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ORIGINAL RESEARCH

Identification of QTLs for Drought-Related Traits in Alien Introgression Lines Derived from Crosses of Rice (*Oryza sativa* cv. IR64) × *O. glaberrima* under Lowland Moisture Stress

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Abstract Drought is a major abiotic stress that limits rice productivity in rain-fed and upland ecosystems. African rice, *Oryza glaberrima*, has low yields but is tolerant to drought and other stresses. We evaluated 513 BC_2F_3 progenies from alien introgression lines (AILs) that were derived from crosses of *Oryza sativa* (IR64) × *O. glaberrima*. They were assessed for yield and other traits when grown under drought at two locations. Such conditions reduced grain production by 59% compared with the recurrent parent (IR64). However, 33 AILs had higher yields, thus demonstrating their potential as genetic material for transferring drought-related traits from *O. glaberrima* to *O. sativa.* A set of 200 AILs was selectively genotyped with 173 simple sequence repeat and sequenced tagged site markers. Molecular analysis showed that a mean of 4.5% of the *O. glaberrima* genome was introgressed in BC_2F_3 AILs. Our analysis revealed 33 quantitative trait loci (QTLs; including 10 novel) for different traits. *O. glaberrima* contributed 50% of the alleles to those newly identified QTLs, with one for grain yield per plant (*ypp9.1*) being new. A QTL at RM208 on chromosome 2 positively affected yield under stress, accounting for 22% of the genetic variation. Our identification of drought-related QTLs for yield and yield components will be useful to future research efforts in marker-assisted selection.

Keywords AIL · Alien introgression line · Drought · *Oryza glaberrima* · *O. sativa* · QTL · SSR · STS

Abbreviations

AIL	Alien introgression line
Chr	Chromosome
IRRI	International Rice Research Institute
LOD	Log-likelihood
LRS	Likelihood ratio statistics
QTL	Quantitative trait locus
SSR	Simple sequence repeat
STS	Sequenced tagged site

Rice is the major food crop for more than one third of the world's population. Its grain production more than doubled between 1996 and 2007, from 252 million tons to about 600 million tons (FAO 2008). However, by 2025, a 25% increase in supply will be necessary to meet growing demand. For example, in African nations, this need is

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driven by both population growth and a shift in consumer preference for rice, especially in urban areas. From 2001 to 2005, the average amount of milled rice produced annually in Sub-Saharan Africa was 8.1 million tons (WARDA 2008). Rice imports into that region now account for 25% of the global total, at an annual cost of more than US $1.5 billion (WARDA 2008). Productivity is affected by both biotic (blast, bacterial blight, sheath blight, brown plant hopper, and stem borers) and abiotic stresses (drought, submergence, salinity, cold, iron or aluminum toxicities, and phosphorous deficiency).

At all stages of rice growth and development, drought is the major stressor, but it has the greatest impact during flowering, when grain formation is suppressed. This results in considerable yield losses under rain-fed and upland ecosystems (Serraj et al. 2009). Studies at the International Rice Research Institute (IRRI) have shown that drought significantly delays peduncle elongation, trapping a very large proportion of the panicle within the flag leaf sheath because expression of cell-wall invertase genes is decreased (Ji et al. 2005). Spikelets that remain within that sheath are usually sterile. This sterility can be of two types: (1) inhibition of starch accumulation in pollen grains or (2) failure of anther dehiscence and/or synchronization with anthesis due to the suspension of septum degradation and stomium breakage (Zhu et al. 2004). Drought that occurs during these processes causes reproductive organs to be damaged.

Though wild species of *Oryza* are phenotypically less desirable than modern varieties, many efforts have been made to develop drought-tolerant varieties using landraces and primitive cultivars of *Oryza sativa*. Some of the most successful examples have utilized *Oryza nivara* genes for resistance to grassy stunt virus (Plucknett et al. 1987), and various genes that confer resistance to brown planthopper, bacterial blight, blast, and tungrovirus, or tolerance to acid sulfate conditions, including cytoplasmic male sterility in rice (Brar and Khush 2006). Likewise, *Oryza spontanea* has been used as the source of wild abortive cytoplasmic male sterility in hybrid rice (Li and Zhu 1988). However, no such transfer from wild species has been made for enhancing drought tolerance. It has been difficult to utilize those genotypes for improving quantitatively inherited traits, e.g., yield, because the superior trait of interest cannot be identified phenotypically in wild accessions.

