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Analysis of drought tolerant QTL linked to physiological and productivity component traits under water-stress and non-stress in rice (*Oryza sativa L.*)

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KEYWORDS

Identify QTL;
drought tolerance;
BILs;

ABSTRACT

The objective of the present investigation was to screen introgressed population for physiological and yield component traits to identify QTL for drought tolerance and discover tolerant lines among top performing BILs for rainfed condition. The breeding material for this study was inter-varietal backcross inbred lines (BILs) of Swarna x WAB 450 developed at Barwale Foundation, Hyderabad, India. 188 BILs along with 10 checks were transplanted in 12□□ pots inside poly house in a randomized block design in 2 replications. Drought stress was imposed at the onset of the reproductive growth phase until grain filling stage by withholding irrigation from the treatment plot until severe leaf rolling was observed; control plot experiment was watered daily till crop maturity: observations were recorded for physiological and productivity related traits. QTL detected for various traits based on composite interval mapping under water stress and the control show significant contribution from the donor parent WAB 450. In this study, 10 QTL were detected for physiological, productivity and related traits under water stress whereas 5 QTL were identified under non stress condition. All identified QTL under water stress for various traits exhibited high phenotypic variance except leaf temperature which was low. WAB 450 contributed all the alleles that increase drought resistance for all QTL identified in this study except two QTL identified for relative water content and spikelet fertility. These QTL might contribute to the maintenance of grain yield through the with drought tolerance.

Introduction

Drought stress was found to be one of the major causes that limit the yield instability (Ozturk *et al.* 2002), and great efforts have been made to breed drought tolerant crop variety. Breeding of new rice cultivar with drought tolerance not only saves a great amount of water but also helps to increase and stabilize the yield. It is therefore scientifically significant to study the genetic basis of drought tolerance and also economically important to explore and utilize new genetic resources of drought tolerance in rice breeding (Xia *et al.*, 2006). Due to the complexity of drought tolerant mechanism in rice, researchers mainly focused on localizing genes that contribute to drought response in a quantitative way using several breeding methods such as marker-assisted selection, marker assisted backcrossing, transgenic technology and so on. Identification of genomic regions with drought-resistant loci, which are useful for marker-assisted breeding of drought tolerance, has been well documented (Crasta *et al.*, 1999; Frova *et al.*, 1999). Several QTL mapping associated with drought tolerance in rice such as root thickness, ratio of root and shoot, root dry weight (Champoux *et al.*, 1995); osmotic adjustment, dehydration tolerance, stoma tolerance, leaf rolling and leaf drying (Lilley *et al.*, 1996; Price and Tomos, 1997; Price *et al.*, 2002) were reported.

Developments of introgressed lines by inter specific hybridization between improved cultivated rice and drought resistant land races have been employed by many rice breeders to explore genetic resources in traditional rice variety. The NERICA varieties provide a good example of how distant hybridization were used to developed drought tolerant cultivars (Jones *et al.*, 1997). Nguyen *et al.* (2010) used SSR technique combined with selective genotyping to map quantitative trait loci (QTLs) associated with drought tolerance in 229 lines (BC_2F_2) derived from the cross between OM1490 / WAB880-1-38-18-20-P1-HB; his report showed that markers associated with drought tolerance were located mostly on chromosomes 2, 3, 4, 8, 9, 10 and 12. Hanamaratti (2007) evaluated Near Isogenic Introgressed Lines (NIILs) of IR 64/Binam (IB) and Teqing/Binam (TB) under non-stress and moisture stress at vegetative stage and reproductive stage for identification of QTLs for physiological and productivity traits. In all, 61 QTLs in IB NIILs and 35 QTLs in TB NIILs were detected ($P<0.01$) for physiological and productivity traits. Hanamaratti (2007) reported that majority of QTLs detected under drought condition, had favourable alleles from Binam.

