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QSY-3 CONTROLS MULTIPLE MORPHOLOGY, PHENOLOGY AND GRAIN YIELD TRAITS IN RICE (*ORYZA SATIVA* L.) UNDER SALINITY STRESS

M. S. K. Khan[1], M. Saeed[2*], S. Perveen[2], M. S. Akram[2] and J. Iqbal[1]

[1]School of Biological Sciences, University of the Punjab, Lahore, Pakistan.
[2]Department of Botany, Faculty of Life Sciences, Government College University, Faisalabad, Pakistan.

[*] Corresponding Author's email: saeed_pbg@gcuf.edu.pk

ABSTRACT

Rice (*Oryza sativa* L.) production, all over the world, is affected by salinity stress. A major quantitative trait locus (QTL), *qSY-3*, linked to rice straw yield was identified in our previous study. Nearest marker to this QTL was RM473D located on rice chromosome 3. Present study was designed to validate this QTL. A representative panel of twenty four rice varieties was used in this study. Panel consisted of salt-susceptible, as well as, salt-tolerant varieties. Panel was cultivated under saline, as well as, normal conditions and traits related to morphology, phenology, and grain yield were scored. Genotyping of the panel was done with 111 polymorphic simple sequence repeats (SSR) markers. Genotypic data were analyzed by STRUCTURE software to identify subpopulations in the panel and TASSEL software for marker-trait association assessment. Marker RM473D was found associated with eight traits including straw yield per plant (SY). Probability (*P*) and phenotypic variance explained (R^2) values for these associations ranged from 0.0001-0.0427 and 23.39%-54.86%, respectively. It appeared that *qSY-3* is a major locus controlling morphology and grain-yield in rice. In future, this locus can be exploited through marker-assisted breeding approach for the development of rice cultivars with good yield potential under salt stress conditions. Our study demonstrated potential of association mapping approach in validating previously identified QTLs.

Key words: association mapping, grain yield, morphology, phenology, quantitative trait locus, rice, salinity.

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop and is ranked at top with respect to its annual consumption as human food. For more than half of the world's population rice is a staple food and provides the maximum (26.2%) calories intake (FAO, 2009). About 1/10 of the world's cultivable land is under rice cultivation. Rice production is affected by a number of biotic and abiotic stresses. About 50% rice yield losses are reported due to abiotic stresses including soil/water salinity (Schleiff, 2008; Ren *et al*., 2010), which is the major problem of world irrigated land (Rengasamy, 2006). Soil/water salinity results in poor plant growth and development. Effects of salinity are manifested in the form of reduced crop yields, which have cultural and socio-economic impacts (Azeem *et al*., 2015; FAO, 2005). The growth and development of rice is disturbed by salinity which damages rice crop at all growth stages (Negrao *et al*., 2013). However, the most salt sensitive stages of rice are maturity and seedling stages (Reddy *et al*., 2017).

In view of increasing world population and the decrease in agricultural land area, appropriate use of saline soils is very important (Saeed *et al*., 2012). Saline soils can be made suitable for cultivation by integrating agronomic, reclamation, breeding and various other approaches (Akram *et al*., 2016; Habib *et al*., 2016). The best way to cope this problem is using salt-tolerant cultivars (Shannon *et al*., 1998; Saeed *et al*., 2012). This approach is economical and sustainable. For the development of salt-tolerant cultivars, molecular mapping approaches are very helpful. With the help of these approaches, DNA markers linked to salt-tolerant traits can be identified (Yano and Sasaki, 1997). These identified DNA markers can then be employed in marker-assisted selection (MAS) to develop salt-tolerant cultivars (Gao and Lin, 2013). In past, a number of quantitative trait loci (QTLs) had been identified in various crop plants by using molecular mapping approaches (Branham *et al*., 2017; Piasecka *et al*., 2017).

In QTL mapping approach, two techniques are in use i.e., a) linkage mapping, b) association mapping (Du *et al*., 2016; Zanke *et al*., 2017). In linkage mapping, bi-parental populations [F_2, back cross (BC), doubled haploid (DH), recombinant inbred lines (RILs), near isogenic lines (NILs) etc.] are used for mapping purposes. In genome-wide association mapping, commercial varieties/lines can be used for mapping purposes. Association mapping has higher resolution compared to linkage mapping (Thornsberry *et al*., 2001; Hirschhorn and Daly, 2005). We identified a major QTL, *qSY-3*, in rice (Khan *et al*., 2016). This QTL was associated with straw yield per plant (SY). It explained 81.56% phenotypic variance (R^2). So, this was a major QTL related to straw yield in rice. Nearest marker to this QTL

was RM473D. In the present study, we planned an association mapping project to confirm and validate this major QTL.

