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POOLED MAPPING OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH DROUGHT TOLERANCE IN RICE (*ORYZA SATIVA* L.) AT SEEDLING STAGE

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ABSTRACT

Drought is among the foremost constraints influencing global rice productivity. The drought tolerance nature of rice is complicated, depending on multiple components and having low heritability. Thus, breeding drought-tolerant varieties is a fundamental way which can be used to increase rice yield in drought. To investigate the genetic basis of seedling tolerance to drought stress of rice (*Oryza sativa* L.), we performed QTL mapping on a big F_2 population of 2600 participants from a cross between the *japonica* rice variety Huaidao 5 (HD5) and the *indica* rice variety 1892S through performing bulked segregant analysis and whole-genome sequencing (BSA-seq). HD5 showed greater tolerance to prolonged drought stress compared with 1892S at seedling stage. By analyzing a pair of opposite DNA pools made from 182 extremely-sensitive seedlings and 182 extremely-tolerant seedlings from the F_2 population using the block regression mapping (BRM) method, we mapped a QTL on chromosome 1, of which the additive effect was estimated to explain 2.20% of the phenotypic variance. We named the QTL *qSLDT1.1* (*q* represents quantitative trait loci, *SL* represents seedling leaf, *DT* represents drought tolerance, *1.1* represents the first one found on chromosome 1), which must be a novel QTL, because no QTLs for rice seedling tolerance to drought stress have been mapped on chromosome 1 before. The information derived from the current research facilitates marker-assisted breeding of drought-resistant lines and positional cloning of the gene conferring drought tolerance in rice.

Key words: Rice, Drought tolerance, QTL mapping, Bulked segregant analysis, Whole-genome sequencing

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INTRODUCTION

It is demonstrated that rice (*Oryza sativa* L.) ranks among the foremost food crops universally, occupying approximately 9% of the arable land on Earth. It offers 21% concentration of the global per capita energy as well as 15% concentration of the per capita protein, supplying nearly 50% of the global population with quality food (Xu *et al.,* 2011). Recently, the rice cultivation worldwide has been challenged by main production constraints, primarily owing to climate change-caused detrimental impacts. Drought is the leading factor of abiotic stress of rice ecosystem, inducing 50% yield loss across the globe (Qin *et al.,* 2011). The occurrences and frequency of such stress is unpredictable and increasing. Around 34 million hectares of rain-fed lowland as well as 8 million hectares of upland rice across Asia frequently suffer drought stress (Venuprasad *et al.,* 2009). Singhal *et al.* (2016) suggested over half of the agricultural land worldwide is going to be challenged by drought stress until 2050. Therefore, high yielding varieties showing drought tolerance need to be bred for sustaining rice yields.

Under the control of many quantitative trait loci (QTLs), the mechanism of drought tolerance is sophisticated (Fleury *et al.,* 2010). Considerable research works have been conducted on identification of genes or QTLs related to drought stress of rice (Huang *et al.,* 2022; Roy *et al.,* 2023). It is known that drought stress influences varying developmental phases of rice. Most of the studies reported concentrate on the role of drought stress on the reproductive development in rice (Mishra *et al.,* 2013; Palanog *et al.,* 2014; Prince *et al.,* 2015; Swamy *et al.,* 2017; Bhattarai *et al.,* 2018; Yadav *et al.,* 2019; Baisakh *et al.,* 2020; Satrio *et al.,* 2021). However, the influence of drought stress on rice seedlings is also important and needs attention. Drought stress at seedling stage is critical for crop establishment under direct seeding conditions. In addition, drought stress at seedling stage could possibly be genetically connected with the drought stress at other developmental stages. Therefore, the study on the genetic foundation of drought stress at

seedling stage has great importance to the breeding of drought-tolerant varieties of rice. Based on five shoot morphological traits: seedling shoot length, seedling dry weight, seedling leaf rolling, seedling leaf drying and seedling drought recovery, Saikumar *et al.* (2014) identified 10 QTLs on chromosomes 3, 5 and 8 for drought tolerance at seedling phase with BILs (BC_1F_6) population of rice. With a BIL population of 143 BC_2F_{20} lines and leaf drying, leaf rolling, leaf number, dry weight of root, dry weight of shoot, maximum root length as well as maximum shoot length index as indicators, Huang *et al.* (2022) mapped 13 QTLs on rice chromosomes 1, 2, 4, 5, 7, 8, 10 and 11 conferring drought tolerance at seedling phase.

