RESEARCH ARTICLE

Mapping quantitative trait loci associated with yield and yield components under reproductive stage salinity stress in rice (*Oryza sativa* L.)

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Abstract

Salinity tolerance in rice is critical at reproductive stage because it ultimately determines grain yield. An F_2 mapping population derived from a Sadri/FL478 cross was exposed to saline field conditions (6–8 dS m^{-1}) after the active tillering stage to identify reproductive stage specific QTLs for salinity tolerance. Genetic linkage map was constructed using 123 microsatellite markers on 232 F_2 progenies. Totally 35 QTLs for 11 traits under salinity stress were detected with LOD > 3, out of which 28 QTLs that explained from 5.9 to 30.0% phenotypic variation were found to be significant based on permutation test. Three major QTL clusters were found on chromosomes 2 (RM423–RM174), 4 (RM551–RM518) and 6 (RM20224–RM528) for multiple traits under salinity stress. Both parental lines contributed additively for QTLs identified for the yield components. A majority of the QTLs detected in our study are reported for the first time for reproductive stage salinity stress. Fine-mapping of selected putative QTLs will be the next step to facilitate marker-assisted backcrossing and to detect useful genes for salinity tolerance at the reproductive stage in rice.

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Introduction

Salinity is one of the major environmental stress that limit the productivity of rice (*Oryza sativa* L.) because of the crop's sensitivity to high concentrations of salt in the soil (Flowers and Yeo 1995). A soil is considered saline if the electrical conductivity of its saturated extract (EC_e, average root-zone EC of saturated soil extract) is above 4 dS m^{-1} (United States Salinity Laboratory Staff 1954). More than 90% of world's rice production comes from Asia, especially south and southeast Asia that has about 21.5 million ha as salt affected, of which 12 million ha are saline and 9.5 million ha are alkaline/sodic (Munns and Tester 2008). Over half of the world's population depends on rice as a staple food crop (Narciso and Hossain 2002). Rice is relatively salt sensitive, and it has a threshold salinity of 3 dS m^{-1}, above which yield loss occurs (Maas and Hoffmann 1977). Maas and Grattan (1999) indicated that rice yield decrease 12% for every unit (dS m^{-1}) increase in EC_e above 3 dS m^{-1}. Rice is relatively tolerant of salt stress during germination, active tillering and toward maturity, and is sensitive during the early seedling and reproductive stages (panicle initiation, anthesis and fertilization) (Zheng *et al.* 2001; Singh *et al.* 2007). Symptoms of salinity stress include white tips of affected leaves, plant stunting, reduced tillering, patchy field growth and in severe cases, plant death. Salinity significantly reduces tiller number per plant, spikelet number per panicle, fertility, panicle length and primary branches per panicle (Heenan *et al.* 1988; Cui *et al.* 1995; Khatun *et al.* 1995; Zeng *et al.* 2002; Sarhadi *et al.* 2012). Reduction in tiller number per plant and spikelet number per panicle were reported to be the major causes of yield loss in one cultivar of rice under salinity stress (Zeng and Shannon 2000). The

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number of spikelets per panicle was determined to be the most sensitive yield component. This component is determined at the early reproductive stage, around panicle initiation (PI) (Counce *et al.* 2000). Rao *et al.* (2008) reported a grain yield reduction by 27, 46 and 50% at an EC_e of 8 dS m^{-1} in tolerant, moderately tolerant and susceptible rice cultivars, respectively. Considerable variation for yield components was reported based on the evaluation of several diverse genotypes for salinity stress response (Yeo and Flowers 1982; Zeng *et al.* 2002; Moradi *et al.* 2003).

QTLs for salinity tolerance

The vast genetic variability reported in rice in response to salinity makes it amenable to genetic manipulation to further enhance its tolerance (Akbar *et al.* 1972; Flowers and Yeo 1981). Breeders have long made use of the high salinity tolerance in landraces such as Nona Bokra and Pokkali. However, negative characters in traditional varieties and numerous complex traits involved in salinity tolerance have presented challenges for conventional breeding to make significant progress and have led to increased interest in molecular breeding methods (Gregorio *et al.* 2002; Ismail *et al.* 2007; Thomson *et al.* 2010). QTL mapping enables the dissection of the genetic control of each tolerance mechanism, opening up the possibility of future efforts to develop varieties with improved salinity tolerance by precisely transferring QTLs into popular varieties and pyramiding multiple relevant QTLs for a particular stress-prone environment (Thomson *et al.* 2010).

