male parent. The F₁ was backcrossed to the female parent to produce the BC₁F₁ which was allowed to self to produce the BC₁F₂. Ten families were generated and evaluated in 2015 Dry Season.

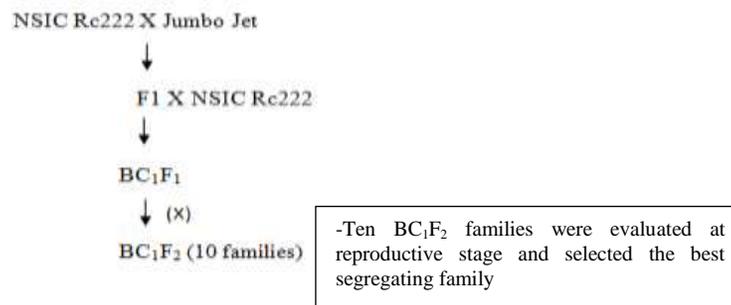


Figure 1. Developmental scheme of BC₁F₂ mapping population derived from NSIC Rc222/ Jumbo Jet genotypes

Reproductive Stage Screening

There were two experimental set-ups, salinized and normal. The salinized experimental set-up was laid out in a randomized complete block design (RCBD) with two replications. On the other hand, the normal experimental set-up was laid out in augmented design. There were two rows of 20 plants each under salinized condition and ten rows of 26 plants each under normal condition. The population of each family under normal condition was larger because this will be used as mapping population. Once the best segregating family was selected under salinized condition, its corresponding family will be genotype under normal condition. Association of the genotype and phenotype will be done on the following generation.

Twenty-one-day-old seedlings in each family were planted with single seedling per hill and a spacing of 20 x 20 cm. Salinization was done two weeks before booting stage at EC 6-8dS m⁻¹ and was maintained until the grains were at the hard dough stage. The salt was applied uniformly between rows after irrigating the field. The EC level was monitored twice a week with the use of a digital data logger (DECAGON EM50) to ensure maintenance of the salinity level. Jumbo Jet, NSIC Rc222 and two check varieties (FL478 and IR29) were included under salinized and normal conditions. FL478 was known to be salt-tolerant while IR29 was known for its susceptibility. Yield and yield components were measured following the Standard Evaluation System (SES) developed at IRRI. Salt tolerance score was recorded at 100 days after transplanting. The modified Standard Evaluation System (SES) of IRRI was used in rating the symptoms of salt toxicity, as shown in Table 1.

Table 1. Standard evaluation system (SES) for scoring of visual salt injury at seedling and reproductive stages in rice

SCORE	OBSERVATION	TOLERANCE
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

Data Analysis

The frequency distribution of the ten BC₁F₂ families (30 individual plants each) for traits associated with salinity tolerance at reproductive stage were

analyzed using Microsoft excel 2010. The analysis of variance was done using SAS Statistical Analysis Version 9.1.3. Also, Pearson's correlation was performed to determine the relationship of SES score to different parameters measured.

Results

Phenotypic Variation among Ten BC₁F₂ families of NSIC Rc222/Jumbo Jet Under Salt Stress (6 dS m⁻¹) at Reproductive Stage

The frequency distribution showed that the two parents were extremely diverse for salt injury scores with Jumbo Jet scoring 3 and NSIC Rc222 scoring 8 (Fig. 2, Table 2). The SES scores recorded from the ten BC₁F₂ families ranged from 3.0 to 8.0 which were close to normal distribution (Table 2). T109284 (BC₁F₂-JJ10-P2) and T109285 (BC₁F₂-JJ10-P3) exhibited high degrees of salinity tolerance with SES score of 4 and 3, respectively, while the most sensitive to salt stress were T109286 (BC₁F₂-JJ15-P1), T109288 (BC₁F₂-JJ17-P1) and T109289 (BC₁F₂-JJ17-P2) scored 8.