Despite the importance of drought as a major constraint to breeding for upland rice ecosystem, not much has been committed to developing drought-tolerant rice cultivars. Most of the high-yielding mega varieties such as IR36, IR64, Swarna, and Samba Mahsuri that are grown in rainfed areas are varieties bred for irrigated ecosystems and they were never selected for drought tolerance (Kumar *et al.*, 2008). Farmers in rainfed ecosystem have no option than to recourse to this choice due to lack of high-yielding and good quality drought-tolerant varieties and risk attached to this choice could be devastating especially in drought years, where such varieties can suffer heavy yield losses in the affected region. It is against this backdrop this study is design to find a lasting solution to the pressing need of farmers in upland rainfed ecosystem. In this study, WAB 450 [one of the accessions of Africa rice varieties (NERICA line)] - upland rice variety known for drought tolerance and blast disease resistance was crossed to Swarna- a popular irrigated lowland mega variety in India. The BILs developed from Swarna x WAB 450 under upland rainfed condition hold a good promise for development of drought tolerant rice cultivars for upland rainfed ecosystem.

Materials and Methods

The breeding materials for this study was inter-varietal backcross inbred lines (Swarna x WAB 450) developed by Barwale Foundation Hyderabad, Andhra Pradesh, India. This introgressed population (in BC_1F_6 generation) was genotyped in Hyderabad at Barwale Foundation rice lab with 58 well distributed polymorphic SSR markers selected from the linkage map developed at Cornel University (Temnykh *et al.*, 2001). Phenotyping was carried out at Rice Research Station of University of Agricultural Sciences Dharwad Karnataka, India. The Research Station is located at latitude of $15^{o}15'$ North and longitude of $70^{o}40'$ East and altitude of 695 meters above Mean Sea Level (MSL) belonging to Agro-climatic Zone No. 8 of Karnataka State. The average rainfall of the Research Station is 1016.20 mm in 75 rainy days distributed mainly during *kharif* (June to October) season. One hundred and eighty eight BILs (BC_1F_6), two parents (Swarna and WAB 450) and 10 local checks were screened in pot under artificially imposed water stress in rain out shelter (poly house) during summer 2011 for physiological and productivity related traits. Observations for traits were taken and data were analysed using Crop Stat developed by IRRI while linkage map was constructed using QTL IciMapping version 3.2 developed by Chinese Academy of Agricultural Sciences.

Result and Discussion

Result of composite interval mapping for all significant QTL under water stress and the control in the pot nursery are presented in Table 1 and Table 2, respectively. In this study, 10 QTL were detected for physiological, productivity related traits under water stress (Table 1) whereas 5 QTL were identified under non stress condition (Table 2). All identified QTL in this investigation were given relevant names according to QTL nomenclature adopted by McCouch, (2008). Out of 10 QTL detected under water stress, two QTL (qRWC3-1 and qRWC6-1) were for relative water content (mapped to chromosome 3 and 6). One QTL (qLT2) for leaf temperature was mapped to chromosome 2. One QTL (qPT7) for productive tillers was mapped to chromosome 7. Two QTL (qPW8 and qPW9) for panicle weight were mapped to chromosome 8 and 9. One QTL (qGN7) for grain number per plant (chromosome 7), Two QTL (qGW3-1 and qGW3-2) for grain weight were mapped to chromosome 3 and one QTL (qTGW1) for thousand grain weight was mapped to chromosome 1 (Table 1). All identified QTL under stress for various traits exhibited high phenotypic variance except leaf temperature which was low (Table 1). QTL (qPT7) for productive tillers under water stress was observed to be overlapping with QTL (qGN7) for grain number per plant on the same chromosome in this study (Table 1). In non-stress pot nursery, 5 QTLs were detected; three for relative water content (qRWC3-2, qRWC6-2 and qRWC6-3) were mapped to two chromosomes (Chromosomes 3 and 6), one QTL (qPT2) for productive tillers was mapped to chromosome 2 and one QTL (qSF2) for spikelet fertility was mapped to chromosome 2. Two QTL flanked by markers (RM489-RM36 and RM253-RM275) for relative water content under non-stress located on chromosome 3 and 6 were also identified under stress treatment with consistent effect (Table 1and 2).