Rice varieties are urgently needed that use water efficiently and are tolerant to drought during different stages of growth, particularly in the reproductive period. Although *indica* rice has only limited genetic variability for drought tolerance, *Oryza glaberrima*, an indigenous African species, shows early plant vigor and resistances to drought, blast, rice yellow mottle virus, nematodes, and the African gall midge (Jones et al. 1997). It has several drought-avoidance mechanisms, including early or synchronized maturation toward the end of a wet season. Dingkuhn et al. (1999) have concluded that the phenological responses of *O. glaberrima* are superior to those of traditional and improved *O. sativa* cultivars from both tropical *japonica* and *indica* subspecies at the photoperiod-sensitive phase and under the hydrological conditions of West Africa. Its thin leaves roll quickly to retain water, and its small-diameter roots efficiently extract water and nutrients because of their close contact with soil particles.

The introduction of high-density molecular linkage maps has facilitated the identification of individual quantitative trait loci (QTLs) associated with yield factors, stress tolerance, disease and insect resistance, and other quality traits in many crop plants (Tanksley 1993). By using molecular markers, such as simple sequence repeats (SSRs), researchers have been able to report traits in rice that are putatively associated with performance under drought, e.g., the morphology, penetrability, and distribution of the root system; osmotic adjustments and dehydration tolerance, including cell membrane stability; early stomatal conductance; visual symptoms of leaf stress (rolling/drying); and the accumulation of abscisic acid (Price et al. 2000). Direct selection for grain yield under controlled drought stress has proven effective in screening for tolerance (Kumar et al. 2008; Venuprasad et al. 2009). Key QTLs have been identified for grain yield (*qtl12.1*) and its components under managed stress environments (Bernier et al. 2007; Kumar et al. 2008). In plant breeding programs, selective genotyping makes it possible to find QTLs using even a limited number of progeny that have been retained after selection. With this approach, QTLs with smaller effects and/or those that are more distant from the nearest marker can be detected. For example, by applying similar methods, Navabi et al. (2009) have assessed data from a population of 436 recombinant inbred rice lines segregating for large-effect QTLs that influence grain yield under drought. Those QTLs have been reliably detected by genotyping as few as 20 selected lines (4.5%).

Here, we describe how molecular markers—SSRs and sequenced tagged site (STS) markers—were used to determine yield and yield-enhancing QTLs from *O. glaberrima* into *indica* variety IR64. Backcross progenies (BC_2F_3) were screened for drought tolerance under field conditions.

Material and Methods

Population Development

From 2005 to 2007, crosses were made in a screenhouse between an elite *indica* rice cultivar, IR64 (female), and two drought-tolerant accessions of *O. glaberrima* (male). Those

males, referred to as RAM 54 and RAM 90 (no IRGC numbers assigned), had been field-tested in Mali, West Africa. Both are traditional cultivars that were collected from the internal delta of River Niger in Mali. A subset of those crosses was used to produce BC_2F_3 alien introgression lines (AILs). Data on agronomic traits were recorded for all BC_2F_3 families. Each BC_2F_3 AIL that was derived from BC_2F_2 was bulk-harvested and used for phenotyping of drought-related traits and for genotyping with SSR and STS markers. Of the 513 AILs tested, 288 were selected from the cross of IR64 × RAM 54, while 225 were obtained from the cross of IR64 × RAM 90.

Field Experimental Design

Those 513 advanced AILs from IR64 × *O. glaberrima* crosses were screened during the dry season (December 2007 to April 2008) for drought tolerance at the reproductive stage. Their selection had been based only on their fertility. During that dry season, "managed" stress was artificially imposed (see below). Screening was conducted on two lowland sites (S1 and S2) at the IRRI experimental fields, Los Baños, Laguna, the Philippines (14° N 121° E, 21 m above sea level). Here, "lowland" referred to a field under flooded, puddled, transplanted, or anaerobic conditions. At the first site, the test population included the recurrent parent, IR64, and four checks with a broad range of drought tolerance (MTU1010, IR55423-01, PSBRc68, and IR77298-14-1-2). At the second site, the same design was used except that only IR64 served as our check. Soil properties, the crop management scheme, and climatic conditions are shown in Table 1.

All sets were laid out in an alpha-lattice design with three replications. Rows were 2 m long and spaced 0.20 m between rows and between hills. One seedling was allowed per hill. Single superphosphate and potassium chloride were used at basal equivalents of 40 kg P ha^{-1} and 40 kg K ha^{-1} along with 120 kg N ha^{-1} in the form of ammonium sulfate. These fertilizers were applied in three even splits, at approximately 20, 40, and 60 days after seeding (DAS). Weeds were controlled initially with pre-emergence herbicides and then by hand-weeding. At both sites, basin irrigation was provided every 4 to 5 days to keep the soil at near field capacity, and tensiometers were installed to monitor the soil water status. Nonstressed trials were those in which plants continued to receive this normal schedule of irrigation. After the seedlings for this control treatment were transplanted (conducted only at Site S1), the field was maintained with approximately 5 cm of standing water before being drained prior to harvest. To induce drought in our stress tests, watering was restricted for 18 days beginning at the flowering stage. Irrigation was withheld until water tension in the top 20 cm of soil reached about 100 kPa, or an equivalent tensiometer reading of 75 cm Hg. These stressed plants were then basin-irrigated until the soil was saturated in the root zone. The stress cycle was then repeated. This irrigation regime resulted in leaf-rolling and tip-burning at the end of each drying cycle. After this 18-day period, the treated plants were re-watered at 10-day intervals until maturity.