Table 2. Mean values of all traits measured in the ten BC₁F₂ families and their parents (NSIC Rc222 and Jumbo Jet) under salt stress at 6 dS m⁻¹ at reproductive stage

TRAIT	PROGENY VALUES						SKEWNESS
	NSIC Rc222	JUMBO JET	MID-PARENT	Min	Max	MEAN OF PROGENIES	
SES	8.00	3.00	5.50	3.00	8.00	5.70	0.27
DFL	93.16	105.57	99.37	84.48	100.51	91.06	0.73
PTH	88.43	109.57	99.00	88.38	120.15	101.94	0.25
NOP	8.20	6.67	7.43	5.28	11.10	9.06	-1.09
NFS	20.50	21.62	21.06	15.78	90.00	52.41	0.11
NSS	100.35	58.63	79.49	47.93	107.85	75.59	0.12
SF	16.50	22.77	19.64	13.77	62.82	36.01	0.53
HGW	1.14	1.31	1.22	1.13	1.44	1.29	-0.04
GY	0.70	1.86	1.28	0.68	5.46	2.87	0.32

^aSES, Salinity evaluation score; DFL, Days to flowering; PTH, Plant height; NOP, Number of panicles; NFS, Number of fertile spikelets; NSS, Number of sterile spikelets; SF, Spikelet fertility; HGW, 100-grain weight; GY, Grain Yield.

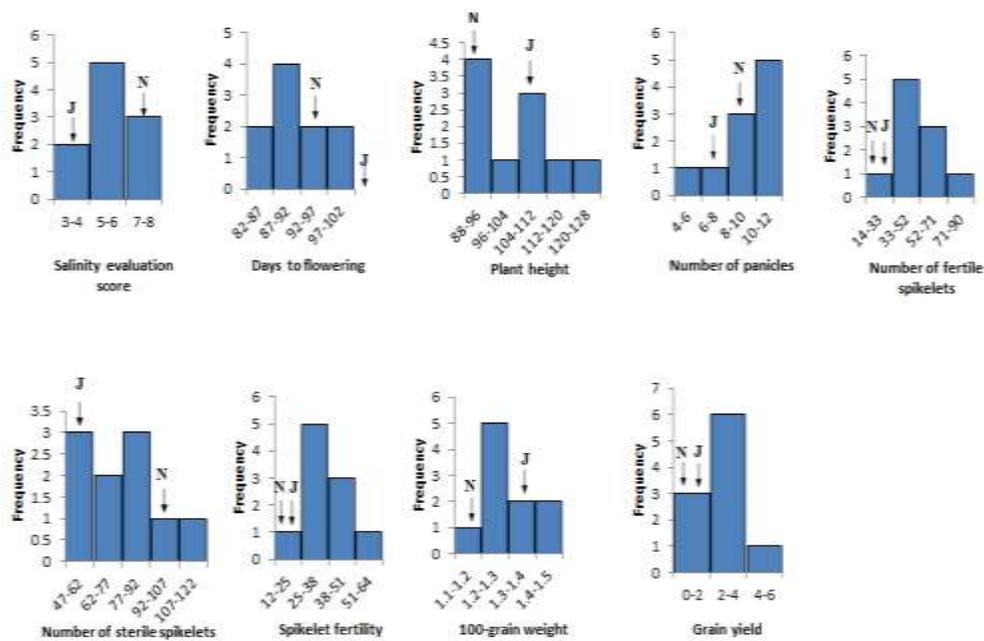


Figure 2. Frequency distribution for yield and yield components of the ten BC₁F₂ families of NSIC Rc222/Jumbo Jet grown under saline condition (N = NSIC Rc222, J = Jumbo Jet)

In addition to salinity evaluation scoring, the following traits were also evaluated at the reproductive stage: days to flowering (DFL), plant height (PTH), number of panicles (NOP), number of fertile spikelets (NFS), number of sterile spikelets (NSS), spikelet fertility (SF), 100-grain weight (HGW) and grain yield (GY). The analysis of variance revealed significant differences between parents and among the ten BC₁F₂ families for almost all traits except for number of panicles and grain yield. The frequency distribution of the ten BC₁F₂ families to the eight morphological traits was approximately normal except for days to flowering and number of panicles which were observed to be skewed towards NSIC Rc222 (Fig. 2, Table 2).

