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Mapping genomic regions associated with yield and drought tolerance indices in recombinant inbred lines of rice

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Abstract

Mapping QTLs with molecular markers can be very useful for plant breeders in agricultural genomics. The identification and introgression of QTLs for grain yield and drought tolerance indices is an efficient approach to improve the drought tolerance of rice varieties. In this study, QTLs controlling some traits associated with grain yield and drought tolerance indices were identified using 150 F_8 lines derived from a cross between Sepidroud and Gharib, under non-stress and stress conditions. The genetic linkage map containing 12 ISSR polymorphic markers, 103 SSR, 1 IRAP marker, 11 REMAP markers and 16 combinations of ISSR markers covered 1005.2 cM of the rice genome and a mean distance between adjacent markers was 4.43 cM. In this experiment, two QTLs with main effects were mapped for SSI and YSI indices, three QTLs with main effects for grain yield under non-stress and stress conditions, TOL, STI, GMP, and YI, four QTLs with main effects for MP and HM. One epistatic QTL was mapped for grain yield under non-stress condition and STI index. The phenotypic variation explained by each main effect QTLs and epistatic QTLs ranged from 3.99 to 25.41% and 6.51 to 18.81%, respectively. Fifteen main effect QTLs, including, *qGY9*, *qGY12a*, *qTOL4, qTOL5*, *qSSI5*, *qSSI6*, *qSTI9*, *qSTII2*, *qMP9*, *qGMP9*, *qGMP12*, *qHM12a*, *qYSI5, qYSI6,* and *qYI12a* as the major QTLs controlling these traits can be considered in rice breeding programs for improving grain yield and drought tolerance after validation. The markers UBC816-2, (Tos2+UBC827)-4, (UBC826+HB12)-6, RM215 and RM5371 located near major QTLs could be used in MAS programs.

***Key words*:** Composite interval mapping, Drought resistance, Molecular markers.

INTRODUCTION

Rice is one of the most important cereals and serves more than half of the world's food, especially in developing countries (Wu *et al.,* 2013). Drought is a major problem limiting the adaptation of high-yielding rice cultivars under drought and dry environments (Lafitte *et al.,* 2007). In Asia, about 8 million hectares of upland and 34 million hectares of rainfed lowland rice are frequently affected by drought stress (Barik *et al.,* 2019). The main method to evaluate the response of cultivars to abiotic stresses such as drought is to evaluate the yield of cultivars under both normal and stress conditions and then to evaluate tolerance and susceptibility indices. Plant adaptation to stress is controlled by genes and plays an important role in stress tolerance and these genes can function under stress and sometimes under non-stress conditions (Bouman and Tuong, 2001). Gene transfer in traits controlled by a single gene has been successful but in multi-gene traits such as drought, it is more complicated. Plant breeders try to find traits that affect yield stability under stress condition. They try to pyramid resistance genes, without these QTLs affecting the yield potential. This strategy generates high-yield and more tolerant cultivars and improves crop yield under drought conditions (Cattivelli *et al.,* 2008). In traditional

methods, the selection was performed based on yield and its stability in different years and environments for genetic improvement of drought tolerance. Therefore, the selection can be performed for secondary traits instead of yield (Manickavelu *et al.,* 2006). To improve drought tolerance, plant breeders must identify traits related to yield stability and then transfer them to high-yielding genotypes. This goal can be achieved using MAS (marker-assisted selection) (Cattivelli *et al.,* 2008). Using permanent and stable populations can be identified stable and important QTLs for drought tolerance. Tolerance genes can be used in breeding programs through MAS and new tolerant varieties of rice can be released (Sabouri *et al.,* 2013).