Trait Evaluation

Plants were randomly selected from each AIL. Data were recorded for the following traits:

1. Days to 50% flowering/heading (days), i.e., the average number of days from seeding until 50% of the panicles had flowered;
2. Plant height (centimeter), average for three plants as measured from the soil surface to the tip of the tallest panicle (awns excluded);
3. Number of tillers per plant (n=6);
4. Percent seed set, calculated as the number of empty/fully filled spikelets divided by the total number of spikelets per panicle (n=6);
5. Grains per plant, i.e., the number of filled spikelets per plant;
6. 100-grain weight (gram), i.e., the average weight of 100 seeds from three samples of bulk-harvested grains from six plants;
7. Yield per plant (gram), for which six hills were bulk-harvested (six plants total) to estimate the grain yield from each plot. Seeds were then dried (50°C), weighed, and adjusted to a moisture content of 14%;
8. Straw dry weight (SDW) for samples taken from a bordered 0.25 m^2 area (six hills) per plot. Collections were made at panicle initiation (approximately 70 DAS) and at maturity. Tissues were dried at 80°C to a constant weight to determine biomass accumulation per plant;
9. Yield-component data for six-hill plots, based on the number of tillers, panicle counts, and oven-dried weight of straw, filled, and unfilled grain;
10. Harvest index, computed as the ratio of grain yield per plant to total harvested biomass per plant; and
11. Leaf-rolling (LR) and leaf-drying (LD), as recorded at weekly intervals after the second stress cycle. Visual scoring (0 to 9) was used according to the "Standard evaluation system for rice" (IRRI 1996). The percent reduction for each trait in stressed plants was calculated relative to the nonstressed control.

Statistical Analysis

Statistical analyses of data for individual traits were performed with SAS version 9.1 (SAS 2003). Line means

Table 1 Soil properties, crop management schemes, and climatic conditions for two study sites at IRRI

	Site S1 (D-block)	Site S2 (UR)
Soil[a]		
Depth of sampling (m)	0–2	0–1
pH (1:1 in water)	6.38	6.48
Org C (%)	1.89	0.94
N (Kjeldahl; %)	0.20	0.07
Olsen P (ppm)	11.5	18.6
Avail K (ME per 100 g)	–	0.58
CEC (cmol kg^{-1})	39.0	28.5
Clay (%)	57.8	36.4
Sand (%)	8.3	27.0
Silt (%)	34.0	36.6
Na (cmol kg^{-1})	0.93	1.25
K (cmol kg^{-1})	1.32	1.44
Ca (cmol kg^{-1})	26.73	14.90
Mg (cmol kg^{-1})	13.50	7.095
Zn (ppm)	0.8	–
B (ppm)	2.0	–
Si (ppm)	172.0	–
Soil texture	Clay	Clay loam
Management		
Establishment method	Transplanting	Transplanting
Water delivery	Irrigated	Irrigated
Sowing date	29 December 2007	2 January 2008
Transplanting date	19 January 2008	23 January 2008
Hydrological conditions	Aerobic, flooding	Aerobic
Climatic conditions		
Total amount of rainfall (mm)	493	534
Estimated evapotranspiration (mm)	669	710

[a] In each field, a composite topsoil sample was collected from five random subsamples at the beginning of the experiment. These were analyzed for pH, total soil organic carbon (Nelson and Sommers 1996), total soil N (Bremner 1996), cation exchange capacity (Sumner and Miller 1996), and soil texture (modified from Koehn 1928). Plant-available Zn, K (NH_4O-Ac extraction), and P (Olsen-P) were determined according to the methods of Ponnamperuma et al. (1981), Helmke and Sparks (1996), and Olsen et al. (1954), respectively

were estimated using the REML option of the SAS MIXED procedure, taking lines as fixed and replicates and blocks within replicates as random. Correlations for trait averages between character pairs were computed at $P<0.05$ in Microsoft Excel™.

DNA Extraction and Genotyping

DNA was extracted from 21-day-old seedlings in the screenhouse according to the protocol of Dellaporta et al. (1983). For both SSR and STS analyses, DNA was diluted to 25 ng μl^{-1} with double-distilled water and was used as our working stock. PCR amplification and detection for SSR and STS markers were conducted on an MJ Thermal Cycler Dyad (384-well alpha unit) and G-storm system (384-well alpha unit). The protocols for both techniques were as described by Chin et al. (2007). Conditions included 100 V and a running time for electrophoresis that ranged from 1.5 to 3.5 h depending on the expected sizes of PCR products for each marker. The procedure for detecting introgression is shown in Fig. 1. Four delineations were made: A', AILs with recurrent parent alleles; B', AILs with alleles from the donor parent; H', AILs heterozygous with alleles from both recurrent and donor parents; and U', AILs with nonparental alleles. To survey DNA polymorphisms in the parents, a set of 464 SSR and 162 STS markers was used to characterize introgression from *O. glaberrima* into *O. sativa*. This involved two lines of *O. sativa*—IR64 (recurrent parent) and IR55423-01—and 12 *O. glaberrima* parents.

