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ORIGINAL PAPER

Fine mapping of QTLs for rice grain yield under drought reveals sub-QTLs conferring a response to variable drought severities

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Abstract Fine-mapping studies on four QTLs, $qDTY_{2.1}$, $qDTY_{2.2}$, $qDTY_{9.1}$ and $qDTY_{12.1}$, for grain yield (GY) under drought were conducted using four different backcross-derived populations screened in 16 experiments from 2006 to 2010. Composite and Bayesian interval mapping analyses resolved the originally identified $qDTY_{2.1}$ region of 42.3 cM into a segment of 1.6 cM, the $qDTY_{2.2}$ region of 31.0 cM into a segment of 6.7 cM, the $qDTY_{9.1}$ region of 32.1 cM into two segments of 9.4 and 2.4 cM and the $qDTY_{12.1}$ region of 10.6 cM into two segments of 3.1 and 0.4 cM. Two of the four QTLs ($qDTY_{9.1}$ and $qDTY_{12.1}$) having effects under varying degrees of stress severity showed the presence of more than one region within the original QTL. The study found the presence of a donor allele at RM262 within $qDTY_{2.1}$ and RM24334 within $qDTY_{9.1}$ showing a negative effect on GY under drought, indicating the necessity of precise fine mapping of QTL regions before using them in marker-assisted selection (MAS). However, the presence of sub-QTLs together in close vicinity to each other provides a unique opportunity to breeders to introgress such regions together as a unit into high-yielding drought-susceptible varieties through MAS.

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Introduction

Living organisms must acquire different biological functions to adapt to changing and hostile environments (Hattori et al. 2009). Diverse environmental conditions lead to the development of high genetic variability in organisms at morphological, anatomical and genetic levels through the course of evolution. Rice is one of the most widely grown food crops in the world. In 2008, a total of 661 million tons of rice was produced from 155.7 million ha (International Rice Research Institute, IRRI 2009a, b). Rice is cultivated in a wide range of environments such as irrigated, rainfed upland, rainfed lowland, flooded and saline, and it faces multiple biotic and abiotic challenges.

Water stress is the biggest challenge for rice productivity in the rainfed rice ecosystem. Rainfed rice occupies about 38% of the total cropped area and contributes 21% to total rice production. In Asia alone, about 34 million ha of rainfed lowland rice and 8 million ha of rainfed upland rice (Huke and Huke 1997) experience drought stress of varying intensities at different stages of the crop almost every year. Drought stress during the cropping season directly affects grain yield (GY), which is particularly devastating at the reproductive stage (Venuprasad et al. 2009b; Lanceras et al. 2004). Recent predictions of climate change suggest a further increase in water deficit in the coming years (Wassmann et al. 2009), leading to an increase in the intensity and frequency of drought (Bates et al. 2008).

Studies have shown the presence of high genetic variability for many physio-morphological traits controlling drought response in rice (Manickavelu et al. 2006); however, progress in breeding for drought tolerance has been slow (Fukai and Cooper 1995). Earlier, the lack of effective selection criteria for traits related to drought tolerance and low heritability of GY under stress were cited as major

reasons for slow progress in breeding (Ouk et al. 2006). Recent studies at IRRI have reported direct selection for GY under stress to be more effective than selection for secondary traits in improving GY under drought in rice (Kumar et al. 2008; Venuprasad et al. 2007, 2008). Using GY under stress as the primary selection criterion, several QTLs contributing yield under drought stress at the reproductive stage have recently been identified (Kumar et al. 2007; Bernier et al. 2007; Venuprasad et al. 2009a; Vikram et al. 2011; Swamy et al., unpublished). For a complex trait such as GY under drought, marker-assisted selection (MAS) could be an efficient strategy to improve current cultivated drought-susceptible varieties (Asins 2002; Bernier et al. 2007).

The identification and introgression of genomic regions with a large and consistent effect on GY under drought presents an opportunity to improve high-yielding but drought-susceptible varieties through MAS of large-effect QTLs (Salekdeh et al. 2002). However, for MAS to be effective, the target QTLs must be free from any undesirable linkage. The large size of the regions encompassing QTLs and the likely presence of undesirable linked genes make it essential to fine-map such regions to facilitate their precise introgression and to identify candidate genes within these QTLs.

Four such QTLs, $qDTY_{2.1}$ flanked by RM521 and RM262 on chromosome 2, $qDTY_{2.2}$ flanked by OSR17 and RM12868 on chromosome 2, $qDTY_{9.1}$ flanked by RM464 and RM24421 on chromosome 9 and $qDTY_{12.1}$ flanked by RM28048 and RM28166 on chromosome 12, were identified at IRRI (Bernier et al. 2007; Venuprasad et al. 2009a; Swamy et al., unpublished). $qDTY_{2.1}$ was identified in a BC_1F_4-derived population from a cross between drought-tolerant parent Apo (*indica*) and susceptible parent Swarna (*indica*). Spanning a region of 42.3 cM between RM521 and RM262 on chromosome 2, this QTL explained a phenotypic variance of 6.2% under rainfed lowland conditions (Venuprasad et al. 2009a). $qDTY_{2.2}$ and $qDTY_{9.1}$ were identified in two different BC_4F_3-derived populations from a cross of drought-tolerant parent Aday sel (*aus*) and susceptible parent IR64 (*indica*). These QTLs span a region of 31.0 and 32.1 cM on chromosomes 2 and 9 and explain a phenotypic variance of 11.2 and 13.0%, respectively, under lowland reproductive-stage drought stress (RS; Swamy et al., unpublished). $qDTY_{12.1}$ was identified under upland conditions in an $F_{3:4}$ population derived from a cross between Vandana with 50% *aus* and 50% *tropical japonica* ancestry (Bernier et al. 2007) and Way Rarem (*indica*). This QTL explained a phenotypic variance of 36.0% under upland RS (Bernier et al. 2007). In our study, four QTLs for GY under drought were fine-mapped using backcross-derived populations to facilitate precise introgression of the QTLs in high-yielding susceptible backgrounds.

Materials and methods

Results from this study were obtained from 16 experiments conducted at the experiment station of the IRRI, Los Baños, Laguna, Philippines, in the dry season (DS) of 2006, 2007, 2008, 2009 and 2010 and the wet season (WS) of 2007, 2008 and 2010. IRRI is located at 14°13′N latitude and 121°15′E longitude, at an elevation of 21 m above mean sea level. The soil type is a Maahas clay loam, iso-hyperthermic mixed typic Tropudalf (Venuprasad et al. 2009a). The sections below describe the details of plant materials used and the methodology adapted to conduct and analyze the experiments.

Plant materials

Backcross populations derived from crosses involving six different parents were used for fine mapping four QTLs for GY under reproductive-stage drought. A BC_1F_4-derived population of 490 lines from a cross between tolerant parent Apo and susceptible parent Swarna was used to conduct the drought-stress trials in DS2006 and DS2007, whereas a set of 193 lines from this population was used to conduct the non-stress trial in DS2007 for the fine mapping of $qDTY_{2.1}$ under lowland conditions. For fine mapping of $qDTY_{2.2}$ and $qDTY_{9.1}$, two different BC_4F_3-derived populations of 288 and 421 lines obtained from a cross between tolerant parent Aday sel and susceptible parent IR64 were screened under lowland stress and non-stress conditions, respectively. Experiments for fine mapping of $qDTY_{2.2}$ were conducted in WS2007 and DS2008, while those for $qDTY_{9.1}$ were conducted in DS2009 and DS2010. A BC_2F_3-derived population of 180 lines from the cross of donor parent Way Rarem and recipient parent Vandana was phenotyped in WS2008, DS2009 and DS2010 under upland reproductive-stage stress conditions for fine mapping of $qDTY_{12.1}$. The trial in DS2009 was affected by heavy rainfall and flooding of the field and is not included in the results. The non-stress trial for this population was conducted in WS2010. Another BC_3F_3-derived population with 470 lines was phenotyped in DS2010 under reproductive-stage stress and non-stress conditions to fine-map $qDTY_{12.1}$.

