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ORIGINAL PAPER

A QTL for rice grain yield in aerobic environments with large effects in three genetic backgrounds

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Abstract A large-effect QTL associated with grain yield in aerobic environments was identified in three genetic backgrounds, Apo/^{2}*Swarna, Apo/^{2}*IR72, and Vandana/^{2}*IR72, using bulk-segregant analysis (BSA). Apo and Vandana are drought-tolerant aerobic-adapted varieties, while Swarna and IR72 are important lowland rice varieties grown on millions of hectares in Asia but perform poorly in aerobic conditions. Two closely linked rice microsatellite (RM) markers, RM510 and RM19367, located on chromosome 6, were found to be associated with yield under aerobic soil conditions in all three backgrounds. The QTL linked to this marker, *qDTY6.1* (DTY, grain yield under drought), was mapped to a 2.2 cM region between RM19367 and RM3805 at a peak LOD score of 32 in the Apo/^{2}*Swarna population. The effect of *qDTY6.1* was tested in a total of 20 hydrological environments over a period of five seasons and in five populations in the three genetic backgrounds. In the Apo/^{2}*Swarna population, *qDTY6.1* had a large effect on grain yield under favorable aerobic ($R^2 \leq 66\%$) and irrigated lowland ($R^2 \leq 39\%$) conditions but not under drought stress; Apo contributed the favorable allele in all the conditions where an effect was observed. In the Apo/IR72 cross, Apo contributed the favorable allele in almost all the aerobic environments in RIL and BC$_1$-derived populations. In the Vandana/IR72 RIL and BC$_1$-derived populations, *qDTY6.1* had a strong effect on yield in aerobic drought stress, aerobic non-stress, and irrigated lowland conditions; the Vandana allele was favorable in aerobic environments and the IR72 allele was favorable in irrigated lowland environments. We conclude that *qDTY6.1* is a large-effect QTL for rice grain yield under aerobic environments and could potentially be used in molecular breeding of rice for aerobic environments.

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Introduction

Rice is cultivated in both anaerobic (lowland) and aerobic (upland) environments. Aerobic cultivation includes both *aerobic rice*, rice grown in favorable upland environments, and *upland rice*, rice grown in unfavorable upland environments. Aerobic rice requires 50% or less of the amount of water used by lowland rice (Castañeda et al. 2002). In the coming years, due to water shortage, cultivation of aerobic rice is expected to expand; this trend has already been observed in Brazil, China, India, and Philippines (Bouman 2001; Lafitte et al. 2002; Wang and Tang 2002; Pinhiero et al. 2006). Aerobic rice is also potentially important in water-short lowland environments (Castañeda et al. 2002; Atlin et al. 2006). High-yielding rice varieties which are bred for the lowland ecosystem generally perform poorly in aerobic soils (Bouman 2001; Lafitte et al. 2002; Atlin et al. 2006; Matsuo et al. 2010). Thus, to reduce water use in rice production and to maintain rice productivity in water-short rice environments, there is an immediate need to accelerate the genetic improvement of rice for aerobic cultivation, which until recently has received little attention compared to lowland rice (Lafitte et al. 2002).

In aerobic rice cultivars, high yield potential and drought tolerance need to be combined (Atlin et al. 2006). As aerobic rice cultivation is most likely to be of interest to farmers in environments with limited or unreliable irrigation, moderate drought tolerance, particularly during the sensitive reproductive stage, is an important trait (Garrity and O'Toole 1994; Atlin et al. 2006). Breeding of high-yielding and drought-tolerant aerobic rice cultivars could be hastened with the identification and deployment through marker-assisted breeding of large-effect QTLs that are associated with both yield potential and grain yield under drought stressed aerobic conditions. To date no such QTLs have been reported in rice. Three previous studies have indicated the presence of large-effect QTLs associated with rice grain yield under drought stress (Bernier et al. 2007; Kumar et al. 2007; Venuprasad et al. 2009b). However, these QTLs were associated with stress tolerance rather than grain yield under aerobic management per se and either had no effect or had a negative effect on yield potential. In this study, we report the identification of a large-effect QTL (*qDTY6.1*) that is associated with grain yield in a broad range of environments varying in moisture conditions and thus has the potential to be used in aerobic rice breeding.