Bulked segregant analysis via deep sequencing (BSA-seq) provides the useful means for QTL mapping. BSA-seq in combination with traditional gene mapping method greatly accelerates the fine mapping of QTLs/genes (Liang *et al.,* 2020). In recent years, BSA-seq has been adopted to mining QTLs in rice (Barik *et al.,* 2019, 2020). Huang *et al.* (2020) proposed a novel statistical approach for BSA-seq, called Block Regression Mapping (BRM). In this study, the BRM method was adopted for mapping QTLs conferring drought tolerance in rice at seedling stage and map a QTL on chromosome 1. The result will facilitate identifying closely linked markers for rice breeding under drought stress, and subsequently fine mapping and cloning of the causal gene in the QTL.

MATERIALS AND METHODS

Mapping population: An F_2 population was developed for QTL mapping from a cross between a *japonica* rice variety Huaidao 5 (HD5) and an *indica* rice variety 1892S, an elite photoperiodic as well as thermo-sensitive male sterile (P/TGMS) line. The preparative experiment suggested a greater tolerance of HD5 to drought stress compared with 1892S at seedling stage.

Seed sowing and planting: With 54 seeds in each box (9 rows × 6 seeds/row), pre-germinated seeds in the mapping population were sown in clean sand within rectangular (38 × 25 ×10 cm^3) plastic turnover boxes. Besides, seedlings grew under the condition of 25°C in the greenhouse. The sand was moistened using tap water each day, and Yoshida nutrient solution (Yoshida *et al.,* 1976) was applied every three days when the seedlings grew to the three-leaf stage. Apart from that, we sowed 100 seeds of every parental line for reference.

Identifying individuals with extreme phenotypes on drought tolerance: Using 25% PEG6000 in the phytotron growth chamber in the cycle of 12 h light (15000 LX) and 12 h dark, seedlings showing uniform growth performance at the three-leaf stage were processed. In addition, seedlings had exposure to 25% PEG6000 for 12 h, and seedlings sowing maximum sensitivity to drought stress (when leaves were wilted at the tip) were identified and collected as the extremely sensitive (ES) individuals. The remaining seedlings were kept in the chamber for 45 h. Meantime, most seedlings were wilted at the leaf tip or even died, while the few seedlings appearing normal were collected as the extremely tolerant (ET) individuals. 50 turnover boxes were chosen in 5 batches, with 10 boxes per batch. For every batch, around 38 ES and 38ET individuals were chosen, each making up of ~7% of total seedlings studied.

Bulking, DNA extraction and sequencing: For bulked segregant analysis, 182 ES individuals and 182 ET individuals were screened for establishing two opposite groups from the 2,600 valid F_2 plants. In each group, a segment of equal weight was sliced from every young leave (formerly preserved in the refrigerator at -80°C) of the group and then all segments were mixed to extract DNA by the CTAB method (Doyle and Doyle, 1987) after moderate modifications. Therefore, two DNA pools were formed, namely, the ES pool and the ET pool. It was found that under standard paired-end 150 bp sequencing library construction protocols, DNA samples of the two parents, HD5 and 1892S, and the two pools received whole-genome resequencing on the Illumina HiSeqX Ten platform.

Investigation of reads and variants: The raw reads in sequenced ES and ET pools were rinsed and trimmed with the BBDuk program for BB Tools (http://jgi.doe.gov/data-and-tools/bbtools/). Paired reads were mapped to the IRGSP-1.0 reference rice genome (http://rapdb.dna.affrc.go.jp) with Burrows-Wheeler Aligner using the Maximal Exact Matches algorithm (BWA MEM) and the alignments were exposed to treatment with SAMTools (Kawahara *et al.,* 2013). Freebayes was employed for calling SNPs and InDels based on default parameters (Garrison and Marth, 2012). For obtaining reliable polymorphic markers, variant (SNP or short InDel) filtering was conducted with custom perl scripts. To prevent serious segregation distortion, only the SNPs or short InDels with allele frequency (AF) values of 0.3 - 0.7 in the population were maintained. Such markers were annotated with the use of snpEff (Cingolani *et al.,* 2012).

