

QTLs associated with yield attributing traits under drought stress in upland rice cultivar of Assam

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ABSTRACT

A total of 94 polymorphic SSR markers were used for genotyping of 90 F_4 plants. Among these, 56 markers fitted for the expected Mendelian ratio of segregation whereas, 38 significantly deviated from it ($P < 0.01$). Only 63 SSR markers could be assigned to 12 linkage groups (LGs) covering a total of 1652.3 cM of the rice genome at an average marker density of 26.22 cM. The number of markers on each linkage group ranged from 3 (LG 10) to 7 (LG 2 and LG 8). A total of 26 QTLs were identified for yield and physiological traits with the phenotypic variation ranging from 0.14% to 26.17%. Among these, 18 QTLs were identified under drought stress and remaining 8 QTLs were detected under irrigated conditions. The QTLs for days to 50% flowering remained consistent in the marker intervals of RM519-RM3331 on chromosome 12 under both hydrological conditions. Four QTLs were localized on the long arm of chromosome 1 between the marker intervals of RM3825-RM243. Thus, with further fine mapping of this region may identify some novel genes associated with drought tolerance which can be used in marker-assisted breeding (MAB) for the development of drought tolerant rice variety.

Key words: Rice, QTLs, yield traits, drought tolerance

INTRODUCTION

Drought is one of the most important abiotic stresses which limit rice production in rainfed environments (Kumar et al., 2016, 2017). Rice is the most important crop in Assam and covers 2.54 million (M) ha of the total 3.3 M ha cropped area. It is a semi-aquatic crop and more prone to losses from drought than other cereals such as wheat and maize, which are better adapted to be grown with less water. In Asia, a total of 23 million ha of rice area is affected by drought of variable intensities. In Assam despite of heavy rainfall, the main rice crop suffers from prolonged drought in *ahu* season and intermittent drought in *sali* season. The popular rice variety Ranjit is widely grown in Assam due to its suitable plant type and high grain yield but it is highly susceptible to drought stress during reproductive stage resulting in significant decline in grain yield. Therefore, it is important to develop variety tolerant to drought stress at reproductive stage.

Previously, secondary traits were used as selection criteria for improving grain yield and drought tolerance in rice (Jongdee et al., 2002; Sinha et al., 2015; Barik et al., 2017). However, desired improvement in grain yield could not be achieved. Therefore, grain yield under drought stress has been suggested as selection criteria in drought breeding (Bernier et al., 2008). By using this approach, many QTLs have been identified (Kumar et al., 2014) and improved breeding lines are developed in Philippines, India, Nepal and Bangladesh (Verulkar et al., 2010).

In the present investigation the local drought tolerant cultivar Banglami was crossed with mega rice variety Ranjit to raise mapping population. The F_4 population was used for identification of QTLs for various yield and physiological traits under drought stress and irrigated conditions. Further these QTLs are to be fine mapped and genes for various drought related traits to be identified for MAB in order to develop drought tolerant rice variety.

MATERIALS AND METHODS

Plant materials and crossing scheme

A tall, early maturing, short bold grains, medium duration (120-130 days) and drought tolerant cultivar, Banglami was used as a female parent. A semi dwarf, long duration (150-155 days), medium slender grains and drought susceptible variety, Ranjit was used as a male parent. The F_1 seeds were obtained from the cross between Banglami and Ranjit. 90 F_2 plants were raised from selfing of true F_1 s. The F_2 plants were harvested and bulked to raise F_3 population. The 90 F_4 lines consisting of 2130 plants were evaluated under drought stress and irrigated conditions.

Phenotyping

The F_4 plants were evaluated for various yield traits following augmented randomized block design under two different hydrological conditions *i.e.*, artificial drought stress created in rainout shelter and non-stress (irrigated) conditions in the experimental field of Regional Agriculture Research Station (RARS) Titabar, during rabi season (December 2015-July 2016). The drought stress was imposed from panicle initiation to panicle emergence period (reproductive stage) by withholding irrigation. The observations on various traits *viz.*, Days to 50 per cent flowering (DTF), Plant height (PH), Number of tillers per plant (NOT), Effective booting tillers per plant (EBT), Panicle length (PL), Number of grains per panicle (NOG), Number of chaff per panicle (NOC), Spikelet fertility (SF), Grain yield per plant (GY) and Relative leaf water content (RLWC) were recorded under both hydrological conditions.