QTL detected for various traits based on composite interval mapping under water stress and the control show significant contribution from the donor parent WAB

Table.1 QTL linked to drought tolerance detected for various traits in Swarna x WAB 450 BILs under water stress in pot experiment based on composite interval mapping

Sl. No.	Trait name	QTL	Chromosome	Position cM	Left Marker	Right Marker	LOD Score	Phenotypic variance (%)	Additive effect
1.	1000 grain weight (g)	qTGW1	1	171.7	RM302	RM529	5.66	56.81	-1.19
2.	Leaf temperature (^{0}C)	qLT2	2	17.3	RM279	RM327	3.51	9.19	-0.43
3.	Relative water content (%)	qRWC3-1	3	41.2	RM489	RM36	9.38	27.22	5.40
4.	Grain weight per plant (g)	qGW3-1	3	116.2	RM563	RM16	3.09	38.80	-3.81
5.	Grain weight per plant (g)	qGW3-2	3	149.2	RM16	RM130	3.11	43.47	-3.62
6.	Relative water content (%)	qRWC6-1	6	108	RM253	RM275	10.10	25.23	-5.16
7.	Productive tillers	qPT7	7	38.2	RM180	RM455	3.38	21.88	-1.05
8	Grain number per plant	qGN7	7	40.2	RM180	RM455	3.52	29.87	-132.14
9.	Panicle weight (g)	qPW8	8	12.1	RM337	RM556	3.26	43.18	-0.58
10.	Panicle weight (g)	qPW9	9	20.3	RM524	RM409	3.81	37.22	-0.77

Table.2 QTL linked to drought tolerance detected for various traits in Swarna x WAB 450 BILs under non- stress in pot experiment based on composite interval mapping

Sl. No.	Trait name	QTL	Chromosome	Position cM	Left Marker	Right Marker	LOD Score	Phenotypic variance (%)	Additive effect
1.	Productive tillers	qPT2	2	101.3	RM327	RM525	4.32	62.09	-2.20
2.	Spikelet fertility	qSF2	2	157.3	RM525	RM112	3.22	33.63	1.96
3.	Relative water content (%)	qRWC3-2	3	29.2	RM489	RM36	4.69	9.37	2.41
4.	Relative water content (%)	qRWC6-2	6	97	RM253	RM275	12.03	50.12	-5.29
5.	Relative water content (%)	qRWC6-3	6	115	RM275	RM340	11.76	42.12	-4.84

450. The negative additive effect (base on the marker data coding for the software used for the analysis) indicated the genetic effect contributed by WAB 450 whereas positive additive effect indicated allelic contribution from Swarna in the BILs' genome. QTL for productive tillers (qPT7) flanked by markers RM180-RM455 under water stress was observed to be tightly linked with QTL for grain number per plant (qGN7) (qGN7) on the same chromosome in this study (Table 1). Two QTL for relative water content flanked by markers RM180-RM455 and RM253-RM275 under non-stress located on chromosome 3 and 6 respectively were also identified under stress treatment with consistent additive effect (Table 1 and 2). Considering the coincidence of QTL on chromosome 3 and 6 with allelic contribution by each parent at both QTL locations, these QTL might contribute to the increased yield potential derived from Swarna x WAB 450 inter cross. WAB 450 contributed all the alleles that increases drought resistance for all QTL identified except QTL for relative water content and spikelet fertility flanked by markers RM498-RM36 and RM525-RM112 located on chromosomes 3 and 2 respectively (Table 1 and 2). These QTL might contribute to the maintenance of grain yield through the control of complex biochemical and physiological processes associated with drought tolerance. These data suggest that unique configurations of multiple alleles may be required for high levels of drought tolerance while maintaining grain yield. Previous studies by Xing *et al.* (2002) and Yu *et al.* (1997) found that epistatic interactions play an important role in determining grain yield and its components in rice, and most of them do not have main effects at the single-locus level. This report probably explains why no QTL was detected for some traits in this study. Phenotypic variation (PVE) explained by detected QTL for different traits ranged from 9.19 to 62.097 per cent. The residual variation that cannot be accounted for by the QTL may be due to epistatic interactions.

Many of the QTL identified for physiological traits except qRWC3-1, qRWC6-1, qRWC6-2, qRWC6-3 and qTGW1 had low LOD score. Threshold LOD score used for detecting QTL in this analysis was 3 and bearing in mind that the LOD score is a measure of the strength of evidence for the presence of a QTL at a particular location. All QTL with a LOD score value more than 3 falls within this confidence interval however, QTL with higher LOD score such as one obtained in this result for qRWC3-1, qRWC6-1, qRWC6-2, qRWC6-3 and qTGW1 are more dependable. Genomic architecture of plant as well as gene interaction with environment determines whether LOD score of any trait will be very low or high. It is expected that a quantitative trait whose expression is govern by many genes should have lower LOD score compare with those having fewer genes; gene into environment interaction also reduces LOD score.

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