Fine mapping of consistent quantitative trait loci for yield under drought stress using rice (*Oryza sativa*) recombinant inbred lines adapted to rainfed environment

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Drought stress is a serious constraint, especially in rainfed rice production, and breeding for drought tolerance by selection based on yield under stress, though effective, is slow Mapping quantitative trait loci (QTLs) for yield and its components under drought stress predominant in rainfed target populations of environment (TPE) will help overcome this limitation. In the present study, a subset of 143 F_8 and F_9 recombinant inbred (RI) lines derived from IR62266-42-6-2 (IR62266), a high-yielding indica ecotype and Norungan, a landrace from TPE, was used to map QTLs for yield and its components under drought predominant in TPE. A large effect yield QTL observed under drought stress in TPE was consistent across two years with a phenotypic variation of 31.3% and 37.9% and additive effect of 629.2 and 424.9 kg/ha Further, this region was fine-mapped to 94.0 kb with positive effect on grain yield under stress.

Keywords: Comparative genomics, drought stress, fine mapping, quantitative trait locus, rice.

RICE (*Oryza sativa* L.) is one of the world's primary staple foods with 730 million tonnes (Mt) harvested in 2012 (ref. 1). It was cultivated in 163 million hectares (M ha) worldwide under varying ecosystems in 2013 (ref. 2). Globally 50% of the rice acreage is rainfed[3]. More than 23 M ha of rice is cultivated under rainfed environment in India[4]. In recent years, the increased occurrence and severity of drought stress has led to a yield decline in rice[5]. In rainfed regions, drought is the major environmental factor that reduces productivity by 13–35% (ref. 6). Developing cultivars with inherent capacity to tolerate drought stress will help improve rainfed rice production[7]. Direct selection for grain yield is an effective approach for the development of drought-tolerant varieties[8–10]. However, conventional breeding for drought resistance is slow in attaining progress due to difficulty in phenotyping large progenies and year-to-year variation in rainfall pattern. Hence, for quantitative traits such as drought tolerance, quantitative trait loci (QTL) mapping followed by marker-assisted breeding (MAB) is considered as an alternate strategy to overcome this limitation[11]. Though several QTLs for yield under drought stress have been mapped in rice[12–16], only few have been successfully deployed in MAB till date[17]. Identifying consistent QTLs for yield under stress with large effect and fine-mapping them will speed up MAB for drought tolerance in rice[12,18]. QTLs with large intervals have the chance of getting undesirably linked genes; hence it is important to fine-map the QTLs so as to introgress the precise genomic segment of interest. Thus, QTLs for yield under drought in upland condition have been fine-mapped recently[19]. However, drought stress differs markedly between upland and rainfed lowlands due to climate and inherent soil characteristics. Thus mapping QTLs under drought predominant in target populations of the environment (TPE) using population derived from locally adapted rice lines is critical[20,21] to further improve the efficacy of MAB. Utilizing unexploited yield-related variabilities and QTLs from new cross combinations, especially involving wild species[22] and landraces is important.

In this study, we report identification of consistent, large-effect QTL for yield under drought stress in TPE, and fine-mapping of the qDTY6.1 region to 94.0 kb using local rice lines adapted to TPE with potential to develop high-yielding, drought-tolerant rice cultivars through MAB.

Materials and methods

Mapping population

Norungan, an *indica* landrace from Tamil Nadu, India, is drought-resistant and possess deep and thick roots with superior hardpan penetrating ability[23]. IR62266, a lowland *indica* ecotype, has shallow and thin root system[23], but has the capacity for osmotic adjustment[24,25]. A subset of 143 F_8 and F_9 RI lines derived from these two parents

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(IR62266 × Norungan) was used in the present study for mapping QTLs for phenology, physio-morpholgical and yield traits under drought in TPE and further fine-map the consistent large-effect QTL for yield under drought.

