RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
50 (4) 430-443, 2018

INHERITANCE AND IDENTIFICATION OF MOLECULAR MARKERS LINKED TO SALT TOLERANCE IN LOWLAND RICE VARIETY 'LLR012'

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SUMMARY

Salinity is one of the most serious factors limiting the productivity of rice worldwide, generally overcome by the effective use of genetically tolerant cultivars. Use of salt tolerant Thai indigenous rice varieties can solve the fail adoption of exotic varieties. A population of 156 $F_{2:3}$ derived from a cross between IR29, salt sensitive variety, and tolerant variety LLR012 was evaluated at the seedling stage under nutrient solution until the NaCl concentration reached 12 dSm^{-1}. Also, the experiment was evaluated under salted field conditions. The salt injury score, root and shoot dry weight, plant height, and tiller/plant seedlings were recorded. Transgressive segregation was determined in all traits due to the quantitative inheritance with the modification of minor or additive genes. Several SSR markers associated with salt tolerance-related traits were identified from both parents. RM313 on chromosome 12 presented the highest regression coefficient (R^2) in the salt injury score (22.97%), root dry weight (22.95%), and shoot dry weight (16.30%). RM413 on chromosome 1 was high in R^2 for root/shoot dry weight (8.36%), plant height (42.66%) and tiller/plant (18.88%). RM 520 on chromosome 3 was high in R^2 for the flowering date (17.34%). The SSR markers associated with the salt tolerance-related traits identified in this study may prove useful for marker-assisted selection, specifically for developing new rice cultivars in breeding programs for salinity tolerance.

Key words: Salt injury, marker-assisted selection, transgressive segregation, QTLs, breeding, root dry weight

Key findings: Both the LLR012 and IR29 contributed to salt tolerance causes of transgressive segregation in all traits. Several SSR markers associated with salt tolerance-related traits were identified due to the quantitative inheritance with modifications of either minor or additive genes.

Manuscript received: May 7, 2018; Decision on manuscript: July 17, 2018; Accepted: August 21, 2018.

Communicating Editor: Dr. C.N. Neeraja

INTRODUCTION

Salinity is one of the more critical forms of environmental stresses limiting the productivity of agricultural crops. More than 800 Mha of land throughout the world is salt-affected by either salinity or the associated condition of sodicity (Rana and Mark, 2008). In Thailand, the largest salt-affected area is the northeastern region, covering approximately 1.84 Mha followed by the coastal saline region of 0.43 Mha, and other regions spanning about 0.06 Mha (Arunin and Pongwichian, 2015). As the largest area for rice production in Thailand (6.72/11.2 Mha of Thai rice production area) (Office of Agricultural Economics, 2015), the northeastern (Isarn) region of Thailand represents up to 75% of the country's rice production. Produced under rainfed lowland areas, a lack of rainfall often results in the absence of water in the field, thereby causing salt particles in the soil to rise to the surface, and thereby increasing the intensity of salinity (Wongsomsak, 1986; Arunin and Pongwichian, 2015).

Rice is a salt-sensitive cereal crop, capable of tolerating salinity at moderate levels of electrical conductivity (4-8 dSm^{-1}), (Akbar, 1986). High salinity may cause delayed seed germination, slow seedling growth, and a reduced rate of seed set leading to a decrease in rice yield (Ruan *et al*., 2011). However, appropriate levels of salinity have also contributed to better quality of product, due to its enhanced aroma found in such premium rice varieties as RD6 and KDML105; identified as geographical indication (GI) varieties of Tung Gula Rong Hai (Summart *et al*., 2010; Zahid *et al*., 2014; Hinge *et al*., 2016). The levels of salt affected soil stress can be neither controlled nor estimated. Therefore, resolving areas of salinity can be achieved via evaporative control through the remaining water level on the soil's surface. However, this method is quite difficult to control, and involves excessive workloads and costs. The tolerant variety has proved to be the best and most sustainable method for salinity stress. Many teams have attempted to develop salt tolerant rice varieties targeting the many salinity affected areas (Zeng *et al*., 2003; Gregorio *et al*., 2002). However, salinity is difficult to predict, and uncertainties in experiments make selections difficult under field conditions.

