

# A Large-Effect QTL for Grain Yield under Reproductive-Stage Drought Stress in Upland Rice

Jérôme Bernier, Arvind Kumar, Venuprasad Ramaiah, Dean Spaner, and Gary Atlin\*

## ABSTRACT

Genetic control of yield under reproductive-stage drought stress was studied in a population of 436 random  $F_3$ -derived lines from a cross between the upland rice (*Oryza sativa* L.) cultivars Vandana and Way Rarem. Screening was conducted under upland conditions at IRRI during the dry seasons of 2005 and 2006. Lines were evaluated in drought stress and nonstress trials in both years to identify QTL contributing to drought resistance. For QTL detection, a set of random lines and the highest and lowest-yielding lines under both stress and nonstress conditions were genotyped by 126 SSR markers. A QTL (*qt12.1*) with a large effect on grain yield under stress was detected on Chromosome 12 in both years. The whole population was genotyped for additional markers on Chromosome 12, allowing QTL localization to a 10.2 cM region between SSR markers RM28048 and RM511. Under stress conditions, the locus also increased harvest index, biomass yield, and plant height while reducing the number of days to flowering. Under nonstress conditions, *qt12.1* did not significantly affect any trait. The additive effect of this QTL on grain yield under stress was 172 kg ha<sup>-1</sup> per year over the 2 yr of testing, representing 47% of the average yield under stress and explaining 51% of the genetic variance. The yield-increasing allele was derived from the susceptible parent, Way Rarem, suggesting an epistatic effect. This is the first QTL reported in rice having a large and repeatable effect on grain yield under severe drought stress in the field.

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**Abbreviations:** Add, Additive effect; Chr, Chromosome; DAS, days after seeding; LOD, logarithm of odds; MAS, marker-assisted selection; PCR, polymerase chain reaction; QTL, quantitative trait loci; SSR, simple sequence repeat.

RICE IS THE most important crop for human consumption, with production on over 150 million hectares yielding almost 600 million megagrams annually (Khush, 2005). Global rice production doubled between 1966 and 1990 (Khush, 1997), but most of this increase came from irrigated areas, where yield growth is now stagnating (Peng et al., 1999). Upland rice, which represents 12% of the total production area (Khush, 1997), is grown almost exclusively by small-holders for household food security but is prone to damage by drought (Babu et al., 2004). Given the high risk of crop loss due to drought, upland rice growers are reluctant to invest in yield-enhancing inputs such as fertilizer, trapping them in a cycle of low productivity (Courtois et al., 2000). By reducing risk and encouraging farmers to invest in yield-increasing inputs, upland rice cultivars with improved drought resistance could result in greater productivity both in drought years and years with adequate rainfall.

Progress in breeding for drought resistance has been slow (Fukai and Cooper, 1995). It has been suggested that the development of drought-resistant varieties could be made more efficient by MAS to introgress alleles of QTL conferring improved drought resistance into the genome of widely used cultivars through backcrossing. When the traits that need to be improved are low in heritability, MAS may be more efficient than phenotypic selection (Asíns, 2002). However, for MAS to be worthwhile, the target

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QTL must have a large and consistent effect on yield (Salekdeh et al., 2002).

Several experiments have reported QTL for yield and yield components under different types of drought stress in rice. Babu et al. (2003) reported five QTL related to grain yield over two different trials conducted in southern India under upland drought stress in the population CT9993/IR62266. In one trial, severe drought stress was applied toward the end of the vegetative stage while in the other, stress was mild, but was applied at the flowering stage. The QTL with the largest effects explained approximately 20 and 28% of the genetic variation for yield in the vegetative and reproductive-stage stress trials, respectively, but none of the yield QTL observed were consistent across trials. Lafitte et al. (2004b), in a population derived from the cross Azucena/Bala, used a drip-irrigation system to precisely withhold water from the beginning of panicle emergence until 50% flowering but failed to identify QTL responsible for increased yield under stress. Lanceras et al. (2004), in the CT9993/IR62266 population, reported on QTL analyses conducted in Thailand in five different water treatments in a single transplanted trial under line-source irrigation. They identified four significant QTL for grain yield under water stress, one of which was consistent in three of the five water treatments and one which was detected twice. The largest-effect QTL identified in this trial explained about 30% of the genetic variance for yield under severe stress. None of those QTL corresponded to those previously identified by Babu et al. (2003) in the same population. Yue et al. (2005) reported a QTL detected in two consecutive years affecting yield, biomass, and harvest index reduction under drought stress in pot experiments. The QTL identified in this experiment (located on Chromosome 9 between markers RM316 and RM219) was consistent and stress-specific but of relatively small effect, explaining only 14 to 25% of the total phenotypic variation (Yue et al., 2005). Of all the QTL identified so far affecting rice yield under drought stress, none are considered to be sufficiently large or consistent to be useful in breeding (Lafitte et al., 2004a).

Despite the large number of QTL reported to affect drought tolerance in upland rice, there have been only a few attempts to introgress drought-tolerance QTL into susceptible genotypes (Courtois et al., 2003; Price, 2002). Large chromosomal regions bearing putative QTL associated with root length in a population derived from a cross between the deep-rooted upland cultivar Azucena and shallow-rooted lowland cultivar IR64 were introgressed into the IR64 background, but the majority of lines carrying the desired introgressions failed to have deeper roots than IR64 (Shen et al., 2001). The reasons for lack of effect of the introgressed segments on root length and yield may be that the target QTL were responsible for a relatively small proportion of the total phenotypic variation in the mapping experiment (5.6–17.7%) or because the introgressed region was large, and therefore

desirable genes within it could be lost because of recombination during backcrossing. Azucena root-related QTL have also been introduced into the *indica* cultivar Kalinga III, but only one of the five target QTL had an effect on root length. Such introgressions have not been reported to consistently improve grain yield under stress (Steele et al., 2006).

Most reported drought-related QTL mapping experiments in rice have employed only five populations (Price et al., 2002). Many of the upland parents used in these populations (e.g., CT9993-5-10-1-M and Azucena) are not considered to be highly drought-tolerant in terms of grain yield under severe drought stress. Many traditional and improved cultivars from drought-prone areas have some tolerance to reproductive-stage drought stress, but they have rarely been used in mapping studies. A more extensive survey of tolerant rice germplasm, currently underway at IRRI, may lead to the identification of lines carrying major genes improving yield under stress. The objective of the present study was to detect QTL with large and repeatable effects on grain yield under reproductive-stage water stress in a cross between an eastern Indian cultivar, Vandana, which produces a relatively high grain yield under severe reproductive-stage stress, and Way Rarem, an Indonesian upland cultivar with high yield potential but poor drought tolerance.

## MATERIALS AND METHODS

### Population Development

The population was derived from a cross between Way Rarem, a drought-sensitive Indonesian upland rice cultivar, and Vandana, an Indian upland rice cultivar that is considered drought-tolerant. Way Rarem belongs to the *indica* varietal group. Vandana's ancestry is 50% tropical *japonica* and 50% *aus*. The two parents differ markedly in the number of days that they take to reach anthesis; Vandana and Way Rarem flower, on average, in 63 and 90 d, respectively, when grown under well-watered conditions during the dry season at IRRI.

Bulk  $F_3$  seeds from this cross were planted at IRRI during the dry season of 2004.  $F_3$  plants were harvested individually and their seeds used to constitute the current mapping population. A total of 436 random  $F_3$ -derived lines were used in the mapping experiment.  $F_{3,4}$  seeds were used in the 2005 experiments and  $F_{3,5}$  seeds harvested from the nonstress treatment of 2005 were used to establish the 2006 trials.

### Phenotyping of the Mapping Population

Field evaluation was conducted under upland conditions at IRRI, Los Baños, Philippines (14°11' N 121°15' E, 21 m above sea level) during the 2005 and 2006 dry seasons. The soil of the IRRI upland farm is a Maahas clay loam (isohyperthermic mixed Typic Tropudalf) (Zhao et al., 2006). In both years, the population was screened in one trial under nonstress conditions and one under stress during reproductive growth and grain filling. For each trial, the design was a two-replicate  $\alpha$ -lattice with 2-m single-row plots. Rows were spaced 0.3 m apart in 2005 and 0.25 m apart in 2006. Six checks with a broad range of drought tolerance (Vandana, Way Rarem, Apo, IR64, IR

55419-04, and IR 74371-54-1-1) were included in the trials and replicated 12 times each. Seeds were dry-direct-seeded in aerobic soil using a seeding density of 2 g per linear meter of row, resulting in a seed rate of approximately 305 seeds m<sup>-2</sup> in 2005 and 365 seeds m<sup>-2</sup> in 2006. Basal applications equivalent to 40 P and 40 K kg ha<sup>-1</sup> were applied in the form of single super phosphate and potassium chloride, and 120 kg ha<sup>-1</sup> of N in the form of ammonium sulfate were applied in three even splits around 21, 42, and 61 d after seeding. Planting dates were 22 Dec. 2004 and 21 Jan. 2006 for the nonstress trials and 11 Jan. 2005 and 7 Jan. 2006 for the stress trials.