Materials and methods

Parental lines and mapping populations

Three large BC_1-derived recombinant inbred line (RIL) populations from the crosses Apo/^{2}*Swarna, Apo/^{2}*IR72 and Vandana/^{2}*IR72 were used in the study. Apo (IR55423-01) is an improved *indica* upland variety with high yield potential and moderate reproductive-stage drought tolerance while Vandana is a highly drought-tolerant upland-adapted line (Venuprasad et al. 2007, 2008). IR72 and Swarna are semi-dwarf high-yielding *indica* lines developed for irrigated and favorable rainfed lowland conditions, respectively. Swarna, in particular, is grown by farmers on over 6 million ha in India, Nepal, and Bangladesh and is considered to have excellent yield potential and quality but is poorly adapted to aerobic conditions, and is highly susceptible to drought damage (Venuprasad et al. 2009b; Verulkar et al. 2010). The Apo/^{2}*Swarna population consisted of 490 $BC_1F_{4:5}$ lines and was previously developed and evaluated to map large-effect QTL associated with lowland drought tolerance (Venuprasad et al. 2009b). The Apo/^{2}*IR72 population was developed by backcrossing IR78877-208-B-1-2, a high-yielding drought-tolerant line from the Apo/IR72 population, to IR72, and consisted of 490 $F_{2:3}$ lines. The Vandana/^{2}*IR72 mapping population consisted of 490 $F_{2:3}$ lines and was developed by backcrossing IR78910-34-B-2-2, a high-yielding drought-tolerant line from Vandana/IR72, to IR72.

Field trials

The study was conducted at the experiment station of the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines, in the dry (DS) and wet seasons (WS) of 2006, 2007, and 2008. IRRI is located at 14°13′N latitude, 121°15′E longitude, and at an elevation of about 21 m above mean sea level. The soil type is a Maahas clay loam, isohyperthermic mixed typic tropudalf. In our study, the term *aerobic* refers to field trials or nurseries conducted under direct-sown, non-puddled, non-flooded, and aerobic conditions in leveled fields; *lowland* refers to flooded, puddled, transplanted, and anaerobic conditions. Trials conducted in lowland irrigated conditions where no stress was imposed are referred to as non-stress trials. Aerobic trials in DS in which drought stress was artificially imposed during the reproductive stage and rainfed trials in WS, in which stress occurred naturally due to periods of low rainfall, are referred to as stress environments.

A subset of the Apo/^{2}*Swarna population (comprising 100–200 random lines) was evaluated in four trials: three under aerobic non-stress conditions (one during WS 2006 and two during DS 2008) and one under aerobic drought stress conditions (during DS 2007). In addition, the two aerobic non-stress experiments conducted during DS of 2006 and 2007 and the two lowland non-stress trials conducted during DS 2007 and 2008 by Venuprasad et al. (2009b) were also used in this study to study the QTL effects. The Apo/^{2}*IR72 population was evaluated under aerobic natural stress condition during the 2006 wet season (WS). The Vandana/^{2}*IR72 population was evaluated under aerobic drought stress during DS 2007. During WS 2008, a subset of the Vandana/^{2}*IR72 (consisting of 250 random lines) population was evaluated in two conditions: aerobic natural stress and aerobic non-stress.

All the trials were laid out as alpha lattice designs, with plot length of 2 m in aerobic trials and 5 m in lowland trials. In the lowland trials, spacing between rows was 0.20 m while in the aerobic trials spacing was 0.25 m. All the trials had two replications and single-row plots. Parental lines and check varieties were always included in the trials.

Management of field trials

In the aerobic trials, dry seed was direct-sown at a rate of 8 g m^{-2} into unpuddled, unflooded, leveled upland fields. In the aerobic non-stress trials conducted during the DS, for the first 4 weeks after sowing (WAS), trials were irrigated by overhead sprinklers once in 3 days for 2–3 h. From the fifth week until harvest, sprinkler irrigation was provided 2–3 times a week for 2 h to reach field capacity. Approximately 40 mm water was added each time and a total of about 800 mm water was added throughout the crop growth. In the Apo/^{2}*Swarna population evaluated in the aerobic trial during DS 2006 and in one of the two DS 2008 aerobic trials, surface irrigation (instead of sprinkler irrigation) was provided twice per week from germination until harvest.

In the aerobic stress trials during the DS, for the first four WAS, the trials were irrigated by overhead sprinklers for 2 h twice weekly. Drought stress was imposed from the tillering stage until maturity by reducing the frequency of irrigation to once a week until harvesting. Tensiometers were installed in the field to monitor soil water tension, allowing irrigation to be withheld until soil water tension reached −30 to −40 kPa at 20 cm soil depth. Repeated stress cycles were conducted to ensure that all entries experienced stress during flowering. In the WS trials, similar to the DS trials, sprinkler irrigation was provided once every 3 days for 2 h duration for the first 4 weeks after sowing (WAS). Once the crop established it was subsequently dependent on rainfall; whenever there was shortage of rainfall, supplemental irrigation by means of sprinklers was provided in the non-stress trials but not in the stress trials. Tensiometers were installed in the field and soil water status was monitored. The stress protocol used in this study is similar to the one regularly used by this group (Bernier et al. 2007; Venuprasad et al. 2007, 2008, 2009b, 2011).

In the lowland non-stress trials, seeds were sown in the nursery and 21-day-old seedlings were transplanted to the main field with a spacing of 10 cm between hills in a row. After transplanting, approximately 5 cm of standing water was maintained in the field until drainage before harvest.