Most previous studies identified QTLs associated with seedling stage salinity tolerance in rice. Salt tolerance has often been found associated with a lower accumulation of sodium (Na^+) in the shoot, but not always (Genc *et al.* 2007). Using Na^+ accumulation and other measures of salt tolerance, major and minor QTLs have been mapped in various crop species including rice (Flowers and Flowers 2005; Jenks *et al.* 2007). A number of mapping studies have identified QTLs associated with salinity tolerance in rice (Singh *et al.* 2007; Haq *et al.* 2010; Singh and Flowers 2010). A major QTL located on chromosome 1 was identified for salt tolerance using F_8 recombinant inbred lines (RILs) of an IR29/Pokkali cross (Gregorio 1997). This major QTL designated *Saltol*, governed Na–K uptake ratio and accounted for 64.3% of the phenotypic variation in salt tolerance. SSR markers RM8094 and RM10745 were suggested for use in marker-assisted selection (MAS) of the *Saltol* QTL (Mohammadi-Nejad *et al.* 2008). Further, an analysis of single feature polymorphisms in the *Saltol* region suggested that FL478 contained >1 Mb DNA fragment from Pokkali at 10.6–11.5 Mb on chromosome 1, flanked by IR29 alleles (Kim *et al.* 2009). A study employing an $F_{2:3}$ population derived from the tolerant indica landrace Nona Bokra and the susceptible japonica Koshihikari identified several QTLs controlling tolerance traits, including major QTLs for shoot K^+ concentration on chromosome 1 (qSKC-1) and shoot Na^+ concentration on chromosome 7 (qSNC-7; Lin *et al.* 2004). The SKC1 gene, which lies within the *Saltol* region, was subsequently cloned and found to encode a sodium transporter that helps to control Na^+ and K^+ homeostasis under salt stress (Ren *et al.* 2005). Ammar *et al.* (2009) used an $F_{2:3}$ mapping population derived from an indica/indica cross of CSR27 (tolerant) and MI-48 (sensitive) to identify QTLs for Na^+, K^+ and Cl^- concentrations in the leaf tissue at the reproductive stage. They detected QTLs for Cl^-, Na–K ratio and Na^+ in the leaf at the reproductive stage on chromosomes 2, 3 and 8, respectively. The three QTLs explained phenotypic variance in the range of 26–52%. Salinity tolerance at the seedling and reproductive stages is only weakly associated (Mishra B., Akbar M. and Seshu D. V. 1990 Genetic studies on salinity tolerance in rice towards better productivity in salt affected soils. Paper presented at the Rice Research Seminar, 12 July 1990, IRRI, Los Baños, Philippines; Moradi *et al.* 2003; Singh and Flowers 2010), suggesting that tolerance at these two stages is regulated by different sets of genes. The reproductive stage is crucial as it ultimately determines grain yield; however, the importance of the seedling stage cannot be ignored as it affects crop establishment. Hence, pyramiding of contributing traits/QTLs conferring tolerance at both stages is needed for developing resilient salt-tolerant cultivars (Moradi *et al.* 2003). The major objectives of this study were to identify and map the major QTLs associated with reproductive stage salinity tolerance in rice.

Materials and methods

Plant materials and population development

The F_2 population from the Sadri/FL478 cross (IR96469) was used for phenotyping under salinity stress at the reproductive stage and the identification of associated QTL regions. The parents were selected based on their performance in a diallel cross analysis under salinity stress (unpublished data). Sadri (IRGC acc. 32329) is an aromatic landrace from northern Iran, susceptible to salinity at the seedling stage but tolerant at the reproductive stage. Sadri is a general name for premium-quality rice landraces from Iran. Before 2001, Sadri landraces were cultivated on more than 60% of the total rice area, because farmers refused to adopt improved varieties on account of their poor cooking quality. FL478 (IR66946-3R-178-1-1) is a recombinant-inbred line of an IR29/Pokkali cross with high seedling stage salinity tolerance, but it is sensitive at the reproductive stage. A total of 232 F_2 plants were developed from the Sadri/FL478 cross and used for phenotyping as well as genotyping.

Phenotyping for yield components under salt stress

The phenotyping was conducted at International Rice Research Institute, Phillipines, under artificially salinized

concrete blocks (8 × 4.8 m and 0.5 m depth). The 21-day-old seedlings of parents and 232 F_2 progenies were transplanted in saline plots. Saline water solution was prepared by dissolving technical grade salt (NaCl) in water to salinize the soil by irrigation of saline water. Salinity stress was imposed initially by irrigating the saline water of EC_e 3–4 dS m^{-1} during transplanting at the seedling stage, which increased to EC_e 6–8 dS m^{-1} after the active tillering stage until maturity. The EC_e of the soil was monitored and adjusted whenever necessary by saline water irrigation. At maturity, data were recorded on each F_2 plant for the following characters: number of days to flowering (DFL), plant height (PHt in cm), number of panicles per plant (PnN), panicle length (PnL in cm), straw dry weight per plant (StDW in g), number of fertile spikelets per plant (FrSp), number of sterile spikelets per plant (StSp), number of total spikelets per plant (TSp), grain yield per plant (GYld in g), percent spikelet fertility (SpFr) and 1000-grain weight (TGW in g).

Construction of a linkage map and QTL mapping

Three-to-four weeks after transplanting, young leaves from all 232 F_2 plants and parents were collected. Genomic DNA samples were extracted by using a modified CTAB protocol (Thomson *et al.* 2006). A parental survey was performed using 450 rice SSR markers. PCR amplification was performed in a 15 μL volume containing 2 μL of 25 ng genomic DNA, 1.5 μL of 10× PCR buffer (containing 100 mM Tris-HCl, pH 8.3, 500 mM KCl and 15 mM $MgCl_2$), 1.25 μL of 1 mM dNTP, 0.5 μL of 50 mM $MgCl_2$, 1 μL each of 5 μM forward and reverse primers and 1 μL of 5 U/μL *Taq* DNA polymerase with 6.75 μL sterile nano-pure H_2O. These items were dispensed in each well along with 1 drop of mineral oil and then covered with PCR plate sealing film. PCR was performed in a G-Storm Thermal Cycler (Model GS1, Gene Technologies, Essex, UK) by initial denaturation at 94°C for 5 min, and then 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 1 min and 30 s and final extension at 72°C for 8 min and storage at 10°C.

PCR was performed on 96-well plates, and the DNA fragments were separated on 8% acrylamide gels (C.B.S. Scientific, Del Mar, USA), and stained with SYBR-Safe (Invitrogen, Carlsbad, USA) for manual allele scoring. Polymorphic markers were used to genotype the F_2 population and to construct a linkage map to cover the entire genome. The linkage map was constructed using Map Manager QTX, vQTXb20 (Manly *et al.* 2001). The phenotypic and genotypic data were analysed by composite interval mapping (CIM) using QGene ver. 4.3.8 (Joehanes and Nelson 2008). Permutations of 10000 iterations were used to determine the threshold of the QTLs in Qgene. Subsequently, the LOD values at $P < 0.05$ were used as the threshold to declare the significance of the QTLs. QTL names were designated following the standard rice QTL nomenclature (McCouch and CGSNL 2008).