Experimental details

Upland, lowland, stress (mild, moderate and severe) and non-stress environments

Throughout this study, the term upland refers to field trials conducted under direct-seeded, non-puddled, non-flooded and aerobic conditions in leveled upland fields, whereas lowland refers to field trials conducted under flooded,

puddled, transplanted and anaerobic conditions. Trials conducted under irrigated conditions with no drought stress imposed are referred to as non-stress trials, while those in which drought stress was imposed during the reproductive stage of the crop are referred to as stress trials. The stress trials are further classified into mild, moderate and severe based on the percentage of yield reduced compared with non-stress trials (Kumar et al. 2008). Under lowland conditions, stress trials showing a yield reduction of 30% or less are termed mild stress (LMiS), those with a reduction from 31 to 65% are termed moderate stress (LMS) and the ones showing a yield reduction above 65% are referred to as severe stress (LSS) trials under lowland conditions. Under uplands, due to the frequent occurrence of drought with higher severity than in lowlands, the trials showing a yield reduction of 40% or less are classified as mild stress (UMiS), those with a yield reduction from 41 to 75% are classified as moderate stress (UMS) and the ones with a 76% or higher yield reduction are classified as severe stress (USS) trials.

Phenotyping of mapping populations

Apo/Swarna BC_1F_4-derived and Aday sel/IR64 BC_4F_3-derived mapping populations were screened under lowland conditions and Vandana/Way Rarem BC_2F_3- and BC_3F_3-derived populations were screened under upland conditions in 2006–2010.

Management of lowland trials

The trials for fine mapping of $qDTY_{2.1}$, $qDTY_{2.2}$ and $qDTY_{9.1}$ were conducted in lowland conditions. All lowland stress and non-stress trials were planted in an α-lattice design with 5-m-long rows at 0.2-m hill and row spacing. For fine mapping of $qDTY_{2.1}$, two stress trials and one non-stress trial were conducted in DS2006 and DS2007 with two replications in single-row plots. Stress trials for $qDTY_{2.2}$ were conducted in WS2007 and DS2008 with three replications in two-row plots, while single-row plots were maintained for the non-stress trial conducted in DS2008. Trials for $qDTY_{9.1}$ were conducted in DS2009 and DS2010 with two replications in single-row plots except for the stress trial conducted in DS2010, which had two-row plots. For all the trials, seeds were sown in a raised-bed nursery and 21-day-old seedlings were transplanted to the main field with each hill containing one seedling. After transplanting, approximately 5 cm of standing water was maintained in the field until drainage before stress initiation at 30 days after transplanting for stress trials, while standing water was maintained up to 10 days before harvest for non-stress trials. Field management of lowland trials was done as described by Venuprasad et al. (2009a).

Management of upland trials

For the fine mapping of $qDTY_{12.1}$, upland trials were conducted in WS2008, DS2009, DS2010 and WS2010 with 180 BC_2F_3-derived lines planted in an α-lattice design with three replications except for an upland non-stress trial conducted in WS2010, which was planted in two replications. Single-row plots 2-m long spaced at 0.25 m apart were maintained for all trials. Another population of 470 BC_3F_3-derived lines was phenotyped in DS2010 to fine-map $qDTY_{12.1}$. Single-row plots of 1.5- and 1.0-m length spaced 0.25 m apart were maintained for stress and non-stress trials. Seeds were dry-direct-seeded in aerobic soil using a seeding rate of 2.5 g per linear meter of row. In all 3 years, stress trials were sprinkler-irrigated twice a week during establishment and early vegetative growth, at 35 days after seeding stress began, and the plots were irrigated only when the soil water tension fell below −50 kPa at 30-cm soil depth. At this soil water potential, most lines wilted and exhibited leaf drying. This type of cyclic stress is reported to be efficient in screening for drought tolerance in populations consisting of genotypes with a broad range of growth duration (Lafitte et al. 2004) and it also ensures that all lines receive adequate stress during reproductive development. The frequency of stress cycles was much higher in the DS than in the WS when the trials were irrigated only in the case of prolonged dry spells that allowed the soil water tension to fall below −50 kPa. Field management of upland trials was done as described by Bernier et al. (2007).

Upland non-stress trials received the same cultural practices as the stress trials except that irrigation was continued twice a week up to 10 days before harvest. The trials were irrigated to field capacity at each irrigation and no flooding was allowed.

Data collection

In all the lowland trials, data on days to 50% flowering (DTF), plant height (PH) at maturity and GY were recorded except in the stress trial for $qDTY_{9.1}$ in DS2009, in which PH was not recorded. DTF was recorded as the number of days from sowing up to the day on which 50% of the plants had flowering tillers. PH of three plants from each plot was measured at maturity from ground level to the tip of the tallest tiller and averaged to get the mean PH for analysis. GY from each plot was harvested at physiological maturity, dried to a moisture content of 14% and weighed (Venuprasad et al. 2009a). This data set was then used to calculate the GY of the genotypes in kg ha^{-1} and was used for analysis. For the upland trials, data for DTF, PH and GY were recorded using the same procedure.

Statistical analysis

The model used for analysis of variance for an α-lattice design was

$$P_{ijk} = M + R_i + B_j(R_i) + L_k + e_{ijk}$$

where P_{ijk} is the measurement recorded on a plot, M is the mean over all plots and R, B, L and e refer to replications, blocks, lines and error, respectively. Data of GY trials for computation of means and standard error of difference (SED) were analyzed using CROPSTAT, taking the effect of replications and blocks within replications as random, and variance components were analyzed by the REML algorithm of PROC MIXED of SAS V.9.1 (SAS Institute Inc. 2004). For the calculation of variance components, the effect of lines was also considered random. Broad-sense heritability was calculated as

$$H = \frac{\sigma^2_G}{\sigma^2_G + \frac{\sigma^2_E}{r}}$$

where H is the broad-sense heritability of a trial, $\sigma^2_G$is the genetic variance, $\sigma^2_E$is the error variance and r is the number of replications in the trial.

Genotyping of mapping populations

Generation of genotypic data

All DNA marker work was conducted in the Molecular Marker Applications (MMA) Lab, Plant Breeding, Genetics, and Biotechnology (PBGB) Division, IRRI. Fresh leaves for all lines were collected and freeze-dried. DNA was extracted from freeze-dried leaf samples by a modified CTAB method in deep-well plates. Polymerase chain reaction (PCR) was performed in 96-well polycarbonate plates by the method described by Panaud et al. (1996). Polyacrylamide gel electrophoresis (Sambrook et al. 1989) was then used for size separation of the amplified DNA fragments using a Mini-Vertical Electrophoresis System (CBS Scientific, model MGV-202-33). The DNA fragments were then stained with SYBR Safe and visualized with a UV trans-illuminator.

Genetic analysis

In this study, rice SSR markers were used to fine-map the previously identified QTL regions for GY under drought. A total of 8 polymorphic SSR markers for $qDTY_{2.1}$, 11 for $qDTY_{2.2}$, 8 for $qDTY_{9.1}$ and 13 for $qDTY_{12.1}$ were added in the different mapping populations in the originally identified QTL region for fine mapping. The markers were taken based on published rice genome maps (IRGSP 2005) and their physical position (Mb) on the *indica* genome (http://www.gramene.org) was taken as a reference and multiplied by a factor of 3.92 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. The Kosambi map function was used for CIM (Kosambi 1944). Bayesian interval mapping (BIM) was performed using the software Q Gene 4.3.10 (Joehanes and Nelson 2008) based on the methods outlined by Sillanpää and Arjas (1998). The software Q Gene 4.3.10 was also used to determine the additive effects of single markers through single-marker regression analysis. Graphical genotyping software GGT 2 (Berloo 2008) was used for construction of a linkage map of F_3-derived populations.

Results

Phenotypic variation for GY and yield-related traits

The means ± SED, percentage yield reduction (YR) and heritability (H) estimates for GY (kg ha^{-1}), PH (cm), DTF and P values in the trials are presented in Table 1. Among the three trials conducted in DS2006 and DS2007 under lowland conditions for the fine mapping of $qDTY_{2.1}$, the severe and moderate stress trials had a mean GY of 524 and 2,069 kg ha^{-1}, with a 90.6 and 63.1% YR, respectively, compared with the non-stress trial, in which a mean GY of 5,557 kg ha^{-1} was recorded. A mean increase of 10.0 and 1.1% for DTF and a decrease of 38.8 and 23.7% for PH were observed in severe and moderate stress trials compared with the non-stress trial. The H estimates ranged from 0.53 to 0.77 for GY, from 0.76 to 0.83 for DTF and from 0.16 to 0.48 for PH.