Detection of the linkage between QTLs and markers, their position on chromosomes, and their effects on yield under non-stress and drought stress conditions in rice have been studied by many researchers (Hittalmani *et al.,* 2003; Bernier *et al.,* 2007; Chakraborty *et al.,* 2011; Vikram *et al.,* 2011; Ghimire *et al.,* 2012; Dixit *et al.,* 2014; Lang *et al.,* 2013; Sabouri *et al.,* 2013; Yadaw *et al.,* 2013; Zhao *et al.,* 2013; Wang *et al.,* 2014; Tian *et al.,* 2015). Yue *et al.* (2005) reported QTLs for yield stability index (YSI) by evaluating the RIL population of Zhenshan 97×IRAT109 using 245 SSR markers under two soil conditions. In their study, in the paddy soil condition, two QTLs on chromosomes 1 and 2 were detected for YSI explaining 9.25% and 12.07% of the total phenotypic variation, respectively. In the sandy field, five QTLs on chromosomes 2, 6, 8, 9, and 10 explained 4.90% to 19.05% phenotypic variation. Bernier *et al.* (2007) mapped QTL for YSI (yield stability index) by evaluating 436 F_3 population lines of Vandana×Way Rarem. In their study, one QTL on chromosome 12 explained 37% phenotypic variation for YSI. Hu *et al.* (2007) mapped QTLs for YSI by evaluating 195 F_9 population lines of Zhenshan 97B×IRAT109. In their study, Four QTLs on chromosomes 1, 4, 5, and 9 explained 6.82% to 19.79% phenotypic variation. Rahimi *et al.* (2014) mapped QTLs linked to drought tolerance indices by evaluating 150 F_5 population lines of Sepidroud×Gharib. For mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), and stress tolerance index (STI), three QTLs were identified on chromosomes 1, 7, and 11 which individually explained 7.11 to 10.60% phenotypic variation. For stress susceptibility index (SSI), yield stability index (YSI), and yield index (YI), two QTLs were identified on chromosomes 1 and 7 which explained 10.80 and 12.53% phenotypic variation, respectively, and for tolerance index (TOL) two QTLs were identified on chromosomes 6 and 7 which explained 6.64% and 9.89% phenotypic variation, respectively. Tiwari *et al.* (2018) mapped QTLs for SSI by evaluating 216 inbred lines of CSR11×MI48. In their study, twenty-one QTLs were identified on chromosomes 1 (three QTLs), 2 (three QTLs), 3 (five QTLs), 5 (two QTLs), 6 (five QTLs), 8, 9, and 12. Bhattarai and Subudhi (2018) mapped QTLs for YSI by evaluating 181 inbred lines of Cocodrie×N-22. In their study, two QTLs were identified on chromosomes 1 and 12 that explained 5% and 5.3% of the phenotypic variation, respectively.

To calculate tolerance indices, genotypes need to be evaluated under both normal and drought-stress conditions, selection of molecular markers associated with drought tolerance indices can be used to select drought-tolerant genotypes at the seedling stages under normal condition.

Few studies of QTL mapping for drought tolerance indices have been conducted in rice, except Rahimi *et al.* (2014) there is no other report in Iranian rice cultivars. This subject aroused our interest in verifying if the QTLs detected for Iranian rice varieties show similar tendencies with external rice varieties. The purposes of the present study were to (a) evaluate and identify QTLs controlling grain yield under non–stress and drought–stress conditions and drought tolerance indices in a F_8 population derived from the cross between two cultivars of rice, Gharib and Sepidroud (b) identify molecular markers associated with drought tolerance indices for the selection of drought-tolerant lines in rice.

MATERIALS AND METHODS

Plant material

A mapping population of 150 RILs (recombinant inbred lines) was derived from a cross between rice cultivars Gharib and Sepidroud in the university of Guilan during 2013-2014. Gharib (as the female parent) is a local cultivar of Guilan province that is a drought-resistant cultivar and Sepidroud (as the male parent) is an improved rice cultivar that is sensitive to drought (Danesh Gilevaei *et al.,* 2018). The experiment was performed using two augment designs under normal irrigation and drought stress environments, separately. Under non–stress environment, rice lines were flood-irrigated until the harvest stage, whereas drought stress was imposed 30 days after transplanting (the maximum tillering stage) by preventing irrigation at the field. For measuring soil

water tension, five gypsum blocks were placed at five points of the field at a depth of about 30 cm in soil. The drought tolerance indices were calculated based on the following equations (Equations 1 to 9):

1. Tolerance Index (TOL) (Rosielle and Hamblin, 1981)

$$TOL = Yp - Ys \quad (1)$$

2. Stress Susceptibility Index (SSI) (Fischer and Maurer, 1978)

$$SSI = \frac{1-\left(\frac{Ys}{Yp}\right)}{SI} \quad (2)$$

$$SI = 1-\frac{\overline{Ys}}{\overline{Yp}} \quad (3)$$

3. Stress Tolerance Index (STI) (Fernandez, 1992)

$$STI = \frac{(Yp)(Ys)}{(\overline{Yp})^2} \quad (4)$$

4. Mean Productivity (MP) (Rosielle and Hamblin, 1981)

$$MP = \frac{Yp + Ys}{2} \quad (5)$$

5. Geometric Mean Productivity (GMP) (Fernandez, 1992)

$$GMP = \sqrt{(Ys)(Yp)} \quad (6)$$

6. Harmonic Mean (HM) (Rosielle and Hamblin, 1981)

$$HM = \frac{2(Yp)(Ys)}{Yp + Ys} \quad (7)$$

7. Yield Stability Index (YSI) (Bouslama and Schapaugh, 1984)

$$YSI = \frac{Ys}{Yp} \quad (8)$$

8. Yield Index (YI) (Gavuzzi *et al.,* 1997)

$$YI = \frac{Ys}{\overline{Ys}} \quad (9)$$

In the above formulas, Ys and Yp are the mean yield of lines under stress and non-stress conditions, respectively. $\bar{Y}s$ and $\bar{Y}p$ are the mean yield of all lines under stress and non-stress conditions, respectively.

