



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 ($DTY_{2.2}$, $DTY_{3.1}$ and $DTY_{8.1}$) 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 high relative 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). Fine-mapping of two QTLs viz., $qDTY_{2.2}$ and $qDTY_{8.1}$ for grain yield (GY) were conducted using backcross derived lines. Composite interval mapping analyses resolved the originally identified $qDTY_{2.2}$ 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 $qDTY_{8.1}$ 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 ($DTY_{2.2}$, $DTY_{3.1}$ and $DTY_{8.1}$) derived from Apo under drought and their physiological response to drought stress. The two QTLs namely $DTY_{2.2}$ and $DTY_{8.1}$ were fine mapped to locate the markers which were close to the QTL region. The original segment of $qDTY_{3.1}$ is 1.4cM 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_{2.2}*, *DTY_{3.1}* and *DTY_{8.1}*.

Green house experiment: The two BIL lines of CB-193 (*DTY_{3.1}* and *DTY_{8.1}*), CB-229 (*DTY_{2.2}*, *DTY_{3.1}* and *DTY_{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 45th 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% agarose gel to adjust the concentration to 25 ng/μL⁻¹. Polymerase chain reaction was performed as described by Panaud *et al.* (1996). Agarose 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.

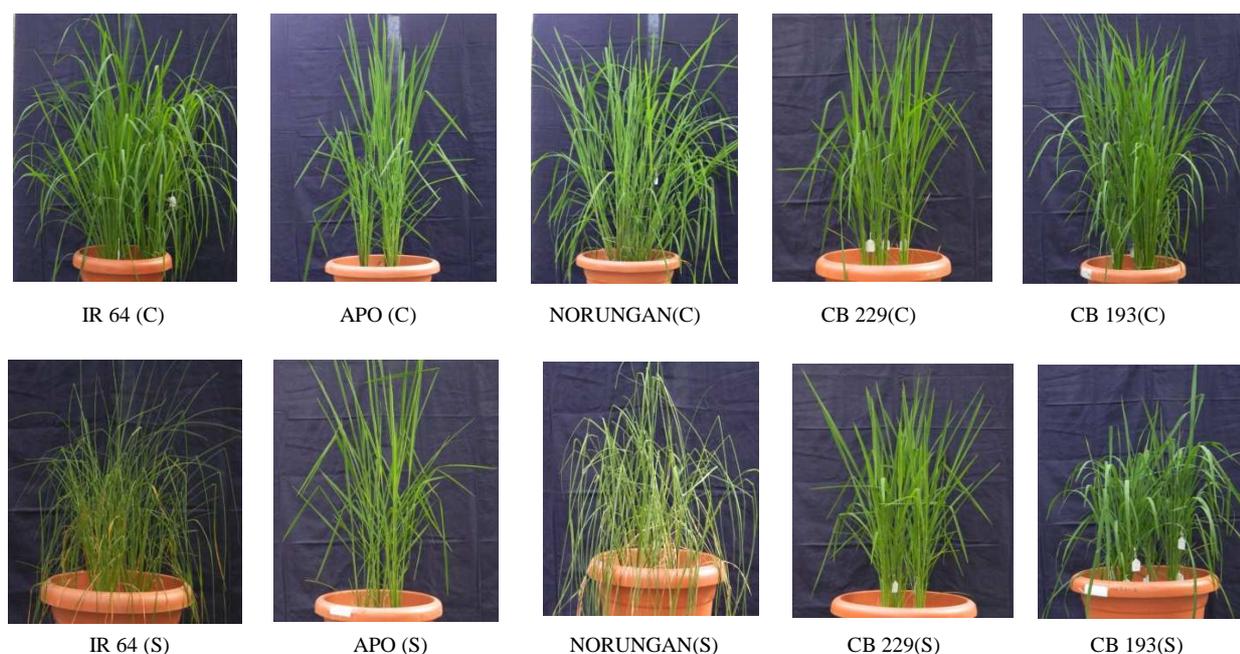


Fig. 1. Parents and BILs under irrigated (C-control) and under drought condition (S- Stress).