QTL Analysis

QTL mapping was performed with 200 BC_2F_3 AILs that comprised 50 high-yielding, 50 low-yielding, 50 random, and 50 unselected lines. Statistical analysis used QTLmapper 2.0 (Wang et al. 2006) and Mapmanager QTX 20 (Manly et al. 2002). Mapping was done for BC_2F_3 data. This process included regression of field performance on the marker genotype and single-point analysis to identify putative single QTLs and to detect epistatic interactions. The latter was evaluated between two loci (E-QTL) by

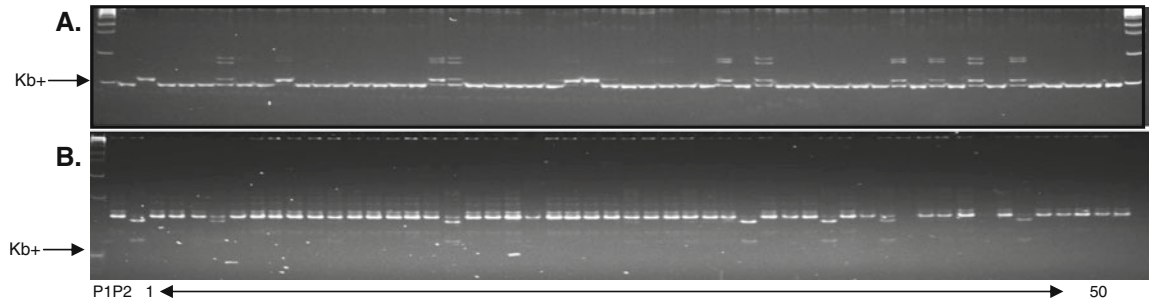

Fig. 1 Characterization of introgression lines using SSR and STS markers for S06053 (**a**) and RM271 (**b**). *P1 O. sativa*, *P2 O. glaberrima*, *1–50* individual AILs

applying QTLmapper 2.0 software. The percent phenotypic variance associated with each significant QTL was calculated from the regression of each marker–phenotype combination to determine the effect of each marker and total phenotypic variation. In addition, we controlled the background genetic variation due to main and epistatic effects of important markers. The locations of these trait-improving QTLs were compared with those identified across rice cultivars and wild species by searching the GRAMENE genomics database (http://www.gramene.org; accessed 1 July 2009). Segregation ratios for individual markers were statistically determined at each marker locus by χ^2 tests for deviations from the expected Mendelian segregation ratio of 3:1. To determine the proportion of alleles from the recurrent and donor parents, genome composition was estimated with the software package GGT version 3.2 (Graphical GenoTypes, http://www.dpw.wau.nl/pv/pub/ggt; van Berloo 2008).

Results

Trait Analysis and Field Performance of AILs

Table 2 presents the phenotypic analysis of different traits for 513 rice AIL populations (BC_2F_3) and the recurrent parent. To determine if their distributions were normal, we calculated skewness. These AILs showed large variations for all traits within each location and between nonstressed and stressed plants. Transgressive segregation was also observed across all locations and treatment conditions (Fig. 2). Induced drought caused significant reductions in all measured traits for the recurrent parent and the AILs. Compared with the control, yield from the IR64 parent was decreased by 59% under drought and by 41% (S1) and 37% (S2) from the AILs. Average yields were similar between the recurrent parent and the AILs at both sites, even though some AILs produced more grain than did the recurrent parent at each site. In all, 33 AILs showed at least a 15% increase over IR64 for three or more yield components.

Selecting for grain yield per plant resulted in a positive response for components such as percent seed set (58%), tiller number (51%), biomass per plant (57%), and harvest index (76%) (Table 3). Although a positive response was also observed between selection for percent seed set and harvest index (59%), no correlation was found between percent seed set and straw dry weight. The highest correlation was noted between biomass (straw weight and grain yield harvested) and straw dry weight (92%), whereas the correlation between yield and days to 50% flowering was not significant (20%).

Polymorphism between *O. sativa* and *O. glaberrima*

Of the 626 molecular marker pairs (464 SSR and 162 STS) that were used to survey polymorphism between *O. sativa* and *O. glaberrima* parents, we found that 188 (40.5%) SSR and 67 (41.3%) STS primer pairs produced polymorphic loci between the parents. In all markers, nonparental and null alleles (i.e., completely lacking a visible band) were coded as missing data. A total of 19 markers (11 SSR and 8 STS) showed polymorphisms between the two donor parents. The heterozygosity in this population was determined according to the banding pattern of co-dominant markers, where both types of alleles could be expressed and scored.