The nonstress trial in 2005 was sprinkler-irrigated twice weekly, resulting in applications of approximately 36 mm water per irrigation, or 1224 mm over the entire growth cycle. Pan evaporation over this period was 637 mm. In 2006, basin irrigation was used once a week, resulting in the application of 92 mm water per irrigation and a total of 1554 mm for the entire season. Pan evaporation totaled 672 mm for the 2006 season. The nonstress trial of 2006 was affected by a moderate stem borer infestation and a typhoon immediately before the majority of the plots were harvested, which resulted in substantial lodging and seed shattering.

In both years, stress trials were sprinkler-irrigated twice weekly during establishment and early vegetative growth, but irrigation frequency was reduced at 56 and 40 d after sowing in 2005 and 2006, respectively. Plots were re-irrigated periodically when soil water tension fell below -50 kPa at a 30-cm soil depth. At this soil water potential, most lines were wilted and exhibited leaf drying. This type of cyclical stress is considered to be efficient in screening for drought resistance in populations consisting of genotypes with a broad range of growth duration (Lafitte et al., 2004b) and ensures that lines of all durations are stressed during reproductive development.

In the stress trial of 2005, the crop received a total of 129 mm of water from the beginning of stress until the end of flowering (from 57–96 DAS), supplying an average of 3.2 mm of water per day while the pan evaporation averaged 6.4 mm per day. There was a total of five irrigation and rainfall events: 46 mm at 66 DAS, 35 mm at 72 DAS, 6 mm at 75 DAS, 33 mm at 82 DAS, and 7 mm at 95 DAS. During that period, the mean daily minimum and maximum temperatures were 23.1 and 33.3°C, with the temperature reaching a maximum of 37.6°C.

The stress trial of 2006 received 167 mm of water between 57 and 104 DAS, resulting in an average of 3.6 mm per day while the average pan evaporation was 6.2 mm per day. There was a total of six irrigation and rainfall events during this period: 41 mm at 63 DAS, 7 mm at 65 DAS, 28 mm at 76 DAS, 15 mm at 82 DAS, 41 mm at 87 DAS, and 35 mm at 96 DAS. During that period, the mean daily minimum and maximum temperatures were 23.9 and 32.5°C, with the temperature reaching a maximum of 34.8°C.

Days to flowering was recorded as the number of days from sowing until 50% of the plants in a plot had flowering tillers. Final plant height was the length in centimeters from the soil surface to the tip of the panicle on the main tiller at maturity. Grain yield under stress and nonstress conditions is reported on an oven-dried basis (0% moisture). Biological yield was sampled by selecting a uniform section 50 cm in length in each plot and harvesting the plants in this section at ground level. Biomass samples were then oven-dried, weighed, and threshed. In

2006, the number of panicles within the biomass sample was recorded in both trials. Harvest index was estimated as the ratio of grain weight to whole plant weight. Flowering delay was calculated as the difference between the 2-yr line mean days to flowering under stress and days to flowering under non-stress. Drought-response index was calculated as the difference between predicted yield under stress based on the number of days to flowering and grain yield under nonstress conditions and the observed grain yield under stress divided by the standard error of the line mean (Bidinger et al., 1987).

Plant height at 4 wk after seeding was measured on four plants per plot in the 2005 nonstress trial, the 2006 stress and nonstress trials, and in another trial using the same population and design sown on 7 Feb. 2006. The last trial could not be reliably drought-stressed because of an early onset of the wet season in 2006, so yield data from this trial have not been used in the current analysis. Data from the four trials where plant height 4 wk after seeding was measured have been combined because the trials were all treated in a similar way at the beginning of the season, before the imposition of stress. Only the data combined over trials were analyzed for this trait.

## DNA Extraction and Amplification

Eight fresh leaves from each line were collected in bulk 21 DAS. The leaves were then freeze-dried and ground in liquid nitrogen. The powdered leaves were then mixed with CTAB (cetyltrimethylammonium bromide) extraction buffer and incubated at 65°C for 30 to 60 min. Chloroform: isoamyl alcohol (24:1) was then added to the mixture. The mixture was then put on a shaker at room temperature for 20 min. Samples were then centrifuged and the upper phase was then transferred into a 2-mL Eppendorf tube. DNA was then precipitated with isopropanol precooled to a temperature of -20°C. The samples were then placed in a -20°C freezer for at least 1 h and then centrifuged in order for DNA to pelletize at the bottom of the tubes. The DNA pellet was then rinsed with 70% (v/v) ethanol and recentrifuged. The ethanol was then drained off and then DNA pellet was left to dry. The DNA was then dissolved in 200 µL of TE (Tris-EDTA) buffer and treated with RNase. DNA was then quantified with a spectrophotometer and subsequently diluted to a final concentration of 10 ng µL<sup>-1</sup> of double-distilled water.

Polymerase chain reaction (PCR) was performed using a volume of 20 µL per reaction. Amplifications were performed using 40 ng of DNA, 1× PCR buffer, 100 µM of dNTPs, 250 µM of oligonucleotide primers, and 1 unit of *Taq* polymerase. This mix was prepared on 96-well polycarbonate plates and the thermocycling used follows the method described by Panaud et al. (1996). After the PCR reaction was completed, 4 µL of 6× loading dye was added to each well. Four microliters of the resulting solution mix was then loaded into an 8% (w/v) polyacrylamide gel for size separation of the amplified DNA fragments using a minivertical electrophoresis system (CBS scientific, model MGV-202-33). DNA fragments were then stained with ethidium bromide and visualized with a UV transilluminator.

## Genetic Analysis of the Mapping Population

A total of 474 SSR markers were screened for polymorphism between the parents. A total of 169 markers showed polymorphism on our gel system out of which we initially selected 121

to be used in the experiment. The markers were taken from previously published rice genetic and sequence maps (IRGSP, 2005; McCouch et al., 2002; Temnykh et al., 2001).

Quantitative trait loci with large effects were initially detected by selectively genotyping (Darvasi and Soller, 1992; Lander and Botstein, 1989) the highest-yielding and lowest-yielding 12% of lines from 2005 stress and nonstress trials. To compensate for the fact that grain yield under stress is negatively correlated with the number of days to flowering under nonstress conditions, selection of lines for inclusion in the tails was done on the basis of stratification into three categories according to the number of days to flowering in the 2005 trial: 60 to 73, 74 to 83, and 84 to 96 d to flowering. In each of those categories, the highest- and lowest-yielding 12% of the lines were selected for genotyping. A total of 39 and 38 lines from the highest- and lowest-yielding tails of the stress treatment and 35 and 38 lines from the highest- and lowest-yielding tails of the nonstress treatment were genotyped as well as 92 random lines. This resulted in a total of 242 lines being genotyped.

Initial QTL analysis of the random lines and the tails of the phenotypic distribution (242 lines) identified a major QTL on Chromosome 12. To increase precision of the position and effect estimate, five markers were added to Chromosome 12 and the whole population (436 lines) was genotyped at a total of nine marker loci on Chromosome 12 (RM3472 to RM3739).

This genetic information was subsequently used to create a linkage map using MapManager QTX (Manly et al., 2001). The marker orders used to create the linkage map corresponded to the published rice genome SSR marker orders (IRGSP, 2005). Map distances were calculated using the 92 random lines exclusively, except in the 10-marker interval (RM3472–RM3739) on Chromosome 12 where the data from all 436 lines was used.

## Statistical Analysis

The model for the combined analysis over years and within irrigation treatments was:

$$P_{ijkl} = M + Y_i + R_j(Y_i) + B_k[R_j(Y_i)] + L_l + LY_{li} + e_{ijkl} \quad [1]$$

where  $P_{ijkl}$  is a measurement recorded on a plot,  $M$  is the mean over all plots and both years, and  $Y$ ,  $R$ ,  $B$ ,  $L$ , and  $e$  refer to years, replicates, blocks, lines and plot residuals, respectively.