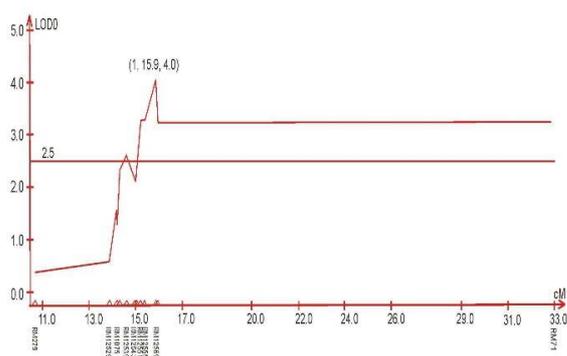


Fig 2a

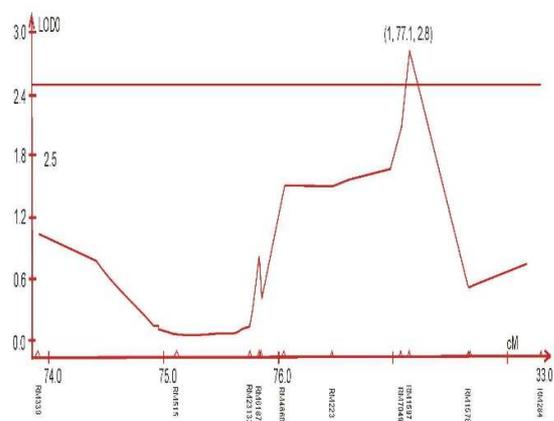


Fig 2b.

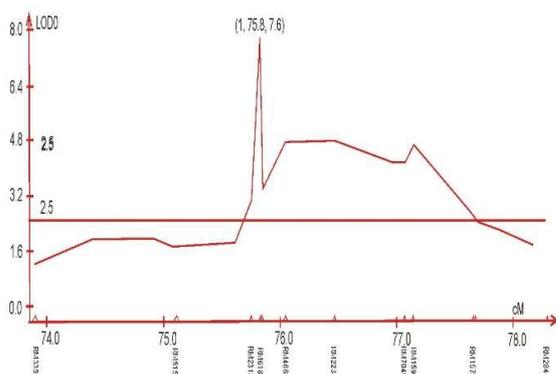


Fig 2c

Fig (a-c). A QTL likelihood curves of LOD score for grain yield (GY) showing significant regions under severe stress. Genetic distance in cM between the markers is indicated on X axis. Horizontal lines correspond to critical LOD value. 2a - DTY 2.2 for number of productive tillers under severe drought; 2b-DTY 8.1 for number of productive tillers; 2c-DTY 8.1 for panicle length

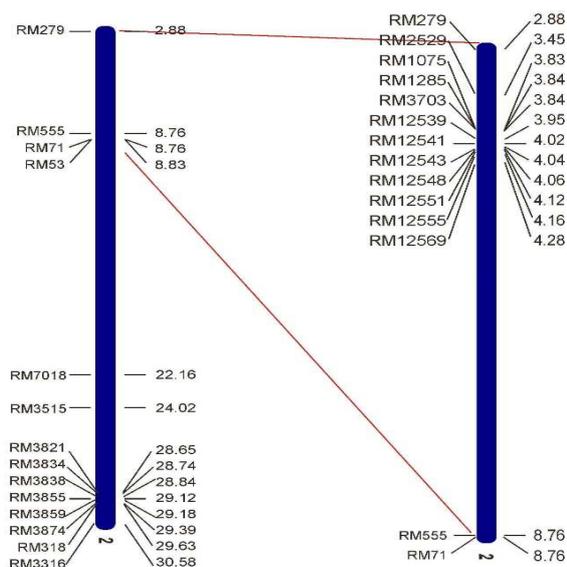


Fig. 3a. Fine mapped region of qDTY2.2.

