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Quantitative trait locus mapping for salt tolerance at maturity stage in indica rice using replicated F_2 population

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Abstract Salinity is one of the major abiotic stresses limiting rice production worldwide. A quantitative trait locus (QTL) mapping study was conducted to identify genomic regions involved in salt tolerance in rice (*Oryza sativa* L.) using F_2 population derived from the cross of indica rice cultivars IR36 (salt susceptible) and Pokkali (salt tolerant). Plant material was phenotyped for morphological and yield-related traits at maturity stage under saline and normal conditions. Plant material was screened for polymorphism using five hundred and fifty-three simple sequence repeat markers, out of which one hundred and eleven were found polymorphic. Six QTLs for different agronomic traits, associated with salt tolerance, were identified. Phenotypic variance explained (R^2) values of QTLs ranged from 11.52 to 81.56 %. Genomic regions having strong correlation with salt tolerance were identified on chromosomes 2, 3, 7, and 8. These genomic regions can be targeted for positional cloning and identification of genes involved in salt tolerance in rice. Identified markers, associated with salt tolerance, can be used in marker-assisted breeding for developing salt-tolerant rice cultivars.

Keywords Quantitative trait locus (QTL) · Rice · Salinity tolerance · Yield

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Introduction

Rice (*Oryza sativa* L.) is the major source of food for human population throughout the world (FAO 2009). Many biotic and abiotic stresses affect rice production worldwide. Abiotic stresses render about 50 % of the total yield losses, among them soil/water salinity is the major limiting factor for rice production (Ren et al. 2010). Approximately 30 % of the total irrigated land of the world is salt-affected (Rengasamy 2006). To feed the increasing human population, it is becoming indispensable to utilize these saline soils either by reclamation practices or by growing salt-tolerant plants (Saeed et al. 2012). Reclamation of saline soils is complicated and costly, but not the permanent solution of the problem. Introduction of salt-tolerant varieties is the realistic approach to obtain better yield under saline conditions (Shannon et al. 1998; Saeed et al. 2012). Salinity affects rice growth and development in varying degrees mostly at all growth stages starting from germination to maturity. In rice, seedling and maturity stages are most sensitive to salinity. By transplanting older seedlings (about 30 days old), sensitivity at seedling stage can be managed. However, sensitivity at maturity stage cannot be escaped and this leads to low rice productivity in saline areas.

With the advent of molecular markers, it is now feasible to identify the chromosomal regions associated with different traits in different crops. QTLs have been detected in maize (Veldboom et al. 1994), wheat (Barakat et al. 2013; Lohwasser et al. 2013), and barley (Takeda and Mano 1997; Lohwasser et al. 2013). With the use of quantitative trait locus (QTL) mapping approach, it is possible to identify the chromosomal regions associated with traits related to salt tolerance in rice (Yano and Sasaki 1997). The genes governing salt tolerance can be independently

traced in a segregating population and pyramided via molecular marker-assisted selection (MAS) in the breeding of new varieties with strong tolerance to salt stress. Thus, in the light of screening the rice genotypes based on morphological and yield-related parameters, DNA-based screening would be beneficial by saving cost, time, and labor. Salinity tolerance is a complex trait and there is limited research carried out to investigate the genetics of salinity tolerance in rice. Most research works regarding salinity tolerance in rice were conducted to determine the effects of salinity on rice production (Nozulaidi et al. 2015) or the effects of salinity by screening genotypes at seedling stage (Khan et al. 1997) or maturity stage (Khan et al. 2013). QTL analysis for salinity tolerance at seedling stage was conducted by a number of researchers in recent years (Lin et al. 2004; Lee et al. 2007; Wang et al. 2011b, 2012a, b; Liang et al. 2014; Bimpong et al. 2014; Krishnamurthy et al. 2015). Rice is sensitive to salinity at the maturity stage also. Keeping in view this fact, the present research project was designed to identify QTLs associated with agronomic traits under salt stress conditions at the maturity stage and explore genomic regions associated with salt tolerance in rice.

Materials and methods

Plant material

Oryza sativa var. Pokkali is a traditional salinity-tolerant rice variety (Xie et al. 2000; Lee et al. 2006), whereas *Oryza sativa* var. IR-36 is sensitive to salinity and performs poorly in saline fields (Faustino et al. 1996). Salinity-sensitive rice variety (IR-36) was crossed with salt-tolerant variety (Pokkali) in 2008 at the School of Biological Sciences, University of the Punjab, Lahore, Pakistan, and F_1 seeds of the cross (IR-36 × Pokkali) were harvested. F_1 seeds of the cross (IR-36 × Pokkali) were sown and F_2 seeds were harvested during 2009. F_2 population was developed by sowing F_2 seeds in 2010.

Construction of salinity blocks

Two salinity blocks were constructed to escape the experimental error and to obtain accurate results. Salinity blocks (each of length 12.20 m × width 6.10 m × depth 1.52 m) with glass roofs were constructed for proper light reception for plant growth and also to avoid dilution of salt by rainfall. Soil was collected from saline areas of District Sheikhupura, Punjab, Pakistan, having electrical conductivity of 6–9 dS m^{-1} and used in the salinity blocks. Leaching, percolation, and surface runoff of soil water were strictly controlled so that electrical conductivity remained constant.