QTL analysis: The marker set was taken for mapping QTLs. Besides, allele frequency difference (AFD) of each marker between the ES and the ET pools could be measured and smoothed by block regression with the BRM method (Huang *et al.,* 2020). Meanwhile, the block size for regression was determined as 20 kb. To measure the AFD curve threshold at the overall (genome-wise) significance level of 0.05, the theoretical allele frequency assumption (= 0.5) in the F_2 population was used.

Concerning every significant AFD peak (candidate QTL), the 95% confidence interval was measured. With the peak AFD value, by adopting the Pooled QTL Heritability Estimator method (Tang *et al.,* 2018), the heritability of every QTL was assessed.

RESULTS

In total, 2,600 F_2 seedlings from the cross between HD5 and 1892S were detected as having drought tolerance. These F_2 seedlings exhibited continuous variation of wilting degree under the drought stress, suggesting that drought tolerance is a quantitative trait.

Whole-genome sequencing of the ET and ES pools and the parents HD5 and 1892S generated ~75.5 to 108.2 million reads of every pool or parental line. Through filtering, 1,984,092 SNPs and 242,848 short InDels were found (Table 1). It could be seen that the average densities of SNP as well as short InDel markers were 5.32 SNP/kb (or one SNP every 188 bp) and 0.65 InDel/kb (or one InDel every 1,538 bp), respectively, which were high enough for QTL mapping.

Using the BRM method, a significant AFD peak was detected on chromosome 1 below the overall (genome-wise) significance level of 0.05 (Figure 1), indicating the existence of a QTL. Apart from that, the QTL was named *qSLDT1.1*. The most probable position of the QTL (the highest point of the peak) was at ~40.50 Mb, with a 95% confidence interval ranging 35.11–43.20 Mb. The AFD peak of the QTL was negative, suggesting that the allele from parent DH5 of the QTL acted to increase drought tolerance. The additive effect heritability of the QTL approached 2.20%, but the dominance effect heritability could not be reasonably estimated due to severe segregation distortion at the QTL.

Table 1 Distributions of SNPs and short InDels identified on different chromosomes.

Chr.	Length (bp)	SNP		Short InDel	
		Number	Density (per kb)	Number	Density (per kb)
1	43,270,923	234,218	5.42	29,969	0.69
2	35,937,250	228,470	6.31	29,381	0.81
3	36,413,819	228,949	6.27	29,433	0.81
4	35,502,694	72,265	2.02	10,316	0.34
5	29,958,434	160,950	5.34	20,314	0.68
6	31,248,787	168,486	5.35	19,386	0.62
7	29,697,621	182,242	6.11	21,256	0.72
8	28,443,022	162,385	5.69	19,278	0.68
9	23,012,720	95,502	4.11	12,514	0.54
10	23,207,287	121,606	5.16	13,603	0.58
11	29,021,106	195,618	6.72	21,854	0.75
12	27,531,856	133,401	4.77	15,439	0.55
Average	373,245,519	1,984,092	5.32	242,743	0.65

