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ORIGINAL PAPER

Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis

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Abstract An $F_{4:5}$ population of 490 recombinant inbred lines (RILs) from the cross Apo/^{2*}Swarna was used to detect quantitative trait loci (QTL) with large effects on grain yield under drought stress using bulk-segregant analysis (BSA). Swarna is an important rainfed lowland rice variety grown on millions of hectares in Asia, but is highly susceptible to drought and aerobic soil conditions. Apo is an aerobic-adapted variety with moderate tolerance to drought. Two rice microsatellite (RM) markers, RM324, and RM416, located on chromosomes 2 and 3, respectively, were shown via BSA to be strongly associated with yield under lowland drought stress. The effects of these QTL were tested in a total of eight hydrological environments over a period of 3 years. The QTL linked to RM416 ($DTY_{3.1}$) had a large effect on grain yield under severe lowland drought stress, explaining about 31% of genetic variance for the trait ($P < 0.0001$). It also explained considerable variance for yield under mild stress in lowland conditions and aerobic environments. To our knowledge this is the first reported QTL that has a large effect on yield in both lowland drought and aerobic environments. The QTL linked to RM324 ($DTY_{2.1}$) had a highly significant effect on grain yield in lowland drought stress ($R^2 =$ 13–16%) and in two aerobic trials. The effect of these QTL on grain yield was verified to be not mainly due to phenology differences. Effects of $DTY_{3.1}$ on yield under stress have been observed in several other rice mapping populations studied at IRRI. Results of this study indicate that BSA is an effective method of identifying QTL alleles with large effects on rice yield under severe drought stress. The

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Apo alleles for these large-effect QTL for grain yield under drought and aerobic conditions may be immediately exploited in marker-assisted-breeding to improve the drought tolerance of Swarna.

Introduction

Drought is a serious problem in rainfed rice, which occupies 50% of total rice area in the world. In Asia alone, about 34 million ha of rainfed lowland and 8 million ha of upland rice (Huke and Huke 1997) are subject to frequent drought stress. Breeding varieties suited to these conditions is an important element in reducing risk and increasing productivity in drought-prone environments, but progress in breeding for drought tolerance in rice has been slow. Given the present global food crisis, improving rice yields in these environments is an important task. Drought tolerance is considered a complex trait. Lack of effective selection criteria for traits related to drought tolerance and low heritability of grain yield under stress are cited as major reasons for slow progress in breeding (Ouk et al. 2006). However, recent reports indicate that in well-managed trials, the heritability of grain yield under drought stress is comparable to that under non-stress conditions, and that direct selection for grain yield under stress is effective (Kumar et al. 2008; Venuprasad et al. 2007, 2008). Another reason for slow progress in breeding has been the failure to identify quantitative trait loci (QTL) with large and consistent effects that could be used for marker-assisted breeding (MAB). Considerable research effort has been devoted, primarily in the widely used populations CT9993/IR62266 and IR64/Azucena, to the mapping of QTL for secondary drought-related traits such as root morphology and osmotic adjustment, but few loci with large effects on either of these traits have been identified (e.g. Babu et al. 2003; Hemamalini et al. 2000; Kamoshita et al. 2002; Tripathy et al. 2000; Yadav et al. 1997; Zhang et al. 2001; Zheng et al. 2000). Progress in mapping QTL for secondary traits associated with drought tolerance is reviewed elsewhere (e.g. Bernier et al. 2008; Price and Courtois 1999; Price et al. 2002), but marker-assisted selection for such QTL has not been successfully used to improve yield under drought stress in rice. A limited number of studies aimed at mapping QTL directly associated with grain yield have been undertaken (Mackill 2003). Two recent reports indicate that QTL with large effects on yield under drought stress may not be uncommon. Bernier et al. (2007) reported a QTL on chromosome 12 in the Vandana/Way Rarem population explaining about 51% of the genetic variance for yield under severe upland drought stress over 2 years. Kumar et al. (2007) reported a major QTL for grain yield under lowland drought stress in the CT9993/IR62266 population on chromosome 1 explaining 32% of the genetic variance for the trait over 2 years. Given these results, it seems worthwhile to expand the effort devoted to identifying donors of alleles with large effects on grain yield under drought stress. Such alleles will be useful in improving understanding of the genetic and physiological basis for differences among rice varieties in performance under drought stress, and may also be useful in MAB. Methods are needed that could permit more potential donors to be screened for the presence of large-effect alleles at reasonable cost, in contrast to research efforts to date that have mainly focused on the intensive study of a few populations.

In expanding efforts to screen germplasm for alleles with large effects on yield under stress, the cost of genotyping is a serious impediment, particularly if conventional QTL analysis [i.e., phenotyping and genome-wide scan of all individuals in a large recombinant inbred lines (RIL) population] is used. Hence, alternate strategies based on selective genotyping (Lander and Botstein 1989; Navabi et al. 2009) warrant testing. Selectively genotyping the phenotypic tails of a population under selection can detect marker–QTL linkage with only a fraction of the genotyping effort required for conventional trait-based QTL analysis, and has been shown to have sufficient power to detect QTL explaining 15% or more of the phenotypic variance for population sizes and broad-sense heritabilities characteristic of cereal breeding programs (Navabi et al. 2009). Bulk-segregant analysis (BSA; Michelmoore et al. 1991) is a particularly efficient form of selective genotyping wherein DNA samples of extreme individuals from each tail of a phenotypic distribution for a given trait are pooled and the two resultant bulks are genotyped. Markers linked to a QTL affecting the trait are expected to be present at different frequencies in the contrasting tails, resulting in polymorphic expression of genotype signals (e.g. bands on an electrophoresis gel) between the two bulks. BSA has been widely used for the genetic analysis of qualitative traits such as disease resistance (e.g. Shen et al. 2003), but not for quantitative traits such as grain yield. However, recently Shashidhar et al. (2005) reported use of BSA to identify markers linked to grain yield in rice. Quarrie et al. (1999) have used BSA to identify QTL for grain yield under drought in maize.

The objective of the present study was to determine if BSA could be used to detect linkage between markers and QTL affecting rice grain yield under drought stress. The population used was a large set of BC_1-derived recombinant inbred lines (RIL) from a cross between the widely grown rainfed lowland cultivar Swarna as recurrent parent and the drought-tolerant, aerobic-adapted cultivar Apo as donor. Apo (IR55423-01) is an improved *indica* upland variety with high-yield potential under aerobic soil conditions (George et al. 2002) and moderate reproductive-stage

drought tolerance (Venuprasad et al. 2007). Swarna, a semi-dwarf high yielding *indica* line, was developed for irrigated and favorable rainfed lowland conditions and is grown by farmers on over 6 million ha in India, Nepal, and Bangladesh. Swarna is considered to have excellent yield potential and quality, but has very poor drought tolerance, and is poorly adapted to direct seeding and aerobic soil conditions, which are often encountered in upper-toposequence fields in rainfed production environments. For example, in 2002, drought stress in eastern India affected 55% of the country's area and 300 million people (Pandey et al. 2007) and many farmers were not able to harvest any grain from drought-affected fields where Swarna was planted. Improving the drought tolerance and aerobic adaptation of Swarna would therefore be of significant benefit to farmers in rainfed environments in South Asia. A second objective of the study was therefore to identify QTL from the drought tolerant and aerobic-adapted donor that may be useful in improving drought tolerance and aerobic adaptation in Swarna and other related lowland-adapted varieties.