Results

Phenotyping of the mapping population

The analysis of variation showed a significantly different response among the F_2 progenies for the 11 evaluated traits under salt stress at the reproductive stage. Considerable effect due to salinity was observed for most of the traits evaluated at that stage.

For all the traits studied, F_2 population variation was beyond the range of their parents, suggesting transgressive segregation and this was observed in both directions for all the traits but to different extents. The distribution within each trait also indicated that these traits are under polygenic control and both parents, Sadri and FL478, contributed genes for these traits. The frequency distribution of yield components of the F_2 population did not fit the normal distribution, except for the distribution of DFL, PHt and PnL, which were close to normal distribution (figure 1). Deviation from the normal distribution could be the result of the influence of some major genes. The phenotypic distributions of all examined traits for the F_2 population displayed a continuous segregation with the skewness ranging from −0.48 to 1.34 indicating quantitative inheritance (table 1). The mean distributions for yield components showed a moderate to high transgressive segregation with values either larger or smaller than those of the parents (figure 1). The mean values of StDW, SrSp and GYld were skewed towards the lower values, while SpFr, TGW and PHt were skewed towards the higher values. Zang *et al.* (2008) reported that the introgression lines (BC_2F_8) derived from an indica/japonica cross (IR64 and Binam are both moderately tolerant of salinity) showed great variation in salinity tolerance.

Correlation among grain yield per plant and yield components

Correlation analysis can provide an understanding of the relationship of grain yield per plant (GYld) and yield components under salinity stress. Correlation analysis revealed that plant height (0.37**), panicle length (0.35**), number of panicles (0.36**), straw dry weight (0.40**), number of fertile spikelets (0.97**), spikelet fertility (0.64**) and 1000-grain weight (0.44**) had a positive and significant contribution to GYld (table 2). Number of days to flowering had a negative and weak correlation with GYld (−0.15*). Number of sterile spikelets had no significant correlation with GYld (here '*' and '**' indicates significance at $P \leq 0.05$ and $P \leq 0.01$, respectively).

Construction of a linkage map and QTL identification

Of the 450 SSR markers used for the parental survey, 155 (34.4%) showed polymorphism between the two parents. These polymorphic markers were then applied in 232 unselected F_2 samples. Out of 155 polymorphic markers, 123 exhibited clear bands. The amplified fragments ranged

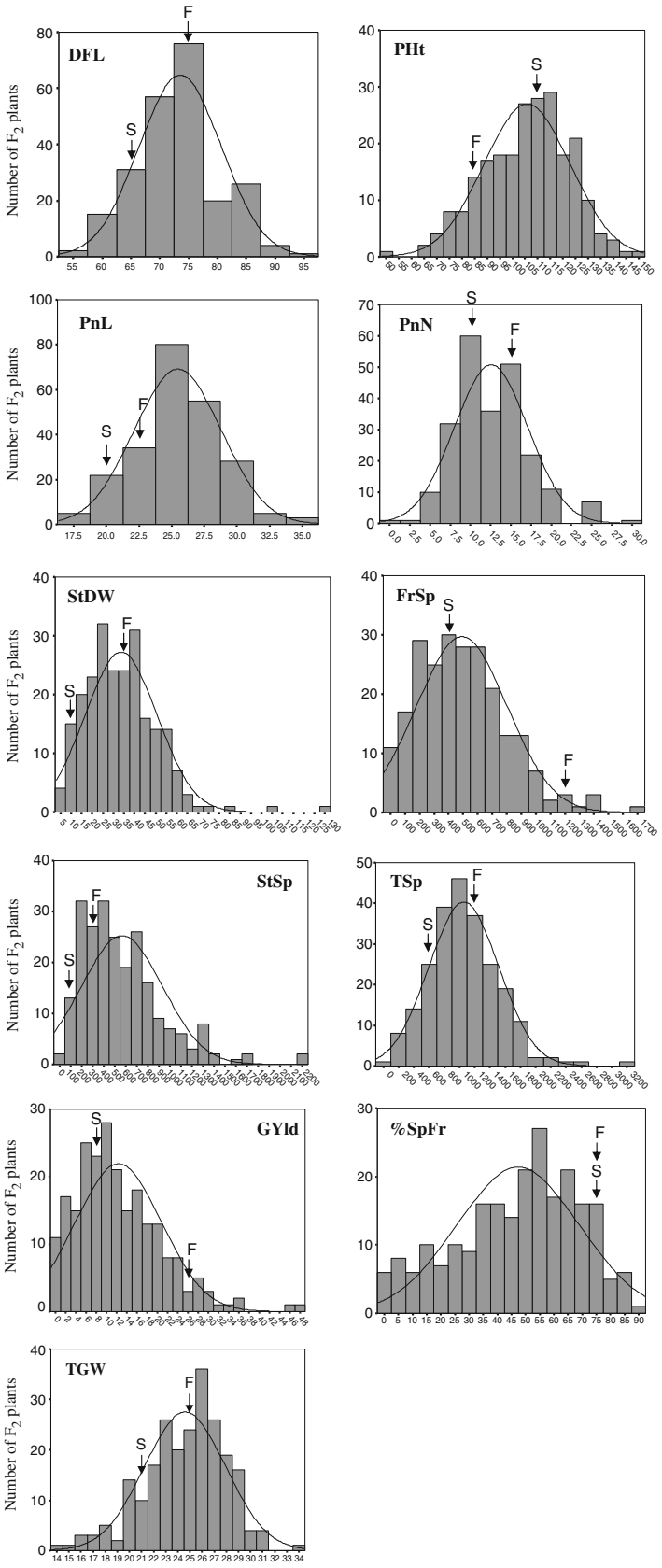

Figure 1. Frequency distribution of yield components of Sadri/FL478 F_2 progenies grown under salt stress at the reproductive stage in artificially salinized field conditions (S, Sadri; F, FL478).