Phenotyping F_2 population for agronomic traits and data analysis

Plants of F_2 population were grown in beds maintaining seedling-to-seedling and row-to-row distances of 2.54 and 7.62 cm, respectively. One hundred and thirteen seedlings of F_2 population were grown and at the age of 30 days each plant was separated into four plantlets by peeling off the tillers. Two identical plantlets (clones) were transplanted in salinity blocks as two replications and two other plantlets were transplanted in normal soil as control. Soil analysis report of saline and normal (control) soil is mentioned in Table 1. Plant-to-plant and row-to-row distances were maintained at 22.86 and 30.48 cm, respectively. Data regarding agronomic traits were collected at maturity stage and only days to 50 % flowering trait was recorded at 50 % flowering stage for each rice F_2 individual grown under saline and normal field conditions. Data were recorded for plant height (PH), number of total tillers $plant^{-1}$ (TTP), number of effective tillers $plant^{-1}$ (ETP), panicle weight (PaW), panicle length (PaL), number of spikelets $panicle^{-1}$ (SpPa), number of unfilled grains $panicle^{-1}$ (UfGPa), number of grains $panicle^{-1}$ (GPa), panicle fertility (%) (PaF), days to 50 % flowering (DFF), days to maturity (DM), grain length (GL), grain width (GW), grain length–

Table 1 Soil analysis report of salinity block and normal field

Parameter	Soil sample collected from salinity block	Soil sample collected from normal field
EC (dS m^{-1})	7.5	2.6
Available sodium (mg kg^{-1} or ppm)	4140	470
Available potassium (mg kg^{-1} or ppm)	130	96
Available calcium (mg kg^{-1} or ppm)	120	60
Available phosphorus (mg kg^{-1} or ppm)	13.9	8.5
pH	8.2	8.0
Organic matter (%)	0.42	0.77
Saturation (%)	40	40
Texture	Loam	Loam

EC Electrical conductivity

Table 2 Statistical parameters for agronomic traits of indica rice F_2 populations grown under normal and saline conditions

Traits	Minimum value		Maximum value		Mean value		Standard deviation		Skewness	
	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline
PH (cm)	73	61	175	120	121.94	90.52	29.66	15.32	0.55	0.21
TTP	6	1	12	12	9.28	5.56	1.28	2.81	0.24	−0.30
ETP	5	1	11	10	8.09	4.51	1.36	2.43	0.27	0.09
PaL (cm)	25	13	34	32	29.25	22.45	1.74	5.68	0.49	−0.40
PaW (g)	4.53	0.50	7.5	7.31	5.53	3.17	0.50	2.12	0.99	0.50
SpPa	245	40	332	305	281	163.32	18.74	77.60	0.90	0.30
UfGPa	8	10	25	39	14	21.77	5.35	6.41	0.96	0.13
GPa	223	14	324	285	267	141.55	22.83	81.31	0.47	0.32
PaF (%)	90.44	35	97.59	96.43	94.91	80.42	2.21	16.04	−0.92	−1.43
DFF	80	82	95	98	86.83	89.35	3.96	3.96	−0.09	−0.11
DM	110	112	125	128	116.83	119.35	3.96	3.96	−0.09	−0.11
GL (mm)	7	6.3	8	7.8	7.51	7.27	0.40	0.34	−0.06	−0.07
GW (mm)	2	1.8	3	3	2.55	2.41	0.39	0.38	−0.17	0.13
GLWR	2.33	2.33	4	4.33	3.03	3.11	0.60	0.59	0.43	0.39
TGW (g)	17	16.5	25	24.5	20.32	19.85	2.53	2.54	0.23	0.23
GY (g plant^{-1})	26.57	0.23	60.59	60.28	43.38	17.60	7.20	17.26	0.23	1.01
SY (g plant^{-1})	44.36	0.7	270.35	269.8	108.06	61.17	54.56	79.21	1.27	1.25
HI (%)	11.48	21.49	83.11	82.33	49.88	45.44	21.63	16.12	−0.16	0.15

PH plant height, *TTP* number of total tillers plant^{-1}, *ETP* number of effective tillers plant^{-1}, *PaW* panicle weight, *PaL* panicle length, *SpPa* number of spikelets panicle^{-1}, *UfGPa* number of unfilled grains panicle^{-1}, *GPa* number of grains panicle^{-1}, *PaF* panicle fertility, *DFF* days to 50 % flowering, *DM* days to maturity, *GL* grain length, *GW* grain width, *GLWR* grain length–width ratio, *TGW* 1000-grain weight, *GY* grain yield plant^{-1}, *SY* straw yield plant^{-1}, *HI* harvest index

Table 3 Analysis of variance estimates for agronomic traits of F_2 population grown under normal versus saline conditions