The study of salt tolerance considers many quantitative traits, in which multiple genes are involved in phenotypes, where multiple environmental conditions are affected. A selection of durable tolerant varieties is therefore difficult, as it requires both skill and the risk of mistakes (Ray and Islam, 2008; Dashti *et al*., 2010). Furthermore, the use of molecular techniques as a marker-assisted selection (MAS) proved to be more accurate, as well as reduced the selection time. The most important characteristics used in the current evaluation and selection of salt tolerant rice include shoot length, plant height, Na^+ and K^+ concentration, root and shoot dry weight at the seedling stage (Flowers *et al*., 2000; Koyama *et al*., 2001; Lin *et al*., 2004; Lee *et al*., 2006), Na^+ and K^+ absorption, and the Na^+/ K^+ Dry weight ratio at the seedling stage (Koyama *et al*., 2001; Akhatr *et al*., 2012). However, the expression of salt-tolerant associated traits is affected by environmental causes, which are problematic in phenotype

evaluations. Therefore, the quantitative trait locus (QTL) associated with salt tolerance in rice has been distributed throughout the rice chromosomes, and identified within all traits.

Donors for salt tolerance are most often deployed from exotic salt-tolerant rice varieties, especially the Indian variety Pokkali (Zeng, 2005; Bhowmik *et al*., 2007; Kavitha *et al*., 2012; Ferreira *et al*., 2015). However, in the breeding program, the use of exotic tolerant rice varieties as donor parents is limited by the genetic linkage drag of the non-agronomic attributes that complicate the selection procedure. Therefore, the use of native or indigenous species as donors proves more suitable due to their high adaptability to specific areas, having been selected for long periods of time within the specified areas. Madee *et al*., 2014; studied salinity and drought tolerance using 40 landrace colored rice varieties at the seedling stage with multivariate cluster analyses, in which the lowland rice variety LLR012 displayed high tolerance to both drought and salt stress, in comparison to the standard Pokkali; which suggests that it could be used as a source of genetic material for breeding rice varieties resistant to salinity.

However, gene actions (genetic inheritance) and heritability of salt tolerance in these newly identified donor varieties remains unclear. Our study therefore aims to determine the heritability of salt tolerance and the identification of a molecular marker linked to salt tolerance in the rice variety LLR012. We feel that the findings of this study can be used as a guideline for the selection of future breeding programs for salt tolerance in rice.

MATERIALS AND METHODS

Plant material

Two rice varieties were used in this study as parental material: IR29 (P_1) and LLR012 (P_2). IR29 is considered a susceptible standard rice variety for salinity tolerance, whereas LLR012 proved to be an indigenous tolerant Thai rice variety (Madee *et al*., 2014). LLR012 was used as a male parent and crossed with IR29 to produce F_1 seeds. A single self-pollinated F_1 plant developed an F_2 population, where bulked pollen from three F_1 plants were backcrossed as the male parents to IR29 and LLR012, in order to develop BC_1P_1 and BC_1P_2 populations, respectively. F_2 individuals were self-pollinated, and their $F_{2:3}$ seeds were used for phenotyping evaluation.

Salt tolerance evaluation

Experiments for the evaluation of salt tolerance parameters were conducted under greenhouse conditions during the dry season (April - June) and wet season (June to August) of 2015, and under field conditions in the wet season (July to November) of 2016.

Greenhouse evaluation

Experiments were conducted in randomized and complete block design (RCBD) with three replications using the parents and progenies of F_1, $F_{2:3}$, BC_1P_1, and BC_1P_2 populations from IR29 and LLR012. The Pokkali variety was used as a standard check for salt tolerance. Experiments during the dry and wet seasons of 2015 were conducted under greenhouse conditions at the Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. Seeds in bags

were pre-germinated by soaking them in water for 24 hours, and incubated in a moist chamber until the seedlings had an average root length of 5 cm. A total of 128 seedlings were transplanted to 50 x 57cm^2 styrofoam sheets with 1.5 cm diameter holes (Gregorio *et al*., 1997). Fertilizer was applied by Yoshida nutrient solution (Yoshida et. al., 1976), and the nutrient solution was changed in three day intervals. After 15 days after seedling, the nutrient solution was mixed with sodium chloride in order to reach the electrical conductivity (EC) of 2 dSm^{-1} for two days. The EC was later increased to 4, 6, 10, and 12 dSm^{-1}, respectively. The salt injury scores (SS) of leaves were recorded following the rice standard evaluation (IRRI, 1996) at 29 DAT. Shoots and roots were then collected and dried; and measured for root dry weight (RDW), shoot dry weight (SDW), and root/shoot ratio (R/S).