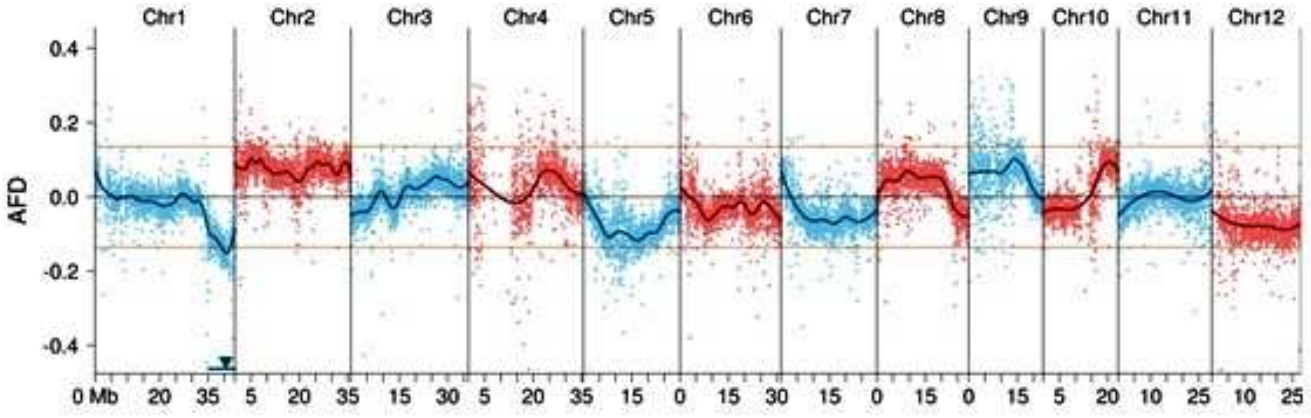

Figure 1 Block regression mapping of QTLs conferring resistance to drought stress of rice. Obviously, the horizontal orange lines suggest the AFD threshold (±0.135) at the overall (genome-wise) significance level of 0.05. The estimated QTL positions are highlighted in filled triangles. The AFD of a marker was measured through subtracting the AF of parent 1892S in the ES pool from that in the ET pool.

Table 2 Reported QTLs in association with drought stress tolerance on chromosome 1 in rice.

Tolerant/sensitive parents	Population	Appraisal period	QTL name	Position (Mb)	References
Kali Aus/IR64	BC_1F_4	Reproductive stage	*qDTY*$_{1.3}$	24.80-36.73	Sandhu *et al.,* 2014
Kali Aus/MTU1010	BC_1F_4	Reproductive stage	*qDTY*$_{1.2}$	7.44-36.73	
			qPH$_{1.1}$	36.73-38.89	
Cabacu/IR64	F_6-BILs	Reproductive stage	*qLRS1.1*	-	Trijatmiko *et al.,* 2014
			qPH1.1	-	
Moroberekan/Swarna	BC_2F_3	Reproductive stage	*qDTH*$_{1.1}$	-	Dixit *et al.,* 2014
Nootripathu/IR20	$F_{8/11}$-RILs	Reproductive stage	-	34.86	Prince *et al.,* 2015
			-	33.05	
			-	23.32	
			-	36.47	
N-22/Cocodrie	F_7-RILs	Reproductive stage	*qPH1.07*	7.05-7.08	Bhattarai *et al.,* 2018
			qPH1.38	38.28-38.61	
			qLRS1.37	37.27-37.37	
			qGY1.42	42.92-42.97	
			qYI1.42	42.98-43.07	
			qHI1.37	37.56-37.74	
Vandana/Cocodrie	$F_{2:3}$	Reproductive stage	*qGYD1.1*	12.90-15.15	Solis *et al.,* 2018
			qGYD1.2	30.70-32.13	
			qGYD1.3	-	
JG88/IR66897B JG88/MR77 JG88/MR167 JG88/SN265	BC_2F_2	Reproductive stage	*QDT1.3* *QDT1.4*	9.86 15.12	Cui *et al.,* 2018
-	-	Vegetative stage	*q1*	22.79-23.32	Hoang *et al.,* 2019
Dular/Swarn	BC_1F_3	Reproductive stage	*qDTY*$_{1.1}$	40.01-40.08	Yadav *et al.,* 2019
			qDTY$_{1.3}$	5.57-5.62	
			qDTF$_{1.2}$	42.65-42.89	
			qPH$_{1.2}$	4.48-4.95	
			qPH$_{1.3}$	38.28-38.61	
AUS 196/IR11N121	BC_1F_3	Reproductive stage	*qDTY*$_{1.1}$	41.76-42.91	
			qDTY$_{1.4}$	25.58-27.77	
			qDTF$_{1.1}$	39.50-41.22	
			qPH$_{1.1}$	38.75-39.38	
IR55419-04/Basmati	F_2	Vegetative stage	*qHt1*	33.05-35.19	Sabar *et al.,* 2019
N22/Cocodrie	$F_{2:3}$	Reproductive stage	*qPN1.1*	-	Baisakh *et al.,* 2020
			qGY1.1	-	
CR 143 – 2-2/ Krishnahamsa	F_7-RILs	Reproductive stage	*qRCC1.1*	34.51-36.47	Barik *et al.,* 2020
			qCHLa1.1	0.21-34.51	
B6144F-MR-6/MH63	ILs	Reproductive stage	*qAER1*	30.55-32.78	Xu *et al.,* 2020
-	-	Tiller stage	-	33.05	Verma *et al.,* 2021
HB/ IR64	F_9-RILs	Vegetative stage	*qCW.D-1*	20.6-21.1	Satrio *et al.,* 2021
			qRSR.RDI-1	25.2-25.7	
			qBB.DRI-1	33.2-33.3	
O. longistaminata/9311	BC_2F_{20}-BILs	Seedling stage	*qDWR1.1*	161.0-162.0	Huang *et al.,* 2022
			qDWS1.1	234.0-235.0	
Banglami/Ranjit	RILs	Reproductive stage	*qPL_1.1*	3.0-34.5	Roy *et al.,* 2023
			qNOC_1.2	6.0-6.2	