Source of variation/trait	Genotype	Salt	Genotype × salt
PH (cm)	2963.73***	170,129.20***	379.03***
TTP	8.48***	2352.76***	20.04***
ETP	4.81***	2166.58***	18.48***
PaL (cm)	30.35***	7829.52***	75.38***
PaW (g)	8.34***	942.03***	5.91***
SpPa	6201.78***	2,347,391.20***	12,916.69***
UfGPa	39.44***	10,256.30***	169.52***
GPa	5954.97***	2,667,973.39***	15,443.20***
PaF (%)	321.82***	35,589.34***	464.59***
DFF	93.63***	1078.21***	0.38^{ns}
DM	93.63***	1078.21***	0.38^{ns}
GL (mm)	0.77^{ns}	9.32**	0.06^{ns}
GW (mm)	0.85^{ns}	3.49^{ns}	0.03^{ns}
GLWR	2.07**	1.20^{ns}	0.04^{ns}
TGW (g)	38.49***	36.73***	0.01^{ns}
GY (g plant^{-1})	307.00***	112,620.10***	742.04***
SY (g plant^{-1})	26,518.65***	372,716.65***	1231.25***
HI (%)	1924.57***	3340.64***	258.59***

PH plant height, *TTP* number of total tillers plant^{-1}, *ETP* number of effective tillers plant^{-1}, *PaW* panicle weight, *PaL* panicle length, *SpPa* number of spikelets panicle^{-1}, *UfGPa* number of unfilled grains panicle^{-1}, *GPa* number of grains panicle^{-1}, *PaF* panicle fertility, *DFF* days to 50 % flowering, *DM* days to maturity, *GL* grain length, *GW* grain width, *GLWR* grain length–width ratio, *TGW* 1000-grain weight, *GY* grain yield plant^{-1}, *SY* straw yield plant^{-1}, *HI* harvest index

ns non-significant; Significant at * $P \leq 0.05$; Significant at ** $P \leq 0.01$; Significant at *** $P \leq 0.001$

Table 4 Correlation coefficients for agronomic traits of indica rice F_2 population grown under normal versus saline conditions

	PH (cm)	TTP	ETP	PaL (cm)	PaW (g)	SpPa	UfGpa	GPa	PaF (%)
PH (cm)	1	−0.71***	−0.77***	−0.78***	0.36***	−0.77***	0.95***	−0.85***	−0.96***
		0.87* **	**0.89*** **	**0.93*** **	**0.95*** **	**0.94*** **	**−0.60*** **	**0.95*** **	**0.80*** **
TTP		1	0.90***	0.63***	−0.22*	0.61***	−0.68***	0.66***	0.69***
			0.97* **	**0.91*** **	**0.87*** **	**0.87*** **	**−0.55*** **	**0.95*** **	**0.83*** **
ETP			1	0.69***	−0.33***	0.68***	−0.72***	0.73***	0.73***
				0.90* **	**0.90*** **	**0.90*** **	**−0.59*** **	**0.91*** **	**0.81*** **
PaL (cm)				1	−0.07^{ns}	0.96***	−0.74***	0.96***	0.78***
					0.94* **	**0.94*** **	**−0.53*** **	**0.94*** **	**0.85*** **
PaW (g)					1	−0.08^{ns}	0.31***	−0.14^{ns}	−0.28**
						0.99* **	**−0.60*** **	**0.99*** **	**0.77*** **
SpPa						1	−0.71***	0.99***	0.75***
							−0.55* **	**1.00*** **	**0.83*** **
UfGpa							1	−0.81***	−1.00***
								−0.61* **	**−0.59*** **
GPa								1	0.85***
									0.84* **
PaF (%)									1
DFF									
DM									
GL (mm)									
GW (mm)									
GLWR									
TGW (g)									
GY (g plant^{-1})									
SY (g plant^{-1})									
HI (%)									