For estimating line means within years, replicates, and blocks within replicates were considered random factors, with lines considered fixed. For estimating line means over years, year effects were also considered random. The results for the yield trial were analyzed by the REML algorithm of PROC MIXED of SAS V.9.1 (SAS Institute, Inc., 2002–2003). Degrees of freedom were computed using the Kenward-Rogers version of the Satterthwaite correction for estimating degrees of freedom (Piepho et al., 2003; SAS Institute, Inc., 2004). The REML algorithm of PROC VARCOMP was used to obtain the variance components required to calculate genetic correlations ( $r_G$ ) among traits, broad-sense heritability ( $H$ ), and proportion of the genetic variance explained by QTL. For variance component estimation, the model described in [1] was used, with all effects considered random. Differences are only discussed when  $p < 0.05$ .

The following formula was used to compute  $H$  across years:

$$H = \frac{\sigma_G^2}{\sigma_G^2 + (\sigma_{GY}^2 / \gamma) + [\sigma_E^2 / (r\gamma)]} \quad [2]$$

(Cooper et al., 1996), where  $\sigma_G^2$  is the genotypic variance,  $\sigma_{GY}^2$  is the genotype  $\times$  year variance,  $\sigma_E^2$  is the plot residual variance, and  $r$  and  $\gamma$  are the number of replicates and years, respectively. For traits measured in 1 yr only, heritability was estimated as:

$$H = \frac{\sigma_G^2}{\sigma_G^2 + [\sigma_E^2 / (r)]} \quad [3]$$

where  $\sigma_G^2$  is the genotypic variance from the single-year analysis. This estimator of broad-sense  $H$  is biased upward by the genotype  $\times$  year interaction variance but was used to approximately compare the relative repeatability of panicle number per  $m^{-2}$  across stress levels as this trait was measured in only a single season (Table 1a and b).

Genetic correlations between traits measured in different environments were computed as:

$$r_{G12} = \frac{r_{p12}}{\sqrt{H_1 \times H_2}} \quad [4]$$

(Cooper et al., 1996) where  $r_{G12}$ ,  $r_{p12}$ ,  $H_1$ , and  $H_2$  are genotypic correlation between traits 1 and 2, phenotypic correlation between the same trait pair, and  $H$  of traits 1 and 2, respectively. This estimation method assumes that the covariance between line means estimated in different trials is entirely caused by correlation of genotypic effects and that there is no environmental covariance. Genetic correlations were reported only when the phenotypic correlation between the two traits was significant ( $p < 0.05$ ).

For genetic correlations between two traits measured in the same environment:

$$r_G = \frac{\text{Cov}_{12}}{\sqrt{\sigma_{G1}^2 \times \sigma_{G2}^2}} \quad [5]$$

(Bernardo 2002), where  $r_{G12}$ ,  $\text{Cov}_{12}$ ,  $\sigma_{G1}^2$  and  $\sigma_{G2}^2$  are the genetic correlation coefficient between traits 1 and 2 within the same trial, genetic covariance of traits 1 and 2, and the genotypic variances of traits 1 and 2, respectively.

## QTL Analysis

Composite interval mapping (CIM) was performed by QTL Cartographer (Wang et al., 2005). The minimal LOD value required to declare a QTL was obtained empirically from 1000 permutation tests (Churchill and Doerge, 1994). The LOD thresholds obtained correspond to an experiment-wise type I error rate of 0.05. The thresholds values obtained for different traits varied from 3.5 to 37.7 with an average of 8.0. The linkage map covered 1591 cM using the Kosambi mapping function, resulting in an average of 1 marker every 12.7 cM (Fig. 1). CIM was conducted separately for means within years and means over the 2 yr.

To estimate the effect of a single marker and calculate the percentage of the genetic variance explained by a marker on a given trait, a model was used in which the line effect in Eq. [1] was partitioned into a component due to the marker ( $A_m$ ) and the residual genetic variation among lines within marker genotypes  $L_i(A_m)$ . The genotype  $\times$  year effect was similarly partitioned. This model is presented below:

**Table 1. (a) Means, ranges, and broad-sense heritabilities for 436 random F<sub>3</sub>-derived lines from Vandana/Way Rarem for agronomic traits under drought stress at flowering and grain-filling stages: IRR1 dry seasons 2005 and 2006. (b) Means, ranges, and broad-sense heritabilities for 436 random F<sub>3</sub>-derived lines from Vandana/Way Rarem for agronomic traits under nonstress conditions: IRR1 dry seasons 2005 and 2006.**

(a)	Grain yield			Biomass yield			Harvest index			Days to flowering			Height			Panicle	Flowering Delay	Drought-response index
	kg ha <sup>-1</sup>									m <sup>-2</sup>								
	2005	2006	Mean	2005	2006	Mean	2005	2006	Mean	2005	2006	Mean	2005	2006	Mean	2006		
Check																		
Apo	268	250	262	5436	8591	7051	0.07	0.04	0.05	100	89	95	73	73	73	347	2.5	0.61
IR55419-04	243	254	236	4614	6659	5608	0.06	0.06	0.06	87	82	85	72	72	72	313	1.2	-0.55
IR 64	28	22	17	2340	4546	3442	0.01	0.02	0.01	108	102	105	47	52	50	130	14.4	-0.63
IR 74371-54-1-1	649	346	499	5313	7885	6590	0.11	0.06	0.09	84	77	81	76	73	75	370	-2.2	0.75
Vandana	539	698	617	5531	6924	6228	0.16	0.17	0.16	63	64	64	70	76	73	350	-0.9	-0.01
Way Rarem	68	117	89	5324	7835	6591	0.03	0.02	0.02	96	87	92	75	74	75	217	1.3	-0.34
Population mean	405	321	363	5759	8189	6972	0.09	0.06	0.07	85	79	82	79	81	80	319	1.9	-0.04
Lowest line	0	0	0	2141	4546	3333	0	0	0.01	60	56	59	60	62	62	112	-14	-2.39
Highest line	1624	1590	1353	11225	13298	12060	0.27	0.23	0.24	118	104	109	95	109	96	536	17.6	4.78
Heritability <sup>†</sup>	0.54		0.70	0.32		0.49	0.56		0.72	0.84		0.91	0.37		0.54	0.38		
LSD <sub>0.05</sub> for comparisons between checks	284	186	241	1876	1695	1800	0.05	0.04	0.04	5	5	5	7	7	7	10		

(b)	Grain yield			Biomass yield			Harvest index			Days to flowering			Height			Panicle	Height 4 wk after seeding			
	kg ha <sup>-1</sup>									cm									m <sup>-2</sup>	Mean
	2005	2006	Mean	2005	2006	Mean	2005	2006	Mean	2005	2006	Mean	2005	2006	Mean	2006				
Check																				
Apo	2499	2295	2380	7568	12598	10012	0.23	0.21	0.22	92	91	92	96	105	100	364	26.9			
IR55419-04	2510	3099	2806	7521	11626	9575	0.3	0.29	0.29	85	82	83	88	94	91	398	29.4			
IR 64	1081	2551	1841	5541	9946	7731	0.22	0.32	0.27	94	87	91	68	76	73	425	23.2			
IR74371-54-1-1	2687	3466	3037	7937	12373	10067	0.35	0.33	0.34	84	82	83	88	96	92	378	28.4			
Vandana	2372	2031	2180	7353	9049	8299	0.27	0.22	0.25	63	63	63	93	95	94	416	29.8			
Way Rarem	1978	2014	2016	9500	9519	9553	0.26	0.2	0.23	89	92	90	97	106	102	234	30.3			
Population mean	2807	2667	2738	9755	12351	11042	0.29	0.23	0.26	80	82	81	104	111	107	337	30.6			
Lowest line	102	684	1318	2425	6428	4783	0.1	0.07	0.07	60	61	62	85	85	90	129	23.8			
Highest line	4563	6293	4738	26845	22714	19487	0.5	0.38	0.4	96	99	96	140	140	127	581	36.3			
Heritability <sup>‡</sup>	0.13		0.23	0.14		0.25	0.19		0.31	0.83		0.91	0.43		0.60	0.32		0.56		
LSD <sub>0.05</sub> for comparisons among checks	732	839	782	3827	3230	3557	0.09	0.07	0.08	3	3	3	7	9	8	12	3.5			

<sup>†</sup>Heritability was calculated as described in Eq. [2], except in the case of panicle number m<sup>-2</sup> where Eq. [3] was used. Heritability was calculated on a line-mean basis over two-replications. All traits were measured over 2 yr, except panicle m<sup>-2</sup> which was measured in a single year.

