

Research Paper

Identification and mapping of QTLs associated with drought tolerance traits in rice by a cross between Super Basmati and IR55419-04

Muhammad Sabar¹⁾, Ghulam Shabir²⁾, Shahid Masood Shah^{*3)}, Kashif Aslam²⁾, Shahzad Amir Naveed⁴⁾ and Muhammad Arif⁵⁾

¹⁾ Rice Research Institute, Kala Shah Kaku, Lahore, Pakistan

²⁾ Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

³⁾ Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus, Abbottabad, 22010, Pakistan

⁴⁾ Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

⁵⁾ National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, 38000, Pakistan

Water stress, in a climate change scenario is one of the major threats for sustainable rice productivity. Combining drought resistance with yield and desirable economic traits is the most promising solution for the researchers. Although several studies resulted in the identification of QTLs for drought resistance in rice, but none of them serve as a milestone. Therefore, there is always a quest to find the new QTLs. The present investigation was carried out to map QTLs involved in drought resistance and yield related parameter in a cross of IR55419-04 and Super Basmati. An F₂ population of 418 individuals was used as the mapping population. The raised nursery was transplanted in lyzimeters. Two extreme sets of tolerant (23 Nos.) and sensitive (23 Nos.) individuals were selected based on total water uptake under water stress conditions. Two hundred thirty microsatellite markers staggered on the whole genome were used for identifying polymorphic markers between the two parents. The selected 73 polymorphic microsatellites were used to genotype individuals and were scattered on a distance of 1735 cM on all 12 linkage groups. QTL analysis was performed by using the WinQTL Cartographer 2.5 V. A total of 21 QTLs were detected using composite interval mapping. The QTLs relating to drought tolerance at the vegetative stage were found on chromosome 1. Novel genomic regions were detected in the marker interval RM520-RM143 and RM168-RM520. The region has a significant QTL *qTWU3.1* for total water uptake. Root morphological trait QTLs were found on chromosome 3. QTLs responsible for additive effects were due to the alleles of the IR55419-04. These novel QTLs can be used for marker assisted breeding to develop new drought-tolerant rice varieties and fine mapping can be used to explore the functional relationship between the QTLs and phenotypic traits.

Key Words: rice, water uptake, mapping population, SSR markers, composite interval mapping.

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. It covers about 9% of the earth's arable land. It is a source of 21% of global human per capita energy and 15% of per capita protein and makes up a quality food for almost half of the world population (Xu *et al.* 2011). Aromatic rice is famous in Asia and has gained broader acceptance in Europe and the United States (Ahmad *et al.* 2005). In an aromatic group of rice, Basmati is very popular and is cultivated solely in the foothills of the Hima-

layas, the Uttar Pradesh and Haryana regions of India and the Punjab province of Pakistan and India (Nagaraju *et al.* 2002). Basmati rice is a very well-known type of rice in the world due to the unique characteristics like long grain, aroma, and intermediate amylose content.

Rice is cultivated in deep water, irrigated, rainfed upland and rainfed lowland (Poehlman and Sleper 1995) whatever the case of its cultivation is, it requires plenty of water to give the optimum yield. The recent climate shift has urged the scientific community to develop strategies to address this issue with the help of scientific advancements. QTL mapping for the different traits and their introgression in cultivated varieties in the past few decades resolved several problems. The main issue with these achievements was their regional acceptability.

Currently number of scientists developed population for

Communicated by Qian Qian

Received May 23, 2018. Accepted November 30, 2018.

First Published Online in J-STAGE on March 28, 2019.

*Corresponding author (e-mail: smasood@cuiatd.edu.pk)

QTL mapping for almost all traits, including drought, submergence, grain quality traits, vascular bundle related traits, and bacterial leaf blight (Bernier *et al.* 2009, Dixit *et al.* 2014, Sandhu *et al.* 2014, Shamsudin *et al.* 2016, Singh *et al.* 2016, Zhai *et al.* 2018). To the best of our knowledge, no study is conducted in which Super Basmati used as one of the parents. Super Basmati is a popular Basmati rice variety developed by Rice Research Institute, Kala Shah Kaku, Punjab (Pakistan) and released in 1996 for general cultivation. The variety is liked by the rice growers of the Punjab province and is being cultivated in the famous Basmati rice belt known as “Kallar Tract”. Super Basmati variety covers major (>80%) area of the total Basmati rice area in the Punjab province. The variety is also very popular among millers, rice traders and consumers for its extra-long grain, excellent taste and typical Basmati aroma. Moreover, it is also preferred in the international market and fetches a considerable amount of foreign exchange for the country since its approval.

Pakistan, in the past few years is facing a challenging situation regarding climatic shift. These shifts resulted in uncertainty and unpredictability for grower of rice. In Pakistan, rice occupies second position after wheat and is grown on an area of 2.3 million hectares. The share of Pakistan in total world rice trade is about 11%, while by quantity it is 8.9%. Rice contributes 5.7% of the total agricultural value and 1.1% to GDP. Therefore, it is of prime importance to increase or at least maintain the yield as the climatic shift is a big challenge for its yield sustainability.

These environmental stresses pave the way for biotic and abiotic stresses. It has been observed that among all stresses drought so far is the main abiotic stress for rice, and many efforts have been done to improve rice yield under drought stress (Guan *et al.* 2010, Xiao *et al.* 2007). Thus, the best approach to increase the rice production is to develop new drought tolerant rice varieties with high yielding potential.