MATERIALS AND METHODS

Association mapping panel and phenotyping for salinity tolerance: Association mapping panel used in this study consisted of 24 rice varieties (Table S1). Seeds of these varieties were provided by Rice Research Institute, Kala Shah Kaku, Lahore, Pakistan. This panel contained salt-susceptible, salt-tolerant and diverse elite commercial cultivars of rice including Pokkali and IR-36, parents of F_2 population in linkage mapping experiment (Khan *et al*., 2016).

Plant material was phenotyped under salt stress and normal field conditions at the research field of School of Biological Sciences, University of the Punjab, Lahore, Pakistan during 2011. Two salinity blocks were constructed. Dimensions of each of these salinity blocks were length 12.20 m × width 6.10 m × depth 1.52 m. These salinity blocks had glass roofs for proper light reception and at the same time these provided hindrance for dilution of salt resulted by rainfall. Glass roofs were constructed at proper height so there was no significant rise in temperature as the salinity blocks were exposed to air from all four uncovered sides. In these salinity blocks soil collected from saline areas of District Sheikhupura, Punjab, Pakistan was utilized. Detail of soil parameters is given in Table S2. During entire period of experiment, electrical conductivity was kept constant by controlling surface runoff, percolation, and leaching of soil water. Rice seedlings were transplanted in salinity block and normal field by following split plot design having three replications. Salinity blocks and normal field were the main plots. In the subplots of main plots, genotypes were assigned. Thus, there were 72 experimental units with 24 varieties replicated three times under both treatments. Each experimental unit measured 0.63 m^2 (0.228 m seedling to seedling distance × 0.305 row to row distance × 9 seedlings). In each experimental unit, of both salinity block and normal field, 30 days old 9 seedlings of each variety were transplanted at spacing of 0.228 m × 0.305 m, thus each variety had 27 seedlings (9 seedlings × 3 replications) per treatment. Panel was grown to maturity. Data were scored for 18 traits related to morphology, phenology, and grain yield. Details of these traits are given in Khan *et al*. (2016). Data was analyzed by COSTAT version 6.303.

Genotyping of association mapping panel and QTL identification: Genotyping of association mapping panel was carried out at the School of Biological Sciences, University of the Punjab, Lahore, Pakistan during 2011. Phenotypic and genotypic data were analysed by genomics and bioinformatics softwares at the Department of Botany, Government College University, Faisalabad, Pakistan during 2011-2015. Fresh leaves from each variety of association mapping panel were collected for DNA extraction. Extraction was done by CTAB method (Doyle and Doyle, 1990). Simple sequence repeats (SSR) markers were used for genotyping of panel. Five hundred and fifty SSR markers were collected from USA. A subset of 10 varieties of panel was used to identify polymorphic SSR markers. This screening yielded 111 polymorphic markers and all 24 varieties were genotyped with these polymorphic markers. The generated genotypic data was analyzed by STRUCTURE v. 2.0 software (Pritchard *et al*., 2000) to identify subpopulations in the panel and TASSEL software (Bradbury *et al*., 2007) for marker-trait associations assessment. During TASSEL program, general linear model (GLM), as well as, mixed linear model (MLM) analysis was performed. Threshold to declare significant associations was set at $P \leq 0.05$.

RESULTS

Trait variation: Marked differences were observed in morphology, phenology and grain yield traits of rice varieties under saline and normal field conditions (Table 1). Some traits showed marked significant reduction under saline conditions. These traits were PH, TTP, ETP, PaL, PaW, SpPa, GPa, PaF, GL, GW, TGW, GY, SY and HI. Analysis of variance indicated that there were significant differences for treatment, genotypes, and treatment × genotype interactions for all traits except non-significant result for PaL and GLWR for salt treatment (Table 2).