Reduction percentage in the eight morphological traits of ten BC₁F₂ families and their parents under salt stress (EC 6 dS m⁻¹) are presented in Table 3. It can be observed that grain yield (91.85%) was the most severely affected trait. Based on the result of the study, grain yield can be reduced from 78.87% (BC₁F₂-JJ10-P3) to 97.57% (BC₁F₂-JJ17-P2).

Table 3. Average of morphological parameters collected at reproductive stage using ten NSIC Rc222/Jumbo Jet BC₁F₂ families grown under normal and saline conditions

	DFL	PTH	NOP	NFS	NSS	SF	HGW	GY
Normal	92.23	93.39	14.39	128.64	24.60	84.23	2.40	30.72
Saline (EC 6 dS m⁻¹)	92.36	98.98	8.48	44.95	71.66	33.66	1.30	2.50
% reduction	-0.15	-5.99	41.09	65.06	-191.26	60.04	45.63	91.85

aDFL, Days to flowering; PTH, Plant height; NOP, Number of panicles; NFS, Number of fertile spikelets; NSS, Number of sterile spikelets; SF, Spikelet fertility; HGW, 100-grain weight; GY, Grain Yield

Number of fertile spikelets (65.06%) and spikelet fertility (60.04%) were the second and third severely affected traits next to grain yield. Number of fertile spikelets can be reduced from 27.88% (BC₁F₂-JJ10-P3) to 88.31% (BC₁F₂-JJ17-P2) while spikelet fertility can be reduced from 12.16% (BC₁F₂-JJ10-P3) to 83.48% (BC₁F₂-JJ17-P2). The average reduction for number of panicles and 100-grain weight were 41.09% and 45.63%, respectively. These reductions were relatively lower compared to the reduction in grain yield, number of fertile spikelets and spikelet fertility. As expected, substantial increase was observed in the number of sterile spikelets (191.26%) as affected by salinity. Moreover, days to flowering (0.15%) also increased under salt stress. However, the increase in days to flowering was too small to be significant showing consistency of the trait under normal and salinized conditions. Interestingly, plant height increased which was not expected for this trait.

Table 4. Correlation coefficients for grain yield and yield components using ten BC₁F₂ families derived from NSIC Rc222/Jumbo Jet grown under salt stress of 6 dS m⁻¹

TRAIT	DFL	PTH	NOP	NFS	NSS	SF	HGW	GY
aSES	-0.48*	-0.74**	-0.35 ^{ns}	-0.48*	-0.01 ^{ns}	-0.42*	-0.52*	-0.67**
DFL	1	0.26 ^{ns}	-0.30 ^{ns}	-0.14 ^{ns}	-0.50*	0.02 ^{ns}	-0.04 ^{ns}	0.12 ^{ns}
PTH		1	0.42*	0.63*	0.31 ^{ns}	0.35 ^{ns}	0.44*	0.62*
NOP			1	0.53*	0.59*	0.37 ^{ns}	0.18 ^{ns}	0.55*
NFS				1	0.11 ^{ns}	0.88**	0.51*	0.84**
NSS					1	-0.26 ^{ns}	-0.00 ^{ns}	-0.03 ^{ns}
SF						1	0.54*	0.81**
HGW							1	0.52*
GY								1

ns: not significant; * and **: significant at P < 0.05 and P < 0.01, respectively.

aSES, Salinity evaluation score; PTH, Plant height; DFL, Days to flowering; NOP, Number of panicles; NFS, Number of fertile spikelets; NSS, Number of sterile spikelets; SF, Spikelet fertility; HGW, 100-grain weight; GY, Grain Yield.

Correlation between Grain Yield and Yield Components

Linear associations between grain yield and yield components under salinity stress were determined through Pearson's correlation coefficient analysis. Grain yield had strong positive correlation with the number of fertile spikelets and spikelet fertility, and had moderate positive correlation with plant height, number of panicles and 100-grain weight (Table 4). On the other hand, moderate negative correlation with SES was observed. Grain yield did not correlate with days to flowering and number of sterile spikelets.