Phenotypic characters such as root dry weight, shoot dry weight, culm length, leaf area etc. have a strong correlation with drought. Many QTLs were identified for drought tolerance related traits such as leaf water potential, root dry weight, plant osmotic potential, root morphology and leaf rolling, shoot dry weight, root hair length, root thickness, deep root mass and total root number (Henry *et al.* 2015, Jongdee *et al.* 2002, Li *et al.* 2015, 2017, Liu *et al.* 2005, Qu *et al.* 2008a, 2008b, Terra *et al.* 2016).

The present study was designed for identification of novel QTLs for enhancement of drought tolerance in a cross between Super Basmati and IR55419-04. In turn, the drought-tolerant introgression lines developed through this research effort will be utilized in the future rice breeding programs in the country. Ultimately, sustainable rice production will bring more opportunities for the prosperity of the country through food security and poverty alleviation.

Materials and Methods

Parental materials and population development

The two parents were selected based on the study reported by Sabar and Arif (2014) in order to develop the mapping population. Super Basmati is an aromatic rice variety sensitive to water stress at the vegetative stage with a long grain. Whereas IR55419-04 is a coarse grain rice variety of the *indica* group. It has a stiff stem, is drought tolerant, but has short grain.

Three dates of parental lines, Super Basmati and IR55419-04, were planted in a crossing block at the research area of the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. Super Basmati was used as a female parent while making a cross with donor IR55419-04. Emasculation of the female plant was done in the afternoon using vacuum emasculator at 500 mmHg and pollination was performed in the next morning with proper tagging. At maturity, successful F₁ seeds were harvested and stored properly. To raise F₁ seedlings, the seeds were placed on a filter paper in petri plates at 30°C in the incubator. One week old seedlings were transferred from the petri plates to the raised wet beds in the field. Genotyping for heterogeneity conformity of the F₁ using two to four polymorphic microsatellites was also done before transplantation. Thirty days after sowing, the seedlings were transplanted in the field. Subsequently, these individuals were allowed to be selfed and the F₂ seeds were harvested at maturity. An F₂ population was used as a mapping population.

Experimental site and F₂ phenotyping

The experiment was conducted in the greenhouse at the International Rice Research Institute (IRRI), Los Banos, Philippines (14°11' N 121°15' E, 21 m above sea level) during the dry season. The bulk seeds harvested from F₁ plants were sown in the seedling trays with covets (cup-shaped) filled 80% with soil. Each covets carried a small hole at the bottom allowing water to enter inside. In each covet, only one seed was dribbled manually and was covered with a thin soil layer. The trays were placed on the flat leveled surface of a tank where the water could remain stagnant so that it could only enter from the covet bottom hole through capillary action. The seedlings were irrigated by maintaining the water level of the tank at 50% covets sink. The raised seedlings were transferred into the pots 20 days after seeding (DAS). The pots were made of 105 cm long 19 cm diameter PVC pipes lined with plastic bags, filled with homogenized IRRI upland farm soil and sealed at the bottom. Each pot was drilled for making three holes near the bottom of the PVC pipe sides to allow drainage. These holes were plugged with rubber corks not allowing the drainage. The lower portion (80%) of the pots was filled with a bulk density equivalent to 1.15 g/cm³ of dry soil by compacting the soil even after every 5 cm during the filling process (Bernier *et al.* 2009). To settle down the soil, the pots were saturated with water for a few days before transplanting the seedlings.

The upper (20%) portion was filled with lowland irrigated soil. The soil level was set aside 5 cm below the edge of the pots. The pots were painted white to reduce the soil temperature from the surroundings. The total number of pots for the F₂ population was 418. For each of the two parental genotypes and a drought-sensitive check variety IR64, there were 30 pots (15 for well-watered and 15 for water stress). The pots were divided into six blocks. Each block was protected with 2.45 cm thick polyester film/polyester fiber non-woven fabric flexible composite material sheet to minimize the heat effect on the surrounding pots. Phosphorus and potassium were applied at 40 kg ha⁻¹ as a basal dose. The equivalent of 40 kg ha⁻¹ of nitrogen was applied at 10 days after transplanting (DAT) of seedlings. The pots of two parents (tolerant and sensitive) were watered thrice a week to maintain the soil near field capacity for the whole duration of the experiment in the well-watered (control) treatment and for 29 days at 40 DAS in the water stress treatment to F₂ population and their two parents.

Phenotypic data collection

The water consumption of water-stressed plants was measured by weighing pots the day following the last irrigation i.e., 41 DAS. During the stress treatment, other readings were recorded at 48, 55, 62 and 69 DAS (once in a week). Evapo-transpiration (water consumption) was calculated as the difference of the initial pot weight and the pot weight at different times (Bernier *et al.* 2009). Soil evaporation was minimized by covering the pots opening with polythene plastic sheets having a hole in the middle for the plant shoot to come out. Aboveground biomass was sampled at 69 DAS by cutting the plants at the soil surface level. The plant harvested shoots were dried by keeping them in the oven at 70°C for 72 hours and their weights were measured with the help of a top loading balance. The data for a number of tillers per plant and plant height were also recorded as described in the revised Standard Evaluation System for rice (SES 2002). For the measurement of the root parameters, the plastic bags were pulled out from the pots and the soil was then cut into three sections i.e., 0–30 cm depth, 30–60 cm depth and 60–100 cm depth. Within every soil section, roots were carefully washed to remove the soil. The washed roots were preserved in plastic bags containing 70% ethanol solution at 4°C until the completion of the scanning process. Root volume, root surface area, root diameter and root length of 60–100 cm depth section were measured by a scanner (EPSON, V700) and the recorded data were analyzed by using WinRHIZO Software, Canada. Roots and shoots were dried by keeping them in oven at 70°C for 72 hours. The root and shoot dry weight was then obtained by weighing on the scale. The leaf area (LA) of the parental genotypes, under well-watered treatment, was measured by the leaf area meter. For leaf area estimation of the F₂ population, a non-destructive method was used. The digital picture of each plant was captured by using a digital camera (Power-Shot G7, Canon) set at one meter distance from the

pot. A paper comprising of 100 cm² area was used as a calibration reference and a three-digit number on white paper was placed just by the side of the top of the pot. The JPG images of the plants were taken at each weighing and were subjected to analysis using ImageJ software.