	DFF	DM	GL (mm)	GW (mm)	GLWR	TGW (g)	GY (g plant^{-1})	SY (g plant^{-1})	HI (%)
PH (cm)	0.86***	0.86***	−0.76***	0.87***	−0.89***	0.80***	−0.62***	0.91***	−0.96***
	0.85* **	**0.85*** **	**−0.66*** **	**0.93*** **	**−0.91*** **	**0.79*** **	**0.89*** **	**0.84*** **	**−0.76*** **
TTP	−0.60***	−0.60***	0.60***	−0.59***	0.64***	−0.57***	0.81***	−0.58***	0.68***
	0.87* **	**0.87*** **	**−0.63*** **	**0.83*** **	**−0.85*** **	**0.72*** **	**0.84*** **	**0.75*** **	**−0.64*** **
ETP	−0.69***	−0.69***	0.61***	−0.73***	0.76***	−0.68***	0.86***	−0.64***	0.79***
	0.86* **	**0.86*** **	**−0.65*** **	**0.86*** **	**−0.87*** **	**0.73*** **	**0.91*** **	**0.84*** **	**−0.70*** **
PaL (cm)	−0.68***	−0.68***	0.58***	−0.73***	0.74***	−0.64***	0.70***	−0.68***	0.79***
	0.91* **	**0.91*** **	**−0.68*** **	**0.90*** **	**−0.93*** **	**0.81*** **	**0.85*** **	**0.78*** **	**−0.70*** **
PaW (g)	0.46***	0.46***	−0.04***	0.60***	−0.49***	0.78***	0.19*	0.34***	−0.35***
	0.87* **	**0.87*** **	**−0.67*** **	**0.94*** **	**−0.91*** **	**0.81*** **	**0.96*** **	**0.93*** **	**−0.79*** **
SpPa	−0.71***	−0.71***	0.54***	−0.75***	0.77***	−0.66***	0.68***	−0.64***	0.81***
	0.87* **	**0.87*** **	**−0.68*** **	**0.91*** **	**−0.90*** **	**0.79*** **	**0.90*** **	**0.90*** **	**−0.71*** **
UfGpa	0.78***	0.78***	−0.75***	0.79***	−0.80***	0.74***	−0.60***	0.91***	−0.91***
	−0.56* **	**−0.56*** **	**0.56*** **	**−0.62*** **	**0.63*** **	**−0.52*** **	**−0.61*** **	**−0.60*** **	**0.60*** **
GPa	−0.76***	−0.76***	0.62***	−0.80***	0.82***	−0.71***	0.70***	−0.74***	0.88***
	0.88* **	**0.88*** **	**−0.69*** **	**0.92*** **	**−0.91*** **	**0.80*** **	**0.95*** **	**0.91*** **	**−0.73*** **
PaF (%)	−0.79***	−0.79***	0.75***	−0.80***	0.81***	−0.74***	0.63***	−0.91***	0.92***
	0.81* **	**0.81*** **	**−0.62*** **	**0.75*** **	**−0.81*** **	**0.71*** **	**0.68*** **	**0.59*** **	**−0.38*** **
DFF	1	1	−0.63***	0.85***	−0.87***	0.80***	−0.47***	0.76***	−0.88***
			−0.62* **	**0.84*** **	**−0.86*** **	**0.78*** **	**0.80*** **	**0.73*** **	**−0.64*** **

Table 4 continued

	DFF	DM	GL (mm)	GW (mm)	GLWR	TGW (g)	GY (g plant^{-1})	SY (g plant^{-1})	HI (%)
DM		1	−0.63***	0.85***	−0.87***	0.80***	−0.47***	0.76***	−0.88***
			−0.63***	**0.84*****	**−0.86*****	**0.78*****	**0.80*****	**0.73*****	**−0.64*****
GL (mm)			1	−0.58***	0.72***	−0.42***	0.61***	−0.70***	0.74***
				−0.61***	**0.75*****	**−0.43*****	**−0.65*****	**−0.62*****	**0.59*****
GW (mm)				1	−0.97***	0.92***	−0.43***	0.77***	−0.88***
					−0.97***	**0.85*****	**0.88*****	**0.84*****	**−0.78*****
GLWR					1	−0.85***	0.53***	−0.77***	0.92***
						−0.82***	**−0.84*****	**−0.78*****	**0.75*****
TGW (g)						1	−0.30***	0.72***	−0.80***
							0.75***	**0.68*****	**−0.55*****
GY (g plant^{-1})							1	−0.50***	0.64***
								0.98***	**−0.79*****
SY (g plant^{-1})								1	−0.90***
									−0.81***
HI (%)									1

PH plant height, *TTP* number of total tillers plant^{-1}, *ETP* number of effective tillers plant^{-1}, *PaW* panicle weight, *PaL* panicle length, *SpPa* number of spikelets panicle^{-1}, *UfGPa* number of unfilled grains panicle^{-1}, *GPa* number of grains panicle^{-1}, *PaF* panicle fertility, *DFF* days to 50 % flowering, *DM* days to maturity, *GL* grain length, *GW* grain width, *GLWR* grain length–width ratio, *TGW* 1000-grain weight, *GY* grain yield plant^{-1}, *SY* straw yield plant^{-1}, *HI* harvest index

ns non-significant; Significant at * $P \leq 0.05$; Significant at ** $P \leq 0.01$; Significant at *** $P \leq 0.001$; Normal-sized font values are of normal field; bold-sized font values are of salinity block

width ratio (GLWR), 1000-grain weight (TGW), grain yield plant^{-1} (GY), straw yield plant^{-1} (SY), and harvest index (%) (HI). PH, PaL, GL, and GW were measured using a measuring scale. GLWR was recorded by dividing GL with GW. TTP, ETP, and GPa were counted. PaW and TGW were recorded using a weighing machine. Days required from germination to 50 % flowering stage was recorded as DFF and days required from germination to maturity stage was recorded as DM. Straw (excluding panicle and roots) for each plant was collected at maturity stage; it was weighed and thus SY was recorded. PaF, GY, and HI were calculated, respectively, as $\text{PaF} = (\text{ETP TTP}^{-1})\,100$; $\text{GY} = \text{ETP GPa (TGW } 1000^{-1})$; and $\text{HI} = (\text{GY SY}^{-1})\,100$. Data were analyzed using the statistical software COSTAT v. 6.303.