Grain yield (GY) showed different type of correlation with other traits under salinity block and normal field data. Traits which showed significant positive correlation with GY included PH, TTP, ETP, PaL, PaW, SpPa, GPa, PaF and HI and traits which showed non-significant positive correlation included GL, GW, TGW and SY. Salinity affected grain formation badly and markedly decreased GY. Plant height (PH) is an important trait with respect to salinity tolerance. Under saline conditions, PH was found positively correlated with GY and this correlation was significant. While under normal field conditions, it had non-significant negative correlation with GY. Straw yield (SY) reflects vegetative growth potential of a plant and is important with respect to both biotic and abiotic stress tolerance of a plant. In this study, SY showed positive correlation with GY under saline conditions, whereas, under normal field conditions it had negative correlation with GY.

Population structure analysis: Population structure analysis, carried out by STRUCTURE v. 2.0 software, identified two sub-populations in the used rice association mapping panel. Two exotic rice varieties i.e.,

IR-36 and Pokkali were assigned to sub-population 1. Sub-population 1 also contained 8 varieties of Pakistan origin. These varieties were IR-6, KS-282, KSK-133, Sathra 278, TN-1, IRP-1, IRP-2, and SN 4365. Sub-population 2 contained 14 rice varieties. All these varieties originated from Pakistan. These varieties were Shaheen Basmati, Basmati 2000, Basmati 198, Super Basmati, Pak Basmati, Basmati 370, Basmati 385, Super Kernal, PB-95, B-515, Basmati 6129, SRI-8, SRI-12, and SHP.

QTL validation: Previously, we identified a major QTL, *qSY-3,* for SY by using linkage mapping (Khan *et al*., 2016). This QTL was positioned on chromosome 3. RM473D was the nearest marker to this QTL. In this study, marker RM473D was found associated with PH-normal, PH-saline, ETP-relative, UfGPa-normal, UfGPa-saline, PaF-normal, GW-normal, GW-saline, GLWR-normal, GLWR-saline, SY-saline, HI-normal, and HI-saline (Table 4). Under GLM and MLM analyses, *P* values for these associations were 0.0001-0.0104 and 0.0001-0.0427, respectively. Under GLM analysis, phenotypic variance explained (R^2) values for these associations ranged from 0.2339 to 0.5486. The highest R^2 value was observed for SY-saline. In this trait, allele of salt-tolerant cultivar, Pokkali, showed a considerable effect of 56.0164. Genetic variance values for these associations were 1.30E-06 to 210.0239. Heritability values for these associations were 1.00E-05 to 1. Results of this study, in combination with Khan *et al*. (2016) findings, demonstrated that *qSY-3* was a major locus which controlled multiple morphological and grain-yield related traits in rice. *qSY-3* might had pleiotropic effects on various parameters. Similar findings were reported by Li *et al*. (2014) in rapeseed (*Brassica napus* L.), where the same QTL affected both seed weight and silique number.

Significant marker-trait associations: In addition to validation of *qSY-3*, some other significant marker-trait associations ($P \leq 0.001$) were also identified in this study (Table 5). These marker-trait associations were identified for SY-saline, GW-normal, GW-saline, GL-normal, GL-saline, and DFF-relative. Linked markers for these associations were RM258, RM254, RM254, RM526, RM526, and RM281, respectively. Phenotypic variance explained (R^2) value for these associations ranged from 33.95% to 64.29%. These marker-trait associations were identified by GLM, as well as, MLM analyses.

Table 1. Statistical parameters for morphological and yield traits of rice varieties grown under normal versus saline conditions.

S. No.	Traits	Mean		Standard Deviation		Skewness	
		Normal	Saline	Normal	Saline	Normal	Saline
1	PH (cm)	125.68	106.5	26.184	23.783	-0.07405	-0.3437
2	TTP	16.139	13.29	3.80556	3.4533	0.4492	0.41276
3	ETP	13.72	11.29	3.9009	2.6289	0.726	0.14788
4	PaL (cm)	26.638	24.83	3.9285	4.7801	0.338	0.1615
5	PaW (g)	2.1509	2.006	0.9072	0.8796	0.2175	0.3017
6	SpPa	127.17	108.94	28.695	35.801	0.1229	0.5326
7	UfGPa	12.93	21.76	4.7803	17.698	0.4227	3.1203
8	GPa	114.24	87.18	28.4138	37.38	0.204	0.7403
9	PaF	89.47	78.95	4.1923	14.9526	-0.6488	-2.0808
10	DFF	86.22	88.555	8.056	7.988	-0.52088	-0.51004
11	DM	116.22	118.56	8.056	7.988	-0.52088	-0.7202
12	GL (mm)	7.957	7.556	1.0818	1.1313	0.5563	0.4523
13	GW (mm)	1.998	1.83	0.34325	0.3111	1.922	1.8821
14	GLWR	4.0804	4.221	0.7847	0.8381	-0.17864	-0.02796
15	TGW (g)	22.282	20.997	2.449	2.5927	0.119	0.1576
16	GY (g/plant)	35.902	20.89	15.168	10.399	0.2998	0.739
17	SY (g/plant)	168.14	108.77	152.595	74.672	3.404	0.6062
18	HI	32.56	29.77	25.335	23.78	1.1704	1.1325