Genotyping and construction of genetic linkage Map

Genomic DNA was extracted following the protocol of Plaschke et al. (1995). For PCR analysis, 10 μ L of reaction mixture consisting 50 ng DNA, 1X PCR buffer, 2.5 mM dNTPs, 20 pM each primer and 1 unit Taq DNA Polymerase enzyme was made for PCR analysis. The amplified products were resolved in 3.5 percent agarose gel stained with ethidium bromide. The 94 polymorphic SSR markers were used for genotyping of 90 F_4 plants. Chi square test was used to compute the segregation pattern of each SSR marker against the expected ratio in F_4 generation at 0.01 probability

level. Linkage analysis was performed by using JoinMap software (Stam, 1993) and WinQTL cartographer 2.5 (Basten et al., 2001) at critical LOD scores of 2.5.

RESULTS AND DISCUSSION

Segregation and linkage analysis of SSR markers

For each SSR marker, the observed segregations were tested against the expected Mendelian ratio in F_4 generation. Among 94 polymorphic markers, 56 (59.57%) fitted for the expected Mendelian segregation, whereas, 38 (40.42%) significantly deviated from it ($P < 0.01$). The observed segregation distortions may be due to the population size of only 90 F_4 plants used in the present study. It is a problem often encountered in mapping studies (Zivy et al., 1992). According to Hackett and Broadfoot (2003), segregation distortion had very little effect both on marker order and map length. Moreover, estimation of recombination fractions among co-dominant markers is less affected by segregation distortion than that of dominant markers (Lorieux et al., 1995). Therefore, all the skewed markers were retained for linkage analysis. Out of 94 polymorphic SSR markers, only 63 SSR markers could be assigned to 12 linkage groups (LGs) covering a total of 1652.3 cM of the rice genome at an average marker density of 26.22 cM. The number of markers on each linkage group ranged from 3 (LG 10) to 7 (LG 2 and LG 8).

QTL analysis

Previously a large proportion of QTLs identification studies targeted secondary traits related to drought tolerance (Lanceras et al., 2004). In the present study, the phenotypic data on yield and physiological traits were utilized for QTL identification using the SSR based genotyping data of 90 F_4 plants. A total of 26 QTLs were identified by composite interval mapping analysis under drought stress and irrigated conditions (Table 1). Three QTLs were identified for days to 50% flowering among these; two QTLs (*qDTF12.1* and *qDTF12.2*) remained consistent in the common marker intervals of RM519-RM3331 on chromosome 12 under two hydrological conditions. It indicated that the QTLs are stable and not essentially affected by environmental factors. A QTL for number of tillers (*qNOT6.1*) was identified on chromosome 6 contributed by susceptible parent Ranjit. There are many reports where the

Table 1. QTL identified under drought stress and irrigated conditions in F₄ lines derived from 'Banglami and Ranjit'