Materials and methods

The study was conducted at the experiment station of the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines, in the dry seasons (DS) of 2006, 2007, and 2008 and the wet season (WS) of 2007. 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.

Definition of aerobic, lowland, stress and non-stress environments

In this article, the term *aerobic* refers to field trials or nurseries conducted under direct-sown, non-puddled, non-flooded, and aerobic conditions in leveled upland 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*, and DS trials in which drought stress was artificially imposed during the reproductive stage are referred to as *stress trials*. Compared to lowland non-stress trials, in all the aerobic trials mild drought stress prevailed throughout the growing season.

Phenotyping in stress and non-stress environments in 2006, 2007 and 2008

A $BC_1F_{4:5}$ population comprising of 490 lines from the Apo/^{2}*Swarna cross was used for the study. To develop the population, Apo and Swarna were crossed and the resultant F_1 was backcrossed to Swarna to produced BC_1F_1s. The BC_1F_1s were selfed and bulked to obtain BC_1F_2 and this procedure of selfing and bulking was continued until BC_1F_4s were obtained. From these BC_1F_4s 500 random $BC_1F_{4:5}$ lines were obtained and 490 of these were used in this study. During DS 2006, in separate experiments, the entire population was evaluated under aerobic and lowland drought stress conditions. These two experiments were repeated during DS 2007 and in addition, a lowland non-stress trial was also conducted using a subset of 200 random lines. In the WS of 2007 and DS 2008, a subset of 100 random lines was evaluated under aerobic conditions and in lowland non-stress conditions, respectively.

All the trials were laid out as alpha lattice designs, with plot length of 5 m in lowland trials and 2 m in aerobic trials. In the lowland trials, spacing between rows was 0.20 m while in aerobic trials spacing was 0.25 m. Two replications were used in all trials except WS 2007 when three replications were used. The number of rows per plot was one in trials conducted during DS 2006 and DS 2007, while those conducted in WS 2007 and DS 2008 consisted of two-row plots.

Management of lowland non-stress trials

In all the lowland non-stress trials, seeds were sown in the nursery and 21-day-old seedlings were transplanted to the main field. One seedling was transplanted per hill at a spacing of 10 cm between hills in a row, except in DS 2007, when two seedlings per hill were transplanted. After transplanting, approximately 5 cm of standing water was maintained in the field until drainage before harvest. Inorganic NPK fertilizer was applied at the rate of 90–30–30 kg ha^{-1}.

Weeds were controlled by application of post-emergence herbicide Sofit (pretilachlor ± safener, 0.3 kg a.i ha^{-1}) 4 days after transplanting (DAT) and by hand weeding. To control stem-borer and other insects, Furadan (carbofuran, 1 kg a.i ha^{-1}) was applied at 5 DAT, followed by Cymbush (cypermethrin, 1 l ha^{-1}) ± Dimotrin (cartap hydrochloride, 0.25 kg a.i ha^{-1}) at 16 DAT. Bayluscide (niclosamide, 0.25 kg a.i ha^{-1}), a molluscicide, was applied to control snails.

Management of lowland stress trials

Initial field establishment and management practices for lowland stress trials in DS 2006 and DS 2007 were similar to the lowland non-stress trials described above. Drought stress was imposed during the reproductive stage by draining water from the paddy at 30 DAT and withholding irrigation until the soil moisture tension reached –70 kPa at

20 cm depth. Severe leaf rolling and firing were observed at this soil moisture level. Fields were then re-irrigated by flash flooding, and drained again after approximately 24 h. This cycle was repeated until harvest. Severe leaf rolling and firing was observed during each stress period. This stress imposition protocol has been developed and used routinely at IRRI (for example, Bernier et al. 2007; Venuprasad et al. 2007, 2008).

Management of aerobic trials

In the aerobic trials, dry seed was direct-sown at a rate of 8 g m^{-2} into unpuddled, unflooded, leveled upland fields. Inorganic P, K, and $ZnSO_4$ fertilizers were each applied at the rate of 40 kg ha^{-1} as a basal dose. N was applied in three splits at the rate of 30–40 kg ha^{-1} per split. Weeds were controlled by application of the pre-emergence herbicide Ronstar (oxadiazon, 0.5 kg a.i ha^{-1}) 5 days after sowing (DAS), and at later stages by hand weeding. Insecticides Furadan (carbofuran, 1 kg a.i ha^{-1}) and/or Cymbush (cypermethrin, 1 l ha^{-1}) ± Dimotrin (cartap hydrochloride, 0.25 kg a.i ha^{-1}) were used to control insect pests when necessary.

In the DS 2006 trial, surface irrigation was applied twice per week from germination until harvest. In DS 2007, and WS 2007 trial, 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 two to three 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. Tensiometers were installed in the field and soil water status was monitored. All aerobic trials were irrigated to the field capacity throughout the experiment.

Rainfall during crop growth was 223, 130, 940, and 355 mm during DS 2006, DS 2007, WS 2007 and DS 2008, respectively, while evaporation was 633, 880, 512, and 600 mm, respectively.

Verifying the effect of QTL on phenology and grain yield under lowland stress in a staggered-planting trial during DS 2008

From the original population of 490 lines a set of 40 lines was chosen for evaluation in a staggered-planting trial. Lines were chosen at random, except for the exclusion of lines with extreme flowering dates and heterozygotes at $DTY_{2.1}$ (DTY, Grain Yield under Drought) and $DTY_{3.1}$, the two QTL affecting yield under lowland drought stress detected in this study. This set consisted of 11 and 14 lines that had Apo and Swarna alleles, respectively at both loci while 6 lines had Apo allele at $DTY_{2.1}$ and Swarna allele at $DTY_{3.1}$, and remaining 9 lines had Swarna allele at $DTY_{2.1}$ and Apo allele at $DTY_{3.1}$. These 40 lines, along with parents and a drought-tolerant check (IR77298-14-1-2-B-10), were evaluated in DS 2008 under lowland stress and non-stress conditions in two-row plots. Management of these experiments was similar to the lowland trials described above ("Materials and methods"; Supplementary Fig. 1).

The non-stress experiment consisted of two replications. In the stress experiment, the complete set of lines was seeded three times at about weekly intervals. Planting 1, which was seeded on 12 December 2007, consisted of three replications. Plantings 2 and 3 consisted of two replications each and were seeded on 20 and 26 December, respectively. The seedlings were planted in the main field when they were 23–27 days old. Plantings 1, 2, and 3 were adjacent to each other in a single field. After transplanting, approximately 5 cm of standing water was maintained in the field. On 15 February, 6 weeks after planting the first set, water was drained and the field was kept dry. Thus, at the time of draining, plantings 1, 2 and 3 were 65, 57, and 51 days old, respectively. In about a month after field drainage, plants were experiencing severe drought stress as observed from leaf drying; tensiometer readings at 20 cm soil depth reached about −70 kPa and the ground water level was more than 80 cm below the surface. The field was irrigated only once following the imposition of stress, on 28 March 28, by flash flooding and draining after a few hours. All the entries flowered between 2 March and 25 April, during which period a total of 50 mm rainfall was received while the pan evaporation was about 290 mm and the ground water depth was, on average, below 70 cm.