PH plant height, *TTP* number of total tillers/plant, *ETP* number of effective tillers/plant, *PaW* panicle weight, *PaL* panicle length, *SpPa* number of spikelets/panicle, *UfGPa* number of unfilled grains/panicle, *GPa* number of grains/panicle, *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, *SY* straw yield/plant, and *HI* harvest index (%).

Table 2. Analysis of variance estimates for morphological and yield traits of 24 rice varieties grown under normal versus saline conditions.

Source of variation/Trait	Block	Treatment	Genotype	Salt × Genotype
PH (cm)	72.22**	13244.17***	3269.54***	484.33***
TTP	34.67*	291.84**	43.94***	35.29***
ETP	34.38*	212.67**	41.62***	24.07***
PaL (cm)	83.03NS	117.36NS	115.51***	5.27***
PaW (g)	0.14NS	0.75*	4.76***	0.03***
SpPa	161.78NS	12506.69***	6139.16***	376.98***
UfGPa	45.67*	2809***	621.77***	389.46***
GPa	35.90NS	27170.03***	5995.83***	672.69***
PaF	19.12*	4012.78***	450.80***	269.85***
DFF	56.51**	196***	384.81***	1.35***
DM	56.51**	196***	384.81***	1.35***
GL (mm)	0.53**	5.51***	7.48***	0.02***
GW (mm)	0.25NS	0.99*	0.64***	0.003***
GLWR	0.53NS	0.72NS	4.01***	0.02***
TGW (g)	2.72**	59.34***	37.89***	0.27***
GY (g/plant)	289.94NS	8238.41**	762.71***	252.39***
SY (g/plant)	47.21**	126849.25***	67339.59***	19244.48***
HI	247.95*	306.89*	3619.82***	7.83***

NS: non-significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$; ***: significant at $P \leq 0.001$.

PH plant height, *TTP* number of total tillers/plant, *ETP* number of effective tillers/plant, *PaW* panicle weight, *PaL* panicle length, *SpPa* number of spikelets/panicle, *UfGPa* number of unfilled grains/panicle, *GPa* number of grains/panicle, *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, *SY* straw yield/plant, and *HI* harvest index (%).

Table 3. Correlation coefficients for morphological and yield traits of rice varieties grown under normal versus saline conditions.