Table 1. Mean values of yield components of Sadri/FL478 F_2 progenies and their parents grown under salt stress at the reproductive stage in artificially salinized field conditions.

Trait	Sadri	FL478	Mid parent	F_2 progenies		
				Range	Mean	Skewness
Days to flowering	63	78	70.5	53–96	73.6	0.11
Plant height (cm)	105	81	93.0	50–151	105.8	−0.29
Panicle length (cm)	20	21	20.5	17–35	25.5	−0.09
Number of panicles	9	14	11.5	1–31	12.6	0.72
Straw dry weight (g)	10.3	33	21.6	3.1–132.1	33.7	1.34
Number of fertile spikelets	364	1108	736.0	10–1747	491.0	0.77
Number of sterile spikelets	95	280	187.5	9–2207	566.2	1.38
Number of total spikelets	459	1388	923.5	52–3169	1057.1	0.68
Grain yield per plant (g)	7.8	27	17.4	0.2–47.6	12.4	1.02
Spikelet fertility %	78.9	79	78.9	1.3–87.6	47.3	−0.40
1000-grain weight (g)	21.3	25	23.2	14.5–33.7	24.6	−0.48

in size from 83 to 299 bp. Linkage maps of 12 chromosomes were created based on genotypic data of 232 F_2's from Sadri/FL478, with 123 SSR primers using the Kosambi mapping function (Kosambi 1944) of Map Manager QTX, vQTXb20 (Manly *et al.* 2001). Marker ordering was based on published microsatellite marker information (Temnykh *et al.* 2001). The total size of the linkage map was 1852 cM, with an average of 15 cM, and the distance between markers ranged from the smallest interval of 0.9 cM on chromosome 3 to the largest interval of 60.6 cM on chromosome 4 (figure 2).

QTLs associated with yield components under salinity stress were identified through interval mapping (IM) and composite interval mapping (CIM; Zeng 1993, 1994) using QGene, ver. 4.3.8 (Joehanes and Nelson 2008). A total of 35 QTLs for 11 yield and yield-related traits were detected on all 12 chromosomes of rice except chromosomes 11 and 12 (table 3). The data were also analysed using CIM by Windows QTL Cartographer 2.5.009 (Wang *et al.* 2011). The results of the QTL analysis from QTL Cartographer were similar to the QGene results. Therefore, just QGene results using CIM are presented here. The detected QTLs individually accounted for 6.2–30.0% of the phenotypic variation. Of all the 35 QTLs, seven accounted for more than 10% of the phenotypic variation. Detected QTLs were named according to the nomenclature suggested by McCouch *et al.* (1997) and McCouch and CGSNL (2008). Here the letter 's' for QTL nomenclature was used for a QTL mapped under saline conditions. A list of putative QTLs flanked by markers along with their LOD values, phenotypic variance and additive and dominance effects appears in table 3. The locations of all identified QTLs on 10 chromosomes of rice are illustrated in figure 2.

Number of days to flowering (DFL): QTL analysis using CIM identified three QTLs on chromosomes 4, 6 and 10 designated as *qDTF4.1s*, *qDTF6.1s* and *qDTF10.1s*, respectively (table 3). *qDTF10.1s* mapped to the interval RM271–RM484 on chromosome 10 with the largest effect explaining 13.3% of the total phenotypic variance. The Sadri allele at *qDTF10.1s* increased days to flowering by 4.8 days. The other two QTLs, *qDTF4.1s* and *qDTF6.1s*, explained 6.9 and 9.1% of the total phenotypic variance and the FL478 allele at these QTLs increased days to flowering by two and three days, respectively. *qDTF4.1s* exhibited

Table 2. Correlation analysis of grain yield and yield components of Sadri/FL478 F_2 progenies under artificially salinized field conditions at the reproductive stage.

Trait	DFL	PHt	PnL	PnN	StDW	FrSp	StSp	TSp	**GYld**	SpFr
PHt	0.16*									
PnL	0.05^{ns}	0.68**								
PnN	-0.08^{ns}	0.27**	0.20**							
StDW	0.19**	0.49**	0.30**	0.68**						
FrSp	-0.10^{ns}	0.38**	0.37**	0.38**	0.40**					
StSp	0.12^{ns}	0.21**	0.14*	0.66**	0.66**	-0.08^{ns}				
TSp	0.03^{ns}	0.43**	0.36**	0.77**	0.79**	0.61**	0.74**			
GYld	**−0.15***	**0.37****	**0.35****	**0.36****	**0.40****	**0.97****	**-0.07^{ns}**	**0.60****		
%SpFr	−0.19**	0.11^{ns}	0.17*	−0.17*	−0.19**	0.66**	−0.68**	-0.09^{ns}	**0.64****	
TGW	−0.38**	0.12^{ns}	0.09^{ns}	0.17*	0.10^{ns}	0.27**	-0.01^{ns}	0.18*	**0.44****	0.23**

*Significant at $P \leq 0.05$; **significant at $P \leq 0.01$; ^{ns}not significant; bold font indicates the correlation of grain yield with yield components.