Genotyping and QTL mapping

Genomic DNA was extracted from the fresh leaf samples of F_8 seedlings and the two parents using a CTAB (cetyl trimethyl ammonium bromide) protocol presented by Saghai Maroof *et al.* (1994). A total of 12 ISSR markers, 103 SSR, 1 IRAP marker, 11 REMAP markers and 16 combinations of ISSR markers distributed on 12 chromosomes were polymorphic between parental lines [primer sequences were received from Gramene (http://www.gramene.org)].

PCR for SSR markers was performed in a total volume of 10 µl of the reaction mixture consisted of 2 µl (5 ng/µl) of template DNA, 0.12 U of Taq polymerase (5 Unit/µl), 0.2 µl of dNTP (2 mM each), 0.6 µl of forward and reverse primer (10 pmol each), 1 µl of 10 X PCR buffer, 0.24 µM of $MgCl_2$, and 5.24 µl of distilled water. Amplification was performed in a Thermo Cycler (Bio-Rad) using the step-cycle program of denaturation at 94 °C for 5 min and afterward following denaturation carried out at 94 °C for 1 min, annealing at 55 to 60 °C for 1 min, and extension at 72 °C for 1 min. Steps 2 to 4 were repeated for 35 cycles, then pursued by a final extension process at 72 °C for 5 min.

PCR for ISSR, the combination of ISSR markers, REMAP and IRAP markers were performed in a total volume of 10 µl consisted of 2 µl (5 ng/µl) of template DNA, 0.12 U of Taq polymerase (5 Unit/µl), 0.12 µl of dNTP (2 mM each), 0.6 µl of primer (10 pmol each), 1 µl of 10 X PCR buffer, 0.24 µM of $MgCl_2$, and 6.24 µl of distilled water. Amplification was performed in a Thermal Cycler (Applied Biosystems, Germany) according to the program of denaturation at 94 °C for 5 min and afterward following denaturation performed at 94 °C for 30 s, annealing at 51 to 56 °C for 30 s, and extension at 72 °C for 5 min. Steps 2 to 4 were repeated for 10 cycles. The PCR products were run on a 3% and 1.9% agarose denaturing gel for SSR and other markers, respectively. Marker bands were revealed by ethidium bromide staining (EtBr).

A linkage map was prepared using QTXb17 Mapmanager (Manly and Olson, 1999), and the genetic distances (cM) were derived by the Kosambi mapping function (Kosambi, 1944). Inclusive composite interval mapping (ICIM) was performed to determine QTL effects such as phenotypic variation explained (PVE), additive effect of the QTL loci, and log-likelihood ratio (LOD) score using QTL IciMapping (Wang, 2009).

One thousand permutation tests were used to calculate significant thresholds for QTL detection and effects.

RESULTS AND DISCUSSION

Linkage map construction

The genetic linkage map consisted of 227 polymorphic markers, which included 12 ISSR markers, 103 SSR, 1 IRAP marker, 11 REMAP markers, and 16 combinations of ISSR markers. The genetic linkage map covered a distance of 1005.2 cM with a mean distance of 4.43 cM between the adjacent markers (Figure 1). Previously, the linkage map was prepared in different mapping populations drived from crosses between Gharib and Sepidrood cultivars (identical parents with our study). Sabouri *et al.* (2010), using 105 SSR markers in the $F_{2:3}$ population, reported the length of the map of 1440.7 cM with a mean distance of 13.73 cM between the adjacent markers. Mardani *et al.* (2013), using 131 SSR and 105 AFLP markers in the $F_{2:4}$ population, reported the length of the map of 2447.7 cM with a mean distance of 10.48 cM between the adjacent markers. Rabiei *et al.* (2015) in $F_{2:4}$ population using 111 AFLP markers and 105 SSR markers reported a map length of 2807 cM with a mean distance of 26.48 cM between the adjacent markers. Rahimi *et al.* (2014) in the F_5 population using 131 SSR and 52 AFLP markers reported a map length of 103.104 cM with a mean distance of 5.81 between the adjacent markers. In the present study, the map length and distance between markers in the genetic map were different from other researchers which could be due to different generations, type and number of markers, and the crossover events in every generation.

QTL mapping

Under non-stress environment, three QTLs on chromosomes 4 [between (Tos2+UBC827)-4 and RM252], 5 [between (UBC826+HB12)-6 and (Tos2+UBC815)-1] and 9 (between RM201 and RM215) were mapped for GY, which explained 5.16, 4.03 and 9.67 of the LOD values and 9.79%, 7.48% and 19.48% of the total phenotypic variation, respectively. The additive effects of the three QTLs were 3.17, 2.77 and 4.46, respectively. The alleles from Sepidroud parent increased GY (Table 1 and Figure 1). Under stress environment, three QTLs on chromosomes 3 (between UBC814-1 and UBC815-2) and 12 (two QTLs) [between UBC816-2 and UBC (816+822)-6 and between RM2197 and RM212] were mapped for GY, which explained 3.27, 12.26 and 4.84 of the LOD values and 3.99%, 22.41% and 5.35% of the total phenotypic variation, respectively. The additive effects were 1.15, 3.27 and 1.33, respectively. The alleles from Sepidroud parent increased GY at two loci (*qGY3* and *qGY12a*) and Gharib parent alleles increased GY at other loci (*qGY12b*). This suggested that alleles for increasing GY were dispersed within the two parents. This result was in accordance with the presence of transgressive segregation for GY in the RIL population. Among the six identified QTLs, two of them (*qGY9* and *qGY12a*) were the major QTLs for the GY. QTLs detected in one environment were not identified in the other, meaning that the QTL effects for GY were environment-specific. Using the MET module in QTL IciMapping software, we analyzed the multi-environment phenotypic values of GY for the RIL population grown in two environments. A total of four QTL×environment interactions were identified and the phenotypic variation explained by QTL×environment interaction ranged from 1.36% to 3.19%. It indicated that QTL×environment interactions were important components for GY although the degree of interactions was low (Table 2).