The three trials used for the fine mapping of $qDTY_{2.2}$ under severe stress, moderate stress and non-stress lowland conditions were conducted in WS2007 and DS2008. A mean GY of 1,392 and 2,015 kg ha^{-1} was recorded for the severe and moderate stress trials, respectively. The YR was 67.8 and 53.6% for the two environments. A mean increase of 1.2% was observed for DTF under severe stress conditions; however, in the moderate stress trial conducted in WS2007, a decrease of 3.4% was recorded for mean DTF. PH decreased by 23.9 and 18.3% under severe and moderate stress conditions, respectively. H estimates for GY, DTF and PH ranged from 0.14 to 0.61, 0.35 to 0.82 and 0.11 to 0.52, respectively (Table 1).

Fine mapping of $qDTY_{9.1}$ was carried out on phenotypic data from three trials conducted in DS2009 and DS2010

Table 1 Experimental details including parents, population size, experimental design, environments and means (*M*), broad-sense heritability (*H*) and *P* values for grain yield (kg ha^{-1}), days to 50% flowering, plant height (cm) and percentage yield reduction (%YR) of trial means under stress versus non-stress conditions

QTL	Population	Parentage		Population size	Experimental design	Season	Environment	Grain yield (kg ha^{-1})			Days to 50% flowering			Plant height (cm)			YR (%)
		Donor	Recipient					*M*	*H*	*P*[a]	*M*	*H*	*P*	*M*	*H*	*P*	
qDTY$_{2.1}$	BC_1F_4-derived	Apo	Swarna	490	50X10 AL	DS2006	LSS	524 ± 332	0.77	****	103 ± 3.7	0.76	****	80 ± 11.9	0.48	****	90.6
				490	100X5 AL	DS2007	LMS	2,069 ± 764	0.53	****	95 ± 2.2	0.83	****	99 ± 30.4	0.23	****	63.1
				193	40X5 AL	DS2007	LNS	5,557 ± 1,100	0.59	****	94 ± 2.8	0.82	****	130 ± 5.6	0.16	****	
qDTY$_{2.2}$	BC_4F_3-derived	Aday sel	IR64	288	60X5 AL	DS2008	LSS	1,392 ± 297	0.43	****	91 ± 1.7	0.52	****	71 ± 3.5	0.44	****	67.8
				288	61X5 AL	WS2007	LMS	2,015 ± 291	0.61	****	86 ± 1.2	0.82	****	76 ± 5.0	0.52	****	53.6
				288	61X5 AL	DS2008	LNS	4,345 ± 660	0.14	NS	89 ± 1.6	0.35	****	95 ± 13.8	0.11	NS	
qDTY$_{9.1}$	BC_4F_3-derived	Aday sel	IR64	421	62X8 AL	DS2010	LSS	764 ± 226	0.25	****	95 ± 2.0	0.50	****	62 ± 4.3	0.20	*	87.5
				421	89X5 AL	DS2009	LMS	1,097 ± 476	0.50	****	88 ± 1.8	0.35	***				83.3
				421	127X4 AL	DS2009	LNS	6,109 ± 1,042	0.33	NS	86 ± 1.7	0.38	****	100 ± 4.3	0.26	**	
qDTY$_{12.1}$	BC_2F_3-derived	Way Rarem	Vandana	180	20X10 AL	DS2010	USS-I	108 ± 73	0.56	****	70 ± 2.6	0.6	****	64 ± 4.3	0.25	**	96.7
				180	20X10 AL	DS2010	USS-II	266 ± 175	0.49	****	68 ± 3.0	0.62	****	66 ± 4.4	0.48	***	91.8
				180	20X10 AL	WS2008	UMS	825 ± 253	0.64	****	65 ± 1.2	0.71	****	106 ± 4.3	0.51	****	74.6
				180	20X10 AL	WS2008	UMiS	2,682 ± 487	0.84	****	65 ± 0.8	0.99	NS	125 ± 4.1	0.88	****	17.5
				180	20X10 AL	WS2010	UNS-I	3,249 ± 744	0.59	****	57 ± 1.4	0.88	****	120 ± 4.6	0.07	NS	
	BC_3F_3-derived	Way Rarem	Vandana	470	50X10 AL	DS2010	USS-III	148 ± 157	0.42	****	79 ± 6.2	0.53	****	70 ± 6.7	0.12	NS	96.2
				470	50X10 AL	DS2010	UNS-II	3,882 ± 1,273	0.38	****	60 ± 23.6	0.76	****	107 ± 2.0	0.11	NS	

AL alpha lattice, *LSS* lowland severe stress, *LMS* lowland moderate stress, *LNS* lowland non-stress, *USS* upland severe stress, *UMS* upland moderate stress, *UMiS* upland mild stress, *UNS* upland non-stress, *NS* non-significant

[a] Probability of difference between genotypes: *, **, ***, **** significant at 5, 1, 0.1, 0.01% *P* levels, respectively

under severe stress, moderate stress and non-stress lowland conditions. A mean GY of 764, 1,097 and 6109 kg ha^{-1} was recorded under severe stress, moderate stress and non-stress conditions, showing a YR of 87.5 and 83.3%, respectively, under severe stress and moderate stress conditions compared with non-stress conditions. The higher YR under moderate stress conditions during DS2009 resulted from insect attack; however, the drought stress was moderate in this season compared with DS2010 as evident from parching groundwater data of 2009 and 2010 (Supplementary Figs. S1a and S1b). Flowering was delayed by 10.7 and 2.0% under severe and moderate stress conditions, while a 37.3% reduction in PH was recorded in severe stress conditions. *H* estimates for GY ranged from 0.25 to 0.50, while those for PH and DTF ranged from 0.20 to 0.26 and 0.35 to 0.50, respectively (Table 1).

qDTY$_{12.1}$ was fine-mapped on the basis of data from seven upland trials conducted between WS2008 and WS2010 under upland severe stress, moderate stress, mild stress and non-stress environments with BC_2- and BC_3-derived populations. Among the trials conducted with BC_2-derived populations, the two stress trials in DS2010 had a mean GY of 108 and 266 kg ha^{-1}, showing a YR of 96.7 and 91.8%, respectively. The moderate stress trial in WS2008 showed a YR of 74.6%, with a mean GY of 825 kg ha^{-1}, while the mild stress trial conducted in the same season showed a mean GY of 2,682 kg ha^{-1}, which was 17.5% less than that of the non-stress trial conducted in WS2010 with a mean GY of 3,249 kg ha^{-1}. The BC_3-derived population had a mean GY of 148 kg ha^{-1} under severe stress conditions with a YR of 96.2%. The *H* estimates for GY, DTF and PH ranged from 0.49 to 0.84, 0.60 to 0.99 and 0.07 to 0.88, respectively, for the BC_2-derived population and from 0.38 to 0.42, 0.53 to 0.76 and 0.11 to 0.12 for the BC_3-derived population (Table 1).

Fine mapping of QTLs

Fine mapping of the four QTLs was carried out through CIM on the respective backcross populations. BIM analysis was also conducted on all stress trials showing a significant effect in CIM to validate the possibility of the presence of multiple sub-QTLs within the original regions. The sections below provide the results of CIM and BIM along with the fine-mapped physical span of the QTLs as compared to the originally identified regions.

Fine mapping of qDTY$_{2.1}$

CIM analysis of markers within *qDTY*$_{2.1}$ showed a region between RM3549 and RM324 having an effect on GY under severe and moderate stress conditions. The LOD peak was detected at 47.8 cM under severe stress and 47.9 cM under moderate stress conditions with RM324 as the closest marker to the peak (Fig. 1a). The QTL explained a phenotypic variance of 6.9 and 2.2% and had an additive effect of 22.7 and 5.3% under severe and moderate stress, respectively (Table 2). The BIM analysis