Genotyping

CTAB method with little modification was used for the genomic DNA extraction from 21 days old leaves of the plants. For measurements of DNA concentration and quality, NanoDrop spectrophotometer (DE 19810 U.S.A.) was used and as per quantification, the working dilutions (25 ng/μl) of the template DNA were prepared.

Polymerase chain reaction (PCR) was performed in 15 μl reaction mix containing 2 μl of 20–25 ng DNA template, 1.5 μl 10x TB buffer [containing 200 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM MgCl₂], 1.2 μl of 1 mM dNTP, 0.80 μl of Taq DNA polymerase (5 Unit/μl) 0.50 μl each of 5 μM forward and reverse primers using a G-Storm GS1 thermal cycler (AlphaMetrix Biotech GmbH, Rodermark, Germany).

Mini vertical polyacrylamide gels for high throughput manual genotyping (CBS Scientific Co. Inc., CA, USA) were used. The samples allowed to run for about 1.5–3.5 hours at 100 volts depended on the size of the PCR fragment. The polyacrylamide gel was stained with SYBR Safe 10% solution in an opaque staining tray with lid for 30 minutes. For visualizing the gel, gel documentation system Alpha Imager 1220 (Alpha Innotech, CA, USA) was used.

Molecular data analysis

Evenly distributed SSR markers were used to identify polymorphic markers to differentiate the two parents. A molecular map was constructed with microsatellites to position the polymorphic markers relative to each other on the map using Map Manager QTX. QTL analysis was performed with microsatellite mapping data using WinQTL Cartographer 2.5 V (Wang *et al.* 2001) to generate the genetic linkage map. Composite interval mapping (CIM) was employed using the selected F₂ plants to locate the more accurate association and precise position of putative QTLs. The QTLs were designated based on the nomenclature procedure suggested by McCouch *et al.* (1997) and McCouch and CGSNL (2008). In the suggested nomenclature, “q” stands for QTL and “Ht, Till, SDW”, refer different traits like plant height, tillers per plant, stem dry weight, the number at the end of the locus name defines the chromosome number on which the QTL has been mapped.

Results

Frequency distribution of physiological and agronomic traits

The two parents and their F₂ population comprising of 418 plants were subjected to severe water stress for four weeks at the vegetative stage (Fig. 1). Whole F₂ population



Fig. 1. F₂ population screening at severe water stress.

gave normal frequency distribution for total water uptake. The mean total water uptake for IR55419-04 was 4956 g, while that of Super Basmati was 3711 g. The variation for total water uptake in the F₂ plants ranged from 2675 to 5743 g. The two extremes i.e., high and low (23 each) were selected on the basis of this trait.

The parental lines, IR55419-04 and Super Basmati achieved the plant height of 111.2 and 100.0 cm, respectively. Selected F₂ plants showed the plant height in the range from 85.4 to 120.2 cm. More number of taller plants (14 out of selected 46 plants) as compared the donor parent IR55419-04 was observed. A high frequency (71.7%) of the tillers per plant in a skewed manner amongst the F₂ individ-

uals showed greater value as compared to sensitive parent Super Basmati. Similarly, the variation for culm length of the selected plants was in the range from 24.9 to 47.0 cm. The frequency of transgressive segregates, for leaf dry weight and stem dry weight was nearly in a normal distribution. The variation in total shoot dry weight of F₂ plants was in the range of 9.4 to 25.7 g showing the transgressive segregation for the parameter. The frequency distribution of this trait seems to follow a bimodal distribution that is negatively skewed (skewness = -0.300). The two parents revealed extreme responses to water stress regarding the leaf drying score, as Super Basmati is drought sensitive (final score = 7) and IR55419-04 is tolerant (final score = 1). Slightly skewed data towards tolerant class was observed for leaf drying score than would be expected in a normal distribution. About 22 individuals of the F₂ population had a range 19.3–319 cm², 24 plants ranged from 319.1–852 cm², and parents IR55419-04 (703.9 cm²) and Super Basmati (312.4 cm²) for leaf area measured. Frequency distribution of each trait among plants of the mapping population and their parents has shown in **Fig. 2**.