For GY, several QTLs have been mapped on chromosome 3 (Bernier *et al.,* 2007; Lang *et al.,* 2013; Yadaw *et al.,* 2013; Wang *et al.,* 2014), chromosome 4 (Hittalmani *et al.,* 2003; Sabouri *et al.,* 2013; Dixit *et al.,* 2014; Pramudyawardan *et al.,* 2018; Descalsota-Empleo *et al.,* 2019), chromosome 5 (Zhao *et al.,* 2013; Zhu *et al.,* 2017), chromosome 9 (Hittalmani *et al.,* 2003) and chromosome 12 (Lang *et al.,* 2013; Wang *et al.,* 2014; Zhu *et al.,* 2017; Descalsota-Empleo *et al.,* 2019). The other QTLs for GY were mapped on chromosomes 1, 2, 6, 7, 8, 10 and 11 by other researchers (Hittalmani *et al.,* 2003; Bernier *et al.,* 2007; Ghimire *et al.,* 2012; Sabouri *et al.,* 2013; Yadaw *et al.,* 2013; Wang *et al.,* 2014). Rabiei *et al.* (2015) identified one QTL on chromosome 3 which explained 10.26% of the PVE for GY ($F_{2:4}$ population derived from the cross between Gharib×Sepidroud). Rahimi *et al.* (2014) identified three QTLs for GY on chromosomes 1, 7, and 11 that explained 9.13% to 11.65% of the PVE under non-stress and drought-stress environments (F_5 population derived from the cross between Gharib×Sepidroud). The difference in observed results may be due to differences in generation, the number and type of markers, and environmental conditions. These results showed that grain yield might be controlled by at least two major QTLs, which accounted for a large portion of the phenotypic variation and several minor QTLs, each accounting for a small portion of the phenotypic variance. In general, we can conclude that the loci controlling this trait are scattered on different chromosomes.

For tolerance index (TOL), three QTLs on chromosomes 4 [between (Tos2+UBC827)-4 and RM252], 5 [between (UBC826+HB12)-6 and (Tos2+UBC815)-1] and 11 (between UBC815-3 and UBC822-2) were mapped which explained 12.10, 15.28 and 7.82 of the LOD values and 4.34%, 5.81% and 3.33% of the total phenotypic variation, respectively.