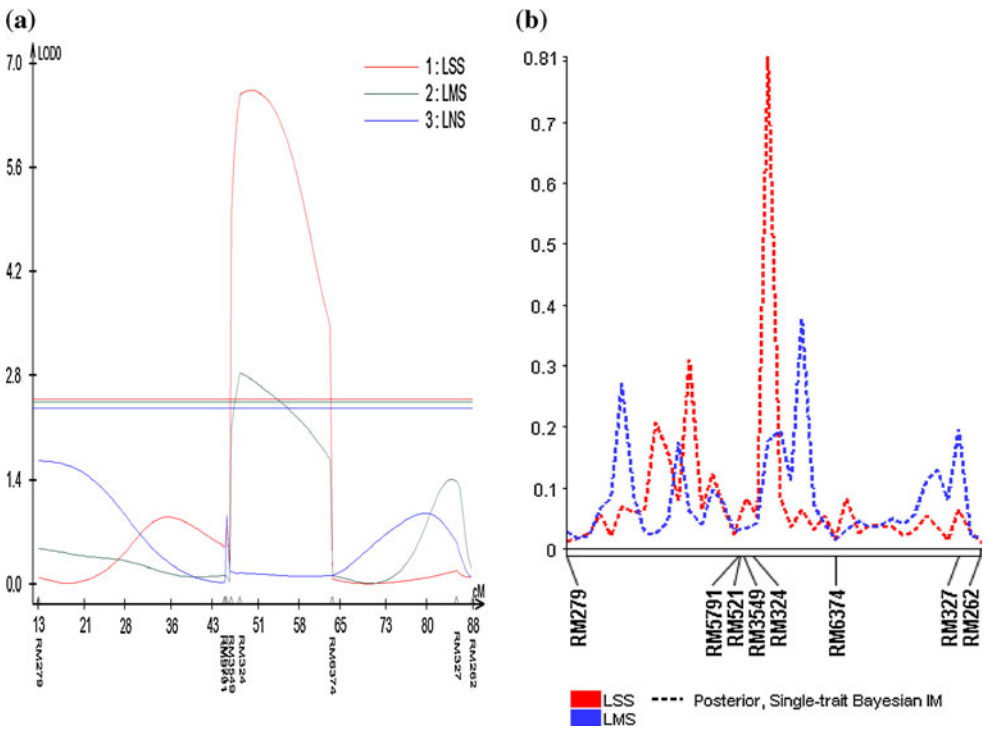

Fig. 1 **a** QTL likelihood curves of LOD score for grain yield (GY) showing significant regions within *qDTY*$_{2.1}$ under severe stress, moderate stress and non-stress lowland conditions. Genetic distance in cM between the markers is indicated on *X* axis. *Horizontal lines* correspond to critical LOD value. **b** *BIM posterior curves* showing QTL peak position within *qDTY*$_{2.1}$ region under lowland severe stress and moderate stress conditions. Marker loci are indicated on *X* axis and *Y* axis corresponds to BIM posterior values

Table 2 Fine-mapped regions within different QTLs for grain yield (kg ha^{-1}) under lowland and upland drought conditions detected through composite interval mapping and their varying effects under different water stress conditions

QTL	Season	Population used	Environment	Fine-mapped region	Interval	Peak position (cM)	Marker closest to the peak	R^2	Add. (%)	LOD
qDTY$_{2.1}$	DS2006	BC_1F_3-derived	LSS	*qDTY*$_{2.1}$	RM3549–RM324	47.8	RM324	6.9	22.7	6.6
	DS2007	BC_1F_3-derived	LMS	*qDTY*$_{2.1}$	RM3549–RM324	47.9	RM324	2.2	5.3	2.8
qDTY$_{2.2}$	DS2008	BC_4F_3-derived	LSS	*qDTY*$_{2.2}$	RM279–RM555	16.7	RM555	10.2	7.0	7.6
qDTY$_{9.1}$	DS2010	BC_4F_3-derived	LSS	*qDTY*$_{9.1B}$	RM24350–RM24390	54.6	RM24350	4.4	7.5	6.2
	DS2009	BC_4F_3-derived	LMS	*qDTY*$_{9.1A}$	RM321–RM566	51.5	RM566	8.9	16.6	7.3
	DS2009	BC_4F_3-derived	LNS	*qDTY*$_{9.1A}$	RM321–RM566	51.9	RM566	4.4	−3.8	3.6
qDTY$_{12.1}$	DS2010	BC_2F_3-derived	USS-I	*qDTY*$_{12.1A}$	RM28130–RM511	54.0	RM511	25.8	−42.8	14.5
				qDTY$_{12.1B}$	RM1261–RM28166	54.8	RM28166	24.2	−42.8	11.7
	DS2010	BC_2F_3-derived	USS-II	*qDTY*$_{12.1A}$	RM28130–RM511	54.0	RM511	28.0	−36.4	13.4
				qDTY$_{12.1B}$	RM1261–RM28166	54.8	RM28166	24.5	−35.0	11.8
	DS2010	BC_3F_3-derived	USS-III	*qDTY*$_{12.1A}$	RM28099–RM28130	52.3	RM28130	20.6	−44.8	23.5
	WS2008	BC_2F_3-derived	UMS	*qDTY*$_{12.1A}$	RM28130–RM511	53.5	RM511	8.1	−13.6	5.1
				qDTY$_{12.1B}$	RM1261–RM28166	54.8	RM28166	6.8	−12.9	6.3
	WS2008	BC_2F_3-derived	UMiS	*qDTY*$_{12.1A}$	RM28130–RM511	54.0	RM511	15.1	−12.3	7.7
				qDTY$_{12.1B}$	RM1261–RM28166	54.8	RM28166	10.2	−10.6	5.3

R^2 percentage of phenotypic variance explained by the marker closest to the peak. *Add.* (%) additive effect as percentage of trial mean for the region, *LSS* lowland severe stress, *LMS* lowland moderate stress, *LNS* lowland non-stress, *USS* upland severe stress, *UMS* upland moderate stress, *UMiS* upland mild stress, *UNS* upland non-stress

Table 3 Flanking markers and span of originally identified and fine-mapped regions within $qDTY_{2.1}$, $qDTY_{2.2}$, $qDTY_{9.1}$ and $qDTY_{12.1}$

QTL	Original QTL		QTL	Fine-mapped region(s)	
	Flanking markers	Length (cM)		Flanking markers	Length (cM)
$qDTY_{2.1}$	RM521–RM262	42.3	$qDTY_{2.1}$	RM3549–RM324	1.6
$qDTY_{2.2}$	OSR17–RM12868	31.0	$qDTY_{2.2}$	RM279–RM555	6.7
$qDTY_{9.1}$	RM464–RM24421	32.1	$qDTY_{9.1A}$	RM321–RM566	9.4
			$qDTY_{9.1B}$	RM24350–RM24390	2.4
$qDTY_{12.1}$	RM28048–RM28166	10.6	$qDTY_{12.1A}$	RM28099–RM511	3.1
			$qDTY_{12.1B}$	RM1261–RM28166	0.4

of this region has shown the presence of a QTL peak near RM324 with a BIM posterior value of 0.81 and 0.38 under LSS and LMS conditions, respectively (Fig. 1b). In terms of physical span, fine mapping of $qDTY_{2.1}$ has resolved the original QTL into a region of 1.6 cM spanning between RM3549 and RM324, within the originally mapped region of 42.3 cM between RM521 and RM262 (Table 3).

Fine mapping of $qDTY_{2.2}$

For $qDTY_{2.2}$, CIM analysis has shown a region between RM279 and RM555 having an effect on GY under severe stress conditions (Fig. 2a). The region explained 10.2% of the phenotypic variance and had an additive effect of 7.0%. The peak of the QTL lay at 16.7 cM, with RM555 as the closest marker to the peak. The region was non-significant under both moderate stress and non-stress conditions (Table 2; Fig. 2a). BIM analysis showed the QTL peak at RM279 with a BIM posterior value of 0.74 (Fig. 2b). CIM of $qDTY_{2.2}$ reduced the original span of 31.0 cM between OSR17 and RM12868 to a region of 6.7 cM between RM279 and RM555 in terms of physical span (Table 3).