Selection of Mapping Population

The T109290 (BC₁F₂-JJ29-P1) was selected as the best segregating family among ten families as shown in Figures 3 and 4.

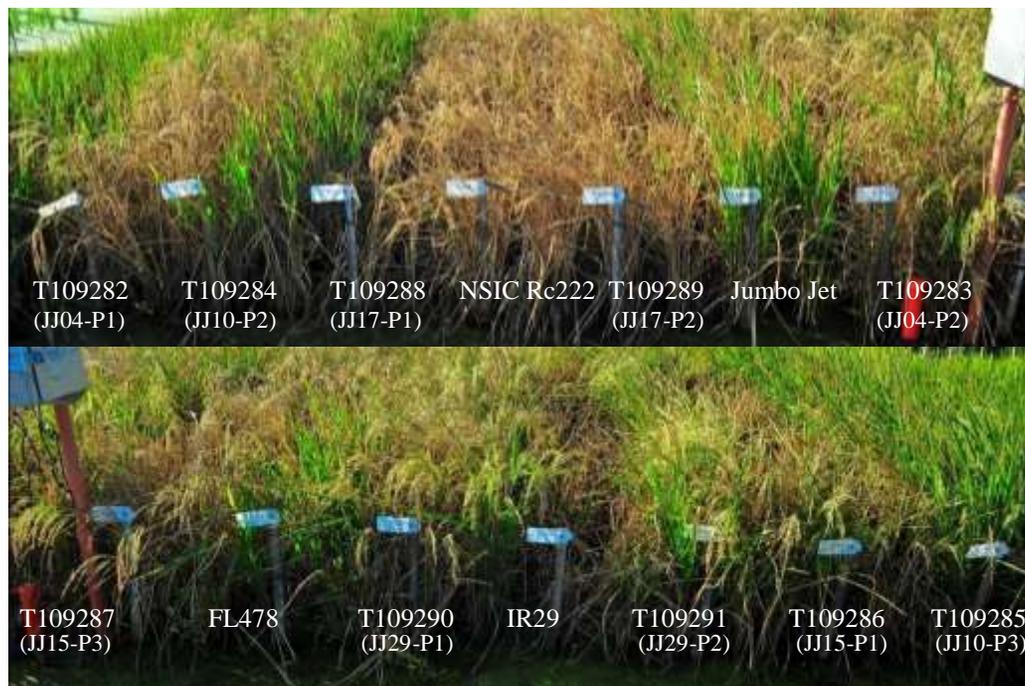


Figure 3. Salinity tolerance screening at reproductive stage using ten BC₁F₂ families of NSIC Rc222/ Jumbo Jet under saline (6 dS/m) condition

The frequency distribution of the 30 lines of T109290 (BC₁F₂-JJ29-P1) for all the traits was approximately normal except for number of sterile spikelets and spikelet fertility which were observed to be moderately skewed to the right (Figure 5). On the other hand, their segregation pattern under salt

stress ranged from 85 to 91 days which were observed to be earlier than their parents. The days to flowering of NSIC Rc222 and Jumbo Jet were 93 and 106 days, respectively.

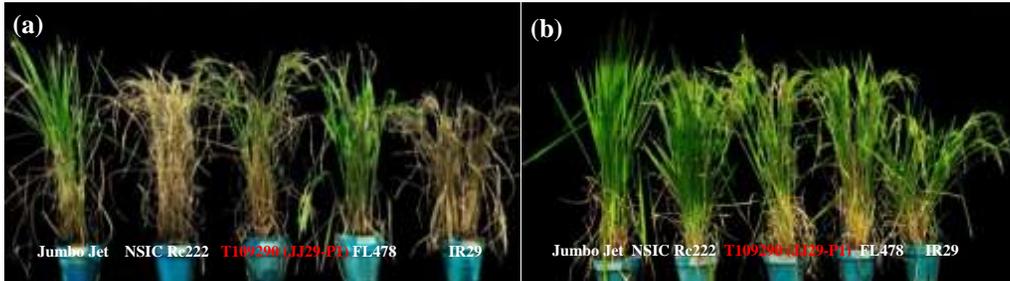


Figure 4. Segregating rice plants within T109290 (BC₁F₂-JJ29-P1) family compared to its parents and checks under (a) salinized and (b) normal conditions