Phenotypic data collection

In all the trials, days to flowering, plant height at maturity, and grain yield were recorded. Days to flowering was recorded when the panicle was exerted 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. In the staggered planting trial during DS 2008, biomass was also harvested from one square meter area for estimation of harvest index. In none of the trials data were collected from lines with poor stand; plots with less than 70% stand were treated as missing.

Genotyping

All molecular marker work was conducted in the Gene Array and Molecular Marker Analysis (GAMMA) Lab, Plant Breeding, Genetics and Biotechnology (PBGB) division, IRRI. Leaf samples of all lines were collected from the first replication in the aerobic experiment of DS

2006. Samples were freeze-dried in a lyophilizer. Miniprep scale DNA extraction was done in deep-well-plates (Axygen scientific, California, USA) via a modified CTAB protocol using a GENO grinder. The quantity and quality of DNA was checked on 0.8% agarose gels and concentration was adjusted to $\sim 20\ \eta g\ \mu l^{-1}$ by comparing with lambda (λ) DNA standards.

From the rice microsatellite (RM) markers (ResGen, Invitrogen Corporation, Huntsville) described in Temnykh et al. (2001), a set of 293 RM markers were selected based on their mapped locations with an average distance of 6 cM between two consecutive markers. PCR amplification was performed as described in Temnykh et al. (2001). PCR products were resolved on 8% non-denaturing polyacrylamide (PAGE) gels as described by Sambrook et al. (1989).

Bulk segregant analysis

From the lowland stress trial of DS 2006, the 20 highest yielding lines ($\sim$4% of the total lines) and 20 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 293 selected RM markers. Markers showing polymorphism in the form of a clearly visible difference in band intensity between the high and low tail in these experiments were selected for genotyping the whole population.

Whole population genotyping

Two marker loci, *RM324* on chromosome 2 and *RM416* on chromosome 3, exhibited clearly differential band intensities in the contrasting tails in the initial BSA analysis in the lowland drought stress phenotyping trials conducted in DS 2006 (Supplementary Fig. 2). The whole population of 490 lines was genotyped for these two markers. RILs were scored according to the parental banding pattern as Apo homozygotes, Swarna homozygotes, or heterozygotes. Whenever null alleles or non-parental bands were observed, they were treated as missing values. To increase precision of the position and effect estimates, more markers were added and the whole population was genotyped at a total of four and five marker loci on chromosomes 2 and 3, respectively. The marker orders used to create the linkage map were assumed from the published rice genome RM marker orders (IRGSP 2005; Temnykh et al. 2001). 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 between them. For markers RM327 and RM262 no physical distances are reported thus for these two the genetic distances reported in Temnykh et al. (2001) were assumed.

Mapping the QTL linked to RM324 and RM416

Mapping of QTL linked to each marker identified as significant via BSA was done using phenotypic data for the trial in which the effect size of the marker was largest. QTL mapping was performed using QTL Network software (Yang et al. 2008), which is based on a mapping methodology outlined by Yang et al. (2007). Briefly, this method analyzes the marker intervals, selects the candidate intervals and uses them as cofactors in a one-dimensional genome scan for putative QTL. The mapping procedures are performed in the framework of a mixed linear model, and an F-statistic based on Henderson method III is used for hypothesis tests. A total of 1,000 permutation tests were used to calculate the critical F-value to control the genome-wise type I error. An experiment-wise significance level of $P < 0.01$ was maintained for QTL detection. For the genome scan, the window size and walk-speed were fixed as 1 and 0.1 cM, respectively. For mapping QTL linked to RM324 ($DTY_{2.1}$) grain yield data from the DS 2006 lowland drought trial were used and the region between RM markers RM327–RM262, spanning 25 cM and containing four RM markers, was scanned. Similarly, to map the QTL linked to RM416 ($DTY_{3.1}$) on chromosome 3, grain yield data from the DS 2006 lowland drought trial were used and the region between RM markers RM15780–RM16030, spanning 16 cM, containing 5 RM marker loci, was scanned.

Statistical analysis

Statistical analysis was performed on individual trials using SAS v9.1.3 (SAS Institute Inc 2004). For the estimation of mean values 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 for a completely random model 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 is the genetic variance, and r the number of replications.

To test the effect of loci on grain yield and related traits, marker loci closest to the peak of the QTL were considered, i.e. RM521 for $DTY_{2.1}$, and RM520 for $DTY_{3.1}$. The

proportion of the genetic variance explained by the QTL (R_G^2) was estimated as the ratio of variance explained by this marker to the total genetic variance for the trait. For the estimation of the mean values 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 BC_1F_4 plants that were heterozygous at the locus in question were omitted from the single-marker analysis.

To remove the effect of differences in days to flowering and then to estimate the mean grain yield for marker classes, in the above model days to flowering in the lowland stress screening environment was introduced as a covariate and the analysis was repeated. In a separate analysis, least squares means for grain yield and days to flowering were obtained on all lines and the population was stratified by dividing it into four subsets based on days to flowering, with sets consisting of lines flowering one or two standard deviations earlier or later than the population mean. Within each of these sets, the grain yields of lines of the two homozygous classes for each marker were compared by *t* test using the PROC GLM procedure. In the staggered planting trial of DS 2008, mean values of marker classes were estimated separately for each of the three stressed planting dates and for the non-stress control.

Results

Performance of parents and progeny

Broad sense heritability (*H*) of grain yield and mean performance of the parents Apo and Swarna and the progenies are presented in Table 1. *H* for grain yield in the trials ranged from 0.55 to 0.74. Yields ranged from 516 and 300 g m^{-2} under non-stress lowland conditions in DS 2007 to 73 and 8 g m^{-2} under severe lowland drought stress in DS 2006 for Apo and Swarna, respectively. In all trials, Apo consistently out-yielded Swarna. Under lowland conditions, Apo out-yielded Swarna 1.7-, 2.5- and 9-fold in non-stress, moderate stress and severe stress trials, respectively. Under aerobic conditions, Apo out-yielded Swarna by 2.5- to 3.6-fold. The yield difference between the cultivars was more evident under increasingly severe stress. It is clear that Apo has a favorable combination of high-yield potential and drought tolerance.

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

Two markers, RM324 and RM416, out of the 293 markers tested were identified by visual observation as having extreme differences in allelic frequency between the high and low phenotypic tails for yield under lowland drought stress in DS 2006 via BSA, and therefore as being putatively linked to QTL for drought tolerance (Supplementary Fig. 2). At these loci, the Apo allele was favored in the high-yielding tail and the Swarna allele was predominant in the low-yielding tail.

Mapping the QTL linked to the markers identified via BSA

Using the phenotypic data for grain yield from the DS 2006 lowland stress trial the QTL linked to RM324 ($DTY_{2.1}$; Fig. 1) and RM416 ($DTY_{3.1}$; Fig. 2) were mapped.