Correlation studies of drought-related parameters

Correlation analysis was performed on 14 different plant traits including roots, shoot, and leaves (**Table 1**). All the root related traits like total root dry weight, deep root length, deep root volume, deep root surface area and deep root diameter were found to have a strong correlation (at P < 0.001) having correlation coefficient in the range of

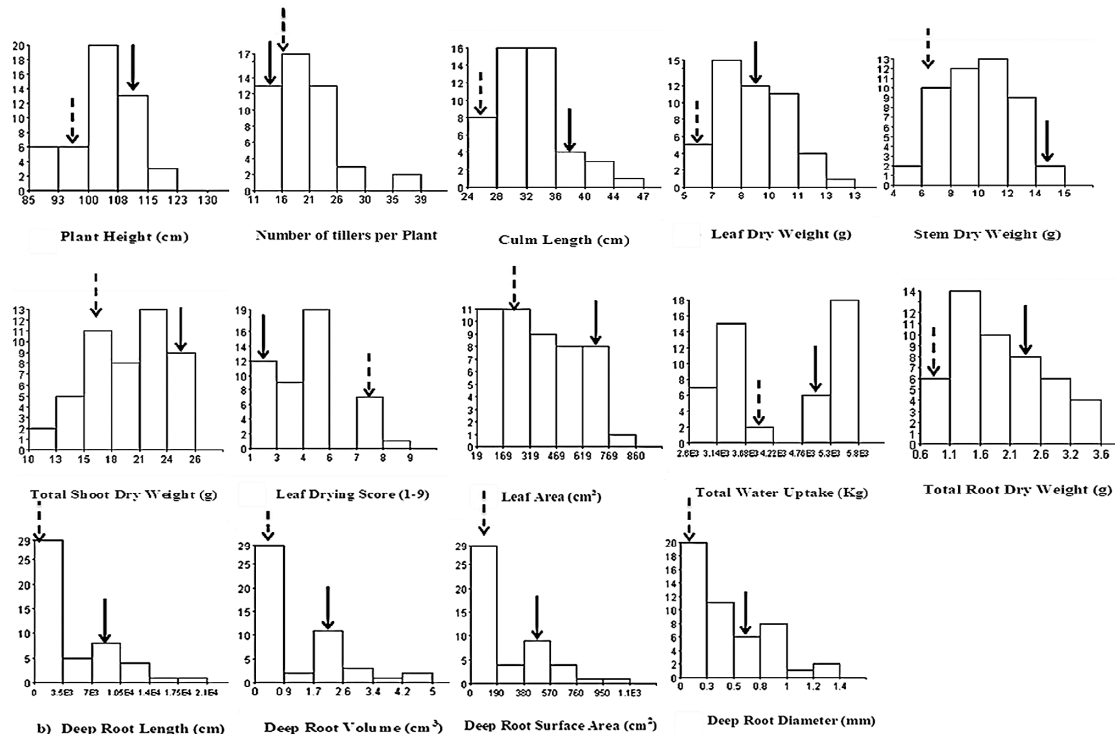


Fig. 2. Frequency distribution of selected two extremes of the F₂ population for water stress-related traits at vegetative stage. Filled and dotted arrows indicate the phenotypic value of the parents IR55419-04 and Super Basmati, respectively.

Table 1. Matrix for Pearson Correlation coefficient (r) showing the simple linear relationship among various drought-related rice plant parameters

	PLHT	TILL	CL	LDW	SDW	TSDW	LFDG	TWU	LFA	TRDW	DRL	DRVOL	DRSA	DRD
PLHT	1													
TILL	-0.264 ^{ns}	1												
CL	0.567 ^{***}	-0.355 [*]	1											
LDW	0.204 ^{ns}	0.511 ^{***}	-0.053 ^{ns}	1										
SDW	0.286 ^{ns}	0.200 ^{ns}	0.441 ^{**}	0.590 ^{***}	1									
TSDW	0.322 [*]	0.298 [*]	0.303 [*]	0.835 ^{***}	0.929 ^{***}	1								
LFDG	-0.100 ^{ns}	0.206 ^{ns}	-0.173 ^{ns}	-0.265 ^{ns}	-0.544 ^{***}	-0.503 ^{***}	1							
TWU	0.172 ^{ns}	-0.195 ^{ns}	0.270 ^{ns}	0.441 ^{**}	0.638 ^{***}	0.667 ^{***}	-0.708 ^{***}	1						
LFA	0.236 ^{ns}	-0.367 [*]	0.258 ^{ns}	0.327 [*]	0.487 ^{***}	0.521 ^{***}	-0.749 ^{***}	0.803 ^{***}	1					
TRDW	0.053 ^{ns}	0.041 ^{ns}	0.368 [*]	0.315 [*]	0.704 ^{***}	0.626 ^{***}	-0.454 ^{**}	0.725 ^{***}	0.424 ^{**}	1				
DRL	0.128 ^{ns}	-0.096 ^{ns}	0.275 ^{ns}	0.413 ^{**}	0.471 ^{***}	0.547 ^{***}	-0.488 ^{***}	0.807 ^{***}	0.587 ^{***}	0.681 ^{***}	1			
DRVOL	0.202 ^{ns}	-0.189 ^{ns}	0.435 ^{**}	0.284 ^{ns}	0.500 ^{***}	0.516 ^{***}	-0.479 ^{***}	0.810 ^{***}	0.563 ^{***}	0.73 ^{***}	0.942 ^{***}	1		
DRSA	0.168 ^{ns}	-0.140 ^{ns}	0.353 [*]	0.361 ^{**}	0.494 ^{***}	0.543 ^{***}	-0.491 ^{***}	0.820 ^{***}	0.587 ^{***}	0.717 ^{***}	0.989 ^{***}	0.981 ^{***}	1	
DRD	0.163 ^{ns}	-0.114 ^{ns}	0.429 ^{**}	0.298 [*]	0.482 ^{***}	0.508 ^{***}	-0.410 ^{**}	0.769 ^{***}	0.470 ^{***}	0.696 ^{***}	0.885 ^{***}	0.952 ^{***}	0.925 ^{***}	1

Abbreviations: PLHT = Plant height; TILL = Tillers per plant; CL = Culm length; LDW = Leaf dry weight; SDW = Stem dry weight; TSDW = Total shoot dry weight; LFDG = Leaf drying; TWU = Total water uptake; LFA = Leaf area; TRDW = Total root dry weight; DRL = Deep root length (Below 60 cm); DRVOL = Deep root volume (Below 60 cm); DRSA = Deep root surface area (Below 60 cm); DRAD = Deep root average diameter (Below 60 cm).