	PH	*TTP*	*ETP*	*PaL*	*PaW*	*SpPa*	*UfGPa*	*GPa*	*PaF*	*DFF*	*DM*	*GL*	*GW*	*GLWR*	*TGW*	*GY*	*SY*
TTP	-0.1541NS **0.3591****	1															
ETP	-0.3429** **0.1735NS**	0.9408** **0.8343****	1														
PaL	0.2524* **0.6683****	0.2942* **0.3241***	0.1744NS **0.1542NS**	1													
PaW	0.1979NS **0.3229****	0.1339 NS **0.1421 NS**	0.0165NS **0.0625NS**	0.5052* **0.5863***	1												
SpPa	0.1579NS **0.4399****	0.4096* **0.4243***	0.2389* **0.2137NS**	0.7386* **0.7258***	0.6067** **0.7470****	1											
UfGPa	0.3784** **0.4249****	-0.2001 NS **0.1600 NS**	-0.2074NS **0.1709NS**	0.2022 NS **0.2301 NS**	0.5353** **-0.0800NS**	0.2699* **0.1651 NS**	1										
GPa	0.0966NS **0.2238NS**	0.4583* **0.3335***	0.2832* **0.1254NS**	0.7275* **0.5909***	0.5327** **0.7573****	0.9856* **0.8857***	0.1031NS **-0.3118****	1									
PaF	-0.2495* **-0.2325***	0.4475* **0.0104 NS**	0.3880** **-0.0716NS**	0.2758* **0.0461 NS**	-0.1565NS **0.3694****	0.3812* **0.2156 NS**	-0.7412** **-0.8953****	0.5241** **0.6292****	1								
DFF	0.2025NS **0.3220****	-0.3448* **0.2374***	-0.3172** **0.2701***	0.0585 NS **0.0248 NS**	-0.4201** **-0.4176****	0.0066 NS **0.0090 NS**	-0.0480NS **0.3520****	0.0152NS **-0.1570NS**	0.0997NS - **0.3452****	1							
DM	0.2025NS **0.3219****	-0.3448* **0.2374***	-0.3172** **0.2701***	0.0585 NS **0.0248 NS**	-0.4201** **-0.4176****	0.0066 NS **0.0090 NS**	-0.0480NS **0.3520****	0.0152NS **-0.1570NS**	0.0997NS - **0.3452****	1*** **1*****	1						
GL	-0.2426* **- 0.2136NS**	-0.1074 NS **-0.1528 NS**	-0.0681NS **0.0896NS**	0.1239 NS **-0.0128 NS**	-0.1786NS **- 0.1915NS**	-0.1876 NS **-0.1769 NS**	-0.4840** **-0.2939***	-0.1088NS **-0.0320NS**	0.2659* **0.2112NS**	0.2929* **0.2928***	0.2929* **0.2928***	1					
GW	0.3130** **0.3289****	-0.1808 NS	-0.1425NS **-0.0526NS**	0.0511 NS **0.1076**	0.4347** **0.4154****	0.0967 NS **0.2021**	0.5950** **0.1819NS**	-0.0047NS **0.1091NS**	-0.4731** - **0.0825NS**	0.0532NS **0.0651NS**	0.0532NS **0.0651NS**	-0.0392NS **0.0249NS**	1				

		-0.0593 NS		**NS**		**NS**										
GL	-0.3681**	0.0008	-0.0028NS	-	-0.4622**	-	-0.7329**	-0.1150NS	0.4867**	0.1712NS	0.1712NS	0.7045**	-0.7140**	1		
WR	**-0.4224****	NS **-0.0695 NS**	**0.0925NS**	0.0217 NS **-0.1460 NS**	**-0.4561****	0.2360* **-0.3009***	**-0.3785****	**-0.1117NS**	**0.2463***	**0.1786NS**	**0.1786NS**	**0.7178****	**-0.6569****			
TG	0.2573*	0.2146	0.2041NS	0.3372*	0.3586**	0.1040	0.0295NS	0.1023NS	0.1091NS	-0.2118NS	-0.2118NS	0.1872NS	0.3791**	-0.1451NS	1	
W	**0.1516NS**	NS **-0.3236***	**-0.2032NS**	* **0.3238***	**0.3887****	NS **0.0503 NS**	**-0.1275NS**	**0.1084NS**	**0.2351***	**-0.2064NS**	**-0.2064NS**	**0.2066NS**	**0.3837****	**-0.1141NS**		
GY	-0.1355NS	0.9103*	0.8485**	0.5066*	0.35186**	0.6171*	-0.1181NS	0.6583**	0.5165**	-0.3195**	-0.3195** -	-0.0504NS	-0.0182NS	-0.0859NS	0.4440**	1
	0.2551*	* **0.5278***	**0.4701****	* **0.6069***	**0.6695****	* **0.8048***	**-0.2428***	**0.8896****	**0.5340****	**-0.0752NS**	**0.0752NS**	**0.1138NS**	**0.1741NS**	**-0.0517NS**	**0.2088NS**	
SY	0.3662NS	-	-0.1111NS	0.2214	-0.1289NS	0.1332	0.2342NS	0.0971NS	-0.0898NS	0.4584*	0.4584*	-0.2897NS	0.0950NS	-0.2980NS	-0.0233NS	-	1
	0.7496***	0.1012 NS **0.5238***	**0.2631NS**	NS **0.3402 NS**	**0.1632NS**	NS **0.3176 NS**	**0.5891****	**0.0253NS**	**-0.4305***	**0.4053***	**0.4053***	**-0.4018NS**	**0.1997NS**	**-0.4463***	**-0.1361NS**	0.0614 NS **0.0404 NS**	
HI	-0.4694**	0.5830*	0.5927**	0.1909	0.2591*	0.2985*	-0.2705*	0.3559**	0.4082**	-0.3578**	-0.3578**	0.3078**	-0.0451NS	0.2034NS	0.1791NS	0.6272*	-
	-0.3605**	* **-0.0880 NS**	**0.1620NS**	NS **0.0877 NS**	**0.2390***	**0.2482***	**-0.3540****	**0.4058****	**0.4360****	**-0.3419****	**-0.3419****	**0.3207****	**-0.0447NS**	**0.2312NS**	**0.2266NS**	* **0.4778*** *	0.5092* **-0.6769*****