Field experiments

The drought experiments were conducted in TPE during rainfed season (September–January) of 2011–12 (DS01) and 2012–13 (DS02) in the experimental fields of Agricultural Research Station, Tamil Nadu Agricultural University (TNAU), Paramakudi, India and during May–October 2013 (FC01) under irrigated (non-stress) condition in Paddy Breeding Station, TNAU, Coimbatore, India. In DS01 and DS02, a subset of 143 F_8 and F_9 recombinant inbred (RI) lines of IR62266 × Norungan respectively, were evaluated along with the parents in a randomized block design with a plot size of 2.0×0.4 m^2 under rainfed (stress) conditions. The trials, DS01 and DS02 were evaluated with two and three replications respectively. Seeds were hand-dibbled into dry soil @ 80 kg/ha. NPK fertilizers were applied at the rate of 50 : 25 : 25 kg/ha. Insect and weed control measures were applied periodically, as required.

The trials, DS01 and DS02 received a total rainfall of 345 and 354 mm respectively, during crop growth. Table 1 gives the site, soil and drought stress characteristics of field trials. There was a dry spell for 30 days coinciding with flowering to grain filling in DS01. Perched soil water-table depth was measured at two-days interval using Peizometers (15 × 100 cm) made of polyvinyl carbonate in DS01. In DS02, there was no rainfall 40 days after emergence (DAE). Hence, one irrigation was given at 61 DAE at flowering stage to save the plants from desiccation. Since there was no rainfall after this irrigation, stress developed and continued till maturity. The drought stress was thus severe in DS02.

In FC01, the subset of 143 F_9 RI lines was evaluated along with the parents under irrigated conditions. Twenty-six-day-old seedlings were transplanted with a spacing of 20 × 10 cm in plots of 1.2×0.2 m^2. The plots were surface-irrigated to field capacity at four days interval. NPK fertilizers were applied at the rate of 100 : 50 : 50 kg/ha.

In all the trials, observations for days to 50% flowering were recorded as the number of days from sowing to flowering in 50% of plants of each RI line. At maturity, plant height, number of tillers and spikelet number were recorded per plant. All the plants in the plot were harvested to record grain yield (kg ha^{-1}). Harvest index was estimated as the ratio of grain yield to the above-ground biomass. Drought score was recorded using leaf rolling at flowering stage during peak stress using 1–9 scale standardized for rice[26].

Genotyping

Leaf samples of RI lines and parents were collected from field-grown seedlings and freeze-dried. DNA was extracted from the leaf tissues using cetyl trimethyl ammonium borate buffer[27]. The quantity and quality of DNA was assessed in 0.8% agarose gel and concentration was adjusted to 50 ng/μl by comparing DNA standards. Polymerase chain reaction (PCR) was performed in a volume of 15 μl in thermal cycler (DNA Engine®, Peltier Thermal Cycler, BIO-RAD, USA or Eppendorf Master Cycler Gradient, Eppendorf, Germany). The reaction mixture contained 1 μM of each primer of (Sigma Aldrich, USA), 100 μM deoxy nucleotide, 1× *Taq* buffer, 0.02 U *Taq* polymerase (Bangalore Genei, India) and 50 ng of template DNA. The PCR steps were as follows: initial denaturation for 5 min at 94°C, 36 cycles of amplification which included denaturation at 94°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 1 min and the final extension step at 72°C for 5 min. A set of 137 SSR markers in the QTL region (between RM585 and RM217) on chromosome 6 was selected from the rice physical map.

Table 1. Site, soil and drought stress characteristics of field trials conducted in rainfed target populations of environment in Paramakudi (DS01 and DS02) and irrigated control in Coimbatore (FC01), India

Particulars	DS01 (2011–2012)	DS02 (2012–2013)	FC01 (2012–2013)
Elevation (m amsl)	40	40	427
Latitude and longitude	9°N, 70°E	9°N, 70°E	11°N, 77°E
Soil texture	Clay loam	Clay loam	Clay
Soil pH	8.1	8.1	8.4
Characterization of stress	Moderate	Severe	Non-stress
Total rainfall during crop period (mm)	345	354	–
Mean temperature (°C)			
Maximum	32.0	33.5	38.9
Minimum	23.5	23.0	26.0
Average relative humidity (%)	83.5	84.0	86.0

DS01, Drought stress 2011–12; DS02, Drought stress 2012–13; FC01, Flooded control 2013.