<sup>‡</sup>Heritability was calculated as described in Eq. [2], except in the case of panicle number m<sup>-2</sup> where Eq. [3] was used. Heritability was calculated on a line-mean basis over two replications. All traits were measure in 2 trials, except height at 4 wk and panicle number m<sup>-2</sup> which were measured in 4 and 1 trials, respectively.

$$P_{ijkl} = M + Y_i + R_j(Y_i) + B_k[R_j(Y_i)] + A_m + A_m Y_i + L_l(A_m) + L_l(A_m) Y_i + e_{ijkl} \quad [6]$$

Variance components were estimated using the REML option of SAS PROC VARCOMP.

## RESULTS AND DISCUSSION

### Genotypic and Phenotypic Variation for Agronomic Traits in Stress and Nonstress Trials

In both environments, yields were lower in the 2006 trials (Table 1a and b). The mean yield reduction due to water stress was 85 and 88% in 2005 and 2006, respec-

tively, indicating very high stress levels. Such high stress levels were desirable because a high percentage reduction of yield is necessary to remove the effect of yield potential and clearly identify lines that are drought-resistant (Babu et al., 2003; Lafitte et al., 2006).

There was a higher average biomass accumulation under stress in the 2006 drought trial than in 2005, but a lower harvest index in that same year resulted in lower grain yield on average. The earlier onset of drought stress in 2006 might be responsible for the lower harvest index. In the nonstress trials, a higher biomass accumulation combined with a lower harvest index also contributed

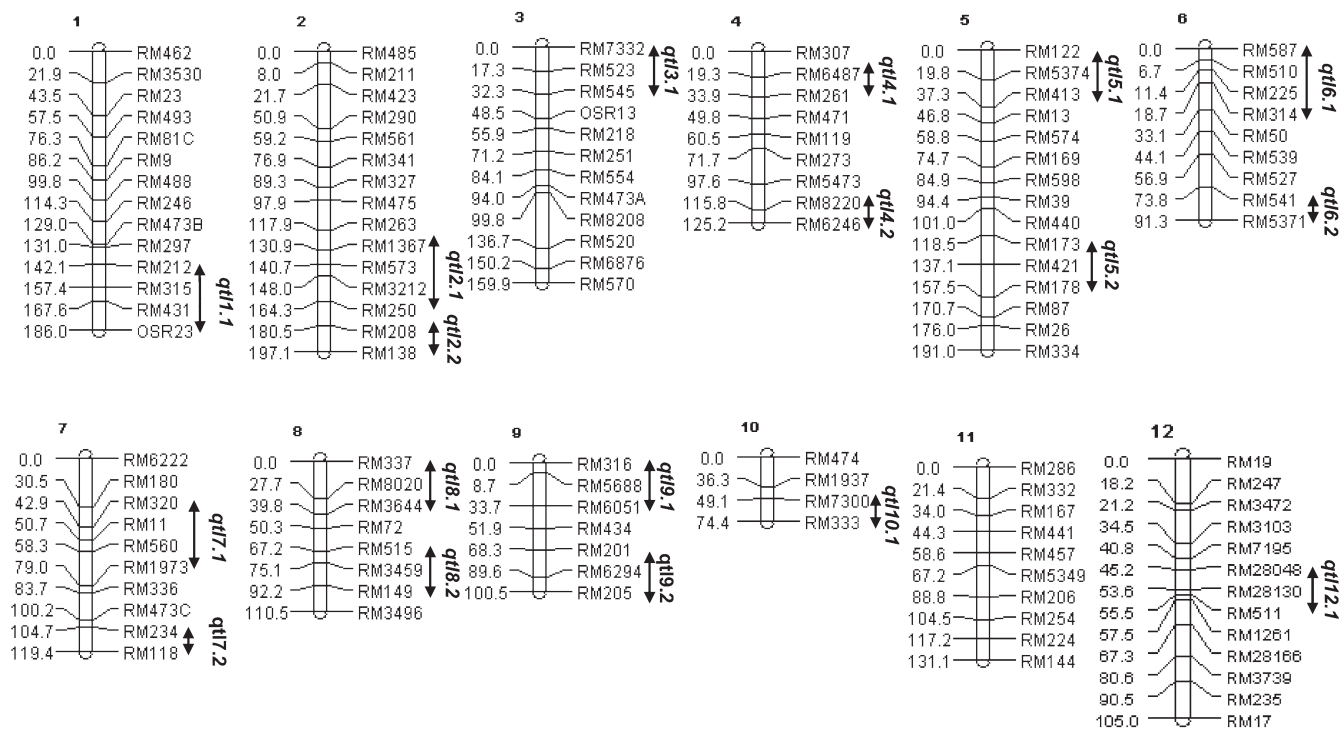


Figure 1. Genetic map locating all QTL intervals identified under drought stress and nonstress conditions in Vandana/Way Rarem.

to slightly lower grain yield in 2006. The lower harvest index in the 2006 nonstress trial can partially be explained by the stem borer (unidentified species) infestation and the typhoon that hit this trial when a lot of material was ready to be harvested.

In all trials, there was a large growth duration difference between Vandana (64 d to flower) and Way Rarem (91 d to flower). The two parents differed for grain yield and harvest index under stress but not under nonstress conditions (Table 1a and b). Those differences do not necessarily imply that Vandana is much more drought resistant than Way Rarem because of the large difference in growth duration. In this experiment, the drought-response index of the parents did not differ significantly, indicating that the difference in grain yield under stress between Vandana and Way Rarem may have been due mainly to their very different phenologies (Table 1a). The parents also differed significantly in the number of panicles produced per m<sup>2</sup>, with Vandana producing many more.

Under nonstress conditions, population means for grain yield, biomass yield, harvest index, final plant height, and plant height at 4 wk were all higher than in either parent. However, the mean number of days to 50% flowering for the population was intermediate to the parental means (Table 1b).

Under severe drought stress conditions, large variation in performance was observed among the lines. Averaged over the 2 yr, a total of 44 lines yielded less than 100 kg ha<sup>-1</sup>, while 10 yielded more than 1000 kg ha<sup>-1</sup>. Population mean biomass yield and plant height under stress were higher than for either parent in both years. Popula-

tion mean flowering delay (1.9 d) was also higher than for either parent, but the average grain yield, harvest index, days to flowering, and panicle number of the population was intermediate to that of the parents (Table 1a). Despite the relatively early onset of stress in 2006, panicle number was not greatly affected by stress, with a reduction of only 5% compared with the nonstress trials. Many lines flowered earlier under stress than under nonstress conditions, some by up to 14 d. Acceleration of flowering under drought stress has been observed previously in some short-duration rice cultivars (Lafitte et al., 2006).

Heritability estimates for the number of days to flowering and plant height were high in all trials. The estimate obtained from the combined analysis over years of grain yield under stress (0.70) was much higher than that obtained under nonstress conditions (0.23) (Table 1a and b). The genotype × year interaction variance for grain yield under stress was low (Table 2), and *H* estimates were relatively high within individual years (Table 1). Biomass yield and harvest index also exhibited a higher level of heritability under drought stress than under nonstress conditions. This demonstrates that the drought screening protocol, which ensured that water-stress was imposed for the entire period of reproductive growth, was highly repeatable. Several factors such as germination problems in both years due to water-logging immediately after sowing, stem borer infestations, and the 2006 preharvest typhoon contributed to the low *H* of the nonstress trials. Although rice commonly is grown in flooded fields, the crop does not always germinate well when direct seeded under anaerobic conditions (Dennis et al., 2000), and stem borers are a major insect pest of rice that cause formation of “dead

**Table 2. Means of homozygous classes and variance partitioning of SSR marker RM511 for traits measured under drought and well-watered conditions in a population of 436 random F<sub>3</sub>-derived lines from Vandana/Way Rarem. IRRD dry season 2005 and 2006.**

