Fine mapping of rice drought QTL and study on combined effect of QTL for their physiological parameters under moisture stress condition

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Abstract: The present investigation was undertaken to study the effect of different yield QTL (DTY2.2, DTY3.1 and DTY8.1) under drought and their physiological response to drought stress. Backcross Inbred Lines (BILs) of IR64 (CB-193 and CB-229) along with IR64, APO and the traditional rice variety Norungan were raised in green house condition under water stress and control to evaluate the effect of the QTL on grain yield. The BIL CB-193 recorded higher photosynthetic rate (22.051), transpiration rate (7.152) and Ci/Ca ratio (0.597) whereas the BIL CB-229 recorded higher water content (80.76%). It was found that the combination of three QTL (CB-229) performed better than the susceptible parent and the line with two QTL (CB-193) under drought stress and control to evaluate the effect of the QTL on grain yield. The BIL CB-229 recorded higher grain yield (GY) were conducted using backcross derived lines. Composite interval mapping analyses resolved the originally identified qDTY2.2 region of 6.7 cM into a segment of 2.1 cM and two sub QTLs at region between RM23132 and RM1578 (75.75 cM-77.66 cM), RM515 and RM1578 (75.11 cM-77.66 cM) were identified in qDTY8.1 region. However this study provides a unique opportunity to breeders to introgress such regions together as a unit into high-yielding drought-susceptible varieties through MAS.

Keywords: Backcross inbred lines, Drought QTL, Fine mapping, Photosynthetic efficiency, Rice

INTRODUCTION

Rice (Oryza sativa L.) is the world’s most important food crop and a primary food source for about half of the world’s population. Rainfed rice occupies 50% of the total rice area in the world. Over 700 million people depend on rainfed rice grown on 50 million ha in Asia. Rice is semi aquatic plant and hence water loving plant. Drought is the major abiotic stress limiting rice production in these areas. Even a shorter spell of drought during reproductive stage has a greater impact on yield loss. In India, drought is major constraint for rice production and accounts for as much as 15 per cent of yield losses (Markandeya et al., 2005). Varieties with high yield potential and improved drought tolerance could reduce risk and help alleviate poverty in drought-prone rice growing areas. Drought tolerant cultivars such as Apo, Vandana, Way Rarem, Nagina 22, and Adaysel have been shown to out-yield susceptible lines such as IR64 and IR72 by several-fold under upland and lowland drought (Venuprasad et al., 2007; Venuprasad et al., 2009b; Bernier et al., 2007; Vikram et al., 2011; Swamy et al., 2011). Crosses between such tolerant and susceptible lines are useful to identify important QTL underlying variation in grain yield under drought stress. Molecular tools facilitate the identification and genomic locations of genes controlling traits related to drought tolerance using quantitative trait loci (QTL) analysis. Larger QTL region results in introgression of unwanted segments. Hence to introgress the precise segment the original QTL region has to be fine mapped and size has to be reduced. The present investigation was undertaken to study the effect of different yield QTL (DTY2.2, DTY3.1 and DTY8.1) derived from Apo under drought and their physiological response to drought stress. The two QTLs namely DTY2.2 and DTY8.1 were fine mapped to locate the markers which were close to the QTL region. The original segment of qDTY2.2 is 1.4 cM hence fine mapping was not required (Venuprasad et al., 2009b). Backcross Inbred Lines (BILs) of IR64 (CB-193 and CB-229) along with IR64, APO and the traditional rice variety Norungan were raised in green house condition under water stress and control to evaluate the effect of the QTL on grain yield.

MATERIALS AND METHODS

Two Backcross Inbred Lines (BILs) of IR64 developed from the cross combination of IR64 X APO along with the parents and Norungan a land race of Tamil Nadu tolerant to drought were used in the investigation.
APO, drought tolerant upland variety, developed at IRRI, is recommended for cultivation under aerobic conditions. Owing to its drought tolerance nature and good performance under aerobic conditions, they serve as important source for mining drought tolerance QTLs. IR64 is a medium duration and high yielding variety but highly susceptible to drought. A set of 50 BILs from the cross between IR64 X APO were generated which carried three mega QTL classes namely DTY\textsubscript{2.2}, DTY\textsubscript{3.1} and DTY\textsubscript{8.1}.