SSR genotyping and QTL identification

DNA was extracted from leaves of parental rice varieties (IR36, Pokkali), F_1 and 113 F_2 individuals. Parental rice varieties were screened for polymorphism using five hundred and fifty-three simple sequence repeat (SSR) markers. One hundred and eleven SSR markers were found polymorphic and used for genotyping the 113 F_2 individuals. The molecular linkage map was constructed using the software JoinMap v.3.0. For the construction of linkage groups, logarithm of odds (LOD) score 3.0 was used. Using WinQTL Cartographer v.2.5 software, QTLs were identified by composite interval mapping (CIM) method. QTL identification was declared significant if LOD score was ≥3.0.

Results

Phenotypic variation

All F_2 individuals showed variation under saline and control treatments with respect to each morphological and yield trait (Table 2). Traits that showed reduction under saline conditions were PH, TTP, ETP, PaL, PaW, SpPa, GPa, PaF, GL, GW, TGW, GY, SY, and HI. Traits that showed increase under saline conditions were UfGPa, DFF, DM, and GLWR. UfGPa was markedly higher under the saline conditions compared to the control. This showed that salinity adversely affected grain formation. GPa was reduced under saline conditions and this trait was significantly responsible for reduced GY under saline conditions (Table 2).

Analysis of variance (ANOVA) showed that significant differences were found for salt, genotypes, and salt × genotype interactions for all the traits except GW and GLWR (Table 3).

PH, TTP, ETP, PaL, PaW, SpPa, GPa, PaF, GL, DFF, and DM showed positive correlation with GY under saline conditions (Table 4). Under normal field conditions, DFF and DM had highly significant negative correlation with GY ($r = -0.47$; $P \leq 0.01$), whereas under saline

conditions these traits had highly significant positive correlation ($r = 0.80$; $P \leq 0.001$). UfGPa had highly significant negative correlation with GY under saline ($r = -0.614$; $P \leq 0.001$) and normal conditions ($r = -0.600$; $P \leq 0.001$). PH had highly significant positive correlation with DFF and DM under both saline and normal conditions. PH also had highly significant positive correlation with GY under saline condition, whereas under normal field conditions it had negative correlation with GY ($r = -0.62$; $P \leq 0.001$). DFF and DM had highly significant positive correlation with TTP, ETP, PaL, SpPa, GPa, PaF, and GY under saline condition, whereas under normal field conditions, the correlation was negative. SY was negatively correlated with GY under control conditions, but under saline conditions SY was positively correlated with GY (Table 4). Frequency distribution of the morphological and yield-related traits showed that a reduction in PH, TTP, ETP, PaL, SpPa, GPa, PaF, GW, GLWR, and GY was observed for greater number of F_2 individuals under saline condition (Fig. 1).

Linkage map construction

Genetic linkage map consisted of 74 SSR markers, spanning 19 linkage groups and covering all 12 rice chromosomes. The abbreviation LG was used to designate specific chromosome such as three linkage groups identified for chromosome 2 were designated LG2_1, LG2_2, and LG2_3. Genetic linkage map covered a total length of 866.43 cM (centiMorgans) (Fig. 2). LG8_1 contained 15 markers in a total length of 106 cM. The average distance between two markers on this linkage group was 7.57 cM. Two closely located markers were RM136 and RM469 located on LG6. Distance between these markers was 1 cM. Overall average distance between the two markers was 11.87 cM (Fig. 2).

QTL identification

Six significant QTLs were identified in this study. Out of these six QTLs, five were identified for relative value