An average of 15% *O. glaberrima* alleles were introgressed into each chromosome (Table 4). When map distances between markers were considered as the basis for estimating the extent of introgression, Chr 9 was the shortest (23 Mbp), while Chr 2 was the longest (43 Mbp). The average distance was 30.92 Mbp for donor-parent chromosome fragments that were introgressed. An average of 8.3 markers per chromosome was found, with Chr 2 having the highest density (11.61) and Chr 9 having the lowest (6.15).

We examined only 173 of 255 polymorphic markers in our molecular analysis of AILs, because most markers had single bands and only a few exhibited multiple bands. Segregation of those 173 markers used in constructing our

Table 2 Agronomic performance of 513 AILs derived from IR64 × *O. glaberrima* under lowland drought conditions

	Location or treatment	Mean	SD	Variance	Kurtosis	Skewness	Range	IR64 (recurrent parent)
Grain yield (g per plant)	S1	8.17	3.46	11.99	0.25	0.39	0.07–19.59	8.54
	S2	7.24	2.92	8.5	0.94	0.7	0.15–19.88	7.65
	No stress[a]	19.77	8.34	69.62	1.14	0.72	0.22–54.02	12.67
Biomass (g per plant)	S1	37.04	10.57	111.67	1.09	0.62	8.16–83.69	23.42
	S2	39.38	10.4	108.1	0.54	0.14	6.16–76.00	26
	No stress	61.8	24.74	612.03	2.84	1.21	9.48–193.53	42.19
Harvest index	S1	0.22	0.07	0.01	−0.01	−0.04	0.00–0.43	0.28
	S2	0.18	0.05	0	0.13	0.16	0.01–0.36	0.18
	No stress	0.33	0.1	0.01	5.53	0.86	0.01–0.98	0.49
Days to heading, i.e., 50% flowering	S1	103.19	7.62	58.01	0.69	0.33	82.20–131.92	103.12
	S2	88.21	10.68	114.15	8.23	–2.49	32.90–105.68	91
	No stress	91.98	8.01	64.16	−0.64	0.15	69.00–113.00	93
Plant height (cm)	S1	95.18	16.08	258.63	−0.19	0.29	54.58–164.56	83.05
	S2	73.23	12.78	163.34	28.16	2.43	16.00–213.32	59.43
	No stress	111.91	24.85	617.71	−0.9	0.14	49.67–175.00	80
Tiller numbers	S1	56.72	13.1	171.5	0.3	0.04	14.92–103.97	59.1
	No stress	76.83	25.63	657.04	−0.03	0.24	10.00–158.00	104
100-seed weight (g)	S1	2.16	0.51	0.26	0.47	−0.46	0.08–3.36	2.5
	No stress	2.82	0.5	0.25	0.32	0.1	1.00–4.50	–
Fertile panicles (%)	S1	71.83	16.03	256.97	0.66	−0.8	5.86–100.00	51.47

[a] Trials with a no-stress treatment were established only at site S1

genetic map was evaluated with a chi-square test for goodness-of-fit. It revealed that for all 12 chromosomes, most markers (93.1%) deviated from the expected Mendelian 3:1 segregation ratio, at a probability of 0.001. Moreover, Chr 2, 3, 4, 7, 8, 9, 11, and 12 showed extreme skewing (Supplementary Table 1, Supplementary Fig. 1). The resultant map consisted of 119 markers (75 SSR and 44 STS).

Identification of Putative and Epistatic QTLs

A total of 23 markers across chromosomes were identified as strongly associated ($P<0.001$) with traits studied at both S1 and S2 (Table 5). To determine empirical significance thresholds for declaring a QTL, 1,000 permutations were done to calculate likelihood ratio statistics (LRS) for each trait. Due to the skewness of our mapping population, which comprised nonidealized lines, we applied statistics of probability (P) for single marker analysis rather than obtaining thresholds for scoring the log of likelihood (LOD) or LRS. QTLs that were identified in this study included:

1. Biomass per plant (bm): Eight QTLs were identified. *O. glaberrima* contributed alleles at Chr 1, 2, 3, 6, and 10. Three QTLs that increased biomass production (Chr 3 and 6) because of the allele contributed by *O. glaberrima* accounted for 29% of the phenotypic variation. Alleles from *O. sativa* contributed to biomass at Chr 1 and 2, with phenotypic variations ranging from 29% to 34%. No QTL for this trait was detected at field site S2.
2. Harvest index (hi): Two QTLs were identified on Chr 2 and 7. An allele from *O. glaberrima* contributed to all of the loci that were involved. Locus RM208 on Chr 2 with the *O. glaberrima* allele contained a major QTL. This finding explained 42% of the phenotypic variation. All of the QTLs were detected at site S1.
3. Plant height (ph): Four QTLs were detected at both S1 and S2. Introgressed alleles were linked with shorter plants at locus RM246 on Chr 2, whereas the *indica* alleles at locus S07050A on Chr 7 contributed favorably to height. One QTL at locus RM208 on Chr 2 with alleles from the *indica* parent accounted for 39% of the phenotypic variation, while *O. glaberrima* alleles at locus RM246 on chromosome 1 described 20% of that variation. Two QTLs were common to field sites S1 and S2.
4. Tiller number (tn): Four QTLs were identified. Locus RM338, on Chr 3, contributed an *indica* allele that accounted for 48% of the phenotypic variation, while locus RM208 (Chr 2) contributed *O. glaberrima* alleles

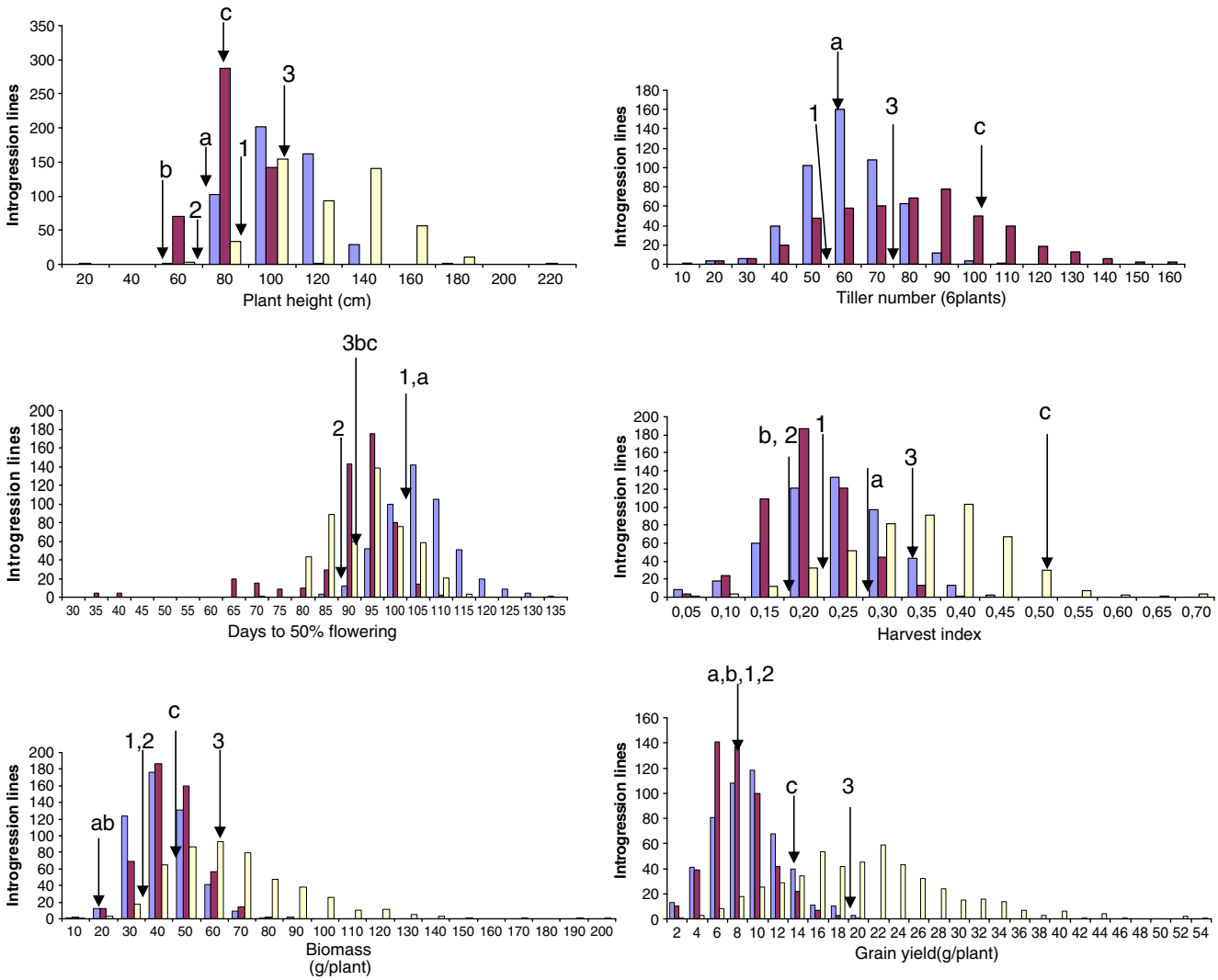

Fig. 2 Frequency distribution of means from BC_2F_3 AILs for each trait. Parental mean (IR64) and AILs at different locations are indicated by *arrow*. *a*, *b*, and *c* are means for IR64 at sites S1, S2, and for unstressed plants, respectively

that accounted for 40% of the detected variation. Individually, *tn2.2* contributed 26%, while *tn7* stood at 14%. The introgressed allele at locus *tn2.1* was associated with increased tiller numbers.