	Grain yield		Biomass yield		Harvest index		Days to 50% flowering		Height cm		Plant height at 4 wk
	Stress	Nonstress	Stress	Nonstress	Stress	Nonstress	Stress	Nonstress	Stress	Nonstress	
<i>Means of homozygous classes</i>											
Vandana homozygotes	216	2745	6367	11039	0.05	0.26	86	82	78	107	30
Way Rarem homozygotes	555	2835	7062	11221	0.10	0.27	79	80	82	108	31
<i>p</i> -value	< 0.0001	NS	< 0.0001	NS	< 0.0001	NS	< 0.0001	NS	< 0.0001	NS	< 0.0001
<i>Variance components (%)</i>											
$\sigma_G^2$	44	10	24	8	44	12	78	78	19	25	22
$\sigma_{QTL}^2$	22	0	10	0	15	0	7	0	7	0	2
$\sigma_{Line(QTL)}^2$	22	10	14	8	29	12	71	78	12	25	20
$\sigma_{G \times Year}^2$	14	22	12	7	8	0	7	9	14	9	13
$\sigma_{Year \times QTL}^2$	2	2	1	0	1	0	0	0	0	0	3
$\sigma_{Year \times Line(QTL)}^2$	12	20	11	7	7	0	7	9	14	9	10
$\sigma_E^2$	42	68	64	85	48	88	15	13	67	66	65
<i>R</i> <sup>2</sup> of RM511 (%)	36	0	21	0	26	0	9	0	16	0	5
Genetic variance explained by RM511 (%)	51	0	40	0	35	0	10	0	36	0	8

hearts” and sterile panicles. Effective chemical control of stem borers is difficult since the insect lives within the rice stems (Ho et al., 2006). Stress trials were planted on different dates compared with nonstress trials and escaped the worst of these problems.

### Genotypic Correlations

The low but positive genetic correlation (0.44) observed between grain yield under stress and nonstress conditions indicates that selection for yield potential should also result in an improvement in yield under drought stress and vice-versa (Table 3). However, this relatively low correlation, combined with the higher heritability for grain yield under stress conditions, indicate that greater gains in yield under drought stress would be obtained by selecting directly within a stress environment than indirectly under nonstress conditions. Factors highly correlated with grain yield under severe stress were days to flower under stress (−0.73) and nonstress (−0.59) as well as harvest index under stress (0.94) and nonstress (0.57). The very strong correlation observed between grain yield under stress and harvest index under stress indicates that the yield differences we observed under drought stress were mostly the result of a large difference in the capacity of plants to maintain seedset under stress, rather than to accumulate biomass. This is consistent with many other reports that the main cause of yield reduction when drought stress is applied around flowering is spikelet sterility (Liu et al., 2006). Under nonstress conditions, the contribution of harvest index to grain yield was lower, although it still remained high, with a genetic correlation 0.73. The reduced correlation of grain yield under water stress with days to flower under stress relative to days to flower under nonstress indicates that lines that

yielded poorly under stress conditions tended to experience flowering delay. Delay in flowering under stress is caused by a combination of slower floral development and reduced panicle elongation rate (Lafitte et al., 2004a). Delayed flowering under drought stress is associated with susceptibility to drought and has previously been shown to be associated with reductions in grain yield and harvest index (Pantuwan et al., 2002).

### QTL Analysis

#### Grain Yield

Results from the QTL analysis are presented in Table 4a for the stress trials and Table 4b for the nonstress trials. A very large QTL for grain yield under stress was detected in the centromeric region of Chromosome 12 (*qtl12.1*) (Fig. 2). The CIM analysis over 2 yr using all 436 lines resulted in the localization of this QTL in the interval between RM28048 (45.2 cM) and RM511 (55.5 cM). The additive effect of the Way Rarem allele at *qtl12.1* was 172 kg ha<sup>−1</sup>, explaining 33% of the total phenotypic variance for grain yield under stress. Significant QTL were also detected under stress conditions in the *qtl12.1* region for biomass yield, harvest index, days to flower, final plant height, flowering delay, drought-response index, and panicle number. Apart from plant height 4 wk after seeding, no traits measured in nonstress environments exhibited a QTL in this region. Surprisingly, the yield-increasing allele at this QTL is contributed by Way Rarem, the more drought-susceptible parent, suggesting an epistatic interaction between this locus and other loci from the Vandana genetic background. We could not, however, detect such an interaction at the digenic level in this population. There are other examples

**Table 3. Genotypic correlations among 2-yr trait means for 436 random F<sub>3</sub>-derived lines from Vandana/Way Rarem in stress and nonstress environments: IRRI dry seasons 2005 and 2006.**

		Grain yield		Biomass yield		Harvest index		Days to 50% flowering		Height Stress
		Stress	Nonstress	Stress	Nonstress	Stress	Nonstress	Stress	Nonstress	
Grain yield	Nonstress	0.44								
Biomass yield	Stress	0.39	0.55							
	Nonstress	NS <sup>†</sup>	0.49	0.65						
Harvest index	Stress	0.94	NS	0.19	-0.33					
	Nonstress	0.57	0.73	-0.26	0.07	0.56				
Days to 50% flowering	Stress	-0.73	NS	0.16	0.35	-0.81	-0.49			
	Nonstress	-0.59	0.21	0.37	NS	-0.73	-0.35	0.98		
Plant Height	Stress	0.22	0.35	0.72	0.53	0.07	NS	-0.05	0.14	
	Nonstress	0.15	0.08	0.31	0.69	-0.25	-0.35	0.21	NS	0.79

<sup>†</sup>NS, not significant at  $p < 0.05$ .

of grain yield under drought stress QTL being contributed by the drought-susceptible parent; however, those are often of small effect and likely to be related to yield potential rather than drought tolerance per se (Lafitte et al., 2004b; Lanceras et al., 2004). In the case of *qtl12.1*, the effect is large and unrelated to yield potential.

This region has been reported to have some effect on drought-related traits in two other studies. QTL for yield reduction under drought stress and for panicle number under stress were reported in an advanced backcrossing QTL experiment (Xu et al., 2005). In this experiment, this region also contributed to grain yield under nonstress conditions, but in our case, the allele is only expressed under stress conditions. Another backcross QTL experiment revealed that this locus contributes to drought-tolerance at the seedling stage using various concentrations of polyethylene glycol (Zhang et al., 2006). In both of these cases, the favorable allele was contributed by the *indica* recurrent parent. *Qtl12.1* also overlaps with the *Pup1* locus, a major phosphorus uptake QTL (Wissuwa et al., 2002).

A total of five genomic regions had significant effects on grain yield under nonstress conditions (*qtl2.1*, *qtl3.1*, *qtl7.1*, *qtl8.1*, and *qtl10.1*). The only region that had significant effects in both years was *qtl2.1*, located near RM3212 on Chromosome 2. This region was also associated with panicle number under stress. The yield increasing allele at this locus was contributed by Vandana and explains about 11% of the phenotypic variation in the combined analysis over years (Table 4b).

### Days to Flower

A large-effect QTL for days to flower under both stress and nonstress conditions was identified on Chromosome 3 near marker RM523 (*qtl3.1*) (Table 4a and b). This QTL had a LOD of 34.4 (stress) and 52.3 (nonstress) for means over 2 yr, resulting in an additive effect of 11.2 and 8.6 d, respectively, for the stress and nonstress trials, with the duration-increasing allele contributed by Way Rarem, the long-duration parent. This region had a significant effect on days to flower in

all four trials as well as on biomass yield under stress and nonstress conditions and plant height under nonstress conditions. The effect measured at this locus is probably caused by the gene *Hd9*, which has previously been fine-mapped within a few centimorgans of RM523 (Lin et al., 2002).

Another QTL (*qtl5.1*) affecting days to flower over more than one trial was significant in the nonstress trial of 2006 as well as in the stress trial of 2005 and for average of the two stress trials. The duration-increasing allele in this case came from Vandana. Two other QTL were detected for days to flower under stress in 2005 and over the 2-yr average (*qtl1.1* and *qtl7.2*). In both cases, the duration-increasing allele was contributed by Way Rarem.

The effect of *qtl12.1* on flowering occurred only under stress conditions. The effect of the Way Rarem allele was to reduce days to flowering under stress in both years by approximately 3 d, or, in other words, to reduce flowering delay due to stress.

### Biomass Yield

As noted above, *qtl12.1* had a large effect on biomass yield under stress. *Qtl3.1* and *qtl6.1* also significantly affected this trait in 2005, but not in 2006. The effect of *qtl3.1* on biomass probably results from the fact that the Way Rarem allele at this locus increases crop duration. In the case of *qtl6.1*, the favorable allele was contributed by Vandana and the increase in biomass is probably a consequence of an increased panicle number, as a QTL for panicle number under stress is located in the same marker interval.