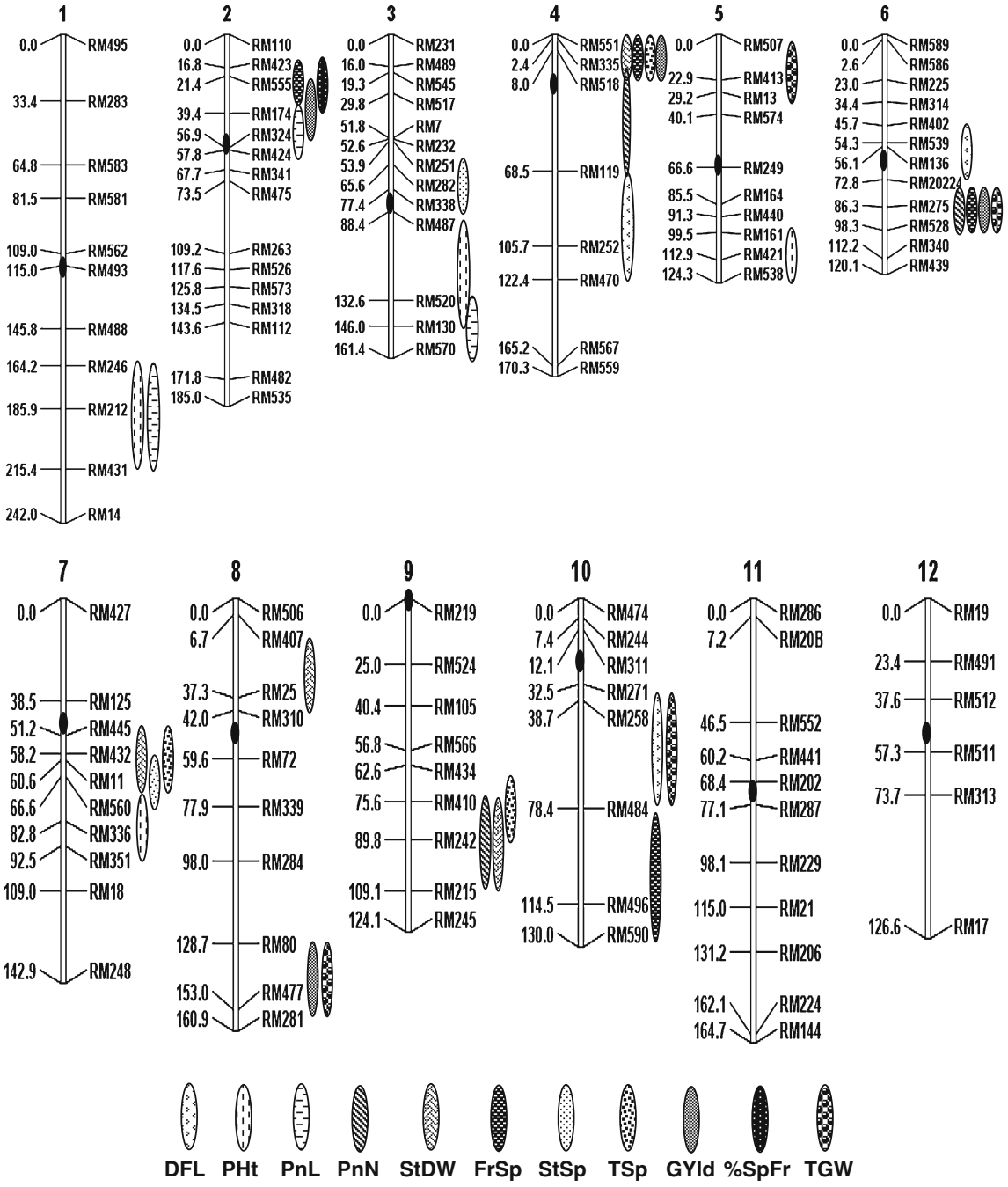

Figure 2. Genetic linkage map showing the location of QTLs for yield components under salinity stress (EC = 6–8 dS m^{-1}) in the Sadri/FL478 F_2 population. Distances are in Kosambi centimorgans. The legends on the chromosomes represent putative regions of QTLs for each trait.

dominance effects and increased DFL by FL478 allele. *qDTF6.1s* exhibited dominance effects and increased DFL by the Sadri allele.

Plant height (PHt): Four loci significantly associated with plant height were detected on chromosomes 1, 2, 3 and 7 using CIM and designated as *qPH1.1s*, *qPH2.1s*, *qPH3.1s* and *qPH7.1s*, respectively (table 3). The loci had R^2 values ranging from 6.6 to 17% and *qPH1.1s*, which was detected near marker RM212 located on the long arm of chromosome 1 had the highest value. The alleles from Sadri increased plant height at *qPH1.1s*, *qPH3.1s* and *qPH7.1s*, while alleles from FL478 increased plant height at *qPH7.1s*.

Panicle length (PnL): Three QTLs were detected on chromosomes 1, 2 and 3 for PnL. *qPL3.1s,* with the largest effect,

Table 3. QTLs detected with a LOD score >3 through composite interval mapping using QGene software for yield components in F_2 population from the cross of Sadri/FL478 under salt stress.