Fine mapping of $qDTY_{9.1}$

CIM analysis of the $qDTY_{9.1}$ region has shown two regions, $qDTY_{9.1A}$ flanked by RM321 and RM566 and $qDTY_{9.1B}$ flanked by RM24350 and RM24390, to be effective under varying stress conditions (Fig. 3a). $qDTY_{9.1A}$ had its peak at 51.5 cM, with RM566 being the closest marker to the peak. This region showed its effect under moderate stress conditions, for which it explained 8.9% of the phenotypic variance and had an additive effect of 16.6%. However, under severe stress conditions, $qDTY_{9.1B}$ was seen to affect GY. The peak of the region was at 54.6 cM, with RM24350 as the closest marker to the peak. This region

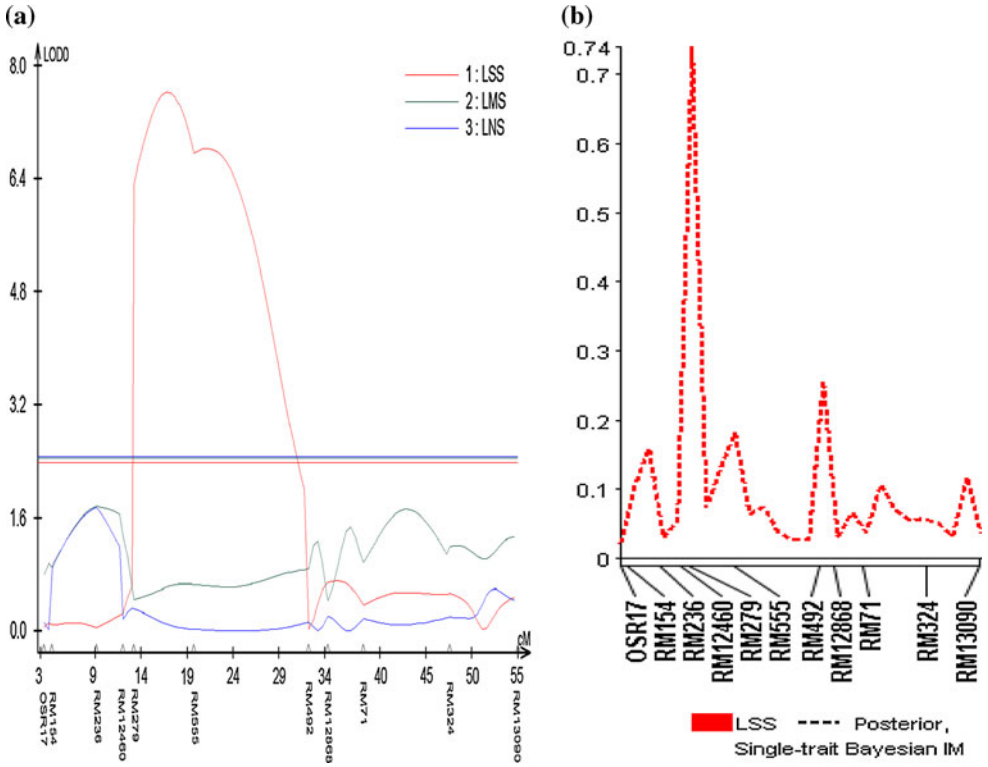

Fig. 2 **a** QTL likelihood curves of LOD score for grain yield (GY) showing significant regions within $qDTY_{2.2}$ under severe stress, moderate stress and non-stress lowland conditions. Genetic distance in cM between the markers is indicated on *X* axis. *Horizontal lines* correspond to critical LOD value. **b** *BIM posterior curves* showing QTL peak position within $qDTY_{2.2}$ region under lowland severe stress conditions. Marker loci are indicated on *X* axis and *Y* axis corresponds to BIM posterior values

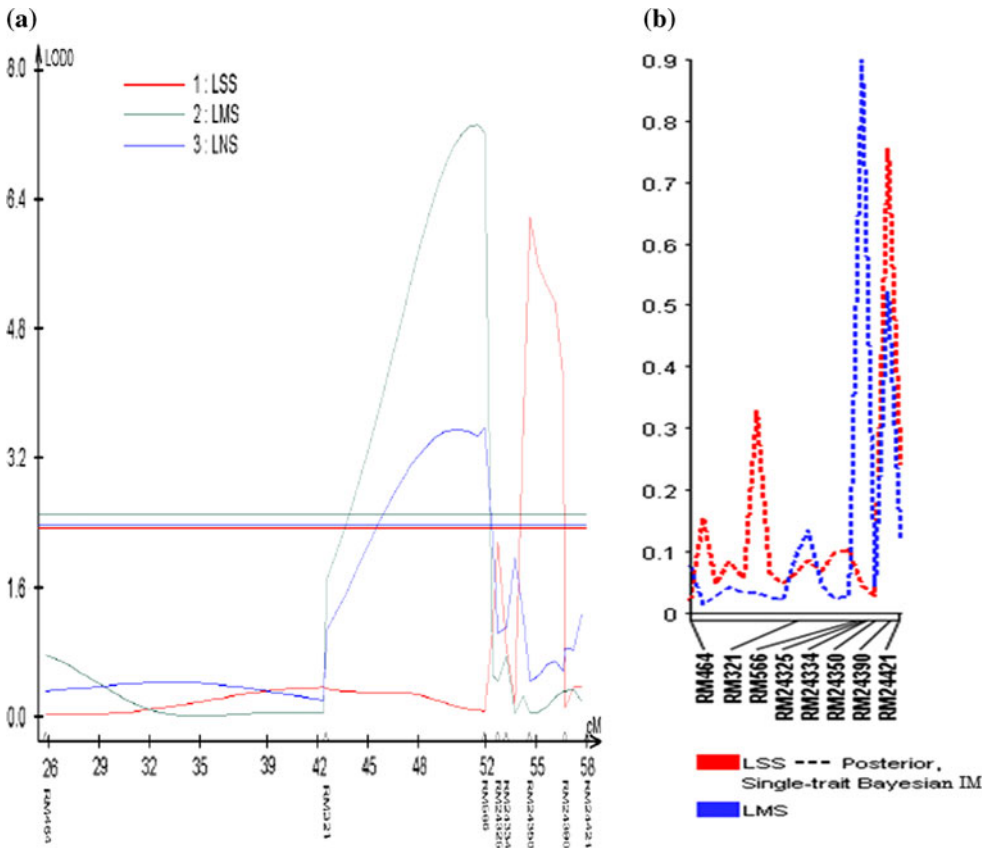

Fig. 3 **a** QTL likelihood curves of LOD score for grain yield (GY) showing significant regions within $qDTY_{9.1}$ under severe stress, moderate stress and non-stress lowland conditions. Genetic distance in cM between the markers is indicated on *X* axis. *Horizontal lines* correspond to critical LOD value. **b** *BIM posterior curves* showing QTL peak position within $qDTY_{2.2}$ region under lowland severe stress and moderate stress conditions. Marker loci are indicated on *X* axis and *Y* axis corresponds to BIM posterior values

explained a phenotypic variance of 4.4% with an additive effect of 7.5%. $qDTY_{9.1A}$ was also significant under non-stress conditions, for which it explained 4.4% of the phenotypic variance and had an additive effect of −3.8% (Table 2). In consistency with CIM results, BIM analysis showed the presence of two QTL peaks within $qDTY_{9.1}$ near RM566 ($qDTY_{9.1A}$) and RM24350 ($qDTY_{9.1B}$) with BIM posterior values of 0.90 and 0.52, respectively, under LMS conditions (Fig. 3b). Under LSS conditions, the QTL peak was seen at RM24350 with a BIM posterior value of 0.76 (Fig. 3b). In terms of physical span, $qDTY_{9.1}$ was resolved into two regions of 9.4 and 2.4 cM between RM321 and RM566 and RM24350 and RM24390, respectively. This QTL originally spanned a region of 32.1 cM between RM464 and RM24421 (Table 3).

Fine mapping of $qDTY_{12.1}$

CIM analysis of $qDTY_{12.1}$ showed the presence of at least two continuous regions, $qDTY_{12.1A}$ between RM28099 and RM511 and $qDTY_{12.1B}$ between RM1261 and RM28166 (Fig. 4a). $qDTY_{12.1A}$ had its peak at 54.0 cM, with RM511 as the peak marker. The peak of $qDTY_{12.1B}$ was at 54.8 cM, with RM28166 as the closest marker to the peak. In the BC_2-derived population, both regions showed their effects in all four stress environments that ranged from severe to mild stress conditions. $qDTY_{12.1A}$ explained 25.8, 28.0, 8.1 and 15.1% of the phenotypic variance in severe stress-I, severe stress-II, moderate stress and mild stress conditions, respectively (Table 2), and had an additive effect of −42.8, −36.4, −13.6 and −12.3%, respectively. $qDTY_{12.1B}$ explained 24.2, 24.5, 6.8 and 10.2% of the phenotypic variance in severe stress-I, severe stress-II, moderate stress and mild stress conditions, respectively, with an additive effect of −42.8, −35.0, −12.9 and −10.6%, respectively. $qDTY_{12.1A}$ also showed its effect in the BC_3-derived population, for which it explained 20.6% of the phenotypic variance under severe stress conditions and had an additive effect of −44.8% (Table 2). All the regions were non-significant in non-stress conditions (Fig. 4a). BIM analysis of this region showed the presence of a highly consistent QTL peak at RM511 ($qDTY_{12.1A}$) with BIM posterior values of 1.0, 0.79 and 0.99 for the BC_2-derived population under USS, UMS and UMiS conditions, respectively (Fig. 4b). However, under USS conditions, another peak near RM28166 ($qDTY_{12.1B}$) was seen with a BIM posterior value of 0.46. $qDTY_{12.1}$ was originally mapped on a 10.6 cM region between RM28048 and RM28166 on chromosome 12. CIM of this QTL with additional markers resulted in two regions of 3.1 and 0.4 cM between RM28099 and RM511 and between RM1261 and RM28166, respectively (Table 3).