Three QTL for biomass yield under nonstress conditions were identified on the basis of the 2-yr mean. One of those loci corresponds to *Hd9* (*qtl3.1*) and is contributed by Way Rarem. A larger-effect QTL is also contributed by Way Rarem (*qtl9.1*), while the one with the smallest effect (*qtl4.1*) was contributed by Vandana.

### Plant Height

A QTL for plant height under both environments contributed by Vandana is located near marker RM315 on

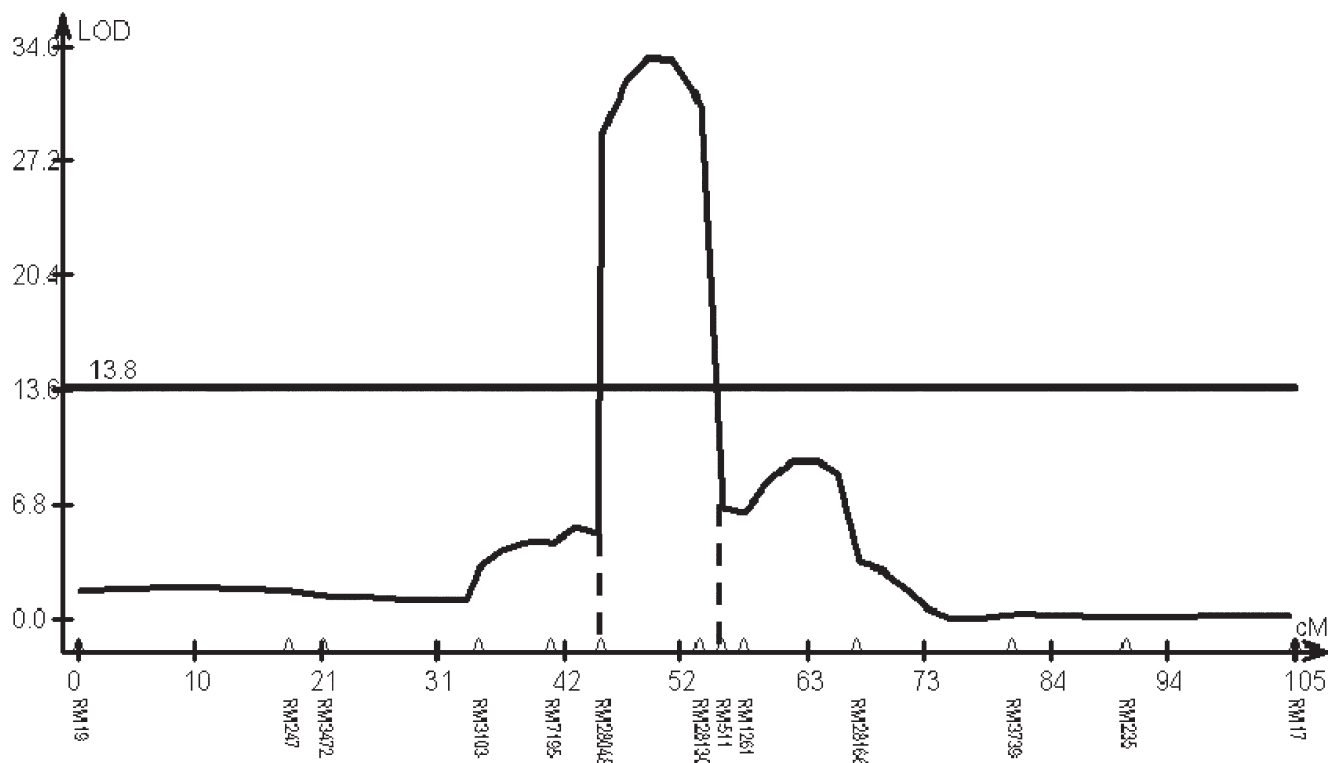


Figure 2. QTL likelihood curves of the LOD score of grain yield under stress for Chromosome 12. The SSR marker locations are listed on the Y axis. The black horizontal line indicates the significance threshold of LOD score 13.8 to detect putative QTLs. The vertical dotted lines indicate the position of *qtl12.1*.

Chromosome 1 (*qtl1.1*). This QTL was identified in both nonstress trials as well as the 2006 stress trial and also affected plant height 4 wk after seeding. Given that RM315 has previously been mapped to the same location as RG220 (Robin et al., 2003) and that RG220 is known to be only 0.3 cM away from *Sd1* (Lin et al., 2002), we assume that the differences in plant height observed here result from polymorphism between the two parents at the *Sd1* locus.

Under nonstress conditions, a further four QTL affecting plant height were identified (*qtl3.1*, *qtl4.2*, *qtl5.2*, and *qtl6.2*). *Qtl3.1* was identified in 2005 only and *qtl4.2* in 2006 only. *Qtl5.2* and *qtl6.2* were significant based on the combined analysis and in both cases; the height-enhancing allele was contributed by Vandana. *Qtl5.2* also showed a significant effect on plant height 4 wk after seeding, but the three other QTL did not. *Qtl12.1* is the only locus that had an effect on plant height under stress conditions only.

Analysis of plant height 4 wk after seeding, on the average of four trials, revealed five significant QTL (*qtl1.1*, *qtl5.2*, *qtl8.2*, *qtl9.1*, and *qtl12.1*). Three of those QTL had been identified to have a significant effect on other traits (*qtl1.1*, *qtl5.2*, and *qtl12.1*), while the last two were detected exclusively for plant height 4 wk after seeding.

### Harvest Index

Only *qtl12.1* had a significant effect on harvest index under stress. Two loci (*qtl2.2* and *qtl8.2*) had significant effects on harvest index under nonstress conditions in 2005, but

none did in 2006 and no loci had significant effects in the combined analysis over the 2 yr.

### Characterizing the Effects of *qtl12.1* through Single-Marker Analysis

To better understand the effects of the major QTL for grain yield under stress identified at *qtl12.1* in the CIM analysis, we performed a single-marker analysis for all traits under both stress and nonstress conditions for RM511, the marker within the *qtl12.1* region with the largest effect on grain yield under stress. Under severe stress, RM511 had a significant effect on grain yield, biomass yield, plant height, days to flower, drought-response index, and flowering delay (Table 2). RM511 had no significant effect on any trait measured in the nonstress environments, except for a very small effect on plant height at 4 wk after seeding, with this locus explaining only 8% of the total genetic variance.

Variance components presented in Table 2 indicate that *qtl12.1* explained a large proportion of the genetic variance for the following stress traits: grain yield (51%), biomass yield (40%), harvest index (35%), and plant height (36%). It contributed to the genetic variance of days to flower under stress (10%) and plant height 4 wk after seeding (8%) to a lesser extent. Genotype  $\times$  year interaction affected all traits except harvest index under nonstress conditions (Table 2). However, in the traits for which *qtl12.1* had a significant effect, the proportion of this interaction explained by *qtl12.1* was very low, with most of the

**Table 4. (a) QTL identified under upland drought-stress conditions at flowering and grain-filling stages in F<sub>3</sub>-derived lines from Vandana/Way Rarem: IRRI, dry season 2005 and 2006. (b) QTL identified under well-watered upland conditions over two consecutive dry seasons in F<sub>3</sub>-derived lines from Vandana/Way Rarem: IRRI, dry season 2005 and 2006.**