To map $DTY_{2.1}$ on chromosome 2, the ~25 cM interval flanked by RM327 and RM262 was scanned and the QTL

Table 1 Grain yield of parents and progenies, standard deviation of progeny grain yield and broad sense heritability (*H*) of grain yield in seven hydrological environments: IRRI DS 2006–2008

Environment	*H* of grain yield	Mean grain yield (g m^{-2})			Standard deviation of progeny grain yield
		Apo (±SE)	Swarna (±SE)	Progeny (±SE)	
Lowland stress					
DS 2006	0.73	72.53 (±21.55)	8.08 (±21.55)	50.92 (±8.20)	42.66
DS 2007	0.55	271.51 (±53.78)	110.52 (±53.78)	204.08 (±23.90)	122.53
Lowland non-stress					
DS 2007	0.58	516.25 (±67.40)	300.25 (±67.40)	563.69 (±8.77)	135.86
DS 2008	0.58	325.50 (±51.34)	NA	408.68 (±8.57)	103.86
Aerobic					
DS 2006	0.74	NA	89.93 (±56.15)	201.06 (±20.95)	110.57
DS 2007	0.70	403.86 (±49.56)	111.87 (±56.21)	215.42 (±19.02)	101.34
WS 2007	0.70	328.16 (±28.29)	133.74 (±28.29)	250.38 (±4.12)	69.35

NA Not available

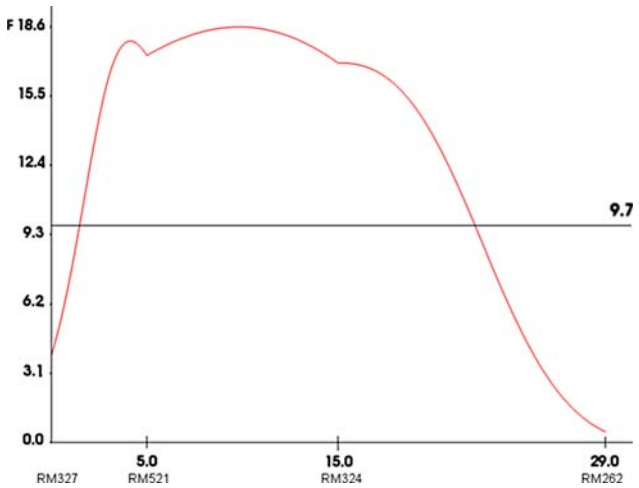

Fig. 1 *F*-statistic curve denoting QTL for grain yield ($DTY_{2.1}$) in DS 2006 lowland drought stress trial on chromosome 2. Genetic distance in cM between two RM markers is indicated on *X*-axis. *Horizontal line* corresponds to critical *F* value ($P < 0.01$)

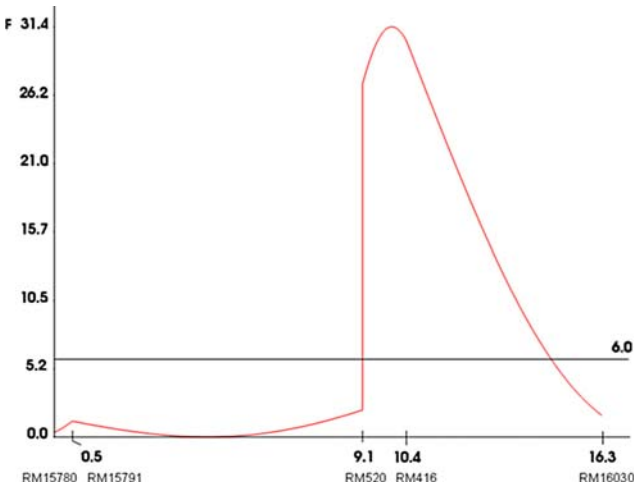

Fig. 2 *F*-statistic curve denoting QTL for grain yield ($DTY_{3.1}$) in DS 2006 lowland drought stress trial on chromosome 3. Genetic distance in cM between two RM markers is indicated on *X*-axis. *Horizontal line* corresponds to critical *F* value ($P < 0.01$)

position was located in the support interval of 2.3–19.8 cM. The QTL peak was localized between RM521 and RM324 at a distance of 4.8 cM from RM521. The mapped QTL had an additive effect of 7.1 g m^{-2} in this trial and was highly significant (*F*-statistic of 18.61; $P < 0.00001$). Similarly, to map $DTY_{3.1}$, the $\sim$16 cM region between RM15791 and RM16030 on chromosome 3 (Fig. 2) was scanned and the QTL position was located in the support interval of 9.1–11.0 cM. QTL peak was located at 10.0 cM and flanked by RM520 (9.1 cM) and RM416 (10.0 cM). The mapped QTL ($DTY_{3.1}$) had an additive effect on grain yield of 13 g m^{-2} under severe lowland stress in DS 2006, which was highly significant (*F*-statistic of 31.44; $P < 0.000001$).

Characterizing the QTL effects on grain yield and related traits

To better understand the phenotypic effects of the two QTL, the marker within the QTL region closest to each peak was subjected to single-marker analysis for yield and related traits in seven experiments conducted over a period of 3 years (2006–2008), including the original mapping experiments.

Effects on grain yield

In both the cases, the marker effect in the whole population was significant in the environment (lowland stress 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 2). Thus, it appears that BSA based on visual differentiation of band intensity between the high- and low-yielding tails comprising 8% of the total population was effective in identifying loci significantly associated with yield under lowland drought stress conditions.

In both the lowland stress trials, $DTY_{2.1}$ and $DTY_{3.1}$ had significant and consistent effects on grain yield. In all the three aerobic trials, $DTY_{3.1}$ had significant and consistent effects on yield. $DTY_{2.1}$ had highly significant effects in two aerobic trials with larger populations (490 lines) but its effect was not seen in WS 2007 aerobic trial with a smaller population size (100 lines). In all the significant allelic contrasts in lowland stress and aerobic environments, the Apo allele was associated with higher yield. In the two lowland non-stress trials, $DTY_{2.1}$ had no effect on yield, while at $DTY_{3.1}$ the Swarna allele was associated with higher grain yield (the effect was significant only in DS 2007). Thus, $DTY_{3.1}$ appears to be a case where opposing alleles are required to optimize performance under stress and non-stress conditions.

$DTY_{3.1}$ had the largest and most consistent effects on yield under lowland stress and aerobic conditions. It explained 31 and 7% of genetic variation in grain yield under severe and mild stress in lowland trials, respectively (Table 3). In the aerobic trials it explained 15–28% genetic variance for grain yield. At $DTY_{3.1}$, the Apo homozygotes significantly outyielded Swarna homozygotes in all the lowland stress and aerobic experiments. In lowland stress, the mean yield advantage of Apo homozygotes ranged from as low as 10% in mild stress to 63% in severe stress; in aerobic trials the yield advantage of Apo homozygotes ranged between 16 and 30% (averaging 22%). This is the first reported QTL to our knowledge that significantly and consistently affects grain

Table 2 Effect of marker genotype on mean grain yield (g m^{-2}) of Apo and Swarna allele homozygotes at the two QTL, based on single-marker analysis for markers nearest each QTL peak, in seven hydrological environments: IRRI DS 2006–2008

Environment	$DTY_{2.1}$ homozygotes for allele type				$DTY_{3.1}$ homozygotes for allele type			
	Apo (±SE)	Swarna (±SE)	P[a]	R^2 (%)[b]	Apo (±SE)	Swarna (±SE)	P	R^2 (%)
Lowland stress								
DS 2006	62.61 (±8.90)	44.53 (±8.79)	****	16.35	78.21 (±9.23)	48.04 (±7.89)	****	30.73
DS 2007	218.51 (±26.51)	191.96 (±26.34)	***	13.23	218.00 (±28.46)	197.63 (±27.11)	*	6.93
Lowland non-stress								
DS 2007	563.45 (±14.57)	544.07 (±13.70)	NS	0	501.13 (±24.97)	554.25 (±10.70)	*	16.97
DS 2008	405.60 (±13.71)	435.10 (±15.88)	NS	6.09	400.21 (±18.45)	416.87 (±13.16)	NS	0
Aerobic								
DS 2006	216.82 (±21.98)	190.48 (±21.63)	**	4.40	246.76 (±25.13)	190.16 (±21.82)	***	17.26
DS 2007	229.51 (±18.50)	204.13 (±18.21)	**	6.66	248.24 (±21.69)	207.04 (±18.91)	***	14.88
WS 2007	245.28 (±8.35)	256.38 (±9.79)	NS	0	273.92 (±10.31)	236.67 (±7.06)	**	27.70