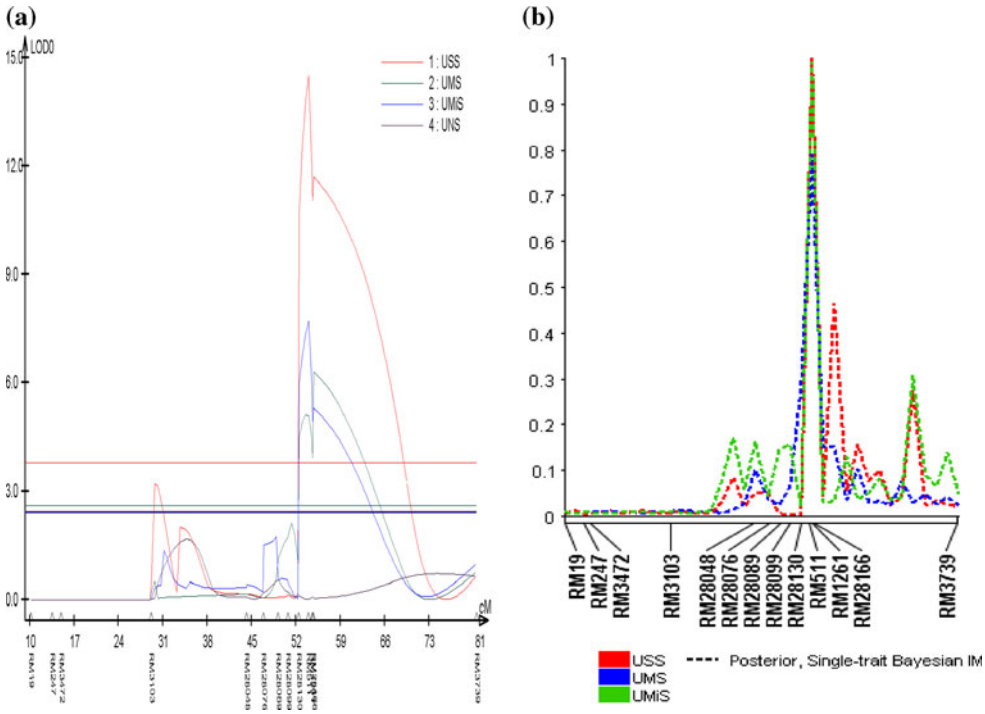

Fig. 4 **a** QTL likelihood curves of LOD score for grain yield (GY) showing significant regions within $qDTY_{12.1}$ under severe stress, moderate stress, mild stress and non-stress upland conditions. Genetic distance in cM between the markers is indicated on *X* axis. *Horizontal lines* correspond to critical LOD value. **b** BIM posterior curves showing QTL peak position within $qDTY_{2.2}$ region under upland severe stress, moderate stress and mild stress conditions. Marker loci are indicated on *X* axis and *Y* axis corresponds to BIM posterior values

Effect of marker loci within the QTLs

Figure 5 shows the additive effect of marker loci and Fig. 6 presents the classes for $qDTY_{2.1}$ (BC_1F_4-derived), $qDTY_{2.2}$ (BC_4F_3-derived), $qDTY_{9.1}$ (BC_4F_3-derived) and $qDTY_{12.1}$ (BC_2F_2-derived), with different segments of the QTLs arranged in the order of increasing GY under varying stress conditions in which the respective QTL has shown a significant effect. All four QTLs showed a decreasing additive effect on GY with decreasing stress. The effect of $qDTY_{2.1}$ reached 20.9% of the trial mean under severe stress conditions at RM3549, while RM324 showed an effect of 20%. Under moderate stress conditions, the highest effect of 5.6% was shown by RM6374, while the peak marker RM324 showed an effect of 5.5% (Fig. 5a). The lines with a donor segment between RM521 and RM324 yielded higher than those with a full region of the QTL from RM521 to RM262, and the lines without the QTLs were the lowest yielding (Fig. 6a) under both severe and moderate stress conditions.

For $qDTY_{2.2}$, RM555 showed the highest additive effect of 6.8% for GY, followed by RM279 with an additive effect of 6.4% under severe stress conditions. The effects of both markers were found to be negative under moderate stress and non-stress conditions (Fig. 5b). All the lines with the fine-mapped segment of the donor genome between RM279 and RM555 were higher yielding than those without this segment under severe stress conditions. The highest-yielding class was the one with the QTL segment between RM154 and RM71; however, the number of lines in this class was only three, not sufficient to draw relevant conclusions (Fig. 6b).

Under severe stress conditions, $qDTY_{9.1B}$ showed the highest additive effect of 8.5% at RM24350, while $qDTY_{9.1A}$ showed an additive effect of 5.9% at RM566. The effect of RM24350 remained the same under moderate stress conditions, while the effect of RM566 increased to 14.2% under moderate stress. Marker RM24334 had a negative effect under both moderate and severe stress conditions (Fig. 5c). $qDTY_{9.1}$ lines with the two fine-mapped segments between RM321 and RM566 ($qDTY_{9.1A}$) and RM24350 and RM24390 ($qDTY_{9.1B}$) without the Aday sel donor segment at RM24334 were the highest yielding under severe stress conditions. RM24334 lies between two fine-mapped regions and it showed a negative effect on GY under both moderate and severe stress. Also, the absence of a donor segment between RM24350 and RM24390 reduced GY under severe stress despite the presence of $qDTY_{9.1A}$ (Fig. 6c; Supplementary Table S1). However, under moderate stress conditions, the lines with $qDTY_{9.1A}$ showed the highest effect, followed by the lines with both segments without RM24334, confirming the negative effect of this locus on GY under drought.

The effect of $qDTY_{12.1}$ increased with increasing stress. RM511, the peak marker for $qDTY_{12.1A}$, showed an additive effect of 45.2, 34.8, 18.4 and 14.4%, respectively, under severe stress-I, severe stress-II, moderate stress and mild stress conditions. RM28166, the peak marker for $qDTY_{12.1B}$, showed an additive effect of 42.6, 33.4, 18.9 and 13.0%, respectively, under severe stress-I, severe