(a)						2005			2006			Combined		
Trait	Chr.	Interval	Marker closest to LOD peak	Position	QTL name	LOD	Add. <sup>†</sup>	R <sup>2</sup>	LOD	Add.	R <sup>2</sup>	LOD	Add.	R <sup>2</sup>
				<b>cM</b>				<b>%</b>			<b>%</b>			<b>%</b>
Grain yield (kg ha <sup>-1</sup> )	12	<i>RM28048-RM511</i> <sup>‡</sup>	RM28130	49	<i>qtl12.1</i>	29.7	221	30	21.2	146	24	34	172	33
Biomass yield (kg ha <sup>-1</sup> )	3	RM523-RM545	RM545	25	<i>qtl3.1</i>				4.8	914	19			
	6	RM587-RM314	RM225	11	<i>qtl6.1</i>				5.2	-425	5	4.8	-233	2
	12	<i>RM28048-RM28166</i>	RM28130	51	<i>qtl12.1</i>	18	765	16	12.5	587	10	23	634	18
Harvest index	12	RM7195-RM28166	RM1261	61	<i>qtl12.1</i>	21.8	0.04	25				22	0.03	26
Days to 50% flowering	1	RM431-OSR23	OSR23	184	<i>qtl1.1</i>	5.4	5.6	15				5.1	4.7	13
	3	<i>RM7332-RM545</i>	RM523	14	<i>qtl3.1</i>	30	12.7	66	29.7	10.1	55	34	11.2	64
	5	RM122-RM5374	RM122	4	<i>qtl5.1</i>	5.1	-4.3	11				4.8	-3.6	9
	7	RM234-RM118	RM118	117	<i>qtl7.2</i>	5.6	6	18				5	4.7	14
	12	<i>RM3103-RM511</i>	RM28048	47	<i>qtl12.1</i>	7.9	-3.3	5	5.8	-2.9	6	7.3	-3.2	6
Plant height at maturity (cm)	1	RM212-RM431	RM315	152	<i>qtl1.1</i>	3.8	-1.8	5				5.1	-1.8	9
	12	<i>RM28048-RM28166</i>	RM1261	51	<i>qtl12.1</i>	9.1	2.2	8	4.9	0.7	1	9.8	1.4	5
Panicle number m <sup>-2§</sup>	2	RM1367-RM250	RM3212	148	<i>qtl2.1</i>				4	-14	3			
	6	RM587-RM314	RM225	11	<i>qtl6.1</i>				4	-22	5			
	12	RM7195-RM28166	RM28130	51	<i>qtl12.1</i>				12.9	26	9			
Flowering delay <sup>¶</sup>	12	RM7195-RM28166	RM28130	51	<i>qtl12.1</i>							19	-2.4	16
Drought-response index	12	RM28048-RM511	RM28130	51	<i>qtl12.1</i>							39	0.89	37

(b)						2005			2006			Combined		
Trait	Chr.	Interval	Marker closest to LOD peak	Position	QTL name	LOD	Add.	R <sup>2</sup>	LOD	Add.	R <sup>2</sup>	LOD	Add.	R <sup>2</sup>
				<b>cM</b>				<b>%</b>			<b>%</b>			<b>%</b>
Grain yield (kg ha <sup>-1</sup> )	2	<i>RM1367-RM250</i>	RM3212	148	<i>qtl2.1</i>	6.3	-223	8	5.4	-167	3	10	-219	11
	3	RM7332-RM545	RM523	14	<i>qtl3.1</i>	8.7	294	11				3.9	189	8
	7	RM320-RM1973	RM11	47	<i>qtl7.1</i>				4.2	-146	2			
	8	RM337-RM3644	RM8020	28	<i>qtl8.1</i>							3.7	173	7
	10	RM7300-RM333	RM7300	57	<i>qtl10.1</i>							4	-97	2
Biomass yield (kg ha <sup>-1</sup> )	3	RM7332-RM523	RM7332	6	<i>qtl3.1</i>							5.4	802	11
	4	RM6487-RM471	RM261	38	<i>qtl4.1</i>							3.8	-415	3
	9	RM201-RM205	RM6294	90	<i>qtl9.2</i>							4.1	979	13
Harvest index	2	RM208-RM138	RM208	186	<i>qtl2.2</i>	3.7	-0.021	9						
	8	RM3459-RM149	RM3459	81	<i>qtl8.2</i>	3.7	0.022	10						
Days to 50% flowering	3	<i>RM7332-RM545</i>	RM523	12	<i>qtl3.1</i>	63.6	9.5	85	24.6	7	51	52.3	8.6	79
	5	RM122-RM5374	RM122	0	<i>qtl5.1</i>				6.9	-2.5	10			
Plant height at maturity (cm)	1	<i>RM315-OSR23</i>	RM431	165	<i>qtl1.1</i>	7.7	-2.6	12	6.7	-3.4	11	10.2	-2.7	16
	3	RM7332-RM523	RM7332	8	<i>qtl3.1</i>	4.6	0.3	0						
	4	RM8220-RM6246	RM8220		<i>qtl4.2</i>				3.9	-0.9	1			
	5	<i>RM173-RM178</i>	RM421	133	<i>qtl5.2</i>	4.6	-2.6	12	3.6	-2.9	7	5.1	-0.2	10
	6	RM541-RM5371	RM5371	86	<i>qtl6.2</i>							3.8	-3.4	19
Plant height 4 wk after sowing (cm) <sup>#</sup>	1	RM212-RM431	RM315	159	<i>qtl1.1</i>							7.5	-0.7	12
	5	RM440-RM421	RM173	125	<i>qtl5.2</i>							4.5	-0.7	10
	8	RM515-RM3459	RM3459	73	<i>qtl8.2</i>							4.4	0.3	2
	9	RM316-RM5688	RM316	0	<i>qtl9.1</i>							4.3	0.6	9
	12	RM28048-RM28166	RM1261	57	<i>qtl12.1</i>							4.5	0.4	3

<sup>†</sup>The additive value is half the difference between the phenotypic value of the two homozygous classes. A positive value indicates that the allele increasing the trait value originates from Way Rarem.

<sup>‡</sup>Loci that are italicized indicate loci that were detected in both years.

<sup>§</sup>Panicle number was counted in 2006 only.

<sup>¶</sup>Flowering delay and drought-response index were only calculated using the combined means over 2 yr.

<sup>#</sup>Plant height 4 wk after seeding was only analyzed using the combined data.

genotype  $\times$  year variance resulting from variation among lines within marker genotype classes. *Qtl12.1* accounted for only 12% of the total genotype  $\times$  year interaction for grain yield under stress and 7% for biomass yield under stress, indicating that this QTL is fairly stable across years and would be suitable for MAS.

## CONCLUSIONS

In this experiment, we identified 18 genomic regions influencing yield or yield component traits in drought-stressed and nonstress environments. We identified a highly significant locus (*qtl12.1*) for grain yield under stress. This QTL explained 51% of genetic variation for the trait and had an additive effect estimated as 172 kg ha<sup>-1</sup> (47% of the trial mean). This is the first report of a QTL with a large and repeatable effect on grain yield under severe drought conditions in a field experiment. The effect of this QTL, located in a 10-cM region between RM28048 and RM511, was observed consistently during two consecutive dry seasons where plants were subjected to severe drought stress at flowering. *Qtl12.1* also had a significant effect on a wide range of other traits under severe stress, including biomass yield, flowering delay, harvest index, plant height, drought-response index, panicle number, and the number of days to 50% flowering. This QTL therefore appears to increase grain yield under stress by increasing the number of panicles, the biomass accumulation, and the harvest index while reducing flowering delay. Since *qtl12.1* had no effect on grain yield under nonstress conditions and is not significantly associated with days to flower under nonstress conditions, it seems to be specifically involved in drought resistance. The fact that this QTL for grain yield under severe stress coincides with QTL for harvest index under stress and flowering delay, but not for days to flowering under nonstress conditions, indicates that the locus affects grain yield under stress primarily through effects on panicle exertion and spikelet fertility under stress and that its effects are due to its effect on plant water status rather than on drought avoidance through earlier flowering. Research is underway to clarify the physiological effects of the locus.

The effect size of this locus in the current population seems to be large enough to support fine-mapping, and the very low genotype  $\times$  year interaction gives this locus a potential for use in MAS to improve the drought tolerance of the eastern Indian upland parent, Vandana. Because this parent is already considered to have good drought tolerance, and is currently grown in the severely drought-prone upland environment of the eastern Indian plateau, an improvement in its drought tolerance could be of significant benefit to farmers in the region who depend on upland rice for food security. Fine-mapping of the locus is currently under way.