NS: non-significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$; Normal-sized font values: *normal field data*; bold-sized font values: *salinity block data*.
PH plant height, *TTP* number of total tillers/plant, *ETP* number of effective tillers/plant, *PaW* panicle weight, *PaL* panicle length, *SpPa* number of spikelets/panicle, *UfGPa* number of unfilled grains/panicle, *GPa* number of grains/panicle, *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, *SY* straw yield/plant, and *HI* harvest index (%).

Table 4. Statistical parameters of *qSY-3* in the association mapping study.

S. No.	Trait	GLM		MLM				
		P	R^2 (%)	*P*	Genetic variance	Marker Effects		Heritability
						IR36 allele	Pokkali allele	
1	PH-normal	0.0016	36.14	0.0031	47.7656	-1.55E+01	15.4573	0.1118
2	PH-saline	0.0003308	42.29	0.0028	188.5231	-1.47E+01	14.6743	0.6813
3	ETP-relative	0.0104	23.39	0.0104	1.30E-06	0.2145	-2.14E-01	1.00E-05
4	UfGPa-normal	0.0083	26.04	0.0427	16.4137	-2.33E+00	2.3288	1
5	UfGPa-saline	0.0096	26.26	-	210.0239	-5.02E+00	5.0211	1
6	PaF-normal	0.0089	23.63	0.0137	10.8363	2.3598	-2.36E+00	1
7	GW-normal	0.0043	27.38	0.013	0.0365	-1.75E-01	0.1753	0.5782
8	GW-saline	0.005	27.96	0.0129	0.0246	-1.62E-01	0.1619	0.4313
9	GLWR-normal	0.0014	25.36	0.0164	0.2614	0.3555	-3.56E-01	1
10	GLWR-saline	0.0015	26.62	0.0081	0.1621	0.4131	-4.13E-01	0.5254
11	SY-saline	0.000047699	54.86	4.77E-05	0.0271	-5.60E+01	56.0164	1.00E-05
12	HI-normal	0.0082	28.68	0.0252	193.9752	12.8542	-1.29E+01	0.4215
13	HI-saline	0.008	28.9	0.0211	149.8715	12.3566	-1.24E+01	0.366

PH plant height, *ETP* number of effective tillers/plant, *UfGPa* number of unfilled grains/panicle, *PaF* panicle fertility (%), *GW* grain width, *GLWR* grain length-width ratio, *SY* straw yield/plant, *HI* harvest index (%), GLM general linear model, MLM mixed linear model, R^2 phenotypic variance explained.

Table 5. Marker-trait associations from GLM and MLM analyses.

Trait	Locus	*P* Marker		R^2 Marker
		GLM	MLM	
SY-saline	RM258	3.62E-06	3.38E-05	0.6429
GW-normal	RM254	2.33E-04	7.39E-04	0.404
GW-saline	RM254	3.52E-04	6.19E-04	0.4055
GL-normal	RM526	4.29E-04	5.25E-04	0.3693
GL-saline	RM526	5.15E-04	8.41E-04	0.3679
DFF-relative	RM281	9.15E-04	9.15E-04	0.3395

DFF days to 50% flowering, *GL* grain length, *GW* grain width, *SY* straw yield/plant, *GLM* general linear model, *MLM* mixed linear model, R^2 phenotypic variance explained.

DISCUSSION

Sub-populations in the rice association mapping panel: Two sub-populations were identified in the rice association mapping panel through STRUCTURE analysis. This analysis grouped rice varieties on the basis of their kinship. Two exotic rice varieties namely IR-36 and Pokkali were grouped in sub-population 1. Eight varieties of Pakistan origin were also included in sub-population 1. It indicated that crossing parents used in the development of these Pakistani rice varieties had shared kinship with IR-36 and Pokkali. Similarly, all the Basmati rice varieties were grouped in the same sub-population 2. Thus, STRUCTURE analysis had the potential to group rice varieties on the basis of their shared characteristics.