[a] Probability of difference between the two homozygote classes; *, **, ***, **** significant at 5, 1, 0.1, 0.01% P levels, respectively, *NS* non-significant

[b] Percentage of genetic variance explained by each marker

Table 3 Effect of marker genotype on mean days to flowering and mean plant height of Apo and Swarna allele homozygotes at the two QTL, based on single-marker analysis for markers nearest each QTL peak, in seven hydrological environments: IRRI DS 2006–2008

Environment	$DTY_{2.1}$ homozygotes for allele type				$DTY_{3.1}$ homozygotes for allele type			
	Apo (±SE)	Swarna (±SE)	P[a]	R^2 (%)[b]	Apo (±SE)	Swarna (±SE)	P	R^2 (%)
Days to flowering								
Lowland stress								
DS 2006	102.79 (±0.68)	104.62 (±0.66)	***	9.46	101.27 (±0.89)	104.43 (±0.65)	****	22.66
DS 2007	95.08 (±0.38)	95.35 (±0.36)	NS	0	94.45 (±0.54)	95.23 (±0.32)	NS	1.83
Lowland non-stress								
DS 2007	94.17 (±0.55)	94.51 (±0.53)	NS	0	92.74 (±0.99)	94.50 (±0.49)	NS	7.36
DS 2008	100.70 (±1.22)	101.86 (±1.29)	NS	1.21	99.88 (±1.29)	101.46 (±1.11)	NS	3.67
Aerobic								
DS 2006	105.70 (±1.63)	107.83 (±1.60)	**	4.40	103.74 (±1.79)	107.93 (±1.51)	***	14.23
DS 2007	96.91 (±0.71)	98.69 (±0.67)	**	5.32	96.52 (±1.08)	98.48 (±0.78)	*	5.44
WS 2007	89.05 (±0.69)	89.80 (±0.78)	NS	0	87.58 (±0.85)	90.33 (±0.64)	**	20.63
Plant height (cm)								
Lowland stress								
DS 2006	82.97 (±1.00)	77.21 (±0.89)	****	24.48	82.58 (±1.83)	79.17 (±1.05)	*	5.20
DS 2007	100.84 (±3.95)	98.55 (±3.94)	**	19.01	98.19 (±3.70)	99.10 (±3.59)	NS	0
Lowland non-stress								
DS 2007	130.28 (±1.39)	129.55 (±1.36)	NS	0	129.78 (±2.52)	130.20 (±2.17)	NS	0
DS 2008	123.66 (±3.94)	122.40 (±4.01)	NS	0	120.64 (±3.88)	123.37 (±3.77)	NS	28.37
Aerobic								
DS 2006	110.76 (±1.64)	108.02 (±1.61)	***	12.46	105.68 (±2.05)	109.27 (±1.77)	**	14.75
DS 2007	102.80 (±2.56)	99.34 (±2.54)	****	16.86	98.36 (±2.84)	100.68 (±2.68)	*	5.40
WS 2007	121.82 (±2.23)	118.31 (±2.30)	**	22.96	116.67 (±2.20)	121.99 (±2.04)	***	49.42

[a] Probability of difference between the two homozygote classes; *, **, ***, **** significant at 5, 1, 0.1, 0.01% P levels, respectively, *NS* non-significant

[b] Percentage of genetic variance explained by each marker

Table 4 Effect of marker genotype, based on single-marker analysis for markers near each QTL peak, on grain yield after covariance adjustment for the effect of flowering date (flowering date from respective trials used as covariate in analysis): IRRI DS 2006 severe lowland stress

Marker	Mean grain yield (g m^{-2}) of homozygotes with allele type			Significance of effect of flowering dates on grain yield
	Apo (±SE)	Swarna (±SE)	P^a	
$DTY_{2.1}$	59.60 (±7.03)	48.48 (±6.95)	****	****
$DTY_{3.1}$	65.96 (±6.18)	51.40 (±5.15)	***	****

[a] Probability of difference between the two homozygote classes; ***, **** significant at 0.1, 0.01 *P* levels, respectively

yield both in lowland drought stress and aerobic environments. $DTY_{3.1}$ seems to be more effective in more stressful environments. $DTY_{2.1}$ had highly significant ($P < 0.0005$) effects in both the lowland stress trials; Apo homozygotes significantly out-yielded Swarna homozygotes by 40 and 14% in severe and mild lowland stress environments, respectively, but no difference was observed on lowland non-stress yield. In DS 2006–2007 aerobic trials it explained 4–7% of the genetic variation in yield.

Effect on yield-related traits

$DTY_{3.1}$ significantly affected days to flowering, and plant height in all the aerobic trials (Tables 3, 4), and in the DS 2006 lowland severe stress trial. $DTY_{2.1}$ significantly affected plant height in all the aerobic and lowland stress trials but effects on days to flowering were not consistent. Both $DTY_{3.1}$ and $DTY_{2.1}$ had no significant effect on any of these traits in both the lowland non-stress trials.

Relation between phenology and yield

Whenever the two QTL significantly affected days to flowering, the Apo homozygotes on average flowered 1.8–4.2 days earlier than Swarna homozygotes. We hypothesized that this early flowering could have caused some of the differences in yield under stress. This hypothesis was tested using three separate approaches: covariance adjustment for flowering date, contrasts between homozygous classes within subsets of lines with similar flowering dates, and imposition of stress at different phenological stages via a staggered-planting experiment.

Covariance analysis

To eliminate the confounding effect of days to flowering on yield under stress, the single-marker analysis for the two loci was repeated with mean days to flowering as cofactor (Table 4) in the DS 2006 trials involving the whole population. Even after covariance adjustment for flowering date, the effect of $DTY_{3.1}$ on grain yield in DS 2006 under severe lowland stress remained highly significant ($P < 0.0006$), as did the effect of $DTY_{2.1}$ ($P < 0.0001$).

Testing the effect of QTL in subsets of lines with comparable flowering dates

In addition to the covariance analysis described above, the effects of $DTY_{2.1}$, and $DTY_{3.1}$ on grain yield in the DS 2006 severe lowland stress trial were analyzed on subsets of lines of comparable flowering dates (Table 5). The effect of these loci is shown not to be due to early flowering alone. In the earliest-flowering subset, flowering two phenotypic standard deviations earlier than the population mean, both the Apo and Swarna homozygotes at $DTY_{3.1}$ have similar mean flowering dates but the Apo homozygotes have significantly higher grain yield ($P < 0.007$), out-yielding Swarna homozygotes by about 43%. At $DTY_{2.1}$, Apo homozygotes significantly ($P < 0.05$) out-yielded Swarna homozygotes in three out of the four flowering-date subsets; the yield advantage of Apo homozygotes ranged between 19 and 77%. The significant differences observed between homozygous classes for these QTL within subsets of lines with similar flowering dates are strong evidence that much of the effect of these loci is independent of their effect on flowering date; if their effect on yield under stress was primarily due to phenology differences, then one would expect no significant differences in yield between homozygous classes within subsets of lines with very similar flowering dates.