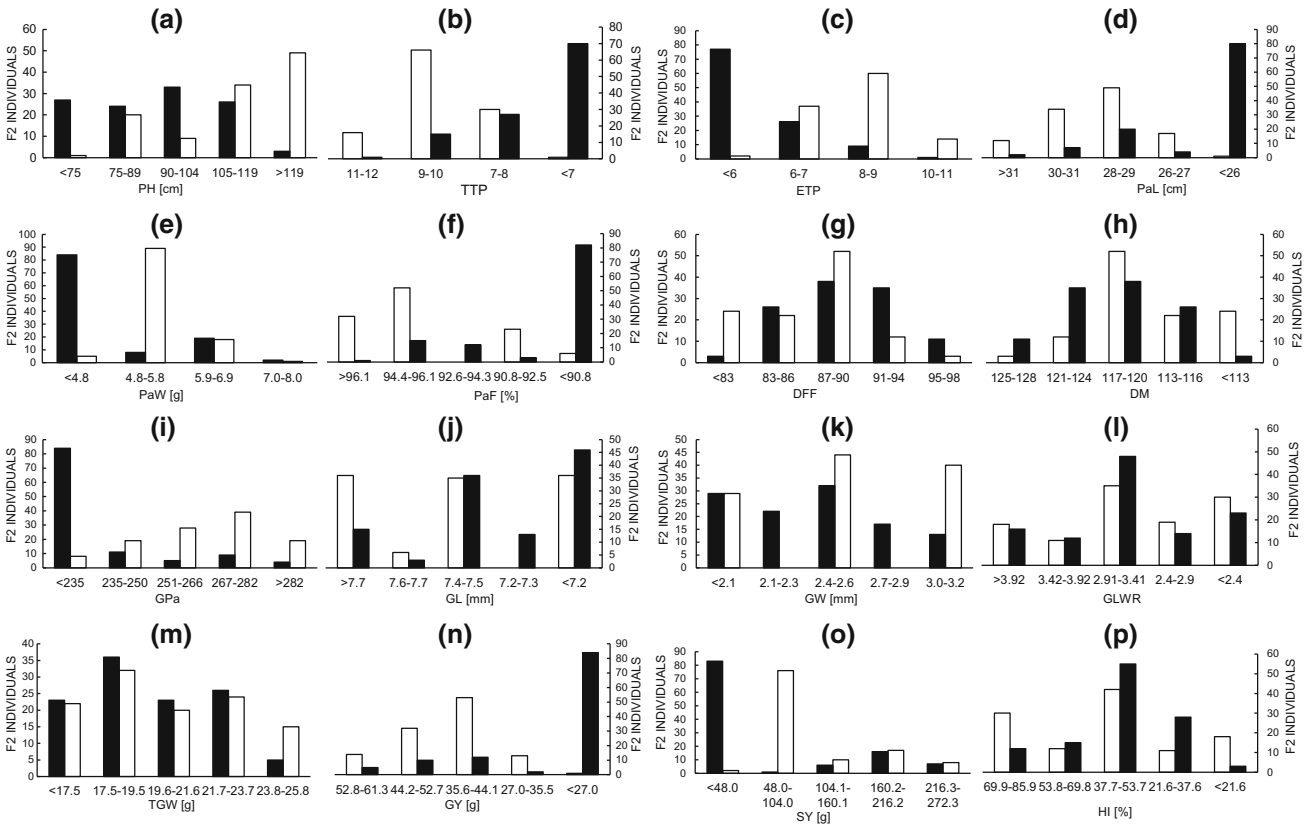

Fig. 1 Frequency distribution of agronomic traits of indica rice F_2 population grown under normal versus saline conditions: plant height (**a**), number of total tillers plant^{-1} (**b**), number of effective tillers plant^{-1} (**c**), panicle length (**d**), panicle weight (**e**), panicle fertility (**f**), days to 50 % flowering (**g**), days to maturity (**h**), number of grain panicle^{-1} (**i**), grain length (**j**), grain width (**k**), grain length–width ratio (**l**), 1000-grain weight (**m**), grain yield (**n**), straw yield (**o**), and harvest index (**p**). *Solid bars* represent saline and *hollow bars* represent normal conditions dataset