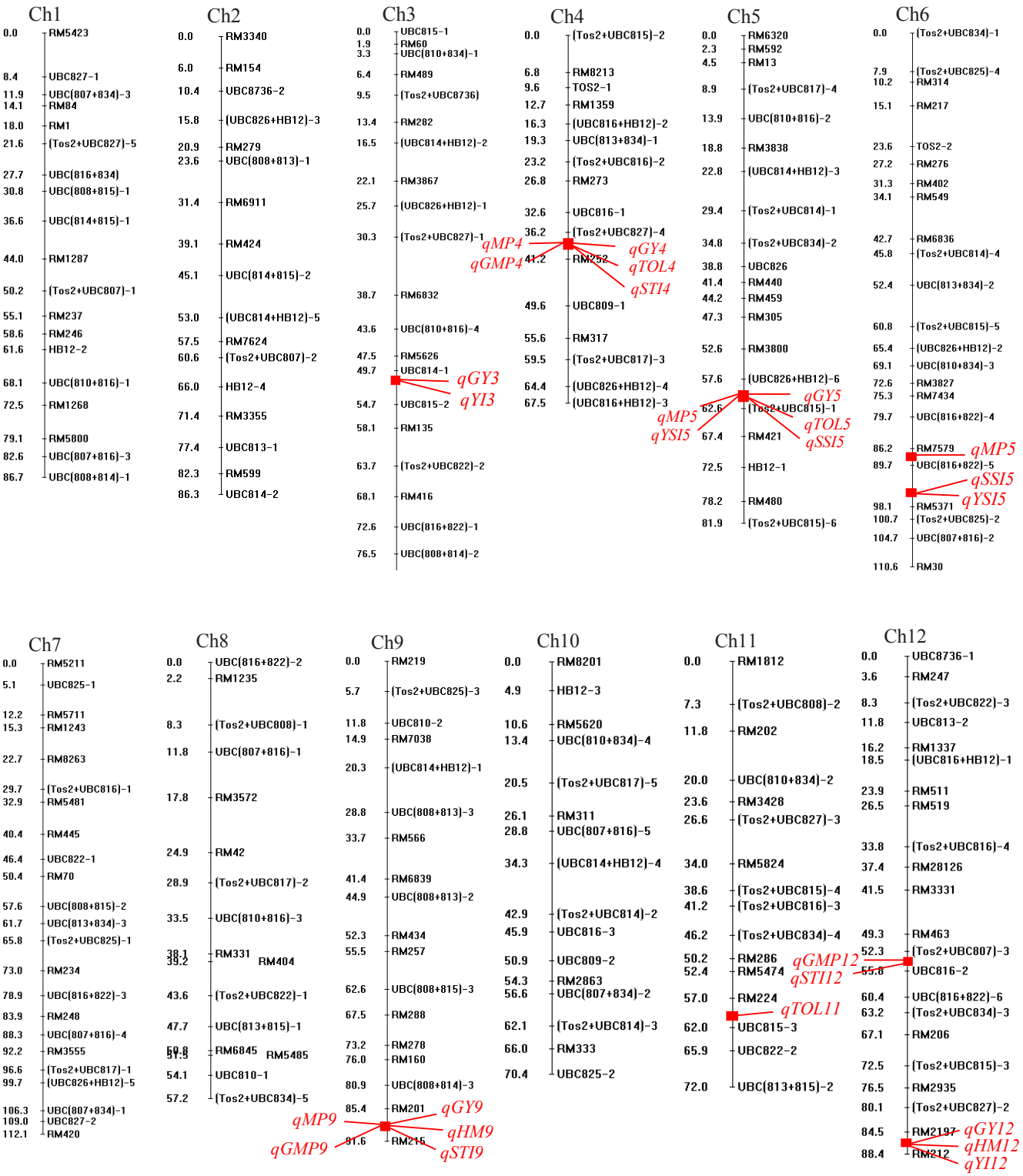

Figure 1. Location of identified QTLs in genetic linkage map for drought tolerance indices and grain yield in rice.

Table 1. Main QTLs detected for yield and drought tolerance indices.

Trait	QTL	Chromosome	Marker interval	QTL position on chromosome	Distance from the QTL	LOD	Add	PVE (%)
YS	*qGY3*	3	UBC814-1-UBC815-2	51.5	1.8	3.27	1.15	3.99
YS	*qGY12a*	12	UBC816-2-UBC(816+822)-6	56	0.2	12.26	3.27	22.41
YS	*qGY12b*	12	RM2197-RM212	88	0.4	4.84	-1.33	5.35
YP	*qGY4*	4	(Tos2+UBC827)-4-RM252	37.5	1.3	5.16	3.17	9.79
YP	*qGY5*	5	(UBC826+HB12)-6-(Tos2+UBC815)-1	61.5	1.1	4.03	2.77	7.48
YP	*qGY9*	9	RM201-RM215	90	1.6	9.67	4.46	19.48
TOL	*qTOL4*	4	(Tos2+UBC827)-4-RM252	37.5	1.3	4.34	1.73	12.10
TOL	*qTOL5*	5	(UBC826+HB12)-6-(Tos2+UBC815)-1	61	3.4	5.81	1.64	15.28
TOL	*qTOL11*	11	UBC815-3-UBC822-2	62	0.0	3.33	-1.44	7.82
SSI	*qSSI5*	5	(UBC826+HB12)-6-(Tos2+UBC815)-1	61	3.4	4.50	0.17	10.87
SSI	*qSSI6*	6	RM5371-(Tos2+UBC825)-2	99	0.9	10.43	-0.27	20.23
STI	*qSTI4*	4	(Tos2+UBC827)-4-RM252	37.5	1.3	3.82	0.10	6.18
STI	*qSTI9*	9	RM201-RM215	91	0.6	7.91	0.14	12.82
STI	*qSTI12*	12	UBC816-2-UBC(816+822)-6	56	0.2	9.35	0.15	15.23
MP	*qMP4*	4	(Tos2+UBC827)-4-RM252	37.5	1.3	5.93	5.30	8.26
MP	*qMP5*	5	(UBC826+HB12)-6-(Tos2+UBC815)-1	61	1.6	3.19	4.31	4.31
MP	*qMP6*	6	UBC(816+822)-5-RM5371	98	0.1	6.29	4.71	8.14
MP	*qMP9*	9	RM201-RM215	90	1.6	12.43	3.74	18.87
GMP	*qGMP4*	4	(Tos2+UBC827)-4-RM252	37.5	1.3	5.62	5.63	7.17
GMP	*qGMP9*	9	RM201-RM215	90	0.6	12.10	0.56	16.87
GMP	*qGMP12*	12	UBC816-2-UBC(816+822)-6	56	0.2	13.88	5.90	18.68
HM	*qHM4*	4	(Tos2+UBC827)-4-RM252	39	2.2	3.70	4.72	4.14
HM	*qHM9*	9	RM201-RM215	91	0.6	7.61	0.50	8.35
HM	*qHM12a*	12	UBC816-2-UBC(816+822)-6	56	0.2	15.04	3.67	18.02
HM	*qHM12b*	12	RM2197-RM212	88	0.4	5.18	-5.91	5.44
YSI	*qYSI5*	5	(UBC826+HB12)-6-(Tos2+UBC815)-1	61	3.4	4.50	-0.08	10.87
YSI	*qYSI6*	6	RM5371-(Tos2+UBC825)-2	99	0.9	10.43	0.12	20.23
YI	*qYI3*	3	UBC814-1-UBC815-2	51.5	1.8	3.27	0.08	4.02
YI	*qYI12a*	12	UBC816-2-UBC(816+822)-6	56	0.2	12.26	0.22	25.41
YI	*qYI12b*	12	RM2197-RM212	88	0.4	4.84	-0.09	5.35