Table 3 Phenotypic correlations among traits from IR64 × *O. glaberrima* introgression lines

Trait*	BM (g per plant)	HI	SDW (g per plant)	TN	% FP	DTH	PH (cm)
Biomass (BM)							
Harvest index (HI)	0.10						
Straw dry weight (SDW)	0.92	−0.26					
Tiller number (TN)	0.53	0.27	0.39				
% fertile panicles (FP)	0.24	0.59	0.00	0.29			
Days to heading, i.e., 50% flowering (DTH)	−0.03	−0.21	0.05	−0.11	−0.21		
Plant height (PH)	0.43	0.14	0.36	0.11	0.26	−0.02	
Grain yield (g, YPP)	0.57	0.76	0.19	0.51	0.58	−0.20	0.33

Sample size n=513

*P=0.05

Table 4 Introgression from *O. glaberrima* into IR64 rice

Chromosome	Total polymorphism	% mean introgression	Range of introgression (%)	Total distance (Mbp)	Density (markers per Chr)
1	18	12.9	3.5–32.1	40.8	11.00
2	18	12.4	0.3–20.9	43.1	11.61
3	23	6.2	1.0–37.2	36.3	9.78
4	18	5.9	0.0–21.0	35.1	9.46
5	10	22.6	5.0–33.0	28.5	7.67
6	12	33.2	7.7–24.5	26.6	7.16
7	13	5.7	0.7–13.0	29.3	7.91
8	16	28.4	3.5–34.7	27.8	7.49
9	16	18.7	0.7–22.2	22.8	6.15
10	9	6.7	1.5–15.7	25.1	6.77
11	15	15.8	1.0–21.8	28.3	7.61
12	5	10.5	3.0–13.0	27.4	7.39
Total	173	179.0		371.1	100.0
Average no. of markers per Chr	14.42				
Mean introgression		14.9		30.9	8.3

Pooled data from both stress and nonstress trials

5. Panicle fertility (ps): Four QTLs were found. Two alleles from *O. glaberrima* and two from *O. sativa* contributed to increased fertility. A major QTL detected at locus RM275 (Chr 6), with alleles from the *indica* parent, accounted for 41% of the phenotypic variation, while locus S10071 (Chr 10), with alleles contributed by the donor parent, was responsible for 39% of that variation.
6. Days to heading (dth): Five QTLs associated with days to 50% flowering were identified on Chr 2, 4, and 10. This was true for both S1 and S2. *O. glaberrima* contributed alleles at three loci and *O. sativa* at two. A QTL identified at locus S10013A (Chr 10), with alleles from *O. glaberrima*, accounted for 28% of the phenotypic variation, while the QTL at locus S10071 (Chr 10), with alleles from the *indica* parent, accounted for 20% of that variation. Even though QTLs *dth2.1* and *dth2.2* had alleles from the donor parents, they were far apart (respective physical locations of 17.45 and 35.14 Mb). One QTL each from those locations on Chr 10 was common to both S1 and S2.
7. Yield per plant (ypp): Six genomic regions were associated with six QTLs. For example, the QTL at locus RM489 on Chr 3, with alleles from its *O. sativa* parent, accounted for 42% of the phenotypic variation, while the QTL at locus RM208 (Chr 2), with alleles from the *O. glaberrima* donor parent, explained 22% of that variation. Four QTLs were identified only from field site S1, whereas the other two were common to both sites.

Our two-way test revealed three significant ($P<0.005$) epistatic interactions (EpQTL). These consisted of six markers across five chromosomes (Table 6). A relatively weak interaction was detected among nonlinked markers (4.23% to 8.25%), which suggested that those QTLs were not highly influenced by other regions of the genome. Two EpQTLs were also found that contained one significant QTL on either side. The other four did not include any of the identified QTLs.

Discussion

O. glaberrima-Derived Alleles Are Associated with Improved Yield

Under induced drought, 513 AILs from the BC_2F_3 population of *O. sativa* × *O. glaberrima* were evaluated for yield and yield components. For stressed plants, some AILs showed transgressive performance to the recurrent parents at both test sites (S1 and S2). This suggested that some positive alleles had been transferred from the *O. glaberrima* donor. Under nonstressed (control) conditions, many AILs, including the tolerant checks, had higher yields than the recurrent parent. Saito et al. (2010) have reported yield increases of more than 20% when using *O. sativa* × *O. glaberrima* progenies. Moreover, yield has been improved by 27% to 84% within BC_2F_2-derived lines from *O. sativa* and wild species of rice, e.g., *Oryza rufipogon* (Moncada et al. 2001) and *Oryza glumaepatula* (Brondani et al. 2002). Thus, the enhanced yields of 49% (S1) and 52% (S2) in our stress trials, when compared with the recurrent parent, are similar to those in previous reports.