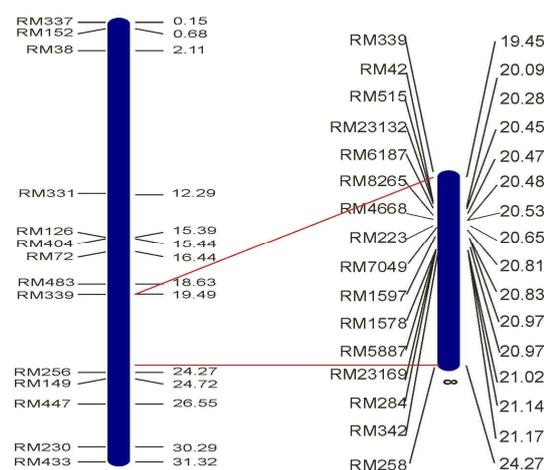


Fig. 3b. Fine mapped region of qDTY8.1.

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 trait through phenotyping and genotyping of large mapping population. Various physiological parameters *viz.*, 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_{3.1}* and *DTY_{8.1}*), CB-229 (*DTY_{2.2}*, *DTY_{3.1}* and *DTY_{8.1}*)] of IR64 both under control and water stress condition (pot culture).

Genotypes	Soil Moisture (%)		Photosynthetic rate (μ mol CO ₂ m ⁻² s ⁻¹)		Stomatal Conductance (mol 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
52 DAS												
CB-193 (2QTL)	96.43	65.56	23.260	22.051*	0.255	0.270*	7.468	7.152*	0.559	0.597	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*	0.591	98.11	97.65*
Norungan	90.82	65.76	21.138	21.543*	0.259	0.271*	7.117	7.182*	0.598	0.606	99.36	97.32
APO	89.39	62.10	20.829	20.090	0.241	0.176	6.751	5.005	0.578*	0.472	99.05	98.31*
IR64	91.73	68.19	22.821	18.107	0.217	0.142	6.084	6.478	0.495	0.397	96.55	96.52
MEAN	91.58	65.14	23.39	20.55	0.26	0.22	7.35	6.50	0.56	0.53	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
58 DAS												
CB-193 (2QTL)	96.43	65.56	32.856	17.382	0.474*	0.247*	16.083	6.519*	0.627*	0.653	98.59*	95.72
CB-229 (3QTL)	78.68	61.53	25.761	20.025*	0.293	0.108	12.098	4.473	0.555	0.178	98.86*	97.22*
Norungan	80.98	60.05	20.928	20.432*	0.201	0.157	8.826	6.922*	0.496	0.384	97.02	97.00*
APO	83.78	54.91	23.302	17.190	0.248	0.236*	10.374	6.288	0.531	0.641	97.88	96.09
IR64	81.16	62.85	21.817	16.293	0.284	0.184	11.297	5.534	0.599*	0.574	97.56	95.60
MEAN	84.21	60.98	24.93	18.26	0.30	0.19	11.74	5.95	0.56	0.49	97.98	96.33
SE	3.16	1.77	2.14	0.83	0.05	0.03	1.22	0.43	0.02	0.09	0.34	0.33
66 DAS												
CB-193 (2QTL)	91.74	41.01	31.614	15.527	0.810*	0.090	13.700	3.807	0.776	0.219	97.11	88.86
CB-229 (3QTL)	88.03	43.52	32.918	19.751*	0.732	*	12.768	4.389*	0.750	0.534	93.42	91.53*
Norungan	69.55	39.69	27.755	17.391*	0.703	0.141	12.051	3.323	0.780	0.441	97.57*	86.32
APO	74.09	36.04	25.086	14.852	0.657	0.112	11.360	4.604*	0.787	0.392	97.35*	87.74
IR64	90.30	49.93	26.577	13.332	0.862*	0.151	12.950	4.499*	0.820	0.581	97.25	88.30
MEAN	82.74	42.04	28.79	16.17	0.75	0.14	12.57	4.12	0.78	0.43	96.54	88.55
SE	4.55	2.31	1.50	1.11	0.04	0.02	0.40	0.24	0.01	0.06	0.78	0.86
72 DAS												
CB-193 (2QTL)	87.25	17.95	30.938	15.465	0.642*	0.141	14.695	3.224	0.734*	0.502	96.20*	82.52
CB-229 (3QTL)	79.08	27.49	32.672	16.657	0.608*	0.074	15.167	3.072	0.702*	0.033	90.64	88.41*
Norungan	75.21	16.28	32.778	13.332	0.498	0.151	14.657	4.499*	0.648	0.581	97.54*	79.18
APO	78.94	30.50	28.294	14.119	0.451	0.113	12.776	2.803	0.669	0.439	92.15	86.38*
IR64	80.10	34.16	29.375	12.352	0.539	0.066	14.657	2.960	0.700	0.177	94.83	79.71
MEAN	87.25	25.28	30.81	14.39	0.55	0.11	14.13	3.31	0.69	0.35	94.27	83.24
SE	1.96	3.50	0.89	0.76	0.03	0.02	0.45	0.30	0.01	0.10	1.27	1.82
77 DAS												
CB-193 (2QTL)	85.02	16.22	19.978	10.286*	0.331	0.110	11.514	4.102*	0.678*	0.556	98.99	72.41
CB-229 (3QTL)	71.96	22.87	18.424	10.473*	0.268	0.066	9.801	2.678	0.646	0.294	99.38*	80.76*
Norungan	70.32	16.89	26.040	4.685	0.363*	0.032	11.941	1.337	0.632	0.351	98.19	63.52
APO	66.68	23.44	20.974	10.951*	0.373*	0.131	12.213	4.923*	0.698*	0.597	99.36*	78.10*
IR64	75.39	27.65	17.937	6.851	0.238	0.047	8.336	1.935	0.620	0.344	99.19	66.93
MEAN	73.87	21.41	20.67	8.65	0.31	0.08	10.76	2.99	0.65	0.43	99.02	72.34
SE	3.12	2.15	1.45	1.23	0.03	0.02	0.74	0.67	0.01	0.06	0.22	3.25