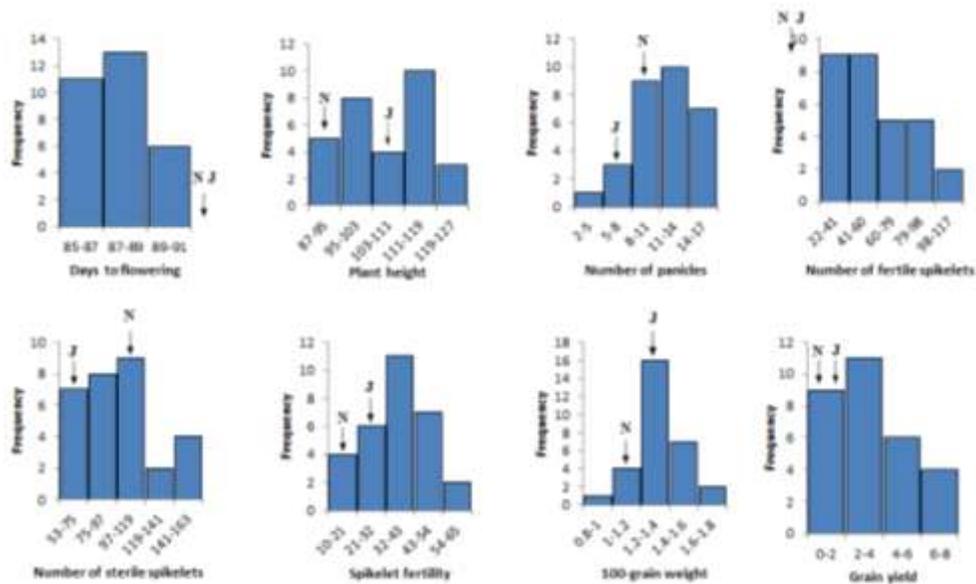


Figure 5. Frequency distribution of yield and yield components among the 30 BC₁F₂-JJ29-P1 plants of NSIC Rc222/Jumbo Jet grown under salinized condition at IRRI, 2015 Dry Season (N = NSIC Rc222, J = Jumbo Jet)

Plant height ranged from 89 to 125 cm and segregated into different phenotypic and genotypic classes. The plant heights of NSIC Rc222 and Jumbo Jet were 88 and 110 cm, respectively. Of the 30 BC₁F₂-JJ29-P1 progenies, five individuals were 87 to 95 cm tall like NSIC Rc222, four plants were around 103 to 111 cm tall similar to Jumbo Jet. Eight individuals were intermediate of

the two parents, ranging from 95 to 103 cm and an example of this is shown in Figure 5. Moreover, 13 individuals were taller than Jumbo Jet.

Number of panicles ranged from 4 to 17. Of the 30 BC₁F₂-JJ29-P1 progenies, one individual had four tillers and three individuals had five to eight tillers, nine plants had eight to eleven tillers, and the remaining 17 individuals had 11–17 tillers. On the other hand, NSIC Rc222 and Jumbo Jet had eight and seven tillers, respectively.

Number of fertile spikelets is an important character known to be highly correlated with grain yield. Number of fertile spikelets in this population ranged from 24 to 115 which were higher than their parents. The number of fertile spikelets of NSIC Rc222 and Jumbo Jet were 21 and 22, respectively.

The lowest number of sterile spikelets ranging from 53-75 was observed in seven individuals which includes Jumbo Jet. On the other hand, nine individuals with number of sterile spikelets ranging from 97-119 just like NSIC Rc222 were observed. Eight individuals were found to be in between the range of the parents. The rest of the individuals got 119 to 163 sterile spikelets which was higher than that of the susceptible parent.