* Significance at $P < 0.05$; ** Significance at $P < 0.01$; *** Significance at $P < 0.001$.

0.725–0.820 with total water uptake. Leaf dry weight, total shoot dry weight, and stem dry weight were the other traits having a significant correlation with total water uptake towards positive side. The relationship among leaf drying (an indicator of water stress level) and root traits was observed to be significant but negative. A strong correlation was observed among all the root traits with each other with positive impact. Although plant height was not significantly associated with root traits but has a strong association with stem length that was positively correlated with all the root traits except deep root length. A negative but significant association between tillers per plant and leaf area was also recorded.

Genetic linkage mapping and QTL analysis

Two hundred thirty SSR markers were used to identify the polymorphism between the two parents. Seventy-three microsatellites (31.7%) scattered on 1735 cM distance on all 12 linkage groups (chromosomes) were found polymorphic (co-dominant).

The identified polymorphic SSR markers were then applied on 46 F_2 individuals of the two extreme classes (23 plants from drought-tolerant extreme and 23 plants from drought-sensitive extreme) in the population. All the 73 polymorphic markers displayed clear banding pattern. A molecular map was constructed with these microsatellite mapping data to position the markers relative to each other on the map using Map Manager QTX.

The total size of the linkage map was 1735 cM and the average interval size was 23.7 cM. Few gaps with large distances were persisted mainly on chromosomes 5, 6, 8 and 9. The analysis was performed with microsatellite mapping data using WinQTL Cartographer 2.5 V (Wang *et al.* 2001) to generate the genetic linkage map.

QTLs identified for different plant traits

Composite interval mapping (CIM) was employed using the F_2 population to locate the more accurate association and precise position of putative QTLs. A total of 21 QTLs were detected using CIM. The QTLs relating to tolerance to water stress conditions at vegetative stage were found on, chromosomes ch1 (*qHt1*), ch2 (*qCmL2*), ch3 (*qLDW3*, *qSDW3*, *qTSDW3*, *qLDW3*, *qTWU3*, *qLA3*, *qTRDW3*, *qDRL3*, *qDRV3*, *qDRSA3*, *qDRD3*, *qDRSA3*, *qDRD3*), ch4 (*qTWU4*), ch6 (*qHt6*), ch9 (*qDRL9*) and ch11 (*qTill11*, *qDRL11*). A summary of the statistics of all significant QTLs (LOD ≥ 3.0) are presented in **Table 2** and are located on chromosomes in **Fig. 3**.

Plant height

Two QTLs *qHt1* and *qHt6* were identified for plant height that explains 19.5%, and 25% of the phenotypic variance (R^2), respectively. QTL *qHt1* on chromosome 1 (LOD value 3.6) was localized at interval RM212-RM265 (peak marker RM212) and IR55419-04 allele contributed in the phenotypic performance. Another QTL *qHt6.2* was present on chromosome 6 at marker interval RM587-RM510 (66.01 cM) showed the phenotypic effect on plant height is coming from IR55419-04 alleles. The LOD value of this QTL was 5.3.

Number of tillers per plant

Only one region was identified with a significant LOD score of 4.0 for the number of tillers per plant on chromosome 11. For this QTL, Super Basmati alleles had a positive effect on tillering ability and explained 19.5% of the phenotypic variation. The QTL *qTill11* for the number of tillers per plant was located between RM202-RM229 marker interval sited at 69.2 cM on chromosome 11.

Culm length

A QTL was spotted on chromosome 2 that was significantly associated with culm length with LOD values 4.7. IR55419-04 alleles contributed (29.5%) for culm length at

Table 2. QTLs detected for water stress tolerance related traits based on composite interval mapping in selected extreme plants of the F₂ population derived from the cross Super Basmati/IR55419-04

Trait name	QTL name	Position	Ch. number	Marker interval	LOD value	R ² (%)	Additive effect
Plant height	<i>qHt1</i>	164.2	1	RM212-RM265	3.6	19.5	-5.6
	<i>qHt6</i>	66.01	6	RM587-RM510	5.3	25.0	-7.6
Tillers per plant	<i>qTill11</i>	69.2	11	RM202-RM229	4.0	19.5	3.1
Culm length	<i>qCmL2</i>	34.0	2	RM110-RM174	4.7	29.5	-0.3
Leaf dry weight	<i>qLDW3</i>	246.3	3	RM520-RM143	3.8	34.0	0.4
Stem dry weight	<i>qSDW3</i>	244.3	3	RM520-RM143	10.7	7.5	-2.4
Total shoot dry weight	<i>qTSDW3</i>	238.3	3	RM520-RM143	11.7	6.9	-4.7
Leaf drying score	<i>qLDS3</i>	234.3	3	RM520-RM143	5.3	4.5	0.7
	<i>qLDS4</i>	109.5	4	RM451-RM559	4.8	12.5	2.2
Total water uptake	<i>qTWU3</i>	238.3	3	RM520-RM143	27.0	9.96	-299.150
	<i>qTWU4</i>	4.4	4	RM119-RM518	5.6	2.45	-236.494
Leaf area	<i>qLA3</i>	234.3	3	RM168-RM520	9.7	17.12	207.195
Total root dry weight	<i>qTRDW3</i>	236.3	3	RM168-RM520	8.3	20.901	-0.778
Deep root length	<i>qDRL3</i>	226.3	3	RM168-RM520	7.2	29.51	-0.010
	<i>qDRL9</i>	50.0	9	RM105-RM410	3.5	9.114	-0.080
	<i>qDRL11</i>	56.0	11	RM202-RM229	3.3	3.857	-0.100
Deep root volume	<i>qDRV3</i>	232.3	3	RM168-RM520	6.7	25.784	-1.829
Deep root surface area	<i>qDRSA3.1</i>	136.7	3	RM16030-RM168	4.1	13.624	48.625
	<i>qDRSA3.2</i>	228.3	3	RM168-RM520	7.7	32.096	-220.250
Deep root diameter	<i>qDRD3.1</i>	136.8	3	RM16030-RM168	4.7	18.346	0.082
	<i>qDRD3.2</i>	242.3	3	RM168-RM520	5.2	25.430	-0.292