Field evaluation

The experiment was laid out in random complete block design (RCBD) with three replications using $F_{2:3}$ populations in the field evaluation at Daeng village, Ban Fang district, Khon Kaen province, in the wet season (July to November) of 2016. Selected seeds from $F_{2:3}$, P_1, P_2, and the check variety (Pokkali) were sown in water for 48 hours. Germinated seeds were sown on seed beds. Thirty-five day old seedlings were transplanted to the field. The plot sizes were 0.75 x 1.25m^2, spaced at 0.25 x 0.25m with 15 plants/plot. IR29 was included as a susceptible check, whereas LLR012 and Pokkali were used as a resistant check. Each check variety was planted between every five plots of the $F_{2:3}$ population plots in order to ensure that salinity occurred uniformly in the experimental field. The fertilizer (23.44 kg/ha of N_2, P_2O_5, and K_2O) was applied at four days after transplanting (DAT), and hand-weeding and chemical application for disease and insect control were practiced as needed. The salt injury scores of leaves were evaluated at 30 DAT (at maximum tilling stage) following the standard evaluation system for rice (IRRI, 1996). The salinity of the field was monitored every seven days with a Waterproof Salt Tester $^{\#}$11. The field was subsequently drained at 41 DAT. Additional data included plant height and tillers number (TN), which were collected at 32 DAT and 66 DAT, respectively. Days to flowering (DTF) were collected when panicle flowering was 50% per plot. All data for the 10 plants per plot were recorded for future analysis.

DNA extraction

Genotyping of the 156 F_2 plants was performed using 68 markers out of 176 SSR markers distributed throughout the rice genome, which were found to be polymorphic between the IR29 and LLR012 varieties. The PCR reactions for SSR markers were carried out in a volume of 10 µl. The PCR component containing 25 ng of genomic DNA, 1X PCR buffer, 1.8 mM $MgCl_2$, 0.2 mM dNTP, 0.2 µM forward and reverse primer, and 0.05 unit *Taq* DNA polymerase (Fermentas). DNA amplification was performed in a DNA Thermal Cycle for five minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 55°C, and two minutes at 72°C; with a final extension of seven minutes at 72°C. The amplification products were

separated by 4.5% polyacrylamide gel electrophoresis.

Data analysis

Broad-sense heritability was computed by the method proposed by Warner, (1952). We determined the phenotypic correlations among traits in all experiments. Marker associations were calculated using the genotypic data of the F_2 population together with phenotypic data of the $F_{2:3}$ populations. The analysis for association between individual SSR markers and salinity tolerance was accomplished by single marker analysis, through a simple regression method (Kearsey and Pooni, 1996).

RESULTS AND DISCUSSION

The traits related to salt tolerance in this study; such as salt injury score, shoot and root dry weight, root/shoot ratio, plant height, days to flowering and tiller number/plant of the $F_{2:3}$ populations followed a continuous distribution pattern (Figure 1A, B, E-J, Figure 2A-D), indicating the quantitative inheritance within the studied traits. In contrast, the backcross populations displayed a mean value towards the backcrossed parent (Figure 1C-D); suggesting the effect of minor or additive genes. Previous studies have suggested that the genetics controlling salinity tolerance are derived from different genetic mechanisms, such as major dominant/recessive genes and polygenic genes, together with additive and dominant effects (Gregorio and Senadhira, 1993).