Effects of salinity on rice morphology and yield: Salinity affects plant growth by disturbing cellular metabolism. This disturbance of the cellular metabolism is translated into reduced plant growth. Genotypes, which have the potential to minimize the damage to their cellular metabolism under saline conditions, tolerate saline conditions. Better morphological performance under saline conditions results in better yield. All parameters studied during this research were important with respect to salinity tolerance in rice. Some parameters were affected more under saline soil compared to others. Most of the morphological (PH, TTP, ETP, PaL, PaW, SY, HI) and grain yield (SpPa, GPa, PaF, GL, GW,

TGW, GY) traits were reduced under saline condition. On the other hand, some of the grain yield and phenology traits (UfGPa, DFF, DM, GLWR) were increased. Under saline conditions, grain formation was most badly affected and a tremendous increase in UfGPa was observed under saline conditions compared to control. Similar findings were reported in the past works (Razzaque *et al*., 2010; Zhou *et al*., 2010). Under stress conditions, in cereals, grain yield is disturbed (Clermont-Dauphin *et al*., 2010; Fitzgerald *et al*., 2010). Reproductive stage is the most affected stage under stress conditions. Genotypes which have the potential to continue sustained grain formation under stress conditions produce better economic yield. Under stress conditions, phenology is most important attribute and genotypes with appropriate phenology development under stress conditions perform better in terms of grain yield (Khatun *et al*., 1995).

QTL validation and association mapping: QTL validation provides authenticity to the identified QTLs. When the association of a marker with a trait of interest is confirmed, it can be employed successfully in the marker-assisted breeding (MAB) program. Genome-wide association mapping approach is a powerful technique which is used extensively in plants now-a-days, including rice (Si *et al*., 2016; Thoen *et al*., 2017), and it has great potential in validating previously identified QTLs (Li *et al*., 2014). For the validation of QTLs, linkage and association mapping have been applied in combination (Li *et al*., 2014; Shi *et al*., 2015; Taguchi-Shiobara *et al*., 2015; Zhao *et al*., 2015; Song *et al*., 2016). Li *et al*. (2014) identified QTLs in rapeseed for silique length (SL) and seed weight (SW) by linkage mapping. Out of the total number of identified QTLs, two co-localized major QTLs, *uq.A09-1* and *uq.A09-3* were detected in all four environments and showed opposite additive-effect direction. These QTLs were validated and fine mapped by regional association analysis with a panel of 576 inbred lines. Shi *et al*. (2015) used linkage mapping to identify QTLs for pod number (PN) and seed number per pod (SN) in rapeseed. Out of the total number of QTLs identified for PN and SN, two major QTLs, *qPN.A06-1* and *qSN.A06-1*, for PN and SN respectively, were colocalised with opposite effects. These QTLs were confirmed by regional association analysis. Song *et al*. (2016) used association mapping followed by bi-parental mapping to identify and validate QTLs governing seed coat color in soybean [*Glycine max* (L.) Merr.]. Taguchi-Shiobara *et al*. (2015) identified marker RM6992 associated with width of the flag leaf (WFL) in rice through genome-wide association and linkage mapping. This marker was located near to the gene *NARROW LEAF 1* (*NAL1*). Zhao *et al*. (2015) employed linkage and association mapping to identify loci for resistance to *Sclerotinia sclerotiorum* in soybean. In the present study, previously identified QTL for straw yield was validated. All these reports highlight the utility of association mapping as a tool to validate QTLs. In the present study, the representative panel of 24 rice varieties was sufficient enough to validate the previously identified QTL as it was identified in both GLM and MLM analyses. It showed that a genetically diverse association mapping panel, even though it might be small, had the potential in validating previously identified QTLs.

Conclusions: We employed genome-wide association mapping approach for validation of our previously identified major QTL, *qSY-3*. This was a major QTL linked to rice straw yield under relative value dataset in the linkage mapping study. In this study, its flanking marker, RM473D was identified associated with eight morphological and yield-related traits including straw yield. From these findings, it was concluded that this locus had pleiotropic effects on a number of morphological traits and this affected rice yield under salt stress conditions. This study also showed that association mapping had high potential to validate QTLs identified by linkage mapping study. Previously, it was considered that a panel consisting of large number of genotypes was required to identify putative associations through association mapping approach. Our study demonstrated that a representative genetically diverse small panel of properly selected genotypes was sufficient enough to validate previously identified QTLs.

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