S. No.	QTLs	Chromosome	Marker Intervals	Position	LOD Score	Additive	R ²
1	<i>qDTF12.2-DS</i>	12	RM519-RM3331	54.60	6.30	-6.03	12.71
2	<i>qDTF3.1-NS</i>	3	RM168-RM186	73.40	5.76	4.98	26.17
3	<i>qDTF12.1-NS</i>	12	RM519-RM3331	52.60	5.31	-5.16	10.25
4	<i>qEBT3.1-DS</i>	3	RM1352- RM1038	15.60	3.11	1.41	16.20
5	<i>qNOT6.1-DS</i>	6	RM400-RM20757	27.00	3.36	-0.14	6.07
6	<i>qPH1.1-DS</i>	1	RM3825-RM1	196.10	3.50	0.23	2.95
7	<i>qPH7.1-DS</i>	7	RM125-RM182	58.40	3.08	2.87	3.33
8	<i>qPL1.1-DS</i>	1	RM5638-RM431	94.70	4.22	0.74	6.86
9	<i>qPL1.2-DS</i>	1	RM431-RM3825	102.50	4.57	0.89	7.38
10	<i>qPL1.3-DS</i>	1	RM1-RM243	255.60	3.47	-0.23	0.46
11	<i>qPL9.1-DS</i>	9	RM296- RM24181	56.00	4.21	0.60	1.05
12	<i>qPL9.2-DS</i>	9	RM24181-RM257	62.10	4.01	0.57	1.01
13	<i>qPL3.1-NS</i>	3	RM186-RM55	75.30	3.61	0.198	3.55
14	<i>qNOG9.1-DS</i>	9	RM296- RM24181	48.00	3.59	2.35	3.20
15	<i>qNOG6.1-NS</i>	6	RM253-RM30	122.50	4.60	-3.66	14.75
16	<i>qNOG6.2-NS</i>	6	RM30-RM217	147.30	4.43	-3.09	14.43
17	<i>qNOG9.2-NS</i>	9	RM242-RM553	199.70	3.30	-24.16	4.48
18	<i>qGY1.1-DS</i>	1	RM256-RM5638	4.00	4.63	0.19	0.14
19	<i>qGY1.2-NS</i>	1	RM3825-RM1	184.60	4.55	-1.46	19.56
20	<i>qGY11.1-NS</i>	11	RM21-RM209	84.70	3.12	-1.59	9.00
21	<i>qRLWC1.1-DS</i>	1	RM1-RM243	234.60	4.46	-1.06	0.89
22	<i>qRLWC6.1-DS</i>	6	RM30-RM217	184.30	2.98	1.97	4.63
23	<i>qRLWC9.1-DS</i>	9	RM296- RM24181	41.00	3.05	4.08	11.07
24	<i>qRLWC9.2-DS</i>	9	RM24181-RM257	78.10	4.79	3.06	8.84
25	<i>qRLWC9.3-DS</i>	9	RM242-RM553	185.20	3.35	9.36	5.92
26	<i>qRLWC11.1-DS</i>	11	RM20-RM206	22.00	4.28	1.66	0.16

DTF- Days to 50% flowering, NOT- No. of tillers per plant, EBT- Effective booting tillers per plant, PH-Plant height (cm), PL- Panicle length (cm), NOG- Number of grains per panicle, GY- Grain yield per plant, RLWC- Relative leaf water content, DS- Drought stress, NS- Non stress (Irrigated conditions), LOD- Logarithm of Odds, R²= % of phenotypic variance.

positive alleles for the trait under drought stress were contributed by the drought-susceptible parents (Lanceras et al., 2004). A QTL for effective booting tillers (*qEBT3.1*) was identified on chromosome 3, explained the highest phenotypic variation of 16.20% under drought stress. The favourable allele in the QTL was contributed by Banglami. Two QTLs were identified for plant height (*qPH1.1*, *qPH7.1*) on chromosome 1 and 7 under drought stress. Similarly on chromosome 1 at a position of 126.40 cM, a QTL for plant height was also reported by Prince et al. (2015) under drought stress condition. Five QTLs were identified for panicle length (*qPL1.1*, *qPL1.2*, *qPL1.3*, *qPL9.1*, *qPL9.2*) under drought stress. Among these, the favourable allele in the four QTLs was contributed by Banglami except for the QTL (*qPL1.3*), which was contributed by Ranjit. Three QTLs were located on chromosome 1 in the position range of 94.70-255.60 cM. Prince et al. (2015) also reported a QTL for panicle length on chromosome 1 at the position of 126.37 cM

under drought stress. However, under irrigated conditions a QTL (*qPL3.1*) was identified for panicle length on chromosome 3 derived from Banglami.

A QTL for number of grains per panicle (*qNOG9.1*) was identified on chromosome 9 under drought stress contributed by Banglami whereas; three QTLs for the similar trait (*qNOG6.1*, *qNOG6.2*, *qNOG9.2*) were identified on chromosome 6 and 9 under irrigated conditions which was contributed by the Ranjit. Higher spikelet fertility (84.88%) was observed in Banglami under drought stress due to existence of well developed deep root system whereas, sharp decline in the spikelet fertility (22.58%) was observed in the shallow rooted variety Ranjit. However, under irrigated conditions higher spikelet fertility of 88.07% was recorded in Ranjit. It indicated that the yield differences observed in the plants under drought stress were mostly due to ability of plants to maintain proper seedset, rather than to accumulate biomass as observed in the present study.