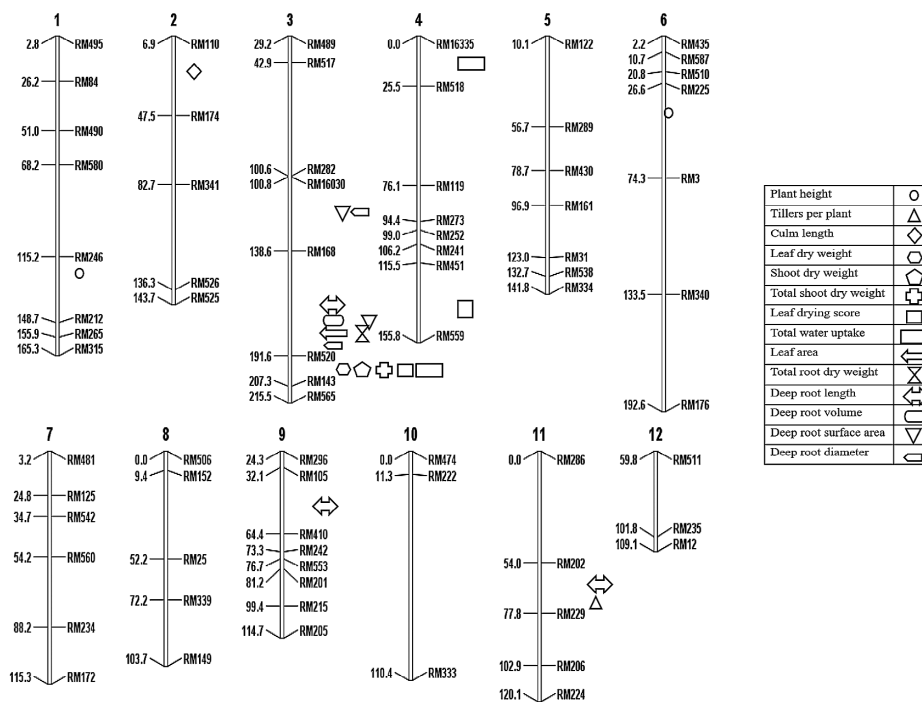


Fig. 3. A genetic linkage map of rice showing detected QTLs on different chromosomes. Chromosome number is at the top, the map distances in centi Morgan (cM) at the left side and SSR markers names at the right side of each linkage group.

chromosome 2. The QTL *qCmL2* was placed between RM110-RM174.

Leaf dry weight

A region was identified on chromosome 3 with LOD scores 3.8 for leaf dry weight. QTL *qLDW3* (Super Basmati allele) had a positive effect and explained 34% of the phenotypic variance for leaf dry weight. The QTL was co-localized in the marker interval RM520-RM143, on the chromosome 3 (246.3 cM).

Stem dry weight

On chromosome 3, one QTL *qSDW3* was associated with stem dry weight with a significant LOD score of 10.7. The marker locus RM520-RM143 was found responsible for this QTL that explained about 7.5% of the phenotypic variation.

Total shoot dry weight

One QTL was located on chromosome 3 that was significantly associated with the total shoot dry weight having

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LOD value 11.7. IR55419-04 allele contributed an additive effect of 4.7 for this QTL with the phenotypic variance of 6.9% and was located between RM520-RM143.

Leaf drying score

Two genomic regions were identified on chromosomes 3 and 4 with LOD scores 5.3 and 4.8 at marker intervals RM520-RM143 and RM451-RM559 for leaf drying score respectively. For the QTL *qLDS3*, Super Basmati alleles had a positive effect with phenotypic variances 4.5% and 12.5% for leaf drying scores on chromosomes 3 and 4, respectively.

Total water uptake

For total water uptake, one genomic region in the marker interval RM520-RM143 was detected with a significant QTL *qTWU3* on the long arm of the chromosome 3 with LOD score 27.0 that explained about 9.96% of the phenotypic variation. Another locus between RM119-RM518 was identified on chromosome 4 that shared about 2.45% in phenotypic variance. The additive effect of both the QTLs was due to the alleles of the IR55419-04 with the values 299.15 and 236.49, respectively.

Leaf area

One QTL *qLA3* was located on chromosome 3 that was significantly associated with leaf area with LOD value 9.7. Super Basmati alleles contributed (207.2) for the QTLs with the phenotypic variance of 17.12% and were located between RM168-RM520.

Total root dry weight

One significant QTL was detected through composite interval mapping on the long arm of chromosome 3 between RM168-RM520 for total root dry weight, with the positive alleles contributed by IR55419-04. This QTL accounted for about 20.1% of the total variation in total root dry weight.