Transgressive segregation was observed in the $F_{2:3}$ populations with trait segregation beyond their parental phenotypes. Transgressive segregation reveals important evidence of the favorable alleles and their effects upon gene introgressions (Brondani *et al*., 2002). The frequent occurrence of transgressive segregation indicated the polygenic inheritance of the trait with at least a few operating genes in an additive way (Arama *et al*., 2000; Takamure and Sano, 2006). Even when the salt tolerance traits in parents were phenotypically similar, the polygenic inheritance of the trait caused the transgressive segregation and relatively high heritability for salt tolerance in the $F_{2:3}$ populations, suggesting the pyramiding of favorable alleles from both the parents (deVicente and Tanksley, 1993). Due to the transgression of the characters, the heritability of traits of this study ranged from a tiller number/plant of 0.44 to days to flowering of 0.99 (Table 1).

The study of salt tolerance involves complex traits with quantitative inheritance and low expressivity. The combination of the effect of genetics, as well as the stage of the rice plant and its environment, make it quite difficult to phenotypically select the desired genotypes within the breeding procedure. Many traits related to salt stress tolerance have been identified and shown effective for indirect selection, which has proven to be a useful alternative in the study on these complicated traits(Flowers *et al*., 2000; Koyama *et al*., 2001; Lin *et al*., 2004; Lee *et al*., 2006; Akhatr *et al*., 2012). In this study, the phenotypic correlations among traits of salinity tolerance in the $F_{2:3}$ populations were very low (Table 2). However, the salt injury scores in the dry season correlated with the salt injury scores

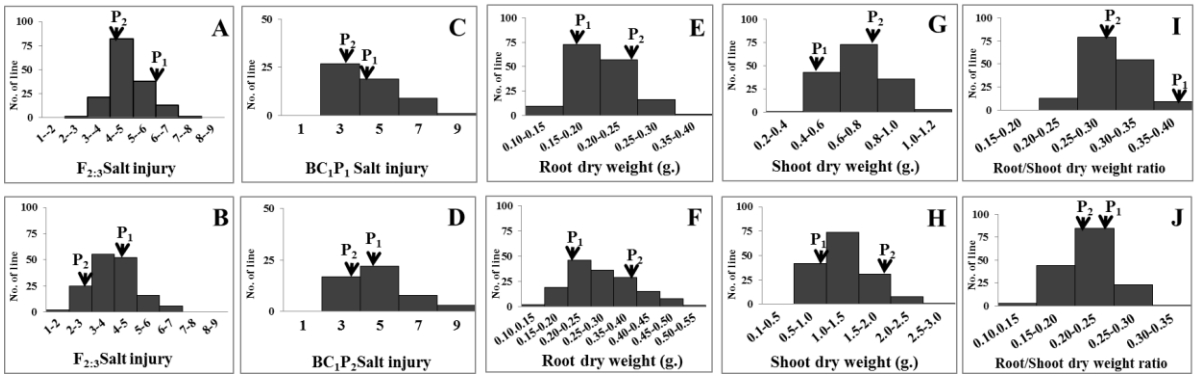

Figure 1. Distribution of traits related to salt tolerance from rice crosses of [IR 29 (P_1) x LLR 012 (P_2)] in greenhouse conditions: A) $F_{2:3}$ salt injury, C) BC_1P_1 salt injury, D) BC_1P_2 salt injury, E) Root dry weight, G) Shoot dry weight, I) Root/Shoot dry weight ratio (A, C, D, E, G, I) in dry season; B) $F_{2:3}$ salt injury, F) Root dry weight, H) Shoot dry weight, J) Root/Shoot dry weight ratio (B, F, H, J) in wet season.

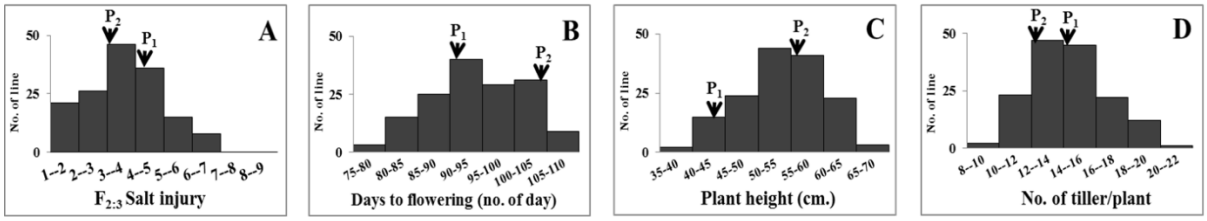

Figure 2. Distribution of traits related to salt tolerance of $F_{2:3}$ populations from rice crosses of IR 29 (P1) x LLR 012 (P2) in field conditions: A) $F_{2:3}$ salt injury, B) Days to flowering, C) Plant height, D) Tiller/plant.