Statistical analysis

Analysis of variance for each trait in different trials was done as mixed models using PROC MIXED procedure of SAS V.9.3 (SAS Institute Inc.)[28]. Combined analysis of the two dry season trials (DS01 and DS02) was done using PROC MIXED procedure of SAS, where the RI lines were kept as fixed and other variables were assigned as random. Broad sense heritability of different traits was estimated within a year for each treatment. The variance components for calculating broad sense heritability for each trait were calculated using SAS program PROC VARCOMP with REML method. PROC CORR and PROC SUMM were used for correlation and summary statistics calculations for all the trials in SAS.

Linkage map and QTL analysis

Linkage maps were constructed from the genotype data using MAPMAKER/EXP 3.0 software[29]. The genetic distance was estimated using the Kosambi map function of the software[30]. Markers were placed onto linkage groups using the group, order and ripple command for checking the critical region with a minimum logarithm of the odds (LOD) score of 3.0 and a maximum recombination fraction of 0.5 and the ordered linkage groups were drawn using MapChart[31]. QTL analysis for each trial was carried out with the line mean of phenotypic traits. Putative QTLs were detected using composite interval mapping (CIM) function of QTL Cartographer 2.5 (ref. 32). The CIM threshold was based on the results of 1000 permutations at 5% significance level[33]. The additive effect and phenotypic variance explained by each QTL (R^2) were estimated at the maximum LOD score. QTLs with LOD score of 3 and above are reported.

Fine-mapping

The grain-yield QTL under drought on chromosome 6 between RM585 and RM217, previously identified from our laboratory[34] and also confirmed in the present study was chosen for fine-mapping. A total of 137 single sequence repeat (SSR) markers in the QTL region (between RM585 and RM217) on chromosome 6 were selected from the Rice Gene Thresher[35], and used in surveying polymorphism between the parents, IR62266 and Norungan. Twenty-seven polymorphic markers (see Supplementary material, Table S1 online) were used in genotyping the 143 RI lines to fine-map the QTL. Graphical genotyping software GGT 2 was used to draw genotypic graphs in RI lines[36].

Comparative genomics

Comparative genomics analysis was performed to identify the syntenic regions in maize, sorghum, pearl millet and barley using the genomics database (www.gramene.org), for the QTL region fine-mapped in this study. Syntenic regions identified were checked for the presence of grain-yield QTLs under stress. QTL information reported for grain yield was collected from the published literature and the Gramene database (www.gramene.org) and compared with the fine-mapped region using the comparative maps available in database.

Results and discussion

Variation in phenology and plant production traits

In this study, the landrace, Norungan, was used as a donor for grain yield under drought to identify consistent large-effect QTLs for plant production traits under drought stress in TPE. In DS01, the soil water table measured using peizometers declined below 80 cm starting from 61 DAE and reduced further to 95 cm (data not shown), coinciding with flowering to grain filling stages. In DS02, there was no rainfall beginning 40 DAE and remained dry till maturity, except for one irrigation given at 61 DAE to save the crop from total desiccation. Considerable variation was observed among the RI lines for phenology and plant production traits under drought stress and non-stress conditions.

The expression of genetic variability for drought-tolerance traits requires a mapping population to be exposed to drought stress predominant in TPE. High-level water stress is desirable to remove the spillover effect of yield potential and identify the lines which are clearly drought-resistant[12,20,37]. Table 2 provides the traits mean, range and broad sense heritability. Grain yield ranged from 31 to 2750 and 66 to 2889 kg/ha with mean of 940 and 644 kg/ha in DS01 and DS02 respectively. About 65–85% reduction in yield is considered severe drought[13]. In the present study, the reduction in mean grain yield was 80.4% and 86.2% in DS01 and DS02 respectively (Table 2). Such severe stress is useful in discriminating the superior lines under drought stress[20]. The mean grain yield in FC01 (non-stress trial) was 4796 kg/ha. Heritability of yield was 0.70, 0.36 and 0.97 in the trails DS01, DS02 and FC01 respectively. Moderate to high heritability of grain yield under drought stress in DS01 and DS02 indicates the suitability of grain yield under drought as a selection criterion[10,13]. Mean days to 50% flowering across the RI lines were 80, 97 and 90 in DS01, DS02 and FC01 respectively. Mean spikelet fertility across the RI lines was 57%, 72% and 84% in DS01, DS02 and FC01 respectively. Since the life irrigation given at flowering stage for the DS02 trial, the spikelet fertility was higher in this trial compared to DS01, but the drought that occurred before and after flowering affected the total grain yield in DS02; thus a lower mean grain yield was observed in DS02. [D1]The mean drought score was 3.8