Rainfall during crop growth was 223, 130, 355, and 860 mm during DS 2006, DS 2007, DS 2008, and WS 2008, respectively, while evaporation was 633, 880, 512, 600, and 498 mm, respectively. In WS 2006, a total of 1,060 mm was received, but due to typhoon ‘Milyeno’ 318 mm of rain was received on a single day (September 28th); as a result, the Apo/^{2}*Swarna population lodged and produced low yields due to grain shattering.

Phenotypic data collection and statistical analysis

In all the trials, days to flowering and grain yield were recorded. Days to flowering were recorded when the panicle was exserted in approximately 50% of the plants in a plot. Grain yield from each plot was harvested at physiological maturity, dried to a moisture content of about 14%, and weighed. Days to flowering were not recorded in the WS 2006 trial in the Apo/^{2}*Swarna population and in WS 2008 trials of Vandana/^{2}*IR72.

Statistical analysis was performed on individual trials using SAS v9.1.3 (SAS Institute Inc. 2004). For the estimation of means for lines, data were analyzed using the REML option of the SAS MIXED procedure where lines were treated as a fixed effect, and replications and blocks within replications as random. Variance components were estimated using the REML option of SAS PROC VARCOMP. Broad sense heritability (H) was estimated as:

$$H = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_E^2}{r}},$$

where σ_E^2 is the error variance, σ_G^2 the genetic variance, and r is the number of replications.

Genotyping and mapping

Leaf samples of all lines were collected from the first replication of the respective field experiments. Samples were freeze-dried and miniprep scale DNA extraction was done in deep-well-plates (Axygen Scientific, Union City, CA, USA) via a modified CTAB protocol using a Geno/Grinder model 2000 (SPEX SamplePrep, Metuchen, NJ, USA). The quantity and quality of DNA was checked on 0.8% agarose gels and concentration was adjusted to ~20 ng μL^{-1} by comparing with lambda (λ) DNA standards.

Ten SSR loci previously linked to grain yield under drought stress in the Apo/IR64 population (RM572, RM6703, RM71, RM3387, RM5686, RM520, RM510, RM256, RM269, and RM511; Venuprasad et al. 2009a), were genotyped in this study. Since RM510 was monomorphic between Vandana and IR72, the tightly linked polymorphic marker RM19367 was used in the Vandana/^{2}*IR72 population. PCR amplification was performed as described in Temnykh et al. (2001). PCR products were resolved on 8% non-denaturing polyacrylamide gels as described by Sambrook et al. (1989). SYBR® Safe DNA gel stain (Invitrogen, La Jolla, CA, USA) was used to stain the gels.

Bulk-segregant analysis (BSA)

In the Apo/^{2}*Swarna population from the aerobic trial of DS 2006, the 30 highest yielding lines (~6% of the total lines) and 30 lowest yielding lines were identified. Equal quantities of leaf tissue from each entry in each tail were bulked and DNA was extracted from the bulk. These two tail bulks were genotyped with the above-selected RM markers. Screening for markers showing polymorphism in the form of a clearly visible difference in band intensity between the high and low tail in this experiment was performed. Similarly, in the Apo/^{2}*IR72 and Vandana/^{2}*IR72 populations, the 20 highest yielding lines and 20 lowest yielding lines from the WS 2006 aerobic natural stress and the DS 2007 aerobic severe stress trials, respectively, were selected and BSA was performed.

Whole population genotyping

A marker locus RM510 (or its tightly linked marker RM19367) exhibited clearly differential band intensities in the contrasting tails in the initial BSA analysis in all the three populations (Supplementary Fig. S1). The whole Apo/^{2}*Swarna population consisting of 490 lines was genotyped for this marker. RILs were scored according to the parental banding pattern as Apo allele homozygotes, Swarna allele homozygotes, or heterozygotes. Whenever null alleles or non-parental bands were observed, they were treated as missing values. Similarly, the whole populations of Apo/^{2}*IR72 and Vandana/^{2}*IR72 comprising 490 lines each were genotyped for RM510 and RM19367, respectively.

To increase precision of the position and effect estimates, more markers were added and the whole Apo/^{2}*Swarna population was genotyped at a total of ten polymorphic marker loci located around, and including the RM510 locus on chromosome 6. The marker orders used to create the linkage map were assumed from the published rice genome RM marker orders (IRGSP 2005; http://www.gramene.org). One million bases on a rice chromosome is approximately equivalent to 4 cM (IRGSP 2005); using this relation the published physical distances between markers (http://www.gramene.org) were used to estimate approximate genetic distances (cM) between them.

QTL mapping

Mapping of QTL linked to RM510 marker identified as significant via BSA was done using phenotypic data for the DS 2006 aerobic trial as the size of effect of RM510 marker was largest in this trial. To map the QTL linked to RM510 (we refer to this QTL as *qDTY6.1*) on chromosome 6, the region between RM19288-RM549, covering ~23 cM and containing 10 RM marker loci, was explored. Composite interval mapping of QTL was performed using QGene version 4.0 software (Nelson 1997; http://www.qgene.org). The critical LOD-value to control the genome-wise type I error was calculated using 1,000 permutation tests. An experiment-wise significance level of $P < 0.01$ was maintained for QTL detection and the genome scan interval was kept at 5 cM.