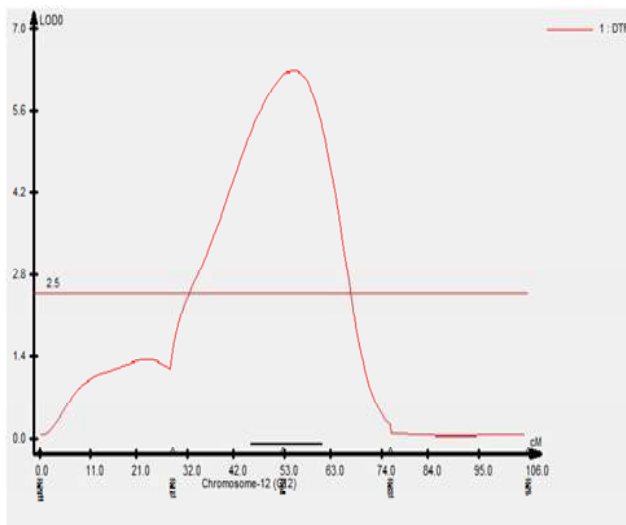


Fig. 1. QTL likelihood curves of the LOD score for days to 50% flowering (*qDTF12.2*) on chromosome 12 under drought stress.

Three QTLs were identified for grain yield, among these a QTL (*qGY1.1*) derived from Banglami was identified on chromosome 1 under drought stress (Fig. 2) whereas, two QTLs (*qGY1.2*, *qGY11.1*) derived from Ranjit were located on chromosome 1 and 11, respectively under irrigated conditions. Vikram et al. (2011) also reported a major QTL for grain yield, *qDTY1.1*, on chromosome 1 flanked by RM11943 and RM431 under drought stress. Genetic information in correlation with physiological traits will also help to develop drought-tolerant rice varieties (Lanceras et al.,

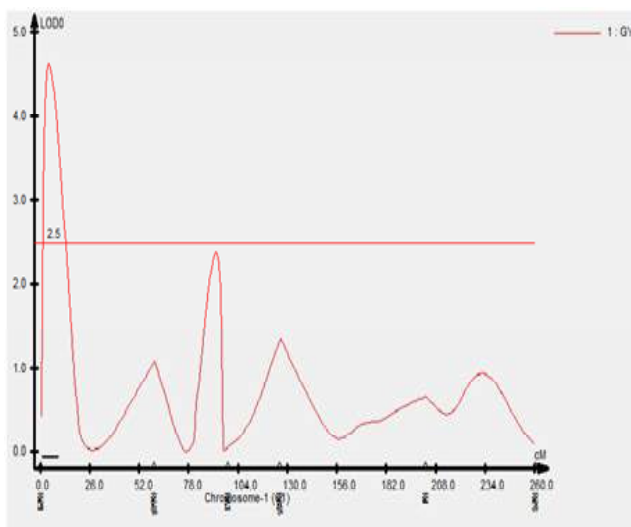


Fig. 2. QTL likelihood curves of the LOD score for grain yield (*qGY1.1*) on chromosome 1 under drought stress.

2004). A total of six QTLs (*qRLWC1.1*, *qRLWC6.1*, *qRLWC9.1*, *qRLWC9.2*, *qRLWC9.3* and *qRLWC11.1*) were identified for relative leaf water content (RLWC) on chromosome 1, 6, 9 and 11 respectively under drought stress. The favourable allele in the five QTLs was contributed by Banglami whereas, for a QTL (*qRLWC1.1*), it was contributed by Ranjit. Under drought stress, higher RLWC (70%) was observed in Banglami whereas, low RLWC (57.14%) was recorded in Ranjit. The maintenance of higher RLWC under water limited conditions is an indicator of drought tolerance (Altinkut et al., 2001; Kumar et al., 2017). In the present study, four QTLs were localized in the long arm of chromosome 1 (184.60-234.60) cM between the marker intervals of RM3825-RM243 under both hydrological conditions. Further, fine mapping of this region by adding more number of markers in the flanking marker intervals of the QTLs may identify some novel genes associated with drought tolerance. The identified QTLs/genes may be used in breeding programme for the improvement in the grain yield of drought susceptible varieties under drought.

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