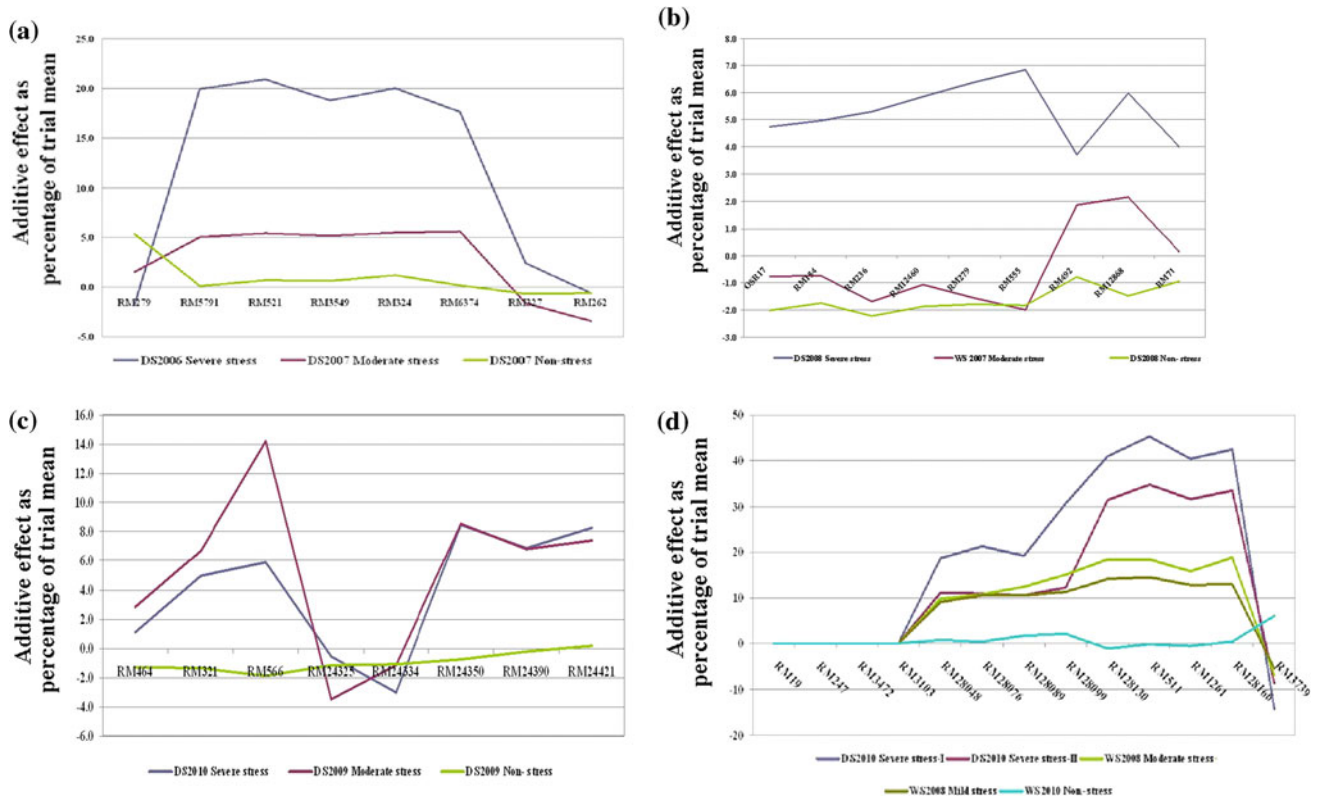

Fig. 5 Additive effect as percentage of trial mean for different markers within $qDTY_{2.1}$ (**a**), $qDTY_{2.2}$ (**b**), $qDTY_{9.1}$ (**c**) and $qDTY_{12.1}$ (**d**) on GY (kg ha^{-1}) under varying drought-stress conditions

stress-II, moderate stress and mild stress conditions (Fig. 5d). The $qDTY_{12.1}$ lines containing the fine-mapped segment of the donor genome between RM28099 and RM28166 with both fine-mapped regions ($qDTY_{12.1A}$ and $qDTY_{12.1B}$) showed the highest GY, followed by the lines containing the originally identified segment (RM28048–RM28166) of $qDTY_{12.1}$. Lines without a fine-mapped segment flanked between RM28099 and RM28166 were lower yielding than those containing this segment under all three stress conditions. However, the lowest-yielding class was the lines without the full complement of QTLs (Fig. 6d; Supplementary Table S2).

Discussion

The drought molecular breeding program at IRRI has identified four QTLs, $qDTY_{2.1}$, $qDTY_{2.2}$, $qDTY_{9.1}$ and $qDTY_{12.1}$, for GY under drought (Bernier et al. 2007; Venuprasad et al. 2009a; Swamy et al., unpublished). It is important to note that all four of these QTL regions increased GY under stress conditions and did not have any effect on GY under non-stress conditions. $qDTY_{12.1}$ explained the highest percentage of phenotypic variance for GY under severe upland drought compared with the three other QTLs. This could be due to the presence of two QTL regions together within $qDTY_{12.1}$ showing a large effect in severe drought stress condition and higher severity of drought under upland condition, which led to higher differences between tolerant and susceptible lines in the population as compared to transplanted lowland conditions. However, $qDTY_{2.1}$, $qDTY_{2.2}$ and $qDTY_{9.1}$ have shown a high and consistent additive effect under severe lowland drought stress despite the low phenotypic variance explained by them. Apart from this, the effect of these QTLs was also seen under aerobic non-stress conditions, leading to increased adaptation of the recipient parents to aerobic conditions and thus making them candidates for MAS. However, these QTLs encompassed large chromosomal segments (Table 3) and needed to be fine-mapped before being used in MAS/MAB (marker-assisted backcrossing). In our 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 a full effect on GY 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

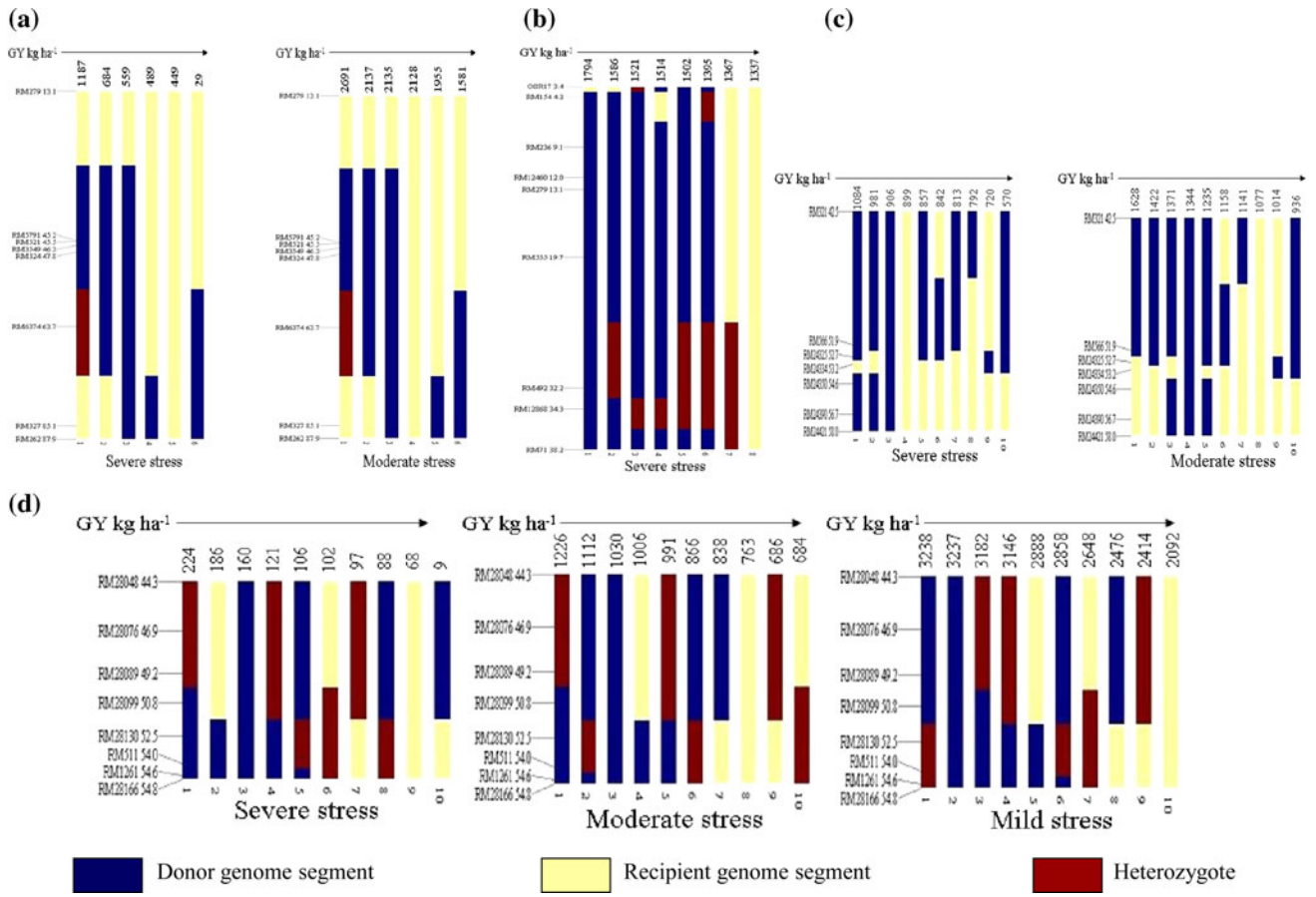

Fig. 6 Line classes with different segments of $qDTY_{2.1}$ (**a**), $qDTY_{2.2}$ (**b**), $qDTY_{9.1}$ (**c**) and $qDTY_{12.1}$ (**d**) in order of increasing mean GY under varying stress conditions when the QTL has shown a significant effect. GY: mean grain yield of lines with respective segment of the QTL; trial standard error of difference (SED) values at 5% level of significance for respective trials—**a** $qDTY_{2.1}$: severe stress, 332 kg ha^{-1}; moderate stress, 764 kg ha^{-1}; **b** $qDTY_{2.2}$: severe stress, 292 kg ha^{-1}; **c** $qDTY_{9.1}$: severe stress, 226 kg ha^{-1}; moderate stress, 476 kg ha^{-1}; **d** $qDTY_{12.1}$: severe stress, 73 kg ha^{-1}; moderate stress, 253 kg ha^{-1}; mild stress, 487 kg ha^{-1}

IR64 and Swarna and also to enhance GY under drought of drought-tolerant cultivar Vandana, a popular upland variety.