Testing the effect of marker

The proportion of the genetic variance explained by the QTL (R^2) was estimated as the ratio between variance explained by RM510 (in Apo/^{2}*Swarna and Apo/^{2}*IR72 populations) or RM19367 (in Vandana/^{2}*IR72 population) marker to the total genetic variance for the trait. For the estimation of the means for different marker classes in a trial, data were analyzed using a model in which marker classes were considered fixed and lines within marker classes, replicates, and blocks within replicates as random, using the REML option of the SAS MIXED procedure. Heterogeneous lines derived from plants that were heterozygous at the locus in question were omitted from the single-marker analysis.

Evaluation of *qDTY6.1* effect in RIL populations

Previously, we had used two rice breeding populations, Apo/IR72 and Vandana/IR72, to evaluate the effectiveness of direct selection under severe artificially imposed drought stress for improving rice grain yield under natural and artificially imposed drought stress (Venuprasad et al. 2007, 2008). These two studies are explained briefly here. A common set of $F_{2:3}$ lines from the two populations were subjected to two cycles of selection (in DS 2003 and 2005) for yield under both non-stress conditions and under artificially imposed stress in a pedigree breeding program. In both the populations, the 40 highest yielding lines in aerobic stress conditions, and the 40 highest yielding lines in lowland non-stress conditions were selected. The four divergently selected sets (stress and non-stress-selected sets within the Apo/IR72, and Vandana/IR72 populations) were evaluated together in replicated trials under aerobic natural stress, aerobic moderate stress, and aerobic severe stress, and irrigated lowland conditions (during WS 2006–DS 2007). The phenotypic data from these previously conducted experiments (Venuprasad et al. 2008) were used to test the effect of QTL identified in the present study. The Apo/IR72 and Vandana/IR72 RIL populations consisting of 80 lines each were genotyped for the RM510 and RM19367 loci, respectively. The effects of marker on grain yield and other related traits were estimated similarly as explained above.

Results

Performance of parents and progeny

Broad sense heritability (*H*) of grain yield in the trials ranged from 0.58 to 0.71 (Supplementary Table S1). In all the aerobic trials, the tolerant parents, Apo and Vandana, consistently out-yielded the susceptible parents, Swarna and IR72. In the aerobic non-stress environments tolerant parents yielded between 387 and 520 g m^{-2}, while susceptible parents yielded between 45 and 147 g m^{-2}. In the aerobic severe stress trials, tolerant parents yielded between 74 and 88 g m^{-2}, while the susceptible parents yielded almost nothing. Average yield of progeny was from 207 to 295 g m^{-2} in aerobic non-stress conditions, while in severe stress it was from 12 to 36 g m^{-2}. Thus, under aerobic conditions, relative to non-stress conditions, progeny showed on average about a 67% reduction in yield in severe stress. During DS, average yield of progeny (BC_1-derived RIL population of Apo/^{2}*Swarna, and RIL populations of Apo/IR72 and Vandana/IR72; data not shown) under irrigated lowland and aerobic non-stress conditions was about 500 and 220 g m^{-2}, respectively; thus, relative to irrigated lowland conditions there was about a 56% yield reduction in irrigated aerobic conditions. Transgressive segregants were observed in all the populations; in the BC_1-derived populations about, 4% of the progeny lines yielded higher than their respective donor parent. Vandana was the earliest flowering parent followed by Apo, IR72 and Swarna; days to flowering of these lines under aerobic non-stress conditions were about 60, 80, 100 and 110, respectively (data not shown). Average days to flowering of the populations were intermediate to that of their two respective parents. Correlation between grain yield and days to flowering was strongly negative in aerobic conditions in all the populations and ranged between −0.49 and −0.69, while under irrigated lowland it was positive and ranged between 0.13 and 0.24 (data not shown).

In the severe stress environment the Apo/^{2}*Swarna population yielded poorly (<12 g m^{-2} trial mean; Supplementary Table S1) and only about 62% of the lines set seeds. In contrast, the two IR72 derived populations (Vandana/^{2}*IR72 and Apo/^{2}*IR72), which were evaluated using the same protocol in adjacent trials, produced higher grain yield (at least 33 g m^{-2}) and all the lines set seeds. The Apo/^{2}*Swarna population is known to do well in lowland drought stress environments (Venuprasad et al. 2009b) and also in aerobic non-stress environments (Table 1). In general, compared to fully irrigated lowland trials the aerobic non-stress trials may all be considered as mild stress environments as there is no standing water throughout the crop growth period and mild stress symptoms were observed on plants prior to irrigation, thus we conclude that the Apo/^{2}*Swarna population is highly susceptible to drought in aerobic environments and for this population we chose the DS 2006 aerobic non-stress trial as the stress environment for BSA and the fully irrigated lowland trials of DS 2007 and DS 2008 were considered non-stress environments.