YS: Grain yield under stress condition, YP: Grain yield under non-stress condition, TOL: Tolerance index, SSI: Stress susceptibility index, STI: Stress tolerance index, MP: Mean productivity, GMP: Geometric mean productivity, HM: Harmonic mean, YSI: Yield stability index and YI: Yield index.
Add: the additive effects of QTLs, the positive values of additive effects mean that Sepidroud alleles had an increasing effect on the studied trait and the negative value showed that Gharib alleles had an increasing effect on the studied trait.
PVE (%): Percentage of phenotypic variance explained by each QTL, the underline marker is close to mentioned QTL.

The additive effects of the three QTLs were 1.73, 1.64, and 1.44, respectively. The alleles from Sepidroud parent increased TOL at two loci (*qTOL4* and *qTOL5*) and Gharib parent alleles increased TOL at other locus (*qTOL11*). Among the three QTLs, *qTOL4* and *qTOL5* showed the major contributions of the phenotypic variation and could be regarded as the major QTLs. Rahimi *et al.* (2014) identified two QTLs for TOL on chromosomes 6 and 7.Their results were consistent with our findings. The difference in observed results may be due to differences in generation, the number and type of markers and environmental conditions.

For stress susceptibility index (SSI), two QTLs on chromosomes 5 [between (UBC826+HB12)-6 and (Tos2+UBC815)-1] and 6 [between RM5371 and (Tos2+UBC825)-2] were mapped which explained 4.50 and 10.43 of the LOD values and 10.87% and 20.23% of the total phenotypic variation, respectively. The additive effects of these QTLs were 0.17 and 0.27, and the alleles from Sepidroud and Gharib parents increased SSI, respectively. Two QTLs, *qSSI5* and *qSSI6* could be regarded as the major QTLs. Rahimi *et al.* (2014) identified two QTLs for SSI on chromosomes 1 and 7 that these results were consistent with our findings. Tiwari *et al.* (2018) mapped 21 QTLs on chromosomes 1 (three QTLs), 2 (three QTLs), 3 (five QTLs), 5 (two QTLs), 6 (five QTLs), 8, 9, and 12. Among these QTLs, *qSSI5* and *qSSI6* were similar to the identified QTLs for SSI in our study.

For STI, three QTLs on chromosomes 4 [between (Tos2+UBC827)-4 and RM252], 9 (between RM201 and RM215) and 12 (between UBC816-2 and UBC (816+822)-6) were mapped which explained 3.82, 7.91 and 9.35 of the LOD values and 6.18%, 12.82% and 15.23% of the total phenotypic variation, respectively. The additive effects of the three QTLs were 0.10, 0.14 and 0.15, respectively. The alleles from Sepidroud parent increased STI. Among the three QTLs, *qSTI9* and *qSTI12* showed the major contributions of the phenotypic variation and could be regarded as the major QTLs. Rahimi *et al.* (2014) identified three QTLs for STI on chromosomes 1, 7, and 11 that their results were consistent with our findings.

For MP, four QTLs on chromosomes 4 [between (Tos2+UBC827)-4 and RM252], 5 [between (UBC826+HB12)-6 and (Tos2+UBC815)-1], 6 [between UBC(816+822)-5 and RM5371], and 9 (between RM201 and RM215) were mapped which explained 5.93, 3.19, 6.29 and 12.43 of the LOD values and 8.26%, 4.31%, 8.14% and 18.87% of the total phenotypic variation, respectively. The additive

Table 2. The main QTLs×environment interaction for grain yield under non–stress and drought stress environments.