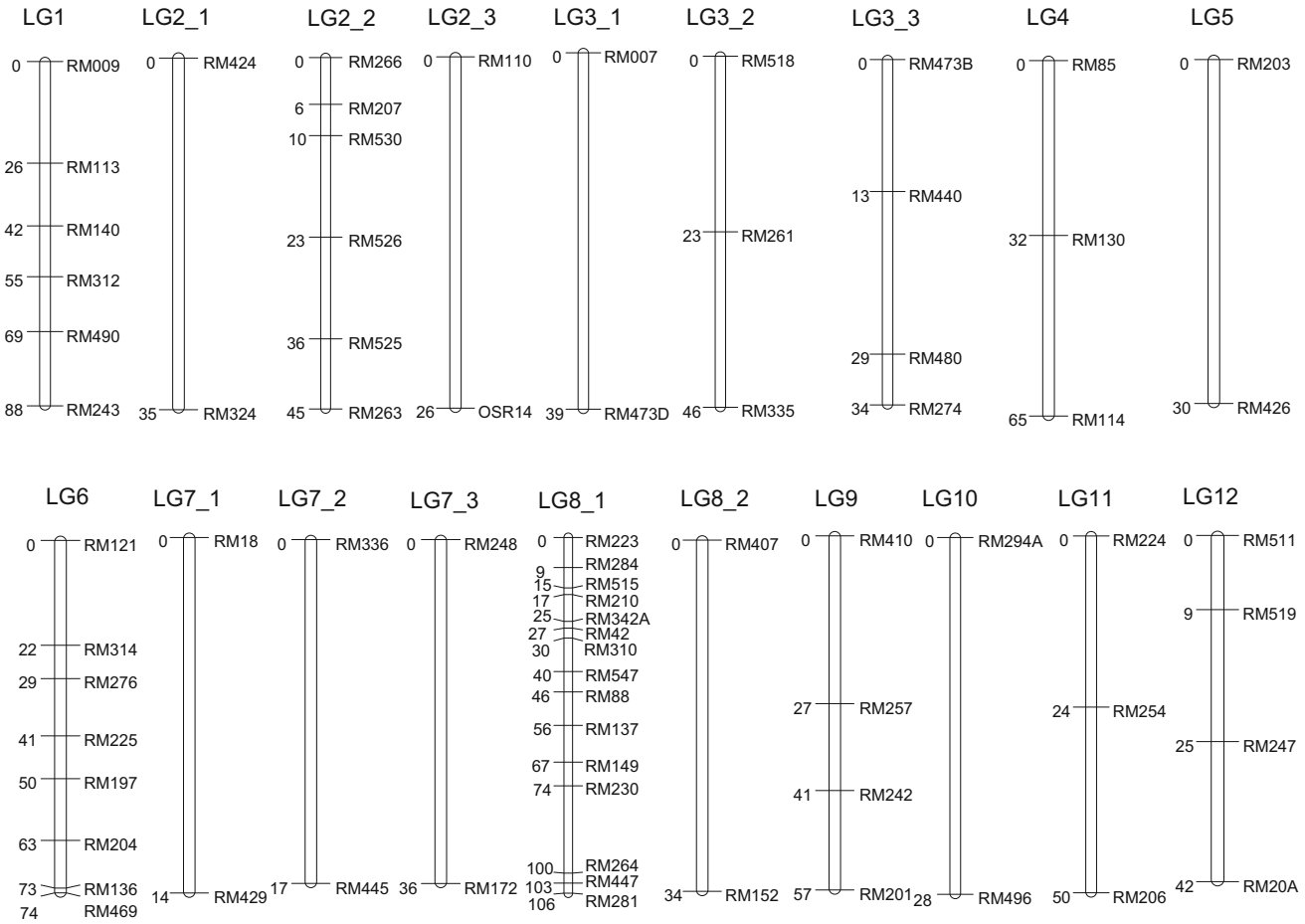

Fig. 2 Linkage map of rice chromosomes 1 to 12

Table 5 Identified QTLs for agronomic traits of indica rice under normal and saline conditions

Trait name	Treatment	QTL	Flanking markers	Chr	a[a]	d[b]	LOD[c]	R^2 (%)	Direction
GLWR	Relative	*qGLWR-2*	RM207-RM530	2	−0.10	−0.07	3.87 (2.35)	14.4	IR36
TGW (g)	Relative	*qTGW-2*	RM526-RM525	2	−0.003	0.01	4.6 (3.42)	15.02	IR36
HI (%)	Relative	*qHI-8*	RM342A-RM42	8	−0.14	−0.05	3.38 (3.32)	11.52	IR36
UfGPa	Saline	*qUfGPa-7*	RM336-RM445	7	4.24	−2.17	3.08 (3.6)	13.85	Pokkali
DM	Relative	*qDM-8*	RM407-RM152	8	−0.002	−0.001	3.12 (50.65)	12.16	IR36
SY (g plant^{-1})	Relative	*qSY-3*	RM007-RM473D	3	0.07	−0.79	14.83 (20.25)	81.56	Pokkali

GLWR grain length–width ratio, *TGW* 1000-grain weight, *HI* harvest index, *UfGPa* number of unfilled grains panicle^{-1}, *DM* days to maturity, *SY* straw yield plant^{-1}

[a] Additive effect; [b] Dominance effect; [c] Logarithm of odds of QTL, threshold LOD of QTL is given within brackets; R^2 phenotypic variance explained

dataset and 1 was identified under saline conditions dataset (Table 5). One QTL (*qGLWR-2*) was identified for GLWR. *qGLWR-2* was identified for relative value dataset. An associated marker with this QTL was RM207 located on chromosome 2. Phenotypic variance explained (R^2) value for this QTL was 14.4 %. The increase in GLWR was contributed by salt-sensitive parent (IR36) allele. One QTL (*qTGW-2*) was identified for TGW. This QTL was identified for relative value dataset. An associated marker with this QTL was RM526 located on chromosome 2. R^2 value for this QTL was 15.02 %. The increase in TGW was due to the allelic contribution of salt-sensitive parent (IR36).

One QTL (*qHI-8*) was identified for HI. *qHI-8* was identified for relative value dataset. An associated marker for this QTL was RM342A located on chromosome 8. R^2 value for this QTL was 11.52 %. The allele responsible for an increase in HI was contributed by salt-sensitive parent (IR36). One QTL (*qUfGPa-7*) was identified for UfGPa. This QTL was identified for saline conditions dataset. An associated marker with this QTL was RM445 located on chromosome 7. R^2 value for this QTL was 13.85 %. The increase in UfGPa was due to the allele from salt-tolerant parent (Pokkali). One QTL (*qDM-8*) was identified for DM. This QTL was identified for relative value dataset. An associated marker with this QTL was RM407 located on chromosome 8. R^2 value for this QTL was 12.16 %. The increase in DM was contributed by the allele from salt-sensitive parent (IR36). A major QTL (*qSY-3*) was identified for SY. This QTL was identified for relative value dataset. An associated marker with this QTL was RM007 located on chromosome 3. R^2 value for this QTL was 81.56 %. Allele responsible for the increase in SY was contributed by the salt-tolerant parent (Pokkali). Additive effect of this locus was 0.07 and the dominance effect was −0.79. An associated marker with this QTL, RM007, will be a suitable candidate for MAS in molecular breeding programs for developing salt-tolerant rice cultivars. The identified major QTLs will give new dimensions to breeding especially with respect to the time required for developing new and improved rice varieties.