Identification of markers linked to yield under aerobic drought stress trials via BSA

The marker RM510 was identified as having extreme differences in frequency between the high and low phenotypic tails in the Apo/^{2}*Swarna and Apo/^{2}*IR72 populations for yield under aerobic conditions during the DS and WS of 2006, respectively (Supplementary Fig. S1). At this locus, the Apo allele was favored in the high-yielding tail and the Swarna/or IR72 allele was predominant in the low-yielding tail. In the Vandana/^{2}*IR72 population, two markers, RM19367 (Supplementary Fig. S1; RM19367 is closely linked to RM510) and RM6703 (not shown), were found to be linked to drought tolerance via BSA. Thus, the region on chromosome 6 around RM510 was common to all three populations and further work and discussion is based only on this region.

Mapping *qDTY6.1*

The QTL for grain yield in Apo/^{2}*Swarna population inferred near RM510 in the DS 2006 aerobic trial was mapped on chromosome 6 (Fig. 1). The QTL peak was located in a 2.2 cM support interval flanked by RM19367 and RM3805 and at a distance of 0.3 cM from RM510. The mapped QTL (*qDTY6.1*) had an additive effect of 534 g m^{-2} in the DS 2006 aerobic trial and was highly significant (LOD = 32.3; threshold LOD at 1% significance level = 3.85).

Characterizing *qDTY6.1* effects on grain yield and days to flowering traits

To better understand the phenotypic effects of QTL *qDTY6.1*, single-marker analysis for yield and related traits was performed using RM510 (or RM19367; markers within the QTL region closest to peak) in the three backcross and two RIL populations. The effects were characterized in a total of 20 field experiments conducted over a period of five seasons (DS 2006–WS 2008) and included the original mapping experiments.

Apo × Swarna genetic background

The marker effect in the whole population was significant in the environment (aerobic trial during DS 2006), where BSA identified a large difference in band intensity, putatively reflecting differences in marker allele frequency, between the two tails (Table 1). Thus, it appears that BSA based on visual differentiation of band intensity between the high- and low-yielding tails comprising 12% of the total population was effective in identifying loci significantly associated with yield under aerobic conditions.

Table 1 Effect of RM510, marker nearest to *qDTY6.1* peak, in Apo × Swarna genetic background on mean grain yield and days to flowering in eight hydrological environments: IRRI DS 2006–DS 2008

Population	Season	Environment	No. of progeny lines	Grain yield (g m^{-2}; mean ± SE)				Days to flowering (mean ± SE)			
				Homozygotes for allele type		P (diff)	R^{2}[c]	Homozygotes for allele type		P (diff)	R^{2}
				Apo	Swarna			Apo	Swarna		
BC_1-derived RIL	DS 2006[a]	Aerobic	490	280.8 ± 23	159.6 ± 23	<0.0001	0.66	100.8 ± 2	110.3 ± 2	<0.0001	0.63
	WS 2006[b]	Aerobic	150	41.6 ± 3	27.8 ± 2	<0.0001	0.38	NA	NA	–	–
	DS 2007	Aerobic	488	218.1 ± 20	217.5 ± 20	NS	0.0	97.9 ± 1	97.9 ± 1	NS	0.00
	DS 2008[a]	Aerobic	96	333.0 ± 12	253.2 ± 12	<0.0001	0.50	90.4 ± 2	92.3 ± 2	NS	0.02
	DS 2008	Aerobic	96	255.3 ± 13	202.6 ± 13	0.002	0.24	92.6 ± 1	95.1 ± 1	NS	0.08
	DS 2007	Aerobic stress	193	11.2 ± 4	11.9 ± 3	NS	0.0	121.9 ± 4	124.7 ± 4	NS	0.02
	DS 2007	Lowland	193	596.3 ± 17	536.0 ± 14	0.002	0.26	94.7 ± 1	94.5 ± 0	NS	0.00
	DS 2008	Lowland	96	437.6 ± 13	379.8 ± 13	0.0009	0.40	100.7 ± 1	100.1 ± 1	NS	0.00