Effect of QTL on phenology and yield under lowland stress in the staggered planting trial of DS 2008

To eliminate the confounding effect of days to 50% flowering on yield under stress, the effects of $DTY_{3.1}$ and $DTY_{2.1}$ on grain yield were analyzed on a set of lines with comparable flowering dates in non-stress conditions (Table 6). This set of lines was planted on three different dates side by side, but stress was initiated on the same date.

In all the three stress sets, similar to the earlier observations ("Performance of parents and progeny"; Table 1), Apo consistently out-yielded Swarna; the difference was more evident under increasingly severe stress (Table 6). The progenies on average yielded 381 g m^{-2} in non-stress but 197, 180, and 112 g m^{-2} in the first, second, and third plantings, which were subjected to mild, moderate, and

Table 5 Effect of $DTY_{2.1}$, and $DTY_{3.1}$, marker genotype, based on single-marker analysis for markers near each QTL peak, on grain yield (g m^{-2}) of sets of lines with comparable flowering dates: IRRI DS 2006 lowland stress

Trait	Mean flowering date of lines in set, in phenotypic standard deviations (σ), relative to the population mean											
	-2σ			$-\sigma$			$\pm\sigma$			$\pm2\sigma$		
	Homozygotes for allele type											
	Apo (±SE)	Swarna (±SE)	P^a	Apo (±SE)	Swarna (±SE)	P	Apo (±SE)	Swarna (±SE)	P	Apo (±SE)	Swarna (±SE)	P
$DTY_{2.1}$												
Days to flowering	97.35 (±0.24)	97.63 (±0.27)	NS	101.54 (±0.19)	101.70 (±0.17)	NS	106.42 (±0.23)	106.74 (±0.17)	NS	110.82 (±0.32)	111.10 (±0.24)	NS
Grain yield	89.37 (±7.14)	68.35 (±7.89)	*	59.69 (±3.45)	49.90 (±3.14)	*	31.80 (±3.07)	27.22 (±2.29)	NS	22.76 (±3.18)	12.71 (±2.43)	**
$DTY_{3.1}$												
Days to flowering	97.11 (±0.30)	97.53 (±0.30)	NS	101.20 (±0.36)	101.69 (±0.15)	NS	106.50 (±0.54)	106.53 (±0.16)	NS	110.80 (±0.60)	110.93 (±0.21)	NS
Grain yield	105.53 (±7.85)	73.97 (±7.85)	**	64.61 (±7.05)	52.92 (±2.96)	NS	25.33 (±7.19)	28.94 (±2.12)	NS	21.54 (±5.66)	13.25 (±2.00)	NS

[a] Probability of difference between the two homozygote class; *, **, *** significant at 5, 1, 0.1% P levels, respectively, *NS* non-significant, *NA* not available

severe flowering-stage drought stress, respectively. Thus, relative to the non-stressed control, there were yield reductions of 48, 53 and 71% in the first, second, and third planted sets, respectively.

In the non-stress control planting, the Apo and Swarna homozygotes at $DTY_{3.1}$ had similar mean flowering dates and yield potential. But under stress, the Apo homozygotes had significantly higher grain yield. Apo homozygotes out-yielded Swarna homozygotes by 19, 42, and 35% in first-, second- and third planted sets, respectively. $DTY_{3.1}$ had no effect on flowering date in the non-stress control, under mild stress and under severe stress, but had a large effect under moderate stress at the second planting date ($P < 0.0006$). Thus, this locus consistently affected yield under stress but not flowering. Similarly, it had no effect on harvest index in non-stress conditions but had a consistently significant effect on harvest index in stress conditions in all the three plantings. No effect of $DTY_{3.1}$ was seen on plant height, panicle number and biomass (data not shown). Whenever the effect was significant, Apo homozygotes had higher yield and harvest index but shorter days to flowering.

Similar effects of $DTY_{2.1}$ were observed; it had no effect on yield or flowering in non-stress conditions, but showed significant effects on yield under stress, with Apo homozygotes out-yielding Swarna homozygotes by 28, 33, and 31% in the first, second and third planted sets, respectively. Significant effect on days to flowering was observed in the first- and second planted sets only.

The results of the staggered-planting trial confirm that these QTL significantly affect grain yield under drought stress, and that the observed effect is not due to phenology per se. Rather, the delayed flowering of Swarna homozygotes in some stress treatments is a direct effect of their susceptibility to drought stress relative to Apo homozygotes.

Discussion

Bulk-segregant analysis was able to identify markers linked to two loci affecting grain yield under severe lowland drought stress (Supplementary Fig. 2). In subsequent analyses of the entire population, effects of both loci were highly significant ($P \leq 0.0005$) and explained 16–31% genetic variance for the trait (Table 2). Thus, it is clear that BSA can identify loci that are associated with grain yield under drought stress in rice, at a considerable savings in genotyping effort and cost, allowing resources to be focused on precise localization of QTL with large effects. Success of BSA in this case probably resulted partly from the population used for in this study. In the present study; a large population (490 lines) derived from parents that

Table 6 Effect of $DTY_{2.1}$ and $DTY_{3.1}$, based on single-marker analysis for markers near each QTL peak, on flowering dates, harvest index and grain yield (g m^{-2}) in a non-stress trial and three stress trials planted in the same paddy on different dates in lowland: IRRI DS 2008

	Control			Stress, set I			Stress, set II			Stress, set III		
	Seeding: 3 January 2008 Transplanting: 29 January 2008			Seeding: 12 December 2007 Transplanting: 04 January 2008			Seeding: 20 December 2007 Transplanting: 16 January 2008			Seeding: 26 December 2007 Transplanting: 20 January 2008		
	Days to flowering	Harvest index	Grain yield	Days to flowering	Harvest index	Grain yield	Days to flowering	Harvest index	Grain yield	Days to flowering	Harvest index	Grain yield
Lines												
Apo (±SE)	89.24 (±1.89)	NA	300.30 (±74.57)	92.17 (±2.24)	0.395 (±0.029)	278.85 (±24.29)	92.53 (±5.35)	0.264 (±0.057)	167.38 (±36.74)	102.91 (±2.80)	0.344 (±0.035)	121.28 (±28.67)
Swarna (±SE)	113.60 (±1.67)	NA	NA	113.67 (±2.24)	0.176 (±0.030)	124.55 (±24.50)	116.38 (±5.35)	0.126 (±0.057)	22.17 (±36.74)	113.94 (±2.89)	0.040 (±0.035)	0 (±29.89)
IR77298-14-1-2-B-10 (±SE)	90.44 (±2.26)	NA	297.80 (±91.32)	93.67 (±3.17)	0.362 (±0.041)	200.68 (±32.94)	86.41 (±7.09)	0.303 (±0.068)	192.85 (±50.65)	85.29 (±3.81)	0.302 (±0.049)	223.71 (±39.55)
Progeny lines homozygous for $DTY_{2.1}$ allele type												
Apo (±SE)	109.02 (±1.27)	0.295 (±0.024)	412.17 (±31.31)	97.94 (±1.00)	0.307 (±0.018)	235.99 (±15.75)	100.03 (±3.92)	0.267 (±0.047)	213.19 (±19.23)	108.02 (±1.95)	0.224 (±0.019)	134.77 (15.78)
Swarna (±SE)	108.64 (±1.12)	0.267 (±0.018)	373.52 (±22.14)	102.65 (±0.71)	0.276 (±0.012)	184.57 (±12.94)	106.21 (±3.66)	0.228 (±0.045)	159.67 (±15.77)	108.36 (±1.76)	0.182 (±0.014)	102.75 (±13.50)
P[a]	NS	NS	NS	***	NS	**	**	NS	**	NS	NS	*
Progeny lines homozygous for $DTY_{3.1}$ allele type												
Apo (±SE)	108.39 (±1.09)	0.270 (±0.024)	389.46 (±24.85)	99.82 (±0.88)	0.314 (±0.011)	214.37 (±11.39)	100.43 (±3.47)	0.278 (±0.048)	211.62 (±16.02)	107.52 (±1.65)	0.220 (±0.013)	128.06 (±10.05)
Swarna (±SE)	109.41 (±1.09)	0.266 (±0.024)	370.76 (±24.89)	102.18 (±0.88)	0.254 (±0.011)	180.02 (±11.40)	107.11 (±3.46)	0.211 (±0.048)	148.50 (±15.98)	108.90 (±1.65)	0.174 (±0.013)	94.52 (±10.09)
P	NS	NS	NS	NS	***	**	**	***	***	NS	**	**