**Green house experiment:** The two BIL lines of CB-193 (DTY\textsubscript{3.1} and DTY\textsubscript{8.1}), CB-229 (DTY\textsubscript{2.2}, DTY\textsubscript{3.1} and DTY\textsubscript{8.1}) were raised under control and water stress condition (pot culture experimentation) in four replication during Summer 2015. The seed materials were grown in plastic pots (30 cm height x 30 cm diameter with drainage hole) filled with three parts of coir pith and one part of natural clay loam soil. Three plants per pots were maintained and were grown in green house under natural temperature. The crop was irrigated till 45\textsuperscript{th} day and there after irrigation was stopped for two replication (till the Relative Water Content reached 60%) and the yield parameters were recorded. The positions of the pots were changed frequently to minimize the micro climate effects. Infrared Gas Analyzer (IRGA), a portable photosynthetic system (LICOR- Model LI 6400 version.5) and used for the measurement of different parameters viz., photosynthetic rate, stomatal conductance, transpiration rate and Ci/Ca ratio. Relative water content was estimated by Weatherley (1950) method and expressed in percentage. The basic principle (Barrs and Weatherley, 1962) of this technique consists essentially in comparing the water content of leaf tissue when fresh leaf sampled with the fully turgid water content and expressing the results on percentage basis. Several biometrical traits were also observed in both under control and moisture stress condition.

**Fine mapping and genotyping**

**Generation of genotypic data:** Fresh leaves for 50 BILs of the cross IR64xApo were collected and freeze-dried. DNA was extracted from freeze-dried leaf samples by a modified CTAB (cetyl tri-methyl ammonium bromide) method (Murray and Thompson, 1980) and the DNA was then quantified on 0.8% agrose gel to adjust the concentration to 25 ng/µL. Polymerase chain reaction was performed as described by Panaud et al. (1996). Agrose gel was used for size separation of the amplified DNA fragments using electrophoresis unit (BioRad 96). The DNA fragments were then visualized using trans-illuminator.

**Genetic analysis:** In this study, rice microsatellite (SSR) markers were used to fine map the previously identified QTL region for grain yield under stress. A total of 13 polymorphic markers for \(qDTY_{2.2}\) and 12 polymorphic markers for \(qDTY_{8.1}\) were added in the originally identified QTL regions in the BILs for fine mapping (Table 4). The markers were taken based on their physical position (Mb) on the indica genome (http://www.ricetogo.org) and multiplied by 2.7 for approximate estimation of cM distances for analysis. Composite interval mapping (CIM) was performed using Windows QTL Cartographer 2.5.009 (Wang et al., 2011). The LOD threshold value was obtained empirically from 1,000 permutation tests (Churchill and Doerge, 1994). The LOD thresholds obtained correspond to an experiment-wise type I error rate of 0.05.

![Fig. 1. Parents and BILs under irrigated (C-control) and under drought condition (S- Stress).](image-url)
RESULTS AND DISCUSSION

Marker assisted selection using consistent yield QTLs under drought could be an alternative approach for improving rice grain yield under drought situations (Swamy et al., 2011; Vikram et al., 2012). QTLs have been identified in the past few years for different drought-related traits through phenotyping and genotyping of large mapping populations. Various physiological parameters such as photosynthetic rate, stomatal conductance, transpiration rate, Ci/Ca ratio and relative water content were measured in BILs namely CB-193 (DTY 3.1 and DTY 8.1), CB-229 (DTY 2.2, DTY 3.1 and DTY 8.1), their parents (IR64 and APO) and check (Norungan) at different intervals (52, 58, 66, 72 and 78 days after sowing) and the results were presented in Table 1. During initial stage of moisture reduction in pot culture at 52 DAS (days after sowing), the BIL CB
Table 1. Estimation of physiological parameters in two Backcross Inbred Lines (CB-193 (DTY$_{1}$ and DTY$_{2}$), CB-229 (DTY$_{2}$, DTY$_{3}$ and DTY$_{4}$)) of IR64 both under control and water stress condition (pot culture).