Table 2. Trait mean, range and broad sense heritability for phenology and plant production traits under drought stress (DS01 and DS02) and non-stress (FC01) conditions for IR62266 × Norungan RI lines and parents

Trait	Trial	Norungan	IR62266	Trial mean	Range	SD	H^2
Days to 50% flowering	DS01	76	80	80	58–95	10.45	0.73
	DS02	105	108	97	61–108	8.41	0.18
	FC01	78	102	90	78–122	8.64	NA
Drought score	DS01	3	2	3.76	1–7	1.84	0.69
	DS02	4	5	4.00	3–9	0.76	0.31
	FC01	–	–	–	–	–	–
Plant height (cm)	DS01	88	66	87	19–149	36.06	0.72
	DS02	56	53	52	22–77	12.65	0.60
	FC01	140	91	100	59–142	20.40	0.68
No. of tillers	DS01	7	5	8	1–19	1.23	0.43
	DS02	5	3	4	2–11	1.12	0.23
	FC01	10	6	11	5–21	2.38	0.79
No. productive tillers	DS01	7	7	8	1–17	3.00	0.34
	DS02	4	3	4	1–9	1.33	0.20
	FC01	10	6	11	3–24	2.49	NA
No. of grains/panicle	DS01	44	46	45	3–100	19.40	0.35
	DS02	74	83	26	9–106	9.75	0.07
	FC01	62	127	82	17–160	31.17	NA
No. of chaffs/panicle	DS01	27	32	32	4–71	13	0.42
	DS02	8	15	9	2–24	3.69	0.10
	FC01	8	5	19	1–64	13.02	NA
Spikelet fertility (%)	DS01	62	66	57	26–83	13	0.40
	DS02	90	90	72	31–90	10.52	0.21
	FC01	89	96	84	51–99	11.50	NA
Grain yield (kg/ha)	DS01	2000	1042	940	31–2750	734	0.70
	DS02	1074	1007	644	66–2889	360	0.36
	FC01	3750	5722	4796	813–13847	2529	0.97
Straw yield (kg/ha)	DS01	6104	9400	7826	1219–64063	444	0.40
	DS02	1127	1735	3491	685–9788	1543	0.27
	FC01	17111	3014	9606	2333–22250	4071	0.99
Total biomass (kg/ha)	DS01	8104	10442	8643	625–65313	5687	0.38
	DS02	2296	3188	4112	981–27675	1730	0.81
	FC01	20861	8736	13608	3403–33569	5698	0.96
HI	DS01	0.25	0.15	0.12	0.01–0.30	0.08	0.55
	DS02	0.40	0.32	0.17	0.01–0.43	0.08	0.99
	FC01	0.18	0.66	0.36	0.04–1.00	0.19	0.97

HI, Harvest index; H^2, Broad sense heritability; DS01, Drought stress 2011–12; DS02, Drought stress 2012–13; FC01, Flooded control 2013; NA, Not available.

and 4.0 in DS01 and DS02 respectively (Table 2). The drought score observed here shows the severity of stress in these trials.

QTL analysis

A total of 51 QTLs were identified in DS01, DS02 and FC01 among which 39 QTLs were observed in rainfed environment and 12 in non-stress environments (Table 3). Grain yield QTLs were identified on chromosomes 1, 6 and 10. Consistent large-effect QTL (qDTY6.1) was observed for grain yield under stress conditions in TPE on chromosome 6 near marker RM2434 with phenotypic variation of 31.3%, 37.9% and 3.6% in DS01, DS02 and FC01 respectively, in these RI lines. The same region was reported to be linked to grain yield[38], biomass, number of panicles[12] and thousand grain weight[39] in rice. QTL for seedling drought tolerance was reported near RM217 region, and the same region harbours genes expressed early under drought stress[40] and Khowaja *et al.*[41] also reported QTLs for drought avoidance in rice in this region. A metaQTL for yield was reported near this region between RM204 and RM136 on chromosome 6 in rice[42].