Spikelet fertility is one of the most important indicators of salinity tolerance at the reproductive stage and is also a direct determinant of yield. It ranged from 12 to 64% in the BC₁F₂-JJ29-P1 population. NSIC Rc222 showed 16.5% spikelet fertility and Jumbo Jet displayed 22.8% spikelet fertility under salt stress. Of the 30 BC₁F₂-JJ29-P1 progenies, four individuals showed 12-21% spikelet fertility similar to NSIC Rc222, and six individuals were observed to have 21-32% spikelet fertility similar to Jumbo Jet. Interestingly, 20 individuals (66.7%) were found to have spikelet fertility ranging from 32-65%.

The pattern for 100-grain weight which ranged from 0.8 to 1.8 g is shown in Figure 5. The lowest 100-grain weight (1.0 g) was observed in one individual and the highest was found in 16 individuals (1.2-1.4 g) which include Jumbo Jet. Four individuals had 1.0-1.2 g, similar NSIC Rc222. Around nine individuals had 1.4 to 1.8 100-grain weight.

Grain yield declined under salt stress and ranged from 0.31 to 7.24 g per plant in this population. The grain yield of NSIC Rc222 and Jumbo Jet was 0.7 and 1.86 g, respectively. Of the 30 BC₁F₂-JJ29-P1 progenies, nine individuals were on the range of their parents. The rest got higher yield than their parents ranging from 2 to 7.24 g.

Discussion

Phenotypic experiment was conducted in order to determine the reaction of the ten BC₁F₂ families derived from NSIC Rc222/Jumbo Jet to salinity stress.

The ten BC₁F₂ families including their parents NSIC Rc222 and Jumbo Jet showed vigorous growth under normal condition whereas significantly different responses were observed in all the lines under salt-stressed condition. Leaf tip burning and curling were observed as early as ten days after salinization especially in susceptible genotypes. Susceptible rice genotypes may have the following symptoms of salt injury: a) white leaf tip followed by tip burning, b) stunted plant growth, c) low tillering, spikelet sterility, d) low harvest index, e) less florets per panicle, f) less 1000 grain yield, g) low grain yield, h) change in flowering duration, i) leaf rolling, j) white leaf blotches, k) poor root growth, and l) patchy growth in the field (Gregorio *et al.*, 1997; Munns, 2002).

The mean and range of all traits of the ten BC₁F₂ families and their parents under salt stress are presented in Table 2. It was observed that the mean values of three morphological traits namely, plant height, number of sterile spikelets and 100-grain weight were very close to the mid-parental value which means that the alleles of both parents have equal contribution for the expression of the said traits. For example, for plant height, the mean value of 10 the BC₁F₂ families was 101.94 and the mid-parental value was 99.00, similarly, the values were 75.59 and 79.49 for the number of sterile spikelets, and 1.29 and 1.22 for the 100-grain weight, respectively. On the other hand, the mean values of the ten BC₁F₂ families were lower than mid-parental values for DFL but higher for NOP, NFS, SF and GY, showing increasing performance of the progenies relative to their parents.

The skewness value of all the traits of the ten BC₁F₂ families ranged from -1.09 to 0.73 showing continuous segregation which indicates quantitative inheritance (Table 2). It can also be observed that NSIC Rc222 flowers 12 days earlier and has more panicles compared to Jumbo Jet. This finding showed that favorable alleles of NSIC Rc222 were expressed by the BC₁F₂ families, resulting in the development of early-maturing high-yielding lines. Moreover, varying degrees of transgressive segregation was also observed for all traits evaluated. This is due to the variation within the ten BC₁F₂ families that fall beyond their parental phenotypes and can be seen in both direction. The distribution within each trait also indicated that these traits are under polygenic control and that NSIC Rc222 and Jumbo Jet are both contributing genes for the expression of these traits.

Similar grain yield reduction but to lesser extent was reported by Rao *et al.* (2008) with yield reduction of 27%, 46% and 50% at an EC of 8 dS/m in tolerant, moderately-tolerant and susceptible rice cultivars, respectively. Yield reductions in salt-affected soils result primarily from the modification of different metabolic processes in plants under salt stress (Eynard *et al.*, 2005). One factor may be the overall control mechanism (before flowering) of sodium

uptake through root properties and its subsequent distribution in different vegetative and floral parts especially in leaves where it causes leaf mortality thereby reduces transportation of total assimilates to the growing region (Munns, 2002). Grain yield is the final product of yield components, hence, reduced grain yield was suggested to be due to reduced yield components. Agronomical traits such as tiller number per plant, spikelet number per panicle, fertility, panicle length and primary branches were significantly affected by salinity (Khatun *et al.*, 1995). Zeng and Shannon (2000) reported that a significant reduction in tiller number per plant and spikelet number per panicle were the major causes of rice yield loss under salinity stress.