NS non-significant, *NA* not available

[a] Surface irrigated

[b] Typhoon affected hence low yields

[c] Genetic variance explained

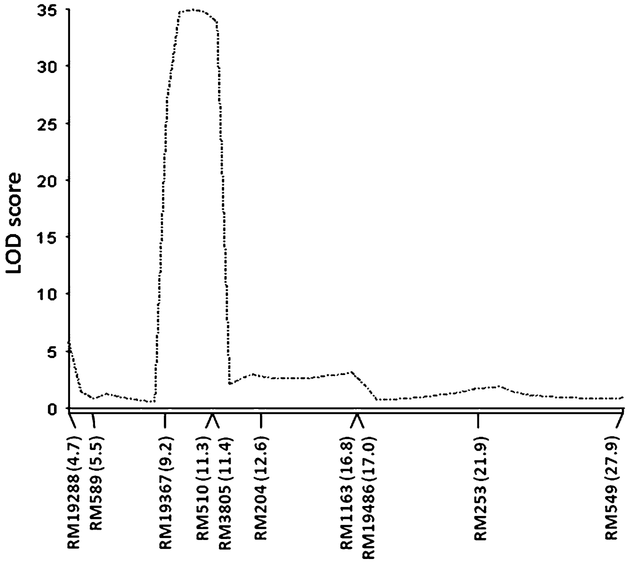

Fig. 1 LOD score curve denoting a QTL on chromosome 6 for grain yield (*qDTY6.1*) in the Apo/^{2}*Swarna population in DS 2006 aerobic trial. Marker names and their genetic positions in cM are indicated on *X*-axis (threshold LOD at 5% = 2.66, 1% = 3.85)

qDTY6.1 explained 66% of the genetic variation in grain yield in favorable dry season aerobic trials where surface irrigation was practiced; the yield advantage of Apo allele homozygotes over Swarna allele homozygotes was 76%. *qDTY6.1* had no effect in the much drier aerobic trial during DS 2007 where sprinkler irrigation was used (130 mm rainfall and 880 mm evaporation). In DS 2008 two aerobic trials were conducted side-by-side, in one trial surface irrigation was used while in the other sprinkler irrigation was used. *qDTY6.1* showed relatively small effect in the sprinkler-irrigated DS 2008 aerobic trial, explaining only 18% of the genetic variation in grain yield ($P < 0.01$), with Apo allele homozygotes out-yielding Swarna allele homozygotes by 19%. But it showed a large effect on grain yield in the surface-irrigated trial of DS 2008, explaining 52% of the genetic variance ($P < 0.0001$), with Apo allele homozygotes out-yielding Swarna allele homozygotes by over 34%. In the two lowland non-stress trials, *qDTY6.1* had a consistent and significant effect and explained 26–39% of genetic variation in grain yield; Apo homozygotes out-yielded Swarna homozygotes by 11–15%, respectively. Thus, across both aerobic and lowland environments, *qDTY6.1* appears to affect yield in favorable environments, with effects being higher under aerobic conditions. This is the first QTL known to us which has large and positive effect on grain yield under both aerobic and lowland irrigated conditions.

qDTY6.1 significantly affected days to flowering in the more favorable aerobic environments, with Apo allele homozygotes flowering about 6 days earlier, on average, than the Swarna allele homozygotes. No significant effect on flowering was seen in lowland conditions (Table 1).

Apo × IR72 genetic background

In the $BC_1F_{2:3}$ population, in the severely stressed aerobic rainfed trial, *qDTY6.1* showed a large effect on yield and days to flowering, explaining 63 and 56% of the genetic variance for the traits, respectively (Table 2). The Apo allele homozygotes significantly out-yielded the IR72 allele homozygotes by over 60% and also flowered about 9 days earlier.

In the RIL population in all the environments tested except under lowland non-stress conditions, the Apo allele homozygotes significantly out-yielded IR72 allele homozygotes on average by 59%; the effect was stronger in severe aerobic stress environments (R^2 up to 55%; Table 2). The Apo allele was significantly associated with early flowering in aerobic and lowland conditions; on average, Apo allele homozygotes flowered early by about 5 days.

Vandana × IR72 genetic background

In the $BC_1F_{2:3}$ population, *qDTY6.1* showed strong effects on yield in both aerobic stress and non-stress trials (Table 3). In both the managed stress trial in DS 2007 and the rainfed stress trial in WS 2008 it explained 40% of the genetic phenotypic variance in grain yield; Vandana allele homozygotes significantly out-yielded IR72 allele homozygotes by 75 and 40% in severe stress (DS 2007) and moderate stress (WS 2008), respectively. In the DS 2007 severe stress trial, a significant effect of this QTL was also seen on days to flowering; Vandana allele homozygotes flowered 8 days earlier than the IR72 allele homozygotes.

In the RIL population, at least in the two aerobic stress environments, Vandana allele homozygotes on average yielded about 25% more than the IR72 allele homozygotes, but the effects were significant only in the severely stressed rainfed aerobic trial of WS 2006 (genetic variance explained was 26%; Table 3). However, in the irrigated lowland conditions IR72 allele homozygotes significantly out-yielded Vandana allele homozygotes by about 16%. In all the above three trials the Vandana allele was significantly associated with early flowering (by ~4.4 days).