[a] Probability of difference between the two homozygote classes; *, **, *** significant at 5, 1, 0.1% P levels respectively, *NS* non-significant, *NA* not available

greatly differed for yield under drought stress was used (Tables 1, 6). Such populations, derived from parents differing greatly in the phenotype of interest, seem more likely to contain large-effect QTL that could be identified by BSA than less diverse populations. A similar population, Vandana/Way Rarem, derived from the cross between a drought susceptible (Way Rarem) and a tolerant parent (Vandana), has been used to identify a large-effect QTL for grain yield under aerobic drought stress (Bernier et al. 2007), although in this case, unusually, the allele conferring tolerance came from the susceptible parent. On the other hand, most populations used to date, e.g. IR64/Azucena, and CT99993/IR62266, were not derived from such contrasting parents (Kumar et al. 2007; Lanceras et al. 2004; Venuprasad et al. 2007) and thus were less successful in identifying large-effect QTL. Another factor affecting the success of BSA could be precision of the stress trial as measured by the broad-sense heritability (*H*) of grain yield under stress. In the present study (Table 1) and in the two previous studies that identified QTL with large effects on yield under drought stress (Bernier et al. 2007; Kumar et al. 2007), heritability of grain yield under stress was above 0.5. Thus, well-managed stress trials, in which the population involves parents that are contrasting in yield under drought stress and in which *H* for grain yield under stress above 0.5 is achieved, appear to offer good prospects for identifying useful QTL for the trait. Our previous studies have shown that even in two-replicate single-row-plot trials, adequate *H* can be realized if the trials are well managed (Bernier et al. 2007; Venuprasad et al. 2007). These results should encourage researchers to use BSA routinely as a cost-effective way to identify QTL alleles that could be used in rice breeding programs for drought tolerance.

Testing the effects of the two identified QTL in a total of eight (Tables 2, 6) hydrological environments over a period of 3 years revealed that the Apo allele at $DTY_{3.1}$ is effective in enhancing yield under stress across lowland and aerobic environments, but may have a negative effect on yield under non-stress lowland conditions relative to the Swarna allele. $DTY_{2.1}$ works mainly in lowland drought stress environments, with a smaller and less consistent effect in aerobic fields, and no effect under non-stress lowland conditions. Introgressing Apo alleles into the Swarna background at both the loci should result in enhanced yield in stress environments, with variable effects on yield potential in non-stress environments.

In general, the aerobic trials may all be considered to be stress environments for Swarna and its BC_1-derived progeny lines, since Swarna is known to perform very poorly under aerobic conditions, and the lowland fully irrigated trials of DS 2007 and DS 2008 may be considered non-stress environments. Considered in this light, it is clear that the two QTL have little or no effect on flowering under non-stress conditions, but have generally significant effects under aerobic and lowland stress environments. Overall, the Apo alleles at both loci exhibited a tendency to reduce days to flowering under stress and aerobic conditions, with this tendency being most marked at $DTY_{3.1}$ under aerobic conditions (Table 3). The fact that both the yield advantage of Apo homozygotes and the flowering delay exhibited by Swarna homozygotes for these loci generally increased with increasing stress indicates that the flowering delay observed is mainly a response to stress. Several researchers have indicated reduced flowering delay as a selection criterion for drought tolerance in rice (Pantuwan et al. 2002; Zou et al. 2008). Thus, Apo alleles at these loci are probably associated with stability of flowering processes, an important drought tolerance trait (Bernier et al. 2008). Bernier et al. (2007) similarly observed that the drought tolerance QTL *qtl12.1* affected both grain yield and days to flowering in stress but not in non-stress environments. Other examples of genes affecting both stress tolerance and flowering have been reported. In *Arabidopsis*, Masle et al. (2005) reported that the *ERECTA* gene influences both drought tolerance and inflorescence differentiation. Further, Kim et al. (2004) reported that a single mutation is responsible for both increased cold tolerance and delayed flowering in *Arabidopsis* and opined that such mutations may have an evolutionary advantage. A similar association in rice between early flowering and drought tolerance has been empirically observed by researchers at IRRI (unpublished data); our results would seem to support this observation. QTL related to heading date (HD) have been reported near $DTY_{3.1}$ (Yano et al. 2001), similarly, QTL for root and yield components under drought are also reported near $DTY_{2.1}$ and $DTY_{3.1}$ (Courtois et al. 2000; Yadav et al. 1997; Yue et al. 2006). So the observed results could be either due to linkage of HD genes with other genes with effects on drought tolerance or due to pleiotrophic effects of the HD genes themselves. Further studies are being conducted to resolve this issue.

Many QTL identified previously as affecting yield under drought stress, both in rice and other species, have turned out to have effects that were highly specific to the background in which they were originally detected (IRRI, unpublished; Maccaferri et al. 2008). However, one of the two regions reported herein as affecting grain yield under stress, $DTY_{3.1}$, has been shown to have a strong influence on grain yield under drought stress in populations derived from the crosses Apo/IR64 (Venuprasad et al. 2009) and IR55419/Way Rarem (IRRI, unpublished data). Thus, these QTL seem to influence grain yield under aerobic and/or drought situation in several genetic backgrounds. Detailed study of the identified regions to characterize their genetic and physiological effects is needed, and may make it

possible to exploit these QTL through MAB in improving rice grain yield under drought stress.

There is considerable evidence from this study and two other published studies (Bernier et al. 2007; Kumar et al. 2007) to support the hypothesis that in rice a few QTL exist which have large effects on grain yield and/or flowering that are unique to particular hydrological conditions (stress vs. non-stress and/or upland vs. lowland). Bulk-segregant analysis can detect such QTL alleles relatively cheaply and quickly, and should aid in rapid screening for large-effect QTL in a larger sample of donors than has been possible previously. It should be possible to exploit these QTL, after fine-mapping and development of gene-based or tightly linked markers, for improving grain yield of valuable varieties such as Swarna in stress-prone environments via marker-assisted backcrossing.