| Genotypes | Soil Moisture (%) | Photosynthetic rate (µmol CO$_2$ m$^{-2}$s$^{-1}$) | Stomatal Conductance (mmol H$_2$O m$^{-2}$s$^{-1}$) | Transpiration Rate (mmol H$_2$O m$^{-2}$s$^{-1}$) | Ci/Ca | Relative Water Content (%) |
|------------|-------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-------|-----------------------------|
|            | C                | S                | C                | S                | C                | S                | C                | S                | C                | S                | C                | S                | C                | S                |
| CB-193 (2QTL) | 96.43            | 65.56            | 23.260           | 22.051*           | 0.255            | 0.270*           | 7.468            | 7.152*           | 0.559            | *                | 98.59            | 95.72            |
| CB-229 (3QTL) | 89.52            | 64.09            | 28.88*           | 20.942           | 0.350*           | 0.252*           | 9.325            | 6.660            | 0.589*           | *                | 98.11            | 97.65*           |
| Norungan   | 90.82            | 65.76            | 21.138           | 21.543*          | 0.259            | 0.271*           | 7.117            | 7.182*           | 0.598*           | *                | 99.36            | 97.32            |
| APO        | 89.39            | 62.10            | 20.829           | 20.090           | 0.241            | 0.176            | 6.751            | 5.005            | 0.578*           | *                | 99.05            | 98.31*           |
| IR64       | 91.73            | 68.19            | 22.821           | 18.107           | 0.217            | 0.142            | 6.084            | 6.478            | 0.495*           | *                | 96.55            | 96.52            |
| MEAN       | 91.58            | 65.14            | 23.39            | 20.55            | 0.26             | 0.22             | 7.35             | 6.50             | 0.56             | *                | 98.33            | 97.10            |
| SE         | 1.29             | 1.00             | 1.45             | 0.69             | 0.02             | 0.03             | 0.54             | 0.40             | 0.02             | 0.04             | 0.49             | 0.45             |

Note: 1993 recorded higher photosynthetic rate (22.051), stomatal conductance (0.270), transpiration rate (7.152) and Ci/Ca ratio (0.597) whereas the BIL CB-229 recorded high relative water content (%). The plants started expressing leaf rolling symptoms at 21 days after drought induction (Fig. 1). When moisture stress increased during 58, 66, 72 and 78 DAS, the line CB-229 registered higher gas exchange parameters espe-
had higher stomatal conductance than IR64. The same ductance when compared to IR64 and the BILs also thetic rate, transpiration rate, stomatal conductance and resistant parent APO and check Norungan. This clearly for all the physiological traits studied.

Under stress condition, the population varied widely photosyntheis, stomatal conductance and transpiration revealed that, the BIL line CB-229 maintained highercially photosynthetic rate and relative water content as resistant parent APO and check Norungan. This clearly showed that the BIL line CB-229 maintained higher internal water status with normal physiological process of photosyntheis, stomatal conductance and transpiration rate and withstand under higher moister stress conditions. Under stress condition, the population varied widely for all the physiological traits studied viz., photosynthetic rate, transpiration rate, stomatal conductance and relative water content. Apo had higher stomatal conductance when compared to IR64 and the BILs also had higher stomatal conductance than IR64. The same kind of results was obtained by Tezera et al. (2002) who reported that higher stomatal conductance would result in higher photosynthetic rate and biomass production. Martinez et al. (2007) also pointed out that higher stomatal conductance may be an enhanced adaption of plants to drought environments. Chen et al. (1995) observed that elevating photosynthetic rate is beneficial to dry matter production and yield. Cao et al. (2001) reported that photosynthetic rate among rice varieties were significant and suggested the net photosynthetic rate as a selection parameter for drought resistant genotypes. The similar result was obtained from this study also and Apo had more photosynthetic rate and relative water content as compared to the other genotypes. The drought QTL was already reported by Venuprasad et al. (2009a) that higher yielding genotypes under drought had greater stomatal conductance and transpiration rate. Sikuku et al. (2010) observed transpiration rate in NERICA rice varieties generally decreased with increase in soil water deficit.