Trait	Chromosome	Position	Marker interval	LOD	AE1[a]	AE2[a]	PVE[b] (A) (%)	PVE[b] (AE) (%)
GY	4	38	(Tos2+UBC827)-4-RM252	5.75	2.78	0.45	2.61	1.36
	5	61	(UBC826+HB12)-6-(Tos2+UBC815)-1	3.52	2.54	0.05	1.69	1.55
	9	90	RM201-RM215	10.69	4.18	0.61	5.72	3.19
	12	56	UBC816-2-UBC(816+822)-6	4.66	-0.10	3.24	2.47	2.80

AE1[a] and AE2[a]: The additive effects of main QTLs under non–stress and drought stress, respectively. The positive values of additive effects mean that Sepidroud alleles had an increasing effect on the studied trait and the negative value showed that Gharib allele had an increasing effect on the studied trait.

PVE[b] (A) and PVE[b] (AE): Percentage of phenotypic variance explained by each main QTLs and the main QTLs×environment interaction, respectively.

effects of the four QTLs were 5.30, 4.31, 4.71 and 3.74, respectively. The alleles from Sepidroud parent increased MP. Among the four QTLs, *qMP9* showed the major contributions of the phenotypic variation and could be regarded as the major QTL. Rahimi *et al.* (2014) identified three QTLs for MP on chromosomes 1, 7 and 11 that their results were consistent with our findings.

For GMP, three QTLs on chromosomes 4 [between (Tos2+UBC827)-4 and RM252], 9 (between RM201 and RM215) and 12 (between UBC816-2 and UBC (816+822)-6) were mapped which explained 5.62, 12.10 and 13.88 of the LOD values and 7.17%, 16.87% and 18.68% of the total phenotypic variation, respectively. The additive effects of the three QTLs were 5.63, 0.56 and 5.90, respectively. The alleles from Sepidroud parent increased GMP. Among the three QTLs, *qGMP9* and *qGMP12* showed the major contributions of the phenotypic variation and could be regarded as the major QTLs. Rahimi *et al.* (2014) identified three QTLs for GMP on chromosomes 1, 7, and 11 that their results were consistent with our findings.

For HM, four QTLs on chromosomes 4 [between (Tos2+UBC827)-4 and RM252], 9 [between (Tos2+UBC827)-4 and RM252] and 12 [between UBC816-2 and UBC (816+822)-6 and between RM2197 and RM212] were mapped which explained 3.70, 7.61, 15.04 and 5.18 of the LOD values and 4.14%, 8.35%, 18.02% and 5.44% of the total phenotypic variation, respectively. The additive effects of the four QTLs were 4.72, 0.50, 3.67 and 5.91, respectively. The alleles from Sepidroud increased the HM at locus *qHM12b* and Gharib alleles increased HM at other loci. Among the four QTLs, *qHM12a* showed the major contributions of the phenotypic variation and could be regarded as the major QTL. Rahimi *et al.* (2014) identified three QTLs for HM on chromosomes 1, 7, and 11 that their results were consistent with our findings.

For YSI, two QTLs on chromosomes 5 [between (UBC826+HB12)-6 and (Tos2+UBC815)-1] and 6 [between RM5371 and (Tos2+UBC825)-2] were mapped which explained 4.50 and 10.43 of the LOD values and 10.87% and 20.23% of the total phenotypic variation, respectively. The additive effects of the two QTLs were 0.08 and 0.12, respectively. The alleles from Gharib and Sepidroud parents increased YSI, respectively. Two QTLs, *qYSI5* and *qYSI6* as the major QTLs could be regarded. Previously, similar results for QTLs of YSI have been reported on chromosome 5 by Hu *et al.* (2007) and chromosome 6 by Yue *et al.* (2005). Several other research studies also mapped QTLs for YSI on chromosomes 1 (Yue *et al.,* 2005; Hu *et al.,* 2007; Rahimi *et al.,* 2014; Bhattarai and Subudhi, 2018), 2 (Yue *et al.,* 2005), 4 (Hu *et al.,* 2007), 6 (Yue *et al.,* 2005), 7 (Rahimi *et al.,* 2014), 8 (Yue *et al.,* 2005), 9 (Yue *et al.,* 2005; Hu *et al.,* 2007), 10 (Yue *et al.,* 2005) and 12 (Bernier *et al.,* 2007; Bhattarai and Subudhi, 2018).

For YI, three QTLs on chromosomes 3 (between UBC814-1 and UBC815-2) and 12 (two QTLs) [between UBC816-2 and UBC (816+822)-6 and between RM2197 and RM212] were mapped which explained 3.27, 12.26 and 4.84 of the LOD values and 4.02%, 25.41% and 5.35% of the total phenotypic variation, respectively. The additive effects were 0.08, 0.22 and 0.09, respectively. The alleles from Sepidroud parent increased YI at two loci (*qYI3* and *qYI12a*) and Gharib parent alleles increased YI at locus (*qYI12b*). Among the three QTLs, *qYI12a* showed the major contributions of the phenotypic variation and could be regarded as the major QTL. Rahimi *et al.* (2014) identified two QTLs for YI on chromosomes 1 and 7 that their results were consistent with our findings.