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References

Babu RC, Nguyen BD, Chamarerk V, Shanmugasundaram P, Chezhian P, Jeyaprakash P, Ganesh SK, Palchamy A, Sadasivam S, Sarkarung S, Wade LJ, Nguyen HT (2003) Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. Crop Sci 43:1457–1469

Bernier J, Kumar A, Venuprasad R, Spaner D, Atlin GN (2007) A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. Crop Sci 47:507–516

Bernier J, Atlin GN, Serraj R, Kumar A, Spaner D (2008) Breeding upland rice for drought resistance. J Sci Food Agric 88:927–939

Courtois B, Mclaren G, Sinha PK, Prasad K, Yadav R, Shen L (2000) Mapping QTLs associated with drought avoidance in upland rice. Mol Breed 6:55–66

George T, Magbanua R, Garrity DP, Tubana BS, Quinton J (2002) Rapid yield loss of rice cropped successively in aerobic soil. Agron J 94:981–989

Hemamalini GS, Shashidar HE, Hittalmani S (2000) Molecular marker-assisted tagging of morphological and physiological traits under two contrasting moisture regimes at peak vegetative stage in rice. Euphytica 112:69–78

Huke RE, Huke EH (1997) Rice area by type of culture: south, southeast, and east Asia. IRRI, Los Baños, Philippines

IRGSP (2005) The map-based sequence of the rice genome. Nature 436:793–800

Kamoshita A, Zhang J, Siopongco J, Sarkarung S, Nguyen HT, Wade LJ (2002) Effects of phenotyping environment on identification of quantitative trait loci for rice root morphology under anaerobic conditions. Crop Sci 42:255–265

Kim HJ, Hyun Y, Park JY, Park MJ, Park MK, Kim MD, Kim HJ, Lee MH, Moon J, Lee I, Kim J (2004) A genetic link between cold responses and flowering time through FVE in *Arabidopsis thaliana*. Nat Genet 36:167–171

Kumar R, Venuprasad R, Atlin GN (2007) Genetic analysis of rainfed lowland rice drought tolerance under naturally-occurring stress in eastern India: heritability and QTL effects. Field Crops Res 103:42–52

Kumar A, Bernier J, Verulkar S, Lafitte HR, Atlin GN (2008) Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. Field Crops Res 107:221–231

Lanceras JC, Pantuwan G, Jongdee B, Toojinda T (2004) Quantitative trait loci associated with drought tolerance at reproductive stage in rice. Plant Physiol 135:384–399

Lander E, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Maccaferri M, Sanguineti MC, Corneti S, Ortega JLA, Salem MB, Bort J, DeAmbrogio E, del Moral LFG, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R (2008) Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. Genetics 178:489–511

Mackill DJ (2003) What molecular tools are available for selection for drought tolerance? In: Fischer KS, Lafitte R, Fukai S, Atlin GN, Hardy B (eds) Breeding rice for drought-prone environments. IRRI, Los Baños, Philippines, pp 55–57

Masle J, Gilmore SR, Farquhar D (2005) The ERECTA gene regulates plant transpiration in *Arabidopsis*. Nature 436:866–870

Michelmoore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Navabi A, Mather DE, Bernier J, Spaner DM, Atlin GN (2009) QTL detection with bidirectional and unidirectional selective genotyping: marker-based and trait-based analyses. Theor Appl Genet 118:347–358

Ouk M, Basnayake J, Tsubo M, Fukai S, Fischer KS, Cooper M, Nesbitt H (2006) Use of drought response index for identification of drought tolerant genotypes in rainfed lowland rice. Field Crops Res 99:48–58

Pandey S, Bhandari HN, Hardy B (2007) Economic costs of drought and rice farmers' coping mechanisms. IRRI, Los Baños, Philippines

Pantuwan G, Fukai S, Cooper M, Rajatasereekul S, O'Toole JC (2002) Yield response of rice (*Oryza sativa* L.) genotypes to drought under rainfed lowlands: 2. Selection of drought resistant genotypes. Field Crops Res 73:169–180

Price AH, Courtois B (1999) Mapping QTLs associated with drought resistance in rice: progress, problems and prospects. Plant Growth Regul 29:123–133

Price AH, Cairns JE, Horton P, Jones RGW, Griffiths H (2002) Linking drought-resistance mechanisms to drought avoidance in upland rice during a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. J Exp Bot 53:989–1004

Quarrie S, Lazic-jancic V, Kovacevic D, Steed A, Pekic S (1999) Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize. J Exp Bot 50:1299–1306

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbour, New York

SAS Institute Inc (2004) SAS OnlineDoc® 9.1.3. SAS Institute Inc, Cary, NC, USA

Shashidhar HE, Vinod MS, Sudhir Naveen, Sharma GV, Krishnamurthy K (2005) Markers linked to grain yield using bulked segregant analysis approach in rice (*Oryza sativa* L.). Rice Genet Newsl 22:69–71

Shen X, Zhou M, Lu W, Ohm H (2003) Detection of Fusarium head blight resistance QTL in a wheat population using bulked segregant analysis. Theor Appl Genet 106:1041–1047

Temnykh S, Declerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. Genome Res 11:1441–1452

Tripathy JN, Zhang J, Robin S, Nguyen TT, Nguyen HT (2000) QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. Theor Appl Genet 100:1197–1202

Venuprasad R, Lafitte HR, Atlin GN (2007) Response to direct selection for grain yield under drought stress in rice. Crop Sci 47:285–293

Venuprasad R, Sta Cruz MT, Amante M, Magbanua R, Kumar A, Atlin GN (2008) Response to two cycles of divergent selection for grain yield under drought stress in four rice breeding populations. Field Crops Res 107:232–244

Venuprasad R, Bool ME, Dalid CO, Bernier J, Kumar A, Atlin GN (2009) Genetic loci responding to two cycles of divergent selection for grain yield under drought stress in a rice breeding population. Euphytica 167:261–269

Yadav R, Courtois B, Huang N, Mclaren G (1997) Mapping genes controlling root morphology and root distribution in a double-haploid population of rice. Theor Appl Genet 94:619–632

Yang J, Zhu J, Williams RW (2007) Mapping the genetic architecture of complex traits in experimental populations. Bioinformatics 23:527–1536

Yang J, Hu CC, Hu H, Yu R, Xia Z, Ye X, Zhu J (2008) QTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics 24:721–723

Yano M, Kojima S, Takahashi Y, Lin H, Sasaki T (2001) Genetic control of flowering time in rice, a short-day plant. Plant Physiol 127:1425–1429

Yue B, Xue W, Xiong L, Yu X, Luo L, Cui K, Jin D, Xing Y, Zhang Q (2006) Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. Genetics 172:1213–1228

Zhang J, Zheng HG, Aarti A, Pantuwan G, Nguyen TT, Tripathy JN, Sarial AK, Robin S, Babu RC, Nguyen BD, Sarkarung S, Blum A, Nguyen HT (2001) Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. Theor Appl Genet 103:19–29

Zheng HG, Babu RC, Pathan MS, Ali L, Huang N, Courtois B, Nguyen HT (2000) Quantitative trait loci for root penetration ability and root thickness in rice: comparison of genetic backgrounds. Genome 43:53–61

Zou GH, Liu HY, Mei HW, Liu GL, Yu XQ, Li MS, Wu JH, Chen L, Luo LJ (2008) Screening for drought resistance of rice recombinant inbred populations in the field. J Integr Plant Biol 49:1508–1516