Severe reduction in number of fertile spikelets and spikelet fertility was observed in the study. Khatun *et al.* (1995) reported that rice seed set is affected by salinization as pollen viability and stigmatic receptivity were reduced upon introduction of artificial seawater. Moreover, they found out that as the concentration of salt is increased, the sodium concentration in the pollen, stigmas, lemmas and paleas also increased. The severe inhibitory effects of salts on fertility may also be due to the differential competition in carbohydrate supply between vegetative growths which hinder its distribution to the developing panicles (Murty and Murty, 1982). The findings of this study suggest that reduction in number of fertile spikelets and spikelet fertility are the primary causes of grain yield reduction.

Salt-stressed rice plants may also have fewer numbers of secondary and tertiary tillers (Prasad *et al.*, 2003). Tillering is directly linked to the number of panicles formed, which is an important determinant of grain yield (Yang *et al.*, 2006). On the other hand, Fabre *et al.* (2005) reported that salinity effects on grain weight were actually brought about by reduced hull size, as evidenced by its dimensions, and therefore took place already before flowering. Spikelet sterility, low harvest index, change in flowering duration and low grain yield are the major symptoms of salinity (Singh and Mishra, 2004). Reduced viability of pollen due to salt stress results in failure of seed set, thus producing sterile spikelets (Abdullah *et al.*, 2001).

Delay in flowering of the BC₁F₂ families was supported by the study of Castillo *et al.* (2003) which observed that salinization during vegetative and reproductive stage of rice delayed flowering and prolonged crop growth duration by five to ten days. The increase in plant height (5.99%) showed an increase in tolerance of BC₁F₂ families in response to salt stress. Based on the correlation analysis data, this study suggests that the number of fertile spikelets and spikelet fertility are the two most important traits contributing to grain yield of rice.

Out of the ten families screened under salinized conditions, clear segregation pattern was observed in four families: (T109282 (BC₁F₂-JJ04-P1), T109287 (BC₁F₂-JJ15-P3), T109290 (BC₁F₂-JJ29-P1) and T109291 (BC₁F₂-JJ29-P2)), showed susceptible and tolerant plants within each family (Fig. 3). These families were scored 5 or rated as moderately tolerant as a whole. However, segregation pattern was most pronounced in T109290 (BC₁F₂-JJ29-P1) making it as the best segregating family suited for use as a mapping population (Fig. 3; 4).

The most tolerant family, T109285 (BC₁F₂-JJ10-P3) had the highest number of fertile spikelets (90), spikelet fertility (62.82%) and 100-grain weight (1.40g) and had the lowest number of sterile spikelets (52.97%). These important traits contribute significantly to grain yield, thus, BC₁F₂-JJ10-P3 also had the highest grain yield (5.46g). Tolerant genotypes tended to exclude salt hence less salt concentration in flag leaves and developing panicles, resulting in higher grain yield (Yeo and Flowers, 1986). In addition, BC₁F₂-JJ10-P3 also had the lowest reduction in terms of number of fertile spikelets (27.88%), spikelet fertility (12.16%), 100-grain weight (38.30%) and grain yield (78.87%). Further evaluation and selection should be done within this family in order to develop new high-yielding salt tolerant varieties.

Acknowledgement

To the University of the Philippines Los Banos (UPLB), International Rice Research Institute (IRRI), Department of Science and Technology - Accelerated Science and Technology Human Resource Development Program-National Science Consortium (DOST-ASTHRDP-NSC) and Central Luzon State University (CLSU) for the support.