Discussion

Previously, Bernier et al. (2007) reported the identification of *qtl12.1*, a large-effect QTL for yield under aerobic, severe drought stress environments, using a selective genotyping approach. In the case of *qtl12.1*, the susceptible parent contributed the positive allele. Here, we have employed a bulked-segregant analysis (BSA) strategy to identify *qDTY6.1*, a large-effect QTL for grain yield under severe (in the Apo/^{2}*IR72 and Vandana/^{2}*IR72 populations) and mild drought stress (in the Apo/^{2}*Swarna population) under aerobic conditions. *qDTY6.1* was mapped to a 2.2 cM interval on chromosome 6 in the Apo/^{2}*Swarna population (Fig. 1) and, in the three populations used, this QTL explained 40–66% of the genetic variation observed for grain yield in aerobic environments (Tables 1, 2, 3). BSA has also been effectively used to identify a large-effect QTL associated with yield under lowland drought stress (Venuprasad et al. 2009b) and, more recently, Salunkhe et al. (2011) showed that BSA could be successfully applied to detect QTL linked to drought tolerance traits in rice (e.g., leaf rolling and drying). Clearly, BSA can be used to identify loci that are linked to grain yield under aerobic drought conditions in rice at a considerable saving in genotyping effort and cost, thus allowing resources to be focused on precise localization of QTL with large effects. As outlined by Venuprasad et al. (2009b), success of BSA in this study could be due to the use of large populations (>400 lines) derived from parents that

Table 2 Effect of RM510, marker nearest to *qDTY6.1* peak, in Apo × IR72 genetic background on mean grain yield and days to flowering in five hydrological environments: IRRI WS 2006–DS 2008

Population	Season	Environment	No. of progeny lines	Grain yield (g m^{-2}; mean ± SE)				Days to flowering (mean ± SE)			
				Homozygotes for allele type		*P* (diff)	R^{2b}	Homozygotes for allele type		*P* (diff)	R^2
				Apo	IR72			Apo	IR72		
BC_1-derived RIL	WS 2006	Aerobic stress	490	42.4 ± 1	26.4 ± 1	<0.0001	0.63	83.5 ± 1	92.29 ± 1	<0.0001	0.56
RIL	WS 2006^a	Aerobic stress	80	73.2 ± 9	43.5 ± 9	<0.0001	0.55	87.6 ± 1	93.78 ± 1	<0.0001	0.53
	DS 2007	Aerobic stress	80	51.8 ± 11	23.9 ± 12	0.004	0.26	93.2 ± 1	99.36 ± 1	0.0008	0.28
	DS 2007	Aerobic	80	241.9 ± 22	185.2 ± 24	0.009	0.28	82.9 ± 1	88.43 ± 1	<0.0001	0.47
	DS 2007	Lowland	80	501.1 ± 32	514.7 ± 34	NS	0.00	80.3 ± 1	82.48 ± 1	0.02	0.14

NS non-significant

[a] Rain-fed trial

[b] Genetic variance explained

Table 3 Effect of RM19367, marker nearest to *qDTY6.1* peak, in Vandana × IR72 genetic background on mean grain yield and days to flowering in seven hydrological environments: IRRI WS 2006–WS 2008

Population	Season	Environment	No. of progeny lines	Grain yield ($g\ m^{-2}$; mean ± SE)				Days to flowering (mean ± SE)			
				Homozygotes for allele type		*P* (diff)	R^{2b}	Homozygotes for allele type		*P* (diff)	R^2
				Vandana	IR72			Vandana	IR72		
BC_1-derived RIL	DS 2007	Aerobic stress	490	41.4 ± 4	23.6 ± 4	<0.0001	0.40	92.9 ± 1	100.6 ± 1	<0.0001	0.55
	WS 2008	Aerobic stress	244	140.1 ± 5	100.2 ± 6	<0.0001	0.40	NA	NA	NA	NA
	WS 2008	Aerobic	244	219.4 ± 7	194.7 ± 8	0.0004	0.26	NA	NA	NA	NA
RIL	WS 2006[a]	Aerobic stress	80	61.2 ± 5	46.8 ± 5	0.02	0.26	84.4 ± 1	89.2 ± 2	0.02	0.14
	DS 2007	Aerobic stress	80	66.9 ± 11	55.4 ± 11	NS	0.05	83.2 ± 2	89.2 ± 2	0.01	0.17
	DS 2007	Aerobic	80	204.1 ± 23	198.3 ± 25	NS	0.00	75.4 ± 1	78.6 ± 1	Ns	0.06
	DS 2007	Lowland	80	458.7 ± 26	530.8 ± 27	0.01	0.26	74.9 ± 1	77.4 ± 1	0.05	0.10

NS non-significant, *NA* not available

[a] Rain-fed trial

[b] Genetic variance explained

greatly differed for yield under aerobic conditions and precision of the stress trial as measured by the broad-sense heritability (*H*) of grain yield.