Fine-mapping studies conducted on $qDTY_{2.1}$, $qDTY_{2.2}$, $qDTY_{9.1}$ and $qDTY_{12.1}$ have not only narrowed the regions containing the QTL, but also resolved two of the four QTLs analyzed ($qDTY_{9.1}$ and $qDTY_{12.1}$) into more than one QTL region within the originally identified region. However, $qDTY_{2.1}$ and $qDTY_{2.2}$ were narrowed to a single interval between RM521 and RM324 and RM279 and RM555, respectively. In the two cases with multiple QTLs within the original QTL region, we observed different loci showing an effect under severe and moderate stress conditions in the case of $qDTY_{9.1}$. In the case of $qDTY_{12.1}$, both the identified loci within the QTL showed an effect under severe stress conditions. Coexisting chromosomal regions/loci imparting tolerance of a varying severity of drought stress provide a unique opportunity to breeders to introgress such regions together as a unit into high-yielding drought-susceptible varieties through MAS/MAB and to develop cultivars possessing increased tolerance of varying stress severities. The presence of sub-QTLs within these QTLs suggests that more than one candidate gene is involved in conferring the large phenotypic effect of QTLs for GY under stress. QTLs affecting maximum root length and root thickness have been reported in the $qDTY_{2.1}$ region (Price et al. 1999; Kamoshita et al. 2002; MacMillan et al. 2006). Swamy et al. (2011) have reported a meta QTL adjacent to $qDTY_{2.1}$ affecting GY under drought. Spanning between RM452 and RM521, this region was reported to contain at least nine candidate genes affecting drought response. Similarly, QTLs for root length within the $qDTY_{2.2}$ region (MacMillan et al. 2006; Kamoshita et al. 2002) and for root thickness (Champoux et al. 1995) adjacent to the $qDTY_{2.2}$ region have been reported. Kamoshita et al. (2008) reported a 37-cM region between R41 and RM215 coinciding with $qDTY_{9.1}$ on chromosome 9 to affect drought response mainly through root traits, but it also shows association with plant water status and GY. Courtois et al. (2009) reported a meta QTL for maximum

root length on chromosome 9 adjacent to $qDTY_{9.1A}$. The study also compiled numerous earlier reports of QTLs for traits, viz., maximum root length, root thickness and root numbers adjacent to or coinciding with $qDTY_{9.1A}$ and $qDTY_{9.1B}$. Similarly, Khowaja et al. (2009) reported clusters of QTLs for root traits adjacent to $qDTY_{9.1B}$. More recently, Uga et al. (2010a, b) reported the presence of two QTLs, *Sta1* and *Dro1*, coinciding with $qDTY_{9.1A}$ and $qDTY_{9.1B}$. These QTLs are reported to distinctly affect stele transversal area (*Sta1*) and maximum root depth (*Dro1*) under drought. These reports not only suggest the presence of two distinct sub-QTLs within this region, but also explain the specific effect of $qDTY_{9.1A}$ under moderate stress and $qDTY_{9.1B}$ under severe stress conditions. In the case of $qDTY_{12.1}$, it was observed that the QTL affected other traits such as PH, DTF, biomass, drought response index (DRI) and flowering delay along with GY under drought stress conditions (Bernier et al. 2007). A meta QTL for GY under drought coinciding with $qDTY_{12.1\mathrm{A}}$ was also reported (Swamy et al. 2011). A total of six putative candidate genes were reported in the $qDTY_{12.1\mathrm{A}}$ region (Swamy et al. 2011). The effect of these regions on a large number of traits related to drought response or GY strongly suggests the presence of more than one gene within these QTLs affecting a wide range of traits under drought.

It is possible that these genes conferring a GY advantage under stress may have undergone strong natural selection to stay together in the course of evolution. These sub-QTLs with a discernible phenotypic effect on GY may affect the same/different physiological traits in response to different severities of stress, leading to a GY advantage. Co-localization of QTLs for GY and DTF and GY and PH has also been reported in earlier studies (Venuprasad et al. 2009a; Vikram et al. 2011). Meta QTLs showing a plieotropic effect on more than one trait under drought have been reported in a Bala × Azucena population (Khowaja et al. 2009). The presence of consistent QTLs affecting multiple traits related to drought response has been reported by Kamoshita et al. (2008).

It is interesting to see that $qDTY_{2.2}$ did not split into more than one region, and unlike the other two QTLs ($qDTY_{9.1}$, $qDTY_{12.1}$), it showed its effect only under severe stress conditions. Similarly, $qDTY_{2.1}$ with a single region showed a large effect under severe stress but a very low effect under moderate stress. These observations further support the fact that while one of the segments of the QTL may have a main effect on GY under drought stress, the presence of multiple sub-QTLs may lead to wider adaptation under varying drought-stress severities.

Results from our fine-mapping studies suggest that drought-tolerance QTLs are complex loci where multiple genes may be working independently or in coordination with each other, leading to an increase in GY under drought. The complex nature of large-effect QTLs for stress tolerance has also been reported for other traits in rice. For example, the *Sub1* region, which confers submergence tolerance, contains three genes, *Sub1A*, *Sub1B* and *Sub1C*, of which *Sub1A* was identified as the primary determinant of submergence tolerance (Xu et al. 2006). However, it is not known whether *Sub1B* and *Sub1C* have any phenotypic effects at a lower severity of submergence (less than 2 weeks) (E. Septiningsih, pers. comm.). Similarly, Ding et al. (2011) have reported a QTL on chromosome 4 controlling root volume per tiller co-segregating with flag-leaf width and spikelet number per panicle. This QTL was resolved into a region of 38 kb with three open-reading frames. Gene clusters that confer resistance to biotic stresses are also reported. The *Pi2/9* locus conferring blast resistance is reported to contain a cluster of NBS-LRR genes (*Pi9*, *Pi2* and *Piz-t*) with different specificities that are all located in a 100-kb region on chromosome 6 (Zhou et al. 2006). A highly conserved cluster of 12 germin-like protein gene members has also been reported on chromosome 8, which confer broad-spectrum disease resistance in rice (Manosalva et al. 2009). Substitution mapping studies on a flowering-time QTL associated with transgressive variation in rice also revealed the presence of multiple sub-QTLs within the region (Thomson et al. 2006). Xie et al. (2008) reported a cluster of GY-related QTLs on the long arm of chromosome 9. A total of seven QTLs for 1,000-grain weight, spikelets per panicle, grains per panicle, panicle length, spikelet density, heading date and PH were identified within the cluster.

Our study also demonstrated that backcross populations that minimize the effect of other background loci are highly suitable for fine mapping of large-effect QTLs for a complex trait such as GY. The development of backcross populations through MAS also ensures the presence of adequate recombination to generate lines with overlapping segments of the QTL. Such mapping resolution is needed to provide a clear picture on the location of the genes responsible for GY response. It also ensures the simultaneous development of near-isogenic lines free from any undesirable linkage drag with the QTL.

In the case of $qDTY_{9.1}$, the presence of a donor genome segment near RM24334 between the two sub-QTLs showed a negative effect on GY under both moderate and severe stress conditions (Fig. 5c). Fine mapping splits the original $qDTY_{9.1}$ region into two, $qDTY_{9.1A}$ and $qDTY_{9.1B}$, and this provides an opportunity for breeders to exclude the negative-effect RM24334 loci while introgressing the $qDTY_{9.1A}$ and $qDTY_{9.1B}$ regions into drought-susceptible varieties. The existence of such regions within conserved drought-responsive regions could account for the low GY potential of most of the traditional drought-tolerant donors/varieties and the low correlation between high GY

potential and high GY under drought (Kumar et al. 2008; Venuprasad et al. 2007). Earlier, a negative effect of the $qDTY_{3.1}$ region on GY under non-stress was reported (Venuprasad et al. 2009a). As with $qDTY_{2.1}$, a donor segment at RM262, a locus within the earlier identified QTL region (RM521-RM262), showed a negative effect on GY under stress conditions. Through fine mapping, the earlier identified region was reduced to RM3549–RM324, thereby successfully excluding the RM262 loci with a negative effect on GY. Our study successfully demonstrates that, through precise fine mapping and a careful study on the contribution of individual loci to GY and related traits, such negative-effect regions can be identified and excluded from marker-assisted introgression.

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