C – Control, S – Moisture Stress, DAS – days after sowing

-193 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-

Table 2a. Yield attributing traits of Backcross Inbred Lines [CB-193 (*DTY_{3.1}* and *DTY_{8.1}*), CB-229 (*DTY_{2.2}*, *DTY_{3.1}* and *DTY_{8.1}*)] of IR64 both under control and water stress condition (pot culture). Define c and S below each table.

Genotypes	DF		PH			NOPT			Per cent Reduction over control
	C	S	C	S	C	S	C	S	
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 (*DTY_{3.1}* and *DTY_{8.1}*), CB-229 (*DTY_{2.2}*, *DTY_{3.1}* and *DTY_{8.1}*)] of IR64 both under control and water stress condition (pot culture).

Geno-types	NFG		SPF		HGW			SPY		Per cent reduction over control		
	C	S	C	S	C	S	C	S				
CB-193 (2QTL)	165	118	28.48	92.7*	66.3	28.48	2	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*	*	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	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.

cially photosynthetic rate and relative water content as resistant parent APO and check Norungan. This clearly revealed that, the BIL line CB-229 maintained higher internal water status with normal physiological process of photosynthesis, stomatal conductance and transpiration rate and withstand under higher moisture 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 adaptation 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 along with BILs when compared to IR64. Araus *et al.* (2002) reported 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 *qDTY_{2.2}* and *qDTY_{8.1}* was done under severe stress wherein the yield traits were recorded. The drought QTL *qDTY_{3.1}* 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 *qDTY_{2.2}* showed a region between RM12529 and RM12571 (13.87cM -

Table 3. Fine-mapped regions within different QTLs for grain yield under stress.