QTLs co-localization

Results showed that QTLs for Yp, TOL, STI, MP, GMP, and HM were co-localized in linkage group 4. QTLs for Ys and YI in linkage group 3, QTLs for Ys, TOL, SSI, MP, and YSI in linkage group 5, QTLs for SSI and YSI in linkage group 6, QTLs for Yp, HM, GMP, MP, and STI in linkage group 9, QTLs for Ys, YI, and HM in linkage group 12, and QTLs for Ys, GMP, STI, HM and YI in linkage group 12 were co-localized. The overlap of QTLs in different traits is due to pleiotropic effects or tight gene linkage. High-density genetic maps are required to determine the nature of pleiotropic effects or gene linkage. The significant correlations (data not shown) among studied traits can be described by these genomic regions containing tight linkage or pleiotropic QTLs.

Hu *et al.* (2007), using 213 SSR markers in 195 rice inbred lines (F_9), mapped *qYSI4* at marker distance of RM273–RM252 on chromosome 4, whereas in the present study, Yp, TOL, STI, MP, GMP, and HM were located on chromosome 4 at the marker distance of (Tos2+UBC827)-4-RM252. Hu *et al.* (2007) and Yue *et al.* (2005) mapped *qYSI9* at the marker distance of RM160–RM215 on chromosome 9, whereas in the present study Yp, STI, GMP, MP, and HM were located on chromosome 9 at the marker distance of RM201-

RM215. Therefore, these regions seem to be suitable candidates for breeding for drought tolerance through MAS as well as for fine mapping of the underlying genes.

Epistatic QTLs

For Yp, a positive additive-by-additive interaction was mapped between genomic regions on chromosome 1 and 9 explaining 6.51% of phenotypic variations. For STI, a negative additive-by-additive interaction was mapped between the genomic regions on chromosomes 9 and 10 explaining 18.81% of phenotypic variations and was identified as a major epistatic QTL (Table 3). Therefore, epistatic effects should be considered in the application of marker-assisted selection (MAS) in rice. Our results were not in agreement with those of Rahimi *et al.* (2014). They reported epistatic interactions on all of the chromosomes except chromosomes 2, 4, and 11 that each explained 3.98% to 18.37% of the total variation.

CONCLUSIONS

The identification and introgression of QTLs for grain yield and drought tolerance indices is an efficient approach to improve the drought tolerance of rice varieties. In our previous study, MP, GMP, STI and HM indices were suggested as suitable indices for recognition of drought-tolerant lines (Danesh Gilevaei *et al.,* 2018). Therefore, after the validation of markers associated with these indices, they can be used in MAS programs in rice. The lines with high GMP, STI, MP and HM values and low SSI and TOL values, recognized as high-yielding drought-tolerant lines. They presented the highest yield under normal environments and good yield under drought conditions. Drought-tolerant rice lines can be adapted in large regions in rain-fed lowland environments where drought is frequent, especially during the reproductive stage. Molecular markers associated with drought tolerance indices can be used to select drought-tolerant lines at the seedling stages under normal condition. Fifteen main effect QTLs containing of *qGY9*, *qGY12a*, *qTOL4, qTOL5*, *qSSI5*, *qSSI6*, *qST19*, *qST1I2*, *qMP9*, *qGMP9*, *qGMP12*, *qHM12a*, *qYSI5, qYSI6* and *qYI12a* can be considered as the major QTLs in rice breeding programs for improving grain yield and drought tolerance after validation. The markers i.e. UBC816-2, (Tos2+UBC827)-4, (UBC826+HB12)-6, RM215 and RM5371 that located near major QTLs are proposed for MAS programs after validation. The results show that some markers are related to several traits important for simultaneous breeding of several traits. The QTLs

Table 3. Epistatic QTLs detected for yield and drought tolerance indices.

Trait	Loci (i)			Loci (j)			LOD	AA[a]	R^{2b} (%)
	Chromosome	Position	Marker interval	Chromosome	Position	Marker interval			
YP	4	46	RM252-UBC809-1	9	81	UBC(808+814)-3-RM201	5.25	2.61	6.51
STI	9	40	RM566-RM6839	10	24	(Tos2+UBC817)-5-RM311	6.07	-0.21	18.81

[a]: Additive-by-additive interaction between two loci: the positive value (+) indicates that the alleles from the two loci have the same increasing or decreasing effect (both have a positive or negative effect) and the negative value (-) indicates that the alleles from two loci have a different effect on the effect of the respective trait (one locus has increasing effect and another locus has a decreasing effect on the trait.

[b]: Percentage of phenotypic variance explained by each epistatic QTL.

identified in this study should also be examined in the other segregating populations or mapping populations to determine the effect of genetic background on the expression of QTLs. The tagging and identification of large-effect QTLs associated with drought tolerance indices will be helpful in the selection of QTLs in early generations with the MAS technique, and will greatly accelerate rice cultivar development for improving drought tolerance. For more precise identification of the significant QTL regions on the chromosome, doing a fine mapping project in the present mapping population is also suggested.

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