Trait	Chromosome number	QTL name	QTL interval	Nearest marker	Peak LOD IM	Peak LOD CIM	PVE (%) IM	PVE (%) CIM	Additive effect (DPE)	Dominant effect (DPE)
Days to flowering	4	*qDTF4.1s*	RM119–RM470	RM252	3.1	**3.6**[a]	5.9	6.9	2.1 (F)	2.2 (F)
	6	*qDTF6.1s*	RM539–RM20224	RM136	5.6	**4.8**	10.6	9.1	3.0 (F)	1.5 (S)
	10	*qDTF10.1s*	RM271–RM484	RM258	7.6	**7.2**	14.0	13.3	4.8 (S)	0.1 (F)
Plant height (cm)	1	*qPH1.1s*	RM246–RM431	RM212	9.7	**9.4**	17.5	17.0	10.0 (S)	3.4 (S)
	3	*qPH3.1s*	RM487–RM130	RM520	6.5	**7.8**	12.0	14.3	7.5 (S)	5.5 (S)
	5	*qPH5.1s*	RM421–RM538	RM538	2.7	3.4	5.2	6.6	5.1 (S)	3.0 (F)
	7	*qPH7.1s*	RM560–RM351	RM336	6.3	**6.8**	11.8	12.6	8.2 (F)	0.4 (F)
Panicle length (cm)	1	*qPL1.1s*	RM246–RM431	RM212	3.2	3.5	6.2	6.8	1.2 (S)	0.4 (S)
	2	*qPL2.1s*	RM174–RM424	RM324	4.4	**3.8**	8.3	7.2	1.6 (S)	1.0 (S)
	3	*qPL3.1s*	RM520–RM570	RM130	4.5	**4.3**	8.5	8.2	1.3 (S)	0.8 (S)
Number of panicles	4	*qPN4.1s*	RM335–RM119	RM518	2.6	**4.6**	5.1	8.6	1.9 (F)	0.6 (S)
	6	*qPN6.1s*	RM20224–RM528	RM275	3.6	**4.9**	6.8	9.2	1.6 (S)	1.7 (S)
	9	*qPN9.1s*	RM410–RM215	RM242	3.2	**4.1**	5.8	7.9	1.8 (F)	1.0 (S)
Straw dry weight (g)	4	*qSTW4.1s*	RM551–RM518	RM335	3.9	**5.1**	7.4	9.6	5.4 (F)	7.1 (S)
	7	*qSTW7.1s*	RM445–RM11	RM432	3.8	**4.2**	7.2	8.0	5.7 (F)	6.2 (S)
	8	*qSTW8.1s*	RM407–RM310	RM25	2.1	3.2	4.1	6.2	5.1 (S)	5.2 (S)
	9	*qSTW9.1s*	RM410–RM215	RM242	2.1	3.3	4.2	6.4	5.8 (F)	3.2 (S)
Number of fertile spikelets	2	*qFRSP2.1s*	RM423–RM174	RM555	3.0	3.3	5.7	6.3	110.5 (S)	21.1 (F)
	4	*qFRSP4.1s*	RM551–RM518	RM335	3.1	**4.4**	6.0	8.3	105.3 (F)	98.5 (S)
	6	*qFRSP6.1s*	RM20224–RM528	RM275	3.1	**4.1**	6.0	7.7	108.5 (S)	60.6 (S)
	10	*qFRSP10.1s*	RM484–RM590	RM496	2.8	3.1	5.3	5.9	114.0 (F)	9.1 (S)
Number of sterile spikelets	3	*qSTSP3.1s*	RM251–RM338	RM282	4.2	**4.8**	8.0	9.2	285.9 (S)	265.0 (F)
	7	*qSTSP7.1s*	RM432–RM560	RM11	3.0	**3.7**	5.8	7.1	47.8 (F)	196.8 (S)
Total spikelets number	4	*qTSP4.1s*	RM551–RM518	RM335	5.5	**6.7**	10.4	12.4	173.5 (F)	184.0 (S)
	7	*qTSP7.1s*	RM445–RM11	RM432	3.6	**4.1**	6.9	7.8	144.0 (F)	173.4 (S)
	9	*qTSP9.1s*	RM434–RM242	RM410	2.6	**3.6**	5.1	6.9	177.7 (F)	47.5 (S)
Grain yield per plant (g)	2	*qGY2.1s*	RM555–RM324	RM174	3.0	**3.6**	5.8	6.9	3.2 (S)	0.5 (S)
	4	*qGY4.1s*	RM551–RM518	RM335	3.1	**4.2**	6.0	7.9	2.7 (F)	1.9 (S)
	6	*qGY6.1s*	RM20224–RM528	RM275	3.5	**4.4**	6.7	8.4	3.2 (S)	1.0 (S)
	8	*qGY8.1s*	RM80–RM281	RM477	3.9	**4.4**	7.4	8.4	3.6 (F)	0.1 (S)
Spikelet fertility	2	*qSPFR2.1s*	RM423–RM174	RM555	4.2	**4.2**	7.9	7.9	9.3 (S)	2.6 (S)
	5	*qSPFR2.5s*	RM440–RM421	RM161	3.1	2.2	6.0	4.2	5.9 (F)	8.6 (F)
	10	*qSPFR2.10s*	RM271–RM484	RM258	3.5	2.7	6.7	5.2	7.2 (F)	19.7 (S)
1000-grain weight (g)	5	*qTGW5.1s*	RM507–RM13	RM413	2.3	**4.0**	4.6	7.6	1.1 (S)	0.3 (F)
	6	*qTGW6.1s*	RM20224–RM528	RM275	2.0	3.2	3.9	6.2	1.0 (S)	0.1 (F)
	8	*qTGW8.1s*	RM80–RM281	RM477	14.8	**18.0**	25.5	30.0	3.0 (F)	1.1 (S)
	10	*qTGW10.1s*	RM271–RM484	RM258	8.9	**9.7**	16.2	17.6	2.4 (F)	0.9 (S)

Peak LOD CIM, logarithm of odds using composite interval mapping; PVE (R^2), percentage of variance explained by the QTL; DPE, direction of phenotypic effect; S, Sadri; F, FL478. [a]QTLs in bold are identified above the $P = 0.05$ threshold using permutation analysis.

explained 8.2% of the total phenotypic variation, and the additive effect of the Sadri allele increased PnL by 1.3 cm. *qPL3.1s* was mapped near marker RM130 located on the long arm of chromosome 3. The other two QTLs, *qPL1.1s* and *qPL2.1s*, explained 6.8 and 7.2% of the total phenotypic variance, respectively, and the Sadri alleles at these QTLs increased PnL (table 3).