Discussion

Behavior of morphological and yield-related traits under salinity stress in rice

Salinity is detrimental to crop production worldwide. Among the cereal crops, rice (*Oryza sativa*) is the most salt sensitive, whereas bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are moderate and most salt-tolerant crops, respectively (Munns and Tester 2008). Salinity affects different agronomic traits like plant height, number of tillers, number of effective tillers, panicle length, panicle weight, number of grains per panicle, grain size, thousand-grain weight, grain yield, straw yield, harvest index, etc. Agronomic traits are affected according to severity of the salinity and susceptibility/tolerance of the crop variety (Dionisio-Sese and Tobita 1998). In the present study, significant differences were found under saline and controlled conditions for each morphological and yield trait of all F_2 individuals (Table 2). Saline conditions affected the metabolic processes involved in grain formation and UfGPa was markedly higher under the saline conditions compared to the controlled conditions. GPa was reduced under saline conditions and was responsible for lower GY under saline conditions. This finding agreed with the previous reports of Razzaque et al. (2010) and Afifi et al. (2010). Razzaque et al. (2010) also reported that percent relative plant height, total tillers, root dry weight, shoot dry weight, and total dry matter were higher in Pokkali and PVSB9, but lower in NS15 under different salinity and supplemental Ca^{+2} levels. These morphological characteristics significantly decreased with increasing salinity levels. Salinity stress caused a decrease in vegetative growth, yield, and yield components of rice (Afifi et al. 2010). Plant yield is also influenced directly or indirectly by a number of other agronomic characters, such as plant height, leaf area, dry matter yield, heading date, lodging resistance, proneness to shattering, and grain yield (Fitzgerald et al. 2010; Saeed et al. 2011). In some studies, effort was made to evaluate genotypic variation with respect to plant phenology and development and it was suggested that this variation might be used for screening genotypes for salinity tolerance (Khatun et al. 1995). Days to 50 % flowering and days to maturity delayed under salinity stress, and these traits were responsible for unfilled grains per panicle, resulting in lower grain yield (Khatun et al. 1995).

QTL identification for salinity stress tolerance and major genomic regions involved in abiotic stress tolerance

QTL identification associated with environmental stresses, yield, and quality is very crucial for the application of map-based cloning and MAS in rice breeding programs (Paterson et al. 1988; Tanksley 1993; Koyama et al. 2001; Kim et al. 2004; Lin et al. 2004; Kwon et al. 2008). Many QTLs related to important traits of rice were detected previously, as for heading date (Li et al. 1995; Yano and Sasaki 1997; Yano et al. 1997, 2001), yield (Lin et al. 1996; Xiao et al. 1996), and disease resistance (Wang et al. 2011a; Kwon et al. 2012). QTLs for salinity tolerance were detected at seedling stage both in indica and japonica rice (Lin et al. 2004; Lee et al. 2006, 2007). In the present work, QTL identification for salinity tolerance in rice at maturity stage was performed. Six QTLs (5 under relative value dataset and 1 under saline conditions dataset) for different agronomic traits were detected. Relative values give an indication of genotypes' potential under stress conditions compared to the control conditions. Important genomic regions correlated with GY were identified on chromosomes 2, 3, 7, and 8. These genomic regions were associated with GLWR, TGW, SY, UfGPa, and DM. TGW, DM, and SY had highly significant positive correlation with GY under saline conditions, whereas under normal conditions these traits were negatively correlated with GY. So these

genomic regions are important with regard to better yield under saline conditions. Previously, genes related to grain yield and grain weight have been identified on chromosomes 2 and 3 in these genomic regions (Song et al. 2007; Suji et al. 2012; Tang et al. 2013). One QTL (*qUfGPa-7*) for UfGPa was detected under saline conditions dataset. The associated marker with this QTL was RM445 located on chromosome 7 (Table 5). This result agreed with the findings of Zhang et al. (1995). Using the F_2 population, derived by crossing salt-tolerant rice mutant (M-20) and sensitive original variety (77–170), a major gene for salt tolerance was identified on chromosome 7 (Zhang et al. 1995). HI had highly significant positive correlation with GY under normal conditions, whereas it had highly significant negative correlation with GY under saline conditions. One QTL (*qHI-8*) was identified for HI located on chromosome 8. A major QTL (*qSY-3*) was identified for SY. Flanking markers of this QTL were RM007 and RM473D located on chromosome 3. Allele for the increase in straw yield came from Pokkali, salt-tolerant parent. Straw yield had positive correlation with grain yield under saline condition, whereas it had negative correlation with grain yield under normal conditions. It implied that this locus was expressed under stress conditions and favored grain yield. A QTL for shoot dry weight under ferrous iron toxicity in rice was previously reported for this interval (Dufey et al. 2012). This locus appeared to control vegetative growth of rice under stress conditions and better vegetative growth later translated to stable yield.

In the present study, six significant QTLs were identified. Out of these six QTLs, five were identified for relative value dataset and 1 was identified under saline conditions dataset. A major QTL (*qSY-3*) was identified for straw yield and the associated marker was RM007 located on chromosome 3. Major QTLs, along with their associated markers, can be good candidates for MAS in molecular plant breeding programs intended for developing salt-tolerant rice cultivars.

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