Deep root length

Three genomic regions co-localized in marker intervals RM168-RM520, RM105-RM410 and RM202-RM229 with LOD values of 7.2, 3.5 and 3.3 respectively were identified on chromosomes 3, 9 and 11, respectively. All the alleles of IR55419-04 contributed positively explaining 29.5%, 9.1% and 3.86% of the total phenotypic variation for deep root length.

Deep root volume

On chromosome 3, one QTL *qDRV3* was associated with deep root volume with significant LOD score of 6.7. The marker locus RM168-RM520 was found responsible for this QTL that explained about 25.78% of the phenotypic variation.

Deep root surface area

The two regions were identified on chromosome 3 with LOD scores 4.1 and 7.7 at marker intervals RM16030-RM168 and RM168-RM520 for deep root surface area, respectively. For the QTL *qDRSA3.1*, Super Basmati allele had a positive effect with phenotypic variance 13.62%, whereas IR55419-04 was the contributor of the alleles for the QTL *qDRSA3.2* with the phenotypic variance of 32.096% on the same chromosome 3.

Deep root diameter

Two significant QTLs were detected on the long arm of chromosome 3 between RM16030-RM168 and RM168-RM520 for deep root diameter with the positive alleles contributed by Super Basmati and IR55419-04, respectively. These QTLs accounted for about 18.35% and 25.43% of the total variation in deep root diameter, respectively.

Discussion

Climate shift is becoming one of the major concern for sustainable agriculture production including rice. To cope with climatic shift, rice breeders have increased their efforts to breed new rice varieties by utilizing currently applied technologies such as marker assisted breeding along with conventional methods. Marker assisted breeding is dependent upon DNA markers tightly linked with identified QTLs for traits of interest such as drought in the climate change scenario. Bi-parental QTL mapping resulted in identification of many QTLs in rice, but to explore potential drought tolerant QTLs is a dire need due to diverse characteristics of Basmati rice.

Physiological and agronomic traits analysis

Screening for drought tolerance targeting the root traits is laborious, complicated and not easy under field conditions due to logistical and methodological issues of root study. Although, hydroponic studies of root phenotyping (Price and Tomos 1997, Steudle 2000, Topp *et al.* 2013) are easy but the results could not be translated successfully in breeding varieties because most of these identified QTLs were detected in quite different environment as experienced in field. That's why we used PVC pipes of bigger diameter with bulk density closer to the field by compacting the soil while filling to make the soil of root zone in accordance with field conditions so that the findings can be translated in improving the drought tolerance.

Drought stress screening of parents and F₂ for physiological and agronomic traits showed that these traits are highly correlated to each other at significant levels of 0.001 and 0.05 (Table 1). Marri *et al.* (2005) reported that plant height has a significant negative correlation with number of tillers, which are in harmony with the finding of current study. The possible reason could be the utilization of maximum energy produce by existing dividing tissue rather than to initiate new division in the form of new tillers. Our results depicting negative but strong association of leaf drying with total water uptake are in agreement with the findings of Courtois *et al.* (2000) and Yue *et al.* (2005). The three traits; deep root length, deep root volume and deep root surface area responded with each other in significantly positive manner as reported by Subashri *et al.* (2009). A positive association of total water uptake with roots related traits i.e., deeper root length, deep root volume and deep root surface area clearly showed that root system of the plant is certainly essential for extraction of water from deeper soil layers under

drought stress conditions. Further, the presence of deep roots under water deficit environment were observed and was the response in search of moisture for maintaining turgor of the tissues (Gowda *et al.* 2011). Many other researchers also reported that under drought stress, genotypes modified root distribution and penetrate in the deeper soil to extract water for their survival and growth (Allah *et al.* 2010, Lobet *et al.* 2014, Puckridge and O'Toole 1980, Uga *et al.* 2008). In addition, a positive and highly significant relationship of shoot dry weight with all deep roots related traits strengthened the finding of Zhang *et al.* (2008). Shoot biomass is an indicator of ultimate grain yield as it translates into the emergence of panicles that bear grains. High shoot biomass under drought indicates the better growth due to higher plant water status maintained through increased water uptake by roots (Levitt 1980). The presence of positive inter relationships of deep root average diameter, deep root length and root dry weight was proved to be obvious under water limited environment as these results are in accordance with the earlier reports (Kano *et al.* 2011, Qu *et al.* 2008a, 2008b). Root architecture adaptations under water deficit conditions plays an important role in developing deeper roots in soil (Bernier *et al.* 2008). Li *et al.* (2005) reported that root diameter and deep root thickness can be used to improve drought tolerance in rice.

Genetic linkage mapping

Genetic map covering 1735 cM genomic region was constructed by 73 SSR markers with an average marker interval of 23.7 cM which is slightly more than the minimum required interval (20 cM) for QTL mapping (Lander and Botstein 1989). However, for selective genotyping more specific markers were used for mapping. Linkage maps of variable lengths of 1781.5, 1645.1 and 1650 cM by using 213 markers, 130 SSR markers and 9303 SNP markers were constructed by Solis *et al.* (2017), Gu *et al.* (2011) and De Leon *et al.* (2016) respectively with different marker spacings. These difference in linkage map length and spacing among studies was results of variation in genetic background of selected parents, marker types used, number of polymorphic markers found and recombination rate in the developed mapping population.