in the wet season under greenhouse conditions, which may be considered as a necessary criteria for primary screening. A similar correlation was found within the salt injury scores in both dry and wet seasons under field conditions, at r = 0.33** and 0.39**, respectively; thereby demonstrating the reliability of this trait. This study depicts the salt injury score as a practical method. Earlier, Suriya-arunroj *et al*. (2005); also found a correlated between salt injury scores with the Na^+/ K^+ ratio in the screening studies of young seedlings under nutrient solution.

Salt injury scores under both seasons correlated with plant height in all conditions. The results indicate that the rice genotype produced taller plants (Asadi and Khiabani, 2007), due to their negative correlation with the tiller number (Table 2). The negative correlation between salt injury score and tiller number indicates that salt sequestration into various parts of the rice lead to more tolerance within the genotype. Salinity caused a decrease in the number of

Table 1. Estimated broad-sense heritability for root dry weight, shoot dry weight, root/shoot ratio, salt injury score, plant height, days to flowering, and tiller number/plant of the $F_{2:3}$ populationsfrom crosses of IR 29 x LLR 012.

Greenhouse /Field Conditions	Experiment	Characters	Heritability
Greenhouse	Dry season	Salt injury score	0.77
		Root dry weight	0.99
		Shoot dry weight	0.97
	Wet season	Salt injury score	0.94
		Root dry weight	0.87
		Shoot dry weight	0.93
		Root/Shoot ratio	0.97
Field	Wet season	Salt injury score	0.73
		Plant height	0.98
		Day to flowering	0.99
		Tiller/plant	0.44

Table 2. Phenotypic correlations among root dry weight (RDW), shoot dry weight (SDW), root/shoot ratio (R/S), salt injury score (SS), plant height (PH), days to flowering (DTF), and tiller number/plant (TN) of $F_{2:3}$ population from crosses of IR 29 x LLR 012 under greenhouse and field conditions.

	Greenhouse								Field			
	Dry season				Wet season				Wet season			
Characters	SS	RDW	SDW	R/S	SS	RDW	SDW	R/S	SS	PH	DTF	TN
SS	1.00											
RDW	-0.12	1.00										
SDW	-0.07	-0.95**	1.00									
R/S	0.10	-0.96**	0.89**	1.00								
SS	0.33**	-0.04	-0.03	0.05	1.00							
RDW	-0.20*	-0.10	0.25**	0.07	-0.27**	1.00						
SDW	-0.19*	-0.11	0.29**	0.03	-0.39**	0.83**	1.00					
R/S	0.06	0.05	-0.10	0.04	0.29**	0.07	-0.42**	1.00				
SS	0.39**	-0.04	-0.08	0.08	0.33**	-0.31**	-0.18*	-0.16	1.00			
PH	0.20*	-0.15	0.13	0.05	0.18*	0.05	0.22**	-0.28**	0.55**	1.00		
DTF	-0.10	0.09	-0.07	-0.08	-0.11	-0.03	-0.04	0.02	0.20*	-0.08	1.00	
TN	-0.15	0.03	-0.003	0.009	-0.08	-0.09	-0.09	0.13	-0.36**	-0.39**	0.07	1.00