RM9 on chromosome 1 harboured major QTLs for grain yield, biomass and straw yield with a phenotypic variation of 38.9%, 37.4% and 34.2% respectively (Table 3). Association of RM9 with yield and yield components under stress has been reported earlier in rice[43–46]. RM5746 on chromosome 12 was linked to days to 50% flowering with a phenotypic variation of 69.77% in DS02. This region was also reported for root volume under drought stress[47]. RM2434 on chromosome 6 and RM5746 on chromosome 12 were linked to drought score showing a phenotypic variation of 72% and 66% in DS01

Table 3. Quantitative trait loci (QTLs) for phenology and plant production traits mapped in IR62266/Norungan recombinant inbred lines under drought stress (DS01 and DS02) non-stress (FC01) conditions

Trait	Trial	Chromosome no.	Marker closest to the peak	Peak position (cM)	Logarithm of the odds (LOD)	Additive effect	R^2
Days to 50% flowering	DS01	12	RM5746	44.01	11.46	1.53	6.5
		6	RM2434	72.83	7.34	–3.42	8.6
	DS02	12	RM5746	50.01	7.26	–10.30	69.7
		6	RM402	41.00	8.94	–3.06	5.5
		6	RM2434	72.83	7.34	–3.42	8.6
	FC01	1	RM8051	17.22	5.56	–5.41	11.9
		12	RM5746	60.01	3.14	1.94	2.8
Drought score	DS01	6	RM2434	86.83	4.40	1.75	1.7
		12	RM5568	48.01	3.78	–0.16	0.5
	DS02	6	RM2434	84.83	3.40	1.12	72.1
		12	RM5746	60.01	4.00	1.21	65.9
Plant height (cm)	DS01	1	RM8051	9.22	4.68	10.31	1.6
		4	RM6314	112.49	9.05	–4.37	2.0
		6	RM402	35.91	6.96	–19.06	8.7
		6	RM7561	26.01	7.46	–10.75	4.2
		10	RM5471	7.8	7.19	–19.27	11.6
		10	RM5095	2.01	8.55	–20.06	9.2
	FC01	6	RM6773	86.80	7.06	5.73	3.8
No. of tillers	FC01	2	RM3512	17.76	3.60	0.59	5.7
		2	RM4472	17.62	3.70	0.58	5.7
		2	RM575	19.80	5.32	0.59	5.5
No. of productive tillers	FC01	2	RM575	21.80	4.15	0.58	4.7
No. of chaffs	FC01	6	RM6773	94.80	3.40	5.13	7.8
Spikelet fertility (%)	DS02	2	RM6942	76.53	3.36	–2.14	3.7
	FC01	6	RM6773	94.80	9.48	–3.79	5.3
Single plant yield (g)	DS01	6	RM6773	118.8	3.44	–0.73	6.4
		10	RM5471	9.8	6.90	–2.51	26.7
	DS02	1	RM9	15.22	4.43	–3.50	23.1
		4	RM6679	72.16	3.90	1.54	13.5
		4	RM6314	86.49	4.03	1.60	14.1
	FC01	6	RM6773	88.88	3.81	5.24	15.1
Grain yield (kg/ha)	DS01	1	RM8051	15.22	3.52	252.69	3.2
		6	RM2434	86.83	3.90	629.2	31.3
		6	RM6773	91.45	3.06	0.04	2.5
		10	RM5471	11.8	6.82	–584.05	25.1
	DS02	6	RM402	41.00	7.14	250.22	16.7
		6	RM2434	78.83	6.90	424.9	37.9
		6	RM6773	93.16	14.10	72.25	3.4
		1	RM9	9.22	13.45	–54.60	38.9
	FC01	6	RM6773	84.80	5.10	638.07	3.6
Straw yield (kg/ha)	DS01	3	RM517	8.01	36.19	0.06	2.6
	DS02	1	RM9	13.22	11.10	0.12	34.2
		11	RM202	0.01	5.35	0.09	15.7
Total biomass (kg/ha)	DS01	6	RM6194	128.72	28.32	–577.85	2.3
	DS02	1	RM9	11.22	15.68	0.07	37.4
		6	RM2434	76.83	11.85	583.00	5.7
		11	RM202	0.01	5.63	0.13	16.4
	FC01	6	RM6194	130.72	4.03	0.06	6.00
HI	DS02	1	RM9	13.22	3.45	–0.04	5.6
		12	RM5746	82.01	3.10	0.03	10.1
	FC01	6	RM2434	72.91	9.86	0.12	16.6