Selection under extreme stress conditions to obtain reliable genotypes may be a potential approach for

Table 5 QTLs identified for eight traits based on single-point analysis of IR64 × *O. glaberrima* (BC_2F_3) AILs

Trait	Name of QTL[a]	Chr	Linked marker	LRS	P^c	PVE (%)	Additive effect	Source
Biomass	*bm1.1*	1	S01022	12.4	0.00042	6	13.07	*IR64*
	bm1.2	1	RM246	82.0	0.00000	34	32.20	*IR64*
	bm1.3	1	S01143A	52.1	0.00000	23	24.34	*O. glaberrima*
	bm2.1	2	S02057B	250.8	0.00000	29	50.57	*IR64*
	bm2.2	2	RM208	213.8	0.00000	34	49.51	*O. glaberrima*
	bm3	3	RM338	250.8	0.00000	71	50.57	*O. glaberrima*
	bm6	6	RM275	250.8	0.00000	71	50.57	*O. glaberrima*
	bm10	10	S10072	55.5	0.00000	24	25.06	*O. glaberrima*
Harvest index	*hi2*	2	RM208	108.0	0.00000	42	17	*O. glaberrima*
	hi7	7	RM134	108.3	0.00000	42	16	*O. glaberrima*
Plant height	*ph1.1*[b]	1	RM246	45.3	0.00000	20	20.50	*O. glaberrima*
	ph2	2	RM208	97.3	0.00000	39	31.12	*IR64*
	ph7.1	7	S07050A	111.0	0.00000	43	32	*IR64*
	ph7.2[b]	7	S07103	18.6	0.00000	9	15.66	*IR64*
Tiller number	*tn2.1*	2	RM208	100.5	0.00000	40	35.70	*O. glaberrima*
	tn2.2	2	RM318	60.0	0.00000	26	24.21	*O. glaberrima*
	tn3	3	RM338	130.5	0.00000	48	38.45	*IR64*
	tn7	7	S07053	29.4	0.00000	14	17.29	*O. glaberrima*
	tn10	10	S10026C	32.4	0.00000	28	26.87	*IR64*
Panicle fertility	*ps2.1*	2	RM318	64.2	0.00000	27	18.48	*IR64*
	ps2.2[b]	2	RM208	94.5	0.00000	38	25.66	*O. glaberrima*
	ps6	6	RM275	105.0	0.00000	41	26.17	*IR64*
	ps10	10	S10071	97.9	0.00000	39	25.83	*O. glaberrima*
Days to heading	*dth2.1*	2	S02057B	45.3	0.00000	20	9.81	*O. glaberrima*
	dth2.2	2	RM208	36.9	0.00000	17	9.13	*O. glaberrima*
	dth4	4	RM349	46.7	0.00000	21	9.88	*IR64*
	dth10.1	10	S10013A	32.2	0.00000	28	9.36	*O. glaberrima*
	dth10.2	10	S10071	43.8	0.00000	20	9.80	*IR64*
Yield per plant	*ypp1*[b]	1	S01143A	60.1	0.00000	26	11.96	*IR64*
	ypp2	2	RM208	73.0	0.00000	22	24.95	*O. glaberrima*
	ypp3	3	RM489	110.7	0.00000	42	14.04	*IR64*
	ypp6	6	RM275	385.2	0.00000	15	25.49	*O. glaberrima*
	ypp8[b]	8	S08107	17	0.00004	8	8.38	*O. glaberrima*
	ypp9	9	RM257	95.0	0.00000	17	13.41	*O. glaberrima*

Chr chromosome, *LRS* likelihood ratio statistics for the association of a trait with this locus, *PVE* percent variation explained

[a] All QTLs were detected only at field site S1 except those marked with [b] that were common to both S1 and S2

[c] Empirical significance thresholds for declaring a QTL at $P=0.001$

Table 6 Epistatic/digenic interactions (EpQTLs) between linked markers affecting traits in IR64 × *O. glaberrima* BC_2F_3 AILS

Trait	Chromosome	Marker A	Chromosome	Marker B	*F* value	*P* value	R^2 (%)
Biomass per plant	2	RM250	8	**S08107**	11.59	0.000	5.78
Yield per plant	8	RM152	11	RM229	9.91	0.000	4.23
Plant height	1	S01054	7	**S07103**	20.61	0.000	8.25

R^2 proportion of total phenotypic variation due to different epistatic interactions

Bold font indicates individual QTLs identified by single-point analysis (see also Table 3). *