* indicates significance at $P < 0.05$
** indicates significance at $P < 0.01$

tillers within sensitive genotypes, due to the reduction of primary tillers in high salinity conditions. In this study, the salt injury score of leaves were noted after the draining of the water from the field experiment at the maximum tiller stage. The IR29 variety scored high tiller numbers under non-saline conditions prior to water drainage. Therefore, when salinity occurred, all plants were completed with the maximum tiller. Most cereal plants are sensitive to salinity during the vegetative and early reproductive stages; yet are less sensitive during the flowering and grain filling stage (Mass and Poss, 1989). The IR29 demonstrated the ability to moderate salt tolerance in the field, due to an occurrence in the maximum tillering stage. The progenies with high tiller numbers were more tolerant to salinity, due to the sequestration mechanism (Zeng and Shannon, 2000; Moradi and Ismail, 2007; Munns and Tester, 2008; Ashraf and Akram, 2009; Gupta and Huang, 2014). Higher tiller numbers have been shown assisted salt tolerance in barley (Islam and Sedgley, 1981), wheat (Zeng and Shannon, 2000), and rice (Saqib *et al*., 2012).

Salinity occurs at the seedling and flowering stage in northeast Thailand due to the salt increase related to low precipitation, high surface evaporation, or drought stress in the growing season. An increased number of tillers may be a favorable characteristic for the salt tolerance adaptation mechanism, resulting in salt dilution in plants (Aslam *et al*., 1989). Farmers in these particular areas employ the transplanted methods to alleviate salinity at the seedling stage, of which rice is the most susceptible (Zeng *et al*., 2003; Singh *et al*., 2004). In this study, we also employed the transplanting method within our field experiments. After the maximum tilling stage, the water in the fields was drained. Salinity had occurred, however, at low levels, due to the increased precipitation at that time. Moreover, the IR29 maintained a greater tiller number than LLR012, and demonstrated greater resistance to salt tolerance through the plants sequestration of salt.

Due to the polygenic characteristics of salt tolerance-related traits and their correlation with one another, the selection of traits related to salt tolerance is complicated (Reddy *et al*., 2017). MAS may prove accurate for salt tolerant genes or QTLs. In this study, due to the limited study of the linkage of markers for salt tolerance due to low polymorphic markers between the parents, single regression analysis was carried out to identify SSR markers associated with salt tolerance in the $F_{2:3}$ populations. All experiments have shown that both parents contributed to the respective salt tolerances (Tables 3 and 4).

Results obtained in the dry season revealed that nine markers located on chromosomes 1, 2, 4, 5, 7, 11 and 12 were associated with SS, SDW, and R/S ($P < 0.05$). Marker RM313 on chromosome 12 showed the highest regression coefficient value ($R^2 = 10.89\%$) for SS in conjunction with the allele contributed from LLR012 (Table 3). During the wet season, 16 markers were identified, located on chromosomes 1, 2, 4, 5, 9 and 12; associated with SS, RDW, SDW and R/S ($P < 0.05$). Marker RM313 on chromosome 12 showed the highest R^2 value (22.95%) for RDW with the allele contributed from IR29 (Table 3). The differences in the

Table 3. Marker mean analysis for salt tolerance in rice of $F_{2:3}$ populations from crosses of IR 29 x LLR 012 under greenhouse conditions.

Seasons	Characters	Marker	Ch.	AA	Aa	aa	R^2	*P*-value
Dry season	Salt injury	RM259	1	5.13	4.83	4.67	4.60	**
		RM431	1	4.47	4.91	4.96	4.27	*
		RM3288	4	4.69	4.75	5.09	3.57	*
		RM307	4	4.72	4.77	5.14	3.11	*
		RM164	5	4.66	4.75	5.14	5.24	**
		RM11	7	4.64	4.77	5.16	5.63	**
		RM313	12	5.41	4.73	4.61	10.89	**
	Shoot dry weight	RM452	2	0.73	0.70	0.65	2.69	*
		RM313	12	0.57	0.70	0.79	6.00	**
	Root/Shoot dry weight ratio	RM206	11	0.30	0.30	0.31	2.67	*
Rainy season	Salt injury	RM431	1	3.63	4.02	4.32	5.90	**
		RM48	2	4.24	4.07	3.8	2.92	*
		RM164	5	3.75	4.09	4.19	2.74	*
		RM219	9	4.12	3.97	3.97	3.65	*
	Root dry weight	RM318	2	0.3	0.28	0.26	3.26	*
		RM307	4	0.3	0.28	0.26	3.58	*
		RM270	12	0.31	0.28	0.25	6.63	**
		RM313	12	0.22	0.28	0.32	22.95	**
	Shoot dry weight	RM431	1	1.12	1.31	1.38	5.63	**
		RM154	2	1.29	1.38	1.12	2.55	*
		RM452	2	1.39	1.26	1.20	3.06	*
		RM307	4	1.24	1.25	1.21	2.76	*
		RM17499	4	1.40	1.27	1.17	4.56	**
		RM17502	4	1.21	1.28	1.38	2.63	*
		RM270	12	1.37	1.29	1.10	4.72	**
		RM313	12	0.98	1.30	1.44	16.3	**
	Root/Shoot dry weight ratio	RM462	1	0.22	0.22	0.25	3.53	*
		RM431	1	0.25	0.22	0.21	8.36	**
		RM302	1	0.24	0.22	0.22	3.20	*
		RM3288	4	0.24	0.22	0.21	4.07	*
		RM6748	4	0.25	0.22	0.22	3.60	*
		RM17499	4	0.21	0.23	0.25	7.64	**
		RM17502	4	0.23	0.23	0.21	2.62	*