Peak position, Marker or position with the highest LOD value in the QTL curve; HI, Harvest index; R^2, Broad sense heritability; DS01, Drought stress 2011–12; DS02, Drought stress 2012–13; FC01, Flooded control 2013.

and DS02 respectively. RM5471 on chromosome 10 was linked to grain yield under stress with the phenotypic variation of 25% in DS01. QTLs for osmotic adjustment capacity[48] and grain weight[41] were reported near RM5471 on chromosome 10 in rice.

Fine mapping of qDTY6.1

The consistent large-effect QTL for yield under drought stress in TPE mapped earlier in these RI lines between the flanking markers RM585 and RM217 on chromosome

6 (qDTY6.1) with an interval of ~3.0 Mb was chosen for fine-mapping[34]. The candidate genomic region was fine-mapped to the smallest fragment of 94.04 kb between RM2434 and RM6773. To define the candidate genomic region for qDTY6.1, 137 SSRs were selected from the interval between RM585 and RM217 based on the physical map position in Rice Gene Thresher[35]. Among these, 27 markers which showed polymorphism between the parents (IR62266 and Norungan) were used in genotyping the 143 RI lines. CIM analysis identified the region flanked by RM2434 and RM6773 on chromosome 6 to be consistently linked to grain yield under stress conditions (Figure 1). qDTY6.1 had its peak at 86.8 cM, close to the marker RM2434. The consistent large-effect qDTY6.1 was fine-mapped between RM2434 (4310.93 kb) and RM6773 (4404.98 kb) with an interval of 94.04 kb (Figure 2). The presence of genomic segment between RM2434 and RM6773 (qDTY6.1) shows a positive effect on grain yield under stress and non-stress conditions.

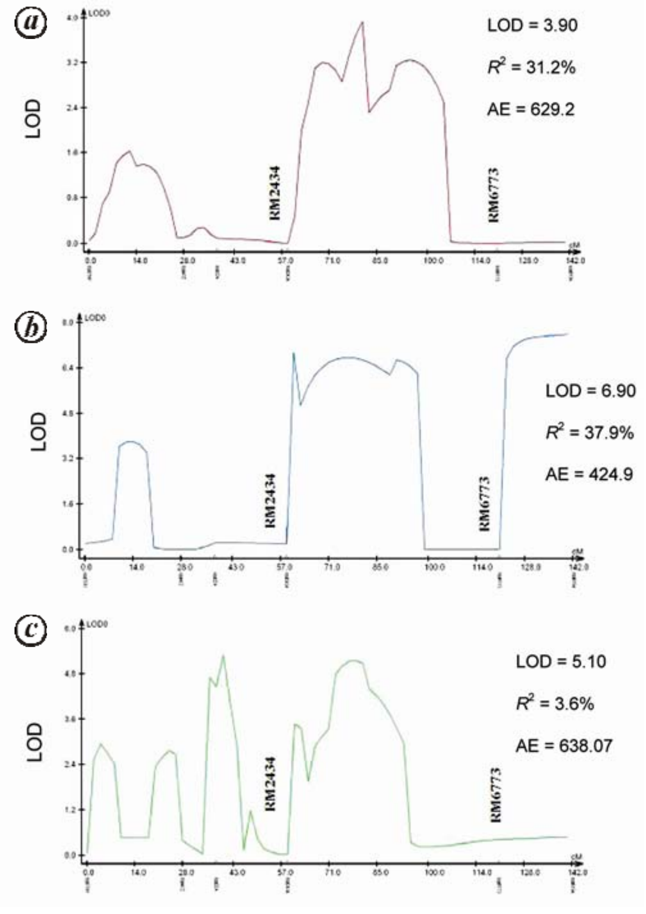

Figure 1. Consistent quantitative trait locus (QTL) peaks on fine-mapped region between RM2434 and RM6773 in stress (DS01 and DS02) and non-stress (FC01) trials: the curve between the mentioned markers is the LOD score for the grain yield QTL, qDTY6.1. R^2 indicates the phenotypic variance explained in percentage. AE, Additive effect. ***a***, Stress condition during the year 2011–12 (DS01). ***b***, Stress condition during the year 2012–13 (DS02). ***c***, Non-stress condition during the year 2013–14 (FC01).