DISCUSSION

Since the beginning of this century, over 310 QTLs for drought tolerance have been recognized from rice (Satrio *et al.,* 2021; Huang *et al.,* 2022; Roy *et al.,* 2023). Most of them are mapped based on the performance during the period of reproductive development. Only 23 QTLs for drought tolerance are mapped at seedling phase. These 23 QTLs are located on chromosomes 1-5, 7, 8 and 10-11 (Saikumar *et al.,* 2014; Huang *et al.,* 2022). However, only two QTLs (*qDWR1.1* and *qDWS1.1*) are located on chromosomes 1, but they are not located within the confidence interval of the *qSLDT1.1*. So the QTL *qSLDT1.1* mapped in this study on chromosome 1 is a novel QTL.

Although only two QTLs for drought tolerance to drought stress at seedling stage in rice have been found on chromosome 1 before, there are a lot of QTLs for drought tolerance at the reproductive phase detected on chromosome 1 (Table 2). Much of the QTLs are located within the confidence interval of the *qSLDT1.1*. In particular, the QTL *QRvd1* for root volume, the QTL *qtl1.1* for plant height at maturity, the QTL $qDTY_{1.1}$ for grain yield, and the QTL $qDTF_{1.1}$ for days to flowering subject to drought stress are relatively close to the peak tip of the *qSLDT1.1*. This implies a possibility of common genetic basis between the drought tolerance of seedling stage and that of reproductive phase, although the position of the *qSLDT1.1* is not precisely estimated due to the wide confidence interval.

Drought tolerance refers to a comprehensive and sophisticated trait that exhibits plant response to drought stress. Numerous traits can suggest the drought tolerance of rice, such as biomass (Saikumar *et al.,* 2014), leaf area (Sabar *et al.,* 2019), panicle length (Nie *et al.,* 2015; Prince *et al.,* 2015; Roy *et al.,* 2023), maximum root length (Huang *et al.,* 2022), and fresh shoot weight (Verma *et al.,* 2021). However, different indicator traits of drought tolerance may result in very different QTLs (Verma *et al.,* 2021; Huang *et al.,* 2022). Therefore, selection of suitable indicator traits is critical to the mapping of drought tolerance QTLs. The current work used the survival state of seedling under drought stress to indicate drought tolerance. Intuitively, survival state must directly reflect the degree of drought tolerance. However, survival state is difficult to be measured quantitatively. Fortunately, BSA-seq only uses the extremely tolerant and the extremely sensitive seedlings, which can be easily identified without the need of quantitative measurement. Therefore, survival state of seedling came out as a suitable indicator trait for BSA-seq. It is not labor-intensive, time-consuming and costly.

Only one QTL with a relatively small additive effect heritability (2.20%) was detected, although a large F_2 population was used. The result suggested that although there was significant difference between the two parental varieties on seedling tolerance to drought stress, the difference was probably controlled by many minor genes of very small effects only. As a very stringent selection intensity (~7%) was used for BSA-seq in this study, the statistical power was not high enough to detect the minor QTLs. To detect these minor QTLs, a larger selection proportion (e.g. 15–20%) of the pools may be required (Magwene *et al.,* 2011).

Conclusion: In this study, we identified a novel drought-tolerant QTL at seedling stage in rice using RBM method based on an F_2 population.

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Authors' Contribution: YW made the experiments and wrote the first draft; WT performed genotyping and processed the data; GZ, CZ, TW and HZ involved in cultivation of rice seedlings and treatment; HW provided the seed samples, created the F_2 population and revised the manuscript.

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