Ch.= chromosome; AA=allele LLR012; Aa= Heterozygous; aa= allele IR29, R^2= regression value. * indicates significance at $P < 0.05$, ** indicates significance at $P < 0.01$

associated markers identified in each season were due to the differences in the severity of salinity stress in each season; in which the dry season proved more stressful than the wet season (Figure 1).

Within the field conditions, molecular markers showed a significant difference in salt injury scores on chromosomes 1, 3, 6, 8, 11 and 12 ($P < 0.05$). The highest possible R^2 for the variance was RM313 on chromosome 12 (22.97%, $P < 0.01$). The most significant difference in PH was found on chromosomes 1 and 11; and marker RM431 on chromosome 1 demonstrated the highest R^2 for PH (42.66%). The significant difference in DTF was six markers on chromosomes 3, 4, 6, 8 and 11($P < 0.05$).The highest R^2 for the variance was RM520 on chromosome 3 (17.34%). The significant difference in TN was eight markers ($P < 0.05$) on chromosomes 1, 2, 4, 8, 9 and 11; and similar to the PH. Marker RM 431 on chromosome 1 had the highest R^2 for this trait 18.88% ($P < 0.01$) (Table 4). Some

Table 4. Marker mean analysis for salt tolerance in rice of $F_{2:3}$ populations from crosses of IR 29 x LLR 012 under field (RS) conditions.

Seasons	Characters	Marker	Ch.	AA	Aa	aa	R^2	P-value
Field	Salt injury	RM431	1	2.39	3.91	4.2	19.5	**
		RM302	1	2.85	3.60	4.47	18.3	**
		RM520	3	3.16	3.76	3.92	3.54	*
		RM115	6	3.26	3.75	3.98	2.98	*
		RM3	6	3.99	3.70	3.34	2.65	*
		RM38	8	3.16	3.77	4.05	4.80	**
		RM286	11	4.09	3.63	3.24	4.51	**
		RM270	12	3.11	3.77	4.41	9.95	**
		RM313	12	4.91	3.64	2.95	22.97	**
	Plant height	RM259	1	56.28	54.05	51.62	7.28	**
		RM431	1	46.64	54.14	58.41	42.66	**
		RM302	1	49.66	53.66	57.48	20.76	**
		RM286	11	55.40	53.72	52.29	2.93	*
	Flowering date	RM520	3	89.84	94.68	98.55	17.34	**
		RM17499	4	93.44	94.29	97.03	2.74	*
		RM115	6	90.41	95.80	97.53	10.75	**
		RM152	8	97.90	94.97	91.00	8.76	**
		RM38	8	89.76	95.80	97.85	14.11	**
		RM254	11	94.08	93.79	97.74	2.95	*
	Tiller/plant	RM431	1	16.00	14.00	13.00	18.88	**
		RM302	1	15.00	15.00	13.00	12.38	**
		RM154	2	14.00	14.00	15.00	3.85	*
		RM307	4	15.00	14.00	14.00	3.45	*
		RM447	8	15.00	14.00	13.00	6.99	**
		RM219	9	15.00	14.00	14.00	7.18	**
		RM206	11	15.00	14.00	14.00	2.72	*
		RM286	11	14.00	15.00	15.00	3.49	*