Number of panicles per plant (PnN): Three QTLs for PnN were detected on chromosomes 4, 6 and 9. *qPN6.1s* had the highest R^2 value (9.2) was mapped to the interval RM20224–RM528 on chromosome 6 and the allele from Sadri increased the number of panicles. The other two QTLs, *qPN4.1s* and *qPN9.1s*, accounted for 8.6 and 7.9% of the phenotypic variation, with the FL478 alleles providing a positive effect on number of panicles (table 3). There is a big gap in *qPN4.1s* intervals, and we need to add more markers in this region to fill this gap.

Straw dry weight per plant (StDW): Four QTLs for straw dry weight were mapped on chromosomes 4, 7, 8 and 9, with R^2 values between 6.2 and 9.6. Among them, *qSTW4.1s* contributed 9.6% of the total phenotypic variation, with the FL478 allele providing an increased effect of 5.39 g. *qSTW7.1s* and *qSTW9.1s*, with the FL478 alleles, also had an increased effect on straw dry weight. *qSTW8.1s* contributed 6.2% of the total phenotypic variation, with the Sadri allele providing a positive effect of 5.05 g.

Number of fertile spikelets per plant (FrSp): In total, four QTLs were detected for number of fertile spikelets. They were designated as *qFRSP2.1s*, *qFRSP4.1s*, *qFRSP6.1s* and *qFRSP10.1s*. *qFRSP2.1s* was identified in the region of RM423–RM174 on chromosome 2, and explained 6.3% of the total phenotypic variation. Another QTL, *qFRSP6.1s* was mapped near marker RM275 on chromosome 6 and contributed 7.7% of the total phenotypic variation. The Sadri alleles had an additive effect at *qFRSP2.1s* and *qFRSP6.1s* loci of 110 and 108 fertile spikelets, respectively. The other two QTLs on chromosomes 4 and 10 accounted for 8.3 and 5.9% of the phenotypic variation. The FL478 alleles had a positive effect at these two loci by 105 and 114 spikelets, respectively. The dominant effects for *qFRSP4.1s* were increased FrSp by the Sadri allele (table 3).

Number of sterile spikelets per plant (StSp): Two QTLs were detected for number of sterile spikelets. *qSTSP3.1s* was detected near marker RM282 located on chromosome 3 and accounted for 9.2% of the phenotypic variation. The Sadri allele had an additive effect of 285 sterile spikelets. The other QTL, *qSTSP7.1s*, located on chromosome 7 near RM11, accounted for 7.1% of the total phenotypic variation. The FL478 allele had an additive effect of 47 sterile spikelets (table 3).

Number of total spikelets per plant (TSp): Three QTLs were detected for TSp on chromosomes 4, 7 and 9, collectively accounting for 27% of the total phenotypic variation. The larger-effect QTL, *qTSP4.1s*, flanked by RM551 and RM518 on chromosome 4, explained 12.4% of the variation. *qTSP7.1s* mapped in the region of RM445–RM11 on chromosome 7 contributed 7.8% of the phenotypic variation. Another QTL, *qTSP9.1s*, mapped near marker RM410 on chromosome 9, contributed 6.9% of the phenotypic variation. The alleles of these QTLs, which increased the number of total spikelets, came from FL478 (table 3).

Grain yield per plant (GYld): Four QTLs were detected for grain yield on chromosomes 2, 4, 6 and 8 and in total explained 31.6% of the phenotypic variation. The GYld QTLs under salinity stress were designated as *qGY2.1s*, *qGY4.1s*, *qGY6.1s* and *qGY8.1s*, respectively (table 3). *qGY2.1s* was detected in the region of RM555–RM324 located on chromosome 2 and accounted for 6.9% of the phenotypic variation. *qGY6.1s* mapped near marker RM275 on chromosome 6 contributed 8.4% of the phenotypic variation. The Sadri alleles at *qGY2.1s* and *qGY6.1s* increased grain yield by 3.2 g per plant. The other two QTLs affecting grain yield per plant, *qGY4.1s* and *qGY8.1s*, were identified on chromosomes 4 and 8, and accounted for 7.9 and 8.4% of the variation. The FL478 alleles by additive effects could increase grain yield at these two loci by 2.7 and 3.6 g per plant, respectively (table 3).

Spikelet fertility per cent (SpFr): Only a single QTL for spikelet fertility was detected near the marker RM555 located on chromosome 2 through CIM. This QTL, *qSPFR2.1s*, accounted for 7.9% of the phenotypic variation. The allele from Sadri could increase SpFr by 9.3%. While using IM, two more QTLs were detected on chromosomes 5 and 10 for spikelet fertility (table 3).

1000-grain weight (TGW): Two QTLs for TGW were detected, collectively explaining 61.4% of the phenotypic variation (table 3). A major QTL, *qTGW8.1s*, with a very large effect was detected under salt stress for TGW. This QTL was located within the RM80–RM281 region on chromosome 8 and accounted for 30% of the total phenotypic variation, and the FL478 allele had an additive effect of 2.95 g to increase TGW. The other large-effect QTL, *qTGW10.1s*, flanked by RM271 and RM484 on chromosome 10, accounted for 17.6% of the total variation. The FL478 allele could increase TGW by 2.36 g. The other two QTLs for 1000-grain weight, *qTGW5.1s* and *qTGW6.1s*, were identified on chromosomes 5 and 6 and accounted for 7.6 and 6.2% of the phenotypic variation. The Sadri alleles could increase TGW at both loci by 1 g.