**Fine mapping:** Fine mapping of qDTY2; and qDTY8; was done under severe stress wherein the yield traits were recorded. The drought QTL qDTY1; was not fine mapped in this study since the closest linked marker was already reported by Venuprasad et al., 2009a. CIM analysis of markers within qDTY2; showed a region between RM12529 and RM12571 (13.87cM -

Table 2a. Yield attributing traits of Backcross Inbred Lines [CB-193 (DTY1; and DTY8;), CB-229 (DTY2; DTY3; and DTY4;)] of IR64 both under control and water stress condition (pot culture). Define c and S below each table.

| Genotypes | DF | PH | NOPT |
|-----------|----|----|------|
| Genotypes | C  | S  | Per cent Reduction over control | C  | S  | Per cent Reduction over control | C  | S  | Per cent Reduction over control |
| CB-193 (2QTL) | 75 | 62* | 17.33 | 95 | 76* | 40.00 | 24 | 13 | 45.83 |
| CB-229 (3QTL) | 75 | 66 | 12.00 | 112 | 95 | 30.36 | 23 | 15* | 34.78 |
| Norungan | 86 | 75 | 12.79 | 154 | 109 | 58.44 | 11 | 9 | 18.18 |
| APO | 81 | 73 | 9.88 | 110 | 87 | 41.82 | 7 | 6 | 14.29 |
| IR64 | 72* | 64 | 11.11 | 97 | 82 | 30.93 | 24 | 11 | 54.17 |
| MEAN | 77.8 | 68 | 114 | 89.8 | 18 | 10.8 |
| SE | 2.52 | 2.55 | 10.70 | 5.72 | 3.70 | 1.56 |

C – Control, S – Moisture Stress

Table 2b. Yield attributing traits of Backcross Inbred Lines [CB-193 (DTY1; and DTY8;), CB-229 (DTY2; DTY3; and DTY4;)] of IR64 both under control and water stress condition (pot culture).

| Genotypes | NFG | SPF | HGW | SPY |
|-----------|-----|-----|-----|-----|
| Genotypes | C  | S  | Per cent reduction over control | C  | S  | Per cent reduction over control | C  | S  | Per cent reduction over control |
| CB-193 (2QTL) | 165 | 118 | 28.48 | 92.7* | 66.3 | 28.48 | 2.6 | 2.41 | 8.02 | 3 | 7.36 | 51.67 |
| CB-229 (3QTL) | 274* | 140 | 48.91 | 89.3 | 66 | 26.09 | 4 | 2.47 | 6.44 | 9* | 9.46* | 50.44 |
| Norungan | 172 | 136 | 20.93 | 89.1 | 66.7 | 25.14 | 3 | 1.83 | 6.93 | 4.56 | 34.20 |
| APO | 259* | 172 | 33.59 | 91.5 | 71.4 | 21.97 | 2.3 | 2.25 | 2.17 | 7.87 | 4.91 | 37.61 |
| IR64 | 157 | 86 | 45.22 | 89.2 | 47.5 | 46.75 | 2.8 | 2.24 | 20.57 | 7 | 4.3 | 73.73 |
| MEAN | 205.4 | 4 | 90.36 | 8 | 22 | 2.41 | 13.1 | 5.72 |
| SE | 25.17 | 0 | 0.74 | 4.14 | 9 | 0.10 | 4 | 0.77 |

C – Control, S – Moisture Stress; DF-days to 50% flowering, PH- plant height, NOPT- number of productive tillers, RL- root length, NFG- number of filled grains, SPF- spiklet fertility, HGW- hundred grain weight, SPY- single plant yield.
The present investigation was undertaken to study the effect of different yield QTL (DTY2.2, DTY3.1 and DTY8.1) derived from Apo under drought and their physiological response to drought stress. The qDTY2.2 showed larger effect under drought but a very low effect under moderate stress. The other two qDTY (3.1 and 8.1) showed its effect only under severe drought stress. It should be possible to exploit these fine mapped QTL after development of gene-based markers, for improving grain yield of valuable varieties such as IR64 in stress prone environments via marker assisted backcross breeding. In this study an attempt was made to narrow down the originally identified QTL regions so as to introgress precisely the smallest possible segment of these QTLs showing profound effect on grain yield under drought while minimizing the chances of introgression of any undesirable linked trait. The successful introgression of the fine-mapped regions of these QTLs by MAS provides an opportunity to improve the drought tolerance of well-adapted, high-yielding but drought-susceptible popular rice varieties.

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