Ch.= chromosome; AA=allele LLR012; Aa= Heterozygous; aa= allele IR29, R^2= regression value. * indicates significance at $P < 0.05$, ** indicates significance at $P < 0.01$

linked markers have been repeatedly detected on chromosomes 1, 4, 6 and 7; whereas none were found on chromosomes 8 and 11, and very few on chromosomes 2, 3, 5, 9, 10 and 12 (Negrao *et al*., 2011); thus indicating that the majority of genes for salt tolerance were located on chromosome 1 (Thomson *et al*., 2010; Platten *et al*., 2013; Reddy *et al*., 2017).

The salt injury scores presented the simplest criteria for the evaluation of a large number of genotypes, and represent the most basic and most studied method (Gregorio *et al*., 2002; Koyama *et al*., 2001; Lin *et al*., 2004; Lee *et al*., 2006). Moreover, Lee *et al*. (2006); detected several QTLs of the visual score of leaf injury symptoms for salinity tolerance at the seedling stage in rice. In this study, salt injury was the trait with the highest possible

explanation for the variances within the field conditions, located on chromosome 1 in the wet season (RS), and chromosome 12 in the dry season (DS) (Table 4). Yen and Lin (2011) identified tightly linked markers of salt tolerance in rice, in which the most significant salt injury scores for salinity tolerance markers were RM 6840 (181.8cM) on chromosome 1, and RM 6732 (73.3-75.8 cM) on chromosome 12.Their findings concur with the results of our present study, in which the same chromosomes and near-linked markers [RM 431 (178.3 cM) and RM 302 (147 cM) on chromosome 1, and RM 313 (65.5 cM) on chromosome 12] were identified, as presented in Tables 3 and 4.

Root dry weight (RDW) was determined to be the most likely explanation for the variance on chromosome 12 in the wet season (Table 3). Lin *et al*. (2004); detected five QTLs for four traits associated with salinity tolerance in roots; and three QTLs for three traits of shoots which altered in various map locations. This study found that several markers located on different chromosomes were linked with shoot growth under salt stress. Shoot dry weight (SDW), on the other hand, was explained by the RM313 on chromosome 12 (Table 3). Koyama *et al*., 2001; evaluated shoot dry weight (SDW) as an indicator of salt tolerance, identifying the linked markers on chromosome 6. The root/shoot dry weight ratio (R/S) was found as the highest possible explanation for the variance on chromosome 1 in the wet season, and on chromosome 12 in the dry season; as well as plant height (PH), which was also found on chromosome 1 (Table 3). Similarly, Bimpong *et al*. (2013); reported two QTLs for plant height, *qPH1.1* and *qPH1.2*; again, located on chromosome 1.

In summary, the rice variety LLR012 was determined to be moderately salt tolerant. The heritability of salt tolerance of most traits was high. Moreover, transgressive segregation was found in all traits, due to the modification of minor or additive gene effects. These findings suggest that a backcrossing method can also be accumulated in the particular genes of several quantitative traits. The SSR markers associated with salt tolerance-related traits were identified in this study, as RM431 on chromosome 1 associated with SS, R/S, PH, and TN; RM313 on chromosome 12, which was closely linked with SS and SDW, and RM520 on chromosome 3, which was identified for DTF. The marker and trait associations proved useful for marker-assisted selections for salinity tolerant rice breeding programs.

ACKNOWLEDGEMENT

This research was supported by the Plant Breeding Research Center for Sustainable Agriculture, the Salt Tolerant Rice Research Group, and the Research Center of Agricultural Biotechnology for Sustainable Economy, Khon Kaen University, Khon Kaen, Thailand. Our gratitude is also extended to the Thailand Research Fund (TRF) (Project code: IRG5780003) and the Faculty of Agriculture at Khon Kaen University for providing financial support for the preparation of our manuscript.

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