References

- Abdullah, Z., Khan, M. A. and Flowers, T. J. (2001). Causes of sterility in seed set in rice under salinity stress. *Journal of Agronomy and Crop Science*. 187:25-32.
- Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances*. 27:84-93.
- Beavis, W. (1998). QTL analyses: power, precision and accuracy. In: Paterson A.H, editor. *Molecular dissection of complex traits*. CRC Press; Boca Raton, FL.
- Castillo, E., To, P. T., Huynh, T. T., Thai, N. H. T. and Tran, T. K. P. (2003). Phenological and physiological responses of a rice cultivar to level and timing of salinity stress. *ACIAR Technical Report*. 52e:89-101.
- Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B. and Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*. 142:169-196.
- Doerge, R. W. (2002). Mapping and analysis of quantitative trait loci in experimental populations. *Nature Reviews, Genetics*. 2:43-52.

- Eynard, A., Lal, R., Wiebe, K. (2005). Crop response in salt-affected soils. *Journal of Sustainable Agriculture*. 27:5-50.
- Fabre, D., Siband, P. and Dingkuhn, M. (2005). Characterizing stress effects on rice grain development and filling using grain weight and size distribution. *Field Crops Research*. 92:11-16.
- Flowers, T. J. (2004). Improving crop salt tolerance. *Journal of Experimental Botany*. 55:307-319.
- Gregorio, G. B., Senadhira, D. and Mendoza, R. D. (1997). Screening rice for salinity tolerance. IRRI Discussion Paper Series no. 22. Manila (Philippines): International Rice Research Institute. pp. 1-30.
- Khatun, S., Rizzo, C. A. and Flowers, T. J. (1995). Genotypic variation in the effect of salinity on fertility in rice. *Plant and Soil*. 173:239-250.
- Mohanrao, M. M. and Deepak, P. (2015). Mapping Population: Types and Method of Production. Retrieved from <http://www.biotecharticles.com/Genetics-Article/Mapping-Population-Types-and-Method-of-Production-3335.html>.
- Munns, R. (2002). Comparative physiology of salt and water stress. *Plant Cell and Environment*. 25:239-250.
- Murty, P. S. S. and Murty, K. S. (1982). Spikelet sterility in relation to nitrogen and carbohydrate contents in rice. *Indian Journal of Plant Physiology*. 25:40-48.
- Prasad, D. K., Asch, F. and Das, U. S. (2003). Salinity effects on tiller and leaf number, leaf appearance rate and leaf duration in irrigated rice. *Agricultural sciences and Resource Management in the Tropics and Subtropics – ARTS – Universität Bonn, Nussallee* [Abstract].
- Rahaman, M. M., Chen, D., Gillani, Z., Klukas, C. and Chen, M. (2015). Advanced phenotyping and phenotype data analysis for the study of plant growth and development. *Frontiers in Plant Science* 6:619. doi: 10.3389/fpls.2015.00619.
- Rao, P. S., Mishra, B., Gupta, S. R. and Rathore, A. (2008). Reproductive stage tolerance to salinity and alkalinity stresses in rice genotypes. *Plant breeding*. 127:256-61.
- Singh, R. K. and Mishra, B. (2004). Role of Central Soil Salinity Research in genetic Improvement of Rice Varieties of India. In: *Genetic Improvement of Rice Varieties of India*. (Eds.), SD Sharma and U Prasada Rao. India: Today and Tomorrow's Printers and Publ. pp. 189-242.
- Yeo, A. R. and Flowers, T. J. (1986). Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. In: *Effect of drought on plant growth. Salts in soils*. Turner, N. C. and Passioura, J. B. (Eds.). CSIRO. Melbourne. Australia. pp. 161-173.
- Yang, G., Xing, Y., Li, S., Ding, J., Yue, B., Deng, K., Li, Y. and Zhu, Y. (2006). Molecular dissection of developmental behavior of tiller number and plant height and their relationship in rice (*Oryza sativa* L.). *Hereditas*. 143:236-245.
- Zeng, L. and Shannon, M. C. (2000). Salinity effects on the seedling growth and yield components of rice. *Crop Science* 40:996-1003.

(Received: 13 September 2018, accepted: 2 November 2018)