QTL analysis by selective genotyping

Selective genotyping is a highly cost-effective and more powerful way than simple interval mapping for identification of novel QTLs controlling the specific trait. There is one disadvantage of this technique is false positive QTLs due to insufficient marker density and small size of the population (Sun *et al.* 2010). Phansak *et al.* (2016) adapted selective genotyping approach in soybean F₂ population for seed protein and oil contents QTLs detection and demonstrated that selective genotyping strategy was highly successful. Keeping in view the power and advantages of the technique, we adopted this approach and 21 QTLs were identified controlling morphological and physiological traits

under study at vegetative stage by choosing 6% of population extremes based on total water uptake during stress period. According to Ayoub and Mather (2002) 5 to 10% population extremes were sufficient for selective genotyping.

In our study, two QTLs for plant height were identified under water stress condition, one on chromosome 1 and one on chromosome 6, however, the LOD value and phenotypic variance of IR55419-04 alleles for QTL *qHt6* were higher than *qHt1*. The QTL *qHt1* identified at the interval of RM212 and RM265 was also detected by Septiningsih *et al.* (2003) at RM315 locus near to identified RM265. Ye *et al.* (2012) has also reported QTLs controlling plant height located on chromosome 1. Two QTLs associated with plant height were detected by using SNP markers on chromosome 6 by McNally *et al.* (2009). The reduction in plant height has been observed as the impact of drought stress at vegetative stage resulting in relative yield penalty (Ahmadikhah and Marufinia 2016). One QTL *qTill1* on chromosome 11 was identified for tiller number per plant at a marker interval of RM202-RM229 which was previously reported by Septiningsih *et al.* (2003) at the same position. The allele of Super Basmati contributed for this locus indicating that tillering ability of this line is either high or tillering was completed before the commencement of earlier than the stress period.

Only one QTL was detected under the severe stress condition (29 days stress) for total shoot dry weight at the lower tip of chromosome 3 at the marker position of RM520. Kamoshita *et al.* (2002) also found the shoot biomass QTL on the lower tip of chromosome 3 by using RFLP and AFLP markers.

Overall, two QTLs were detected controlling leaf drying under a severe drought condition on chromosomes 3 and 4 with LOD scores of 5.3 and 4.8, respectively. Leaf drying QTL on the chromosome 3 was found between the interval of marker RM520-RM143 with a peak at RM520. Similar QTL was reported by Yue *et al.* (2005) at the locus RM520. They also identified other QTLs for this trait on different chromosomes.

Three QTLs (*qDRL3*, *qDRL9* and *qDRL11*) were identified for deep root length (below 60 cm) on chromosomes 3, 9 and 11 under stress condition. Qu *et al.* (2008a) reported deep root length QTL on the tip of chromosome 6 at the marker position of RM454 very near to RM520. The QTL affects deep root length was identified 10 cM distance at RM278 marker position apart from the detected QTL *qDRL9* at marker interval RM105-RM410. Kamoshita *et al.* (2002) reported deep root length on chromosome 11 at RM167 marker position near to identified QTL *qDRL11* at an interval of RM202-RM229. Yue *et al.* (2005) also reported similar QTL on chromosome 11 at the same marker position of RM 229.

One QTL for deep root volume (below 60 cm) was identified on chromosome 3 of the selected extremes lines based on the total water uptake. In contrast, root volume QTL was identified by Qu *et al.* (2008a) using SSR markers

at chromosome 4. Yue *et al.* (2005) also reported deep root volume under drought QTL on chromosome 4 at RM470 marker position at the similar position of RM451.

Maximum number of QTLs were found at RM520 marker locus under the severe drought stress condition for leaf dry weight, stem dry weight, total shoot dry weight, leaf drying, total water uptake, leaf area, total root dry weight, deep root length, deep root volume, deep root surface area and root diameter. All these QTLs were not previously reported at this locus, however, this locus was identified for yield under drought in different previous studies (Yue *et al.* 2005).

In the net shell, composite interval mapping resulted in the identification of 21 QTLs. A major genomic region at RM520 was detected with a significant QTL (*qTWU_{3,1}*) for total water uptake (LOD = 27.0) and some associated root morphological traits on chromosome 3. This major QTL can be used for MAS to develop new drought-tolerant rice varieties. The major water uptake QTL linked with RM520 needs this research push up for the identification of underlying genes and to explore the functional relationship between the QTLs and phenotypic trait.

Rice genotypes having deep roots and higher root diameter produce more aboveground biomass that helps in survival under water stress conditions. In earlier studies, root related traits have been suggested to be of high consideration due to the fact that plant extracts water and nutrition from the soil layers through roots. High root numbers, deep root length and deep root volume enhance physical strength, improved root system architecture, increased nutrients/soil moisture absorption from deeper layers, penetration ability, hydraulic conductivity, root system size and branching density thus minimizing the risk of deficient water status of the plant tissues and resulting in increased tolerance under drought through avoidance mechanism (Kanbar *et al.* 2009, Steele *et al.* 2007, Venuprasad *et al.* 2002). This study had shown that total water uptake and root-related traits are of high importance under water stress environment and their identified QTLs must be given a real focus for the improvement of drought tolerance in future rice varieties. Recycling of drought and floods in a shorter period of time is the outcome of prevailing climate change scenario. Thus, developing water stress tolerant plant varieties is a big challenge for the breeders to meet the higher food demand and ensure food security to human beings. Our findings will be helpful to solve this situation.

Acknowledgments

We thank Higher Education Commission, Pakistan for providing financial support. We also acknowledge the technical support, assistance and guidance during this research work by Dr. Rachid Serraj, Senior Scientist, Crop and Environmental Sciences Division, IRRI, Philippines. Valuable suggestions of Dr. Henry Amelia, the leader of Drought Physiology Group at IRRI, Philippines are also acknowledged.

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