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## References

- Asíns, M.J. 2002. Review: Present and future of quantitative trait locus analysis in plant breeding. *Plant Breed.* 121:281–291.
- Babu, R.C., J.X. Zhang, A. Blum, T.H.D. Ho, R. Wu, and H.T. Nguyen. 2004. HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) via cell membrane protection. *Plant Sci.* 166:855–862.
- Babu, R.C., B.D. Nguyen, V. Chamarek, P. Shanmugasundaram, P. Chezhan, P. Jeyaprakash, S.K. Ganesh, A. Palchamy, S. Sadasivam, S. Sarkarung, L.J. Wade, and H.T. Nguyen. 2003. Genetic analysis of drought resistance in rice by molecular markers: Association between secondary traits and field performance. *Crop Sci.* 43:1457–1469.
- Bernardo, R. 2002. Breeding for quantitative traits in plants. Stemma Press, Woodburn, MN.
- Bidinger, F.R., V. Mahalakshmi, and G.D.P. Rao. 1987. Assessment of drought resistance in pearl millet [*Pennisetum americanum* (L.) Leeke]. II Estimation of genotype response to stress. *Aust. J. Agric. Res.* 38:49–59.
- Churchill, G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* 138:963–971.
- Cooper, M., I.H. DeLacy, and K.E. Basford. 1996. Relationship among analytical methods used to analyse genotypic adaptation in multi-environment trials. p. 193–224. *In* M. Cooper and G. L. Hammer (ed.) *Plant adaptation and crop improvement*. Univ. Press, Cambridge, UK.
- Courtois, B., G. McLaren, P.K. Sinha, K. Prasad, R. Yadav, and L. Shen. 2000. Mapping QTL associated with drought avoidance in upland rice. *Mol. Breed.* 6:55–66.
- Courtois, B., L. Shen, W. Petalcorin, S. Carandang, R. Mauleon, and Z. Li. 2003. Locating QTLs controlling constitutive root traits in the rice population IAC 165  $\times$  Co39. *Euphytica* 134:335–345.
- Darvasi, A., and M. Soller. 1992. Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. *Theor. Appl. Genet.* 85:353–359.
- Dennis, E.S., R. Dolferus, M. Ellis, M. Rahman, Y. Wu, F.U. Hoeren, A. Grover, K.P. Ismond, A.G. Good, and W.J. Peacock. 2000. Molecular strategies for improving waterlogging tolerance in plants. *J. Exp. Bot.* 51:89–97.
- Fukai, S., and M. Cooper. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. *Field Crops Res.* 40:67–86.
- Ho, N.H., N. Baisakh, N. Oliva, K. Datta, R. Frutos, and S.K. Datta. 2006. Translational fusion hybrid Bt genes confer resistance against yellow stem borer in transgenic elite vietnamese rice (*Oryza sativa* L.) cultivars. *Crop Sci.* 46:781–789.

- IRGSP. 2005. The map-based sequence of the rice genome. *Nature* 436:793–800.
- Khush, G.S. 1997. Origin, dispersal, cultivation and variation of rice. *Plant Mol. Biol.* 35:25–34.
- Khush, G.S. 2005. What it will take to feed 5.0 billion rice consumers by 2030. *Plant Mol. Biol.* 59:1–6.
- Lafitte, H.R., A. Ismail, and J. Bennet. 2004a. Abiotic stress tolerance in rice for Asia: Progress and the future. In T. Fischer et al. (ed.) *New directions for a diverse planet: Proceedings of the 4th International Crop Science Congress, Brisbane, Australia.*
- Lafitte, H.R., A.H. Price, and B. Courtois. 2004b. Yield response to water deficit in an upland rice mapping population: Associations among traits and genetic markers. *Theor. Appl. Genet.* 109:1237–1246.
- Lafitte, H.R., Z.K. Li, C.H.M. Vijayakumar, Y.M. Gao, Y. Shi, J.L. Xu, B.Y. Fu, S.B. Yu, A.J. Ali, J. Domingo, R. Maghirang, R. Torres, and D.J. Mackill. 2006. Improvements of rice drought tolerance through backcross breeding: Evaluation of donors and selection in drought nurseries. *Field Crops Res.* 97:77–86.
- Lanceras, J.C., G.P. Pantuwan, B. Jongdee, and T. Toojinda. 2004. Quantitative trait loci associated with drought tolerance at reproductive stage in rice. *Plant Physiol.* 135:384–399.
- Lander, E.S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185–199.
- Lin, H., M. Ashikari, U. Yamanouchi, T. Sasaki, and M. Yano. 2002. Identification and characterization of a quantitative trait locus, *Hd9*, controlling heading date in rice. *Breed. Sci.* 52:35–41.
- Liu, J.K., D.Q. Liao, R. Oane, L. Estenor, X.E. Yang, Z.C. Li, and J. Bennett. 2006. Genetic variation in the sensitivity of anther dehiscence to drought stress in rice. *Field Crops Res.* 97:87–100.
- Manly, K.F., R.H. Cudmore, Jr., and J.M. Meer. 2001. Map Manager QTX, cross-platform software for genetic mapping. *Mamm. Genome* 12:930–932.
- McCouch, S.R., L. Teytelman, Y. Xu, K.B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing, Q. Zhang, I. Kono, M. Yano, R. Fjellstrom, G. DeClerck, D. Schneider, S. Cartinhour, D. Ware, and L. Stein. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Res.* 9:199–207.
- Panaud, O., X. Chen, and S. McCouch. 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). *Mol. Gen. Genet.* 252:597–607.
- Pantuwan, G., S. Fukai, M. Cooper, S. Rajatasereekul, and J.C. O'Toole. 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.
- Peng, S., K.G. Cassman, S.S. Virmani, J. Sheehy, and G.S. Khush. 1999. Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. *Crop Sci.* 39:1552–1559.
- Piepho, H.-P., A. Büchse, and K. Emrich. 2003. A hitchhiker's guide to mixed models for randomized experiments. *J. Agron. Crop Sci.* 189:310–322.
- Price, A.H. 2002. QTLs for root growth and drought resistance in rice. p. 563–584. In S. Mohan Jain et al. (ed.) *Molecular techniques in crop improvement.* Kulwer Academic Publisher, Norwell, MA.
- Price, A.H., J.E. Cairns, P. Horton, H.G. Jones, and H. Griffiths. 2002. Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: Progress and new opportunities to intergrade stomatal and mesophyll responses. *J. Exp. Bot.* 53:989–1004.
- Robin, S., M.S. Pathan, B. Courtois, H.R. Lafitte, S. Carandang, S. Lanceras, M. Amante, H.T. Nguyen, and Z. Li. 2003. Mapping osmotic adjustment in an advanced back-cross inbred population of rice. *Theor. Appl. Genet.* 107:1288–1296.
- Salekdeh, G.H., J. Siopongco, L.J. Wade, B. Ghareyazie, and J. Bennett. 2002. A proteomic approach to analyzing drought- and salt-responsiveness in rice. *Field Crops Res.* 76:199–219.
- SAS Institute, Inc. 2002, 2003, 2004. SAS online doc 9.1.3. SAS Institute Inc., Cary, NC.
- Shen, L., B. Courtois, K.L. McNally, S. Robin, and Z. Li. 2001. Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. *Theor. Appl. Genet.* 103:75–83.
- Steele, K.A., A.H. Price, H.E. Shashidar, and J.R. Witcombe. 2006. Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theor. Appl. Genet.* 112:208–221.
- Temnykh, S., G. DeClerck, A. Lukashova, L. Lipovich, S. Cartinhour, and S. McCouch. 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.
- Wang, S., C.J. Basten, and Z.B. Zeng. 2005. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (<http://statgen.ncsu.edu/qlcart/WQTLCart.htm>; verified 8 January 2007)
- Wissuwa, M., J. Wegner, N. Ae, and M. Yano. 2002. Substitution mapping of *Pup1*: A major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. *Theor. Appl. Genet.* 105:890–897.
- Xu, J.L., H.R. Lafitte, Y.M. Gao, B.Y. Fu, R. Torres, and Z.K. Li. 2005. QTLs for drought escape and tolerance identified in a set of random introgression lines of rice. *Theor. Appl. Genet.* 111:1642–1650.
- Yue, B., W. Xue, L. Xiong, X. Yu, L. Luo, K. Cui, D. Jin, Y. Xing, and Q. Zhang. 2005. Genetic basis of drought resistance at reproductive stage in rice: Separation of drought tolerance from drought avoidance. *Genetics* 172:1213–1228.
- Zhang, X., S. Zhou, Y. Fu, Z. Su, X. Wang, and C. Sun. 2006. Identification of a drought tolerant introgression line derived from Dongxiang common wild rice (*O. rufipogon* Griff.). *Plant Mol. Biol.* 62:247–259.
- Zhao, D.L., G.N. Atlin, L. Bastiaans, and J.H.J. Spiertz. 2006. Cultivar weed-competitiveness in aerobic rice: Heritability, correlated traits, and the potential for indirect selection in weed-free environments. *Crop Sci.* 46:372–380.