QTL clusters: Four QTL clusters were observed on chromosomes 2, 4, 6 and 9. The first QTL cluster, flanked by RM423 and RM174 on the short arm of chromosome 3, contained three QTLs for FrSp, SpFr and GYld (figure 2). The allele from Sadri at these loci increased FrSp, SpFr and GYld. The second QTL cluster was flanked by RM551 and RM518 on the short arm of chromosome 4, including *qSTW4.1s*, *qFRSP4.1s, qFRSP4.1s*, *qTSP4.1s* and *qGY4.1s*. The FL478 allele of these QTLs could increase StDW, FrSp, TSp and GYld. The third QTL cluster was located in the interval of RM20224–RM528 on the long arm of chromosome 6. It contained QTLs for PnN, FrSp, GYld and TGW (figure 2). The Sadri allele had a positive effect on all four traits. The fourth QTL cluster was associated with RM242 on chromosome 9, including *qPN9.1s*, *qSTW9.1s* and *qTSP9.1s*. The allele from FL478 at this region could increase PnN, StDW and TSp. This region has been reported as a QTL cluster for yield components (Xie *et al.* 2008).

QTLs for correlated traits were often mapped in the same chromosomal regions (Veldboom *et al.* 1994). This trend was observed in our study. For example, StDW and GYld were correlated and had two QTLs, *qSTW4.1s* and *qGY4.1s*, which were found at the same map locations on chromosome 4. TGW and GYld also showed a high correlation and had two QTLs, *qTGW8.1s* and *qGY8.1s*, in a similar location on chromosome 8 (table 2). In both cases, the directions of the correlations were consistent with that of the effects of the QTLs on the traits. These colocalized QTLs could be attributed to pleiotropy or tightly linked genes.

The significant threshold of each putative QTL detected by CIM was reconfirmed using 10,000 permutations for the

presence of each QTL across the 12 chromosomes (Churchill and Doerge 1994). The significance level of the detected QTLs had average LOD scores greater than 3.6 with $P < 0.05$. Using permutations, 28 QTLs out of the 35 detected QTLs were reconfirmed. QTLs reconfirmed through these permutations may potentially be useful as fine-mapping targets for map-based gene cloning and functional analysis and also for breeding by marker-assisted backcrossing (MABC).

Discussion

Both parental lines, Sadri and FL478, contributed additive effects for QTLs for days to flowering, plant height, number of panicles, straw dry weight, number of fertile spikelets, number of sterile spikelets, grain yield per plant and 1000-grain weight. Sadri contributed additive effects in the QTLs for panicle length and spikelet fertility, while FL478 contributed additive effects in the QTLs for total spikelet number. A majority of the QTLs detected in our study are reported for the first time for the yield components of rice under reproductive stage salinity stress.

Moreover, identifying additional salt tolerance-related QTLs from different donors would give rice breeders a wider option in combining superior QTLs into one genetic background using gene-pyramiding techniques, since the commonly used donor parents for salinity tolerance seem to possess a few superior tolerance traits (Ismail *et al.* 2007). Combining genes for tolerance at all developmental stages will facilitate the development and release of new robust rice varieties with substantially higher salt tolerance (Thomson *et al.* 2010). Most QTL mapping studies have been limited to seedling-stage tolerance because phenotyping for salinity tolerance at reproductive stage is very tedious and time-consuming. There have been only few reports on QTL analysis of rice salt tolerance at the reproductive stage but mostly unpublished (Singh and Flowers 2010). Islam (2004) evaluated 80 RILs of IR29/Pokkali at the reproductive stage using salinized water (EC = 5 dS m^{-1}) at the IRRI greenhouse and reported a QTL on short arm of chromosome 4 for per cent reduction for biomass weight and another QTL for per cent reduction in filled grain weight on chromosome 7 flanked by RM445 and RM11. In our study, a QTL was mapped on the short arm of chromosome 4 for straw dry weight, which is similar to the finding of Islam (2004). Palao *et al.* (Palao C. D. C., Vina C. B., Gregorio G. B., Thomson M. J. and Singh R. K. 2012 Identification of major QTLs for salinity tolerance at the reproductive stage in rice (*Oryza sativa* L.), Abstract in the first IRRI Young Scientists Conference, IRRI, Los Baños, Philippines, 8–9 November 2012) used 201 F_2 plants from IR64/IR4630-22-2-5-1-3 for QTL mapping under salinity stress at the reproductive stage in rice in greenhouse conditions. F_2 plants were exposed to salinity stress at EC_e 10 dS m^{-1} before the booting stage. Thirty-four QTLs were detected for traits such as plant height, panicle length, single-grain weight, straw weight, root dry weight, %Na, %K and Na–K ratio. But their study found some unique QTLs for plant height, panicle length and straw weight, which did not coincide with the QTLs found in our study. This may be attributed to the different populations used and screening conditions employed.

Our study supported claims from previous studies that the genes governing salinity tolerance at the seedling and reproductive-stage are very different. No QTLs were detected on the short arm of chromosome 1 where the *Saltol* QTL and SKC1 have been reported. This reconfirms that the genes and QTLs underlying the mecahnism for seedling and reproductive stages salinity are very different and pooling of both mechanisms in one background could be a possible answer for salinity tolerance throughout crop growth with sustainable grain yield.

The Sadri/FL478 F_2 population showed a good combination of seedling and reproductive stage salinity tolerance. This population has seedling and reproductive-stage tolerance genes from FL478 and reproductive-stage tolerance genes from Sadri. The F_3 population of Sadri/FL478 is currently available. This population will be screened under salinity stress at both the seedling and reproductive stages and salt tolerant progenies will proceed to advanced generations to breed salt-tolerant rice lines. The advanced population could be used to validate QTL effects for MABC. Fine-mapping of selected QTLs will help to identify closely linked markers for use in MABC to supplement the breeding programmes.

The knowledge of QTLs for salt tolerance will be a valuable tool in future plant breeding programmes for producing high-yield rice varieties for salt-affected environments. Pyramiding salt-tolerance QTLs using MAS will be useful for the development of new varieties with high salt tolerance for salinity-affected regions (Lin *et al.* 2004). QTLs identified for both the seedling and reproductive stages could be used to combine genes controlling different physiological mechanisms into a single genetic background to enhance salt tolerance of rice.

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