QTL-Fine mapped region	Associated trait under stress	Marker Inerval	Peak position	Marker closest to the peak	R ² (%)	Add%	LOD score
<i>qDTY2.2</i>	Productive tillers	RM12529- RM12571	15.9cM	RM12569	19.0	28.5	4
<i>qDTY8.1</i>	Productive tillers	RM23132- RM1578	77.1cM	RM1597	23.0	15.3	2.9
<i>qDTY8.1</i>	Panicle length	RM515- RM1578	75.8cM	RM6187	43.0	42.8	7.6

Table 4. List of polymorphic markers used for fine mapping.

Chromosome 2		Chromosome 8	
Markers	Position (cM)	Markers	Position (cM)
RM279	10.67	RM339	73.9
RM12529	13.87	RM515	75.11
RM1075	14.20	RM23132	75.75
RM1285	14.21	RM6187	75.83
RM3703	14.31	RM8265	75.85
RM12539	14.61	RM4668	76.05
RM12543	14.98	RM223	76.47
RM12548	15.05	RM7049	77.07
RM12551	15.21	RM1597	77.14
RM12555	15.39	RM1578	77.66
RM12569	15.86	RM5887	77.67
RM12571	15.95	RM284	78.29
RM71	33.0		

15.95cM) having an effect on GY under drought stress condition. The peak was detected at 15.9 cM under stress conditions with RM12569 as the closest marker to the peak at LOD 4 (Fig. 2a and 3a). The QTL explained a phenotypic variance of 19.0% and had an additive effect of 28.5 (Table 3). Similarly analysis of *qDTY8.1* exhibited two sub QTLs at region between RM23132 and RM1578 (75.75 cM- 77.66 cM), RM515 and RM1578 (75.11 cM-77.66 cM) for the traits *viz.*, panicle length and number of productive tillers under drought conditions with RM1597 and RM6187 as the closest markers to the peak respectively (Fig 2b and 2c & 3b). The peak was detected at 77.1 and 75.8 cM under stress conditions with LOD of 2.9 and 7.6 respectively. The QTL explained a phenotypic variance of 23.1% and 43% and had an additive effect of 15.3 and 42.8 (Table 3) under drought condition.

In the present investigation, several biometrical traits were observed in all genotypes under study which exhibited that, the BIL CB-229 had recorded higher number of productive tillers, root length, number of filled grains per panicle, spikelet fertility percentage, hundred grain weight and grain yield under moisture stress condition when compared to the BIL CB-193. This could be due to the presence of two QTL regions together within *qDTY8.1* showing larger effect in drought stress condition. However, *qDTY2.2*, *qDTY3.1* and *qDTY8.1* have shown a high and consistent additive effect under severe drought condition. The *qDTY2.2* exhibited higher effect on increased number of tillers

and *qDTY8.1* had an effect on panicle length and number of productive tillers thus increasing the yield under drought. Since, the QTL *DTY2.2* was responsible for root length (MacMillan *et al.*, 2006; Kamoshita *et al.*, 2002) and root thickness (Champoux *et al.*, 1995, Dixit *et al.*, 2012). In this study also, the BIL CB-229 possessing QTL *DTY8.1* was associated with grain yield under stress condition. The similar result was already reported by Vikram *et al.* (2012). But the significant advantage of CB-193 was earliness in flowering and reduced plant height. Both the BILs of IR64-193 (*DTY3.1* and *DTY8.1*) and IR64-229 (*DTY2.2*, *DTY3.1* and *DTY8.1*) were earlier in flowering with reduced plant height, which possessed *DTY3.1* responsible for flowering date and plant height (Venuprasad *et al.*, 2009a). Ding *et al.* (2011) reported complex nature of large-effect QTLs for stress tolerance working on a QTL on chromosome 4 controlling root volume per tiller co-segregating with flag-leaf width and spikelet number per panicle.

Conclusion

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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