The positive alleles were contributed by the landrace, Norungan. Thus the region qDTY6.1 is a potential candidate for improving rainfed rice yields through deployment in MAB and/or map-based cloning of underlying genes.

Rice Gene Thresher[35] was used to mine this region with stress gene catalogue and identified 21 genes that were upregulated under drought stress condition. Plastocyanin, vacuolar proton pyrophosphatase, acyl-CoA-binding protein and phosphate-induced protein-1 are Dayhoff's curated stress protein family present in the qDTY6.1 region. These proteins are related to photosynthesis[49,50], development of larger root system with increased auxin transport or higher auxin concentration in the root system with increased yield under dryland condition. ACPB2 was induced by abscisic acid (ABA) and drought. ABA-mediated reactive oxygen species (ROS) production in guard cells promotes stomatal closure, reduces water loss and enhances drought tolerance. Overexpression of ACBP2 improves drought tolerance and the mutant acpb2 is more sensitive to drought stress[51].

Comparative genomics of qDTY6.1

The evolutionary relationship among cereals is a known fact and the syntenic relationship can be used to find the homologous regions, which are useful in identifying their significance in stress adaptation in cereals. We compared the fine-mapped qDTY6.1 region for synteny among other cereals. The qDTY6.1 region was syntenic with maize (see Supplementary material, Figure S1 *a* online), pearl millet, sorghum (see Supplementary material, Figure S1 *b* online) and barley. This region was also found in

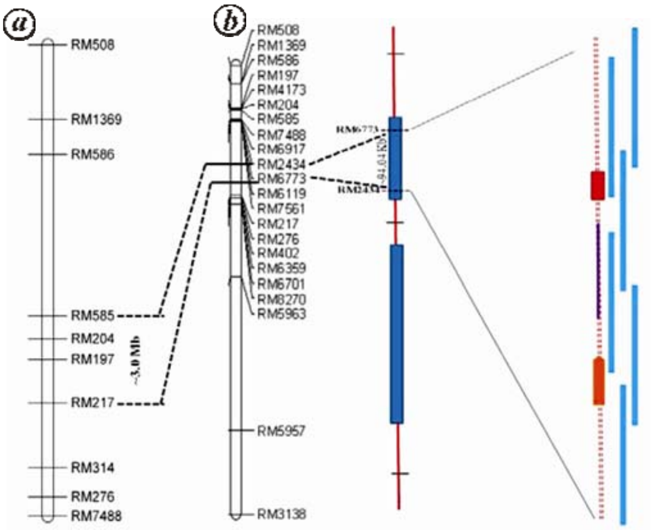

Figure 2. Location of qDTY6.1 on chromosome 6. (***a***) Linkage map constructed in a previous study[34] and distance in physical map position. ***b***, Fine-mapped region (qDTY6.1) between the markers RM2434 and RM6773 and its narrowed distance in the physical map.

maize on chromosome 9 near the marker umc109 in bin-9.02, sorghum on linkage group F, HHU37a, HHU37b and HHU37c, pearl millet on chromosome 2, Xpsr490(Ss1) and barley on chromosome 7H near marker ABC255 in bin-6. The syntenic regions in maize[52], pearl millet[53], sorghum[54] and barley[55,56] were reported for grain yield under drought stress. In sorghum, the syntenic region was linked to photosynthesis-related vacuolar H+translocating pyrophosphatase (see Supplementary material, Figure S1 *b* online).

Conclusion

To sum up, a consistent large-effect QTL (qDTY6.1) for yield under drought predominant in TPE was further confirmed and fine-mapped for application in MAB using rice lines derived from a local land race, Norungan. This region is also syntenic among cereals, indicating that drought adaptation mechanisms underlying this QTL have been conserved across cereals. qDTY6.1 is a potential candidate for improving grain yield under water stress in rice and other cereals as well. Further cloning of genes in this region may be useful in improving the production of rice and other cereal under water-limited environments.

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