Testing the effects of the identified QTL in a total of eight hydrological environments over a period of 3 years in the Apo/^{2}*Swarna population revealed that the Apo allele at *qDTY6.1* is effective mainly in favorable aerobic (surface irrigated) environment, but its effect is reduced with increasing stress (in sprinkler-irrigated trials; Table 1). It also has a large effect in irrigated lowland environments but has no effect in lowland drought stress conditions (data not shown). Introgressing Apo alleles into the Swarna background at this locus should result in enhanced yield in aerobic and lowland non-stress environments. To our knowledge, this is the first large-effect QTL reported that positively affects both rice yield potential, and yield under aerobic conditions. In the Apo and IR72 backgrounds, the effect of the QTL was studied using two populations, RIL and $BC_1F_{2:3}$. In both, the Apo (aerobic-adapted parent) allele was significantly associated with higher yield under aerobic stress and non-stress conditions (Table 2), while in irrigated lowland conditions the Apo and IR72 alleles were associated with similar yields. In the Vandana and IR72 genetic background, the Vandana (aerobic-adapted parent) allele was associated with higher yield in aerobic stress and non-stress environments, the effect being larger in stress environments and significant in four out of six aerobic trials. The IR72 (lowland adapted parent) allele was associated with higher yield in lowland irrigated conditions, indicating that different alleles are effective in different hydrological environments. Thus, it appears that in this QTL region the Apo allele has an advantage over the Vandana allele as it is not associated with a reduction in yield potential. The *qDTY6.1* region reported here has also been found to affect yield under stress in at least three other populations: Vandana/Way Rarem (Bernier et al. 2007), Apo/IR64 (Venuprasad et al. 2009a) and Vandana/IR64 (Venuprasad 2004). Thus, this QTL seems to influence grain yield under aerobic and/or drought conditions in several genetic backgrounds.

qDTY6.1 seems to have a significant effect on flowering under lowland non-stress conditions (except in the Apo/^{2}*Swarna population), aerobic non-stress environments (in Apo and IR72 genetic background), and aerobic stress environments (in the two IR72-derived populations) (Tables 1, 2, 3). Overall, the drought-tolerant parent alleles (Apo and Vandana) at this locus exhibited a tendency to reduce days to flowering under aerobic conditions. In close proximity to *qDTY6.1*, a major QTL affecting heading date, HD3A (located at a distance of 2.939 Mb from top; RM510 is located at 2.831 Mb), has been reported (Yano et al. 2001; http://www.gramene.org). Thus, the observed effect of *qDTY6.1* on flowering could be due to linkage of *qDTY6.1* with HD3A or alternatively could be due to a pleiotropic effect of HD3A itself. Genes affecting both abiotic stress tolerance and flowering are known in plants (Kim et al. 2004; Masle et al. 2005). Further, the two large-effect QTLs previously identified for rice yield under drought stress were known to affect flowering but they were not associated with any previously known HD genes

(Bernier et al. 2007; Venuprasad et al. 2009b). Hence, further studies are needed to ascertain the relationship between *qDTY6.1* and HD3A.

qDTY6.1 was shown to work in several genetic backgrounds and, importantly for rice production in South and Southeast Asia, in the background of the rice mega-varieties Swarna, IR72, and IR64. Introgression of *qDTY6.1* into these mega-varieties via marker-aided backcrossing is expected to improve both aerobic adaptation and yield potential of these varieties. However, caution has to be exercised as alleles from some donors could reduce yield potential (as seen with the Vandana allele in this study). Swarna is a highly popular variety grown over large areas in Asia due to its high yield potential and quality, and therefore any improvements in aerobic adaptation of Swarna could benefit rainfed-rice farmers. Further, introgression of *qDTY6.1* is expected to reduce the heading duration of these mega-varieties by up to 9 days in aerobic conditions. This is an added advantage as any reduction in duration could reduce risk due to late-season drought or early monsoon withdrawal. Short duration has been recognized as a critical trait for water savings in aerobic systems (Atlin and Lafitte 2002). Detailed study of the identified region to characterize its genetic and physiological effects is needed, and may make it possible to exploit this QTL through marker-assisted breeding to improve rice grain yield under aerobic environments. It has to be noted that though lines homozygous for the Apo allele significantly out-yielded the lines homozygous for the Swarna allele, rarely does their mean yield out-yield the donor parent Apo. This suggests that Apo possibly has additional QTL affecting aerobic rice yield and the same is reported earlier (Venuprasad et al. 2009a). Further efforts are therefore needed to characterize QTLs that could be combined with *qDTY6.1* to further increase the productivity of these mega-varieties under aerobic conditions. In tropics, aerobic rice yields are considerably lower than that of flood irrigated lowland rice; in this study, about 50% mean yield reduction was observed while others have reported up to 32% reduction (Bouman et al. 2005; Peng et al. 2006). Understanding the molecular mechanism of action of *qDTY6.1* may increase our understanding of plant adaptation to aerobic versus anaerobic conditions.

We conclude that QTL *qDTY6.1* is associated with a favorable combination of high yield potential, high harvest index (data not shown), shorter maturity, and tolerance to moderate levels of water stress, all of which are important traits in aerobic systems (Atlin and Lafitte 2002) and thus will be useful in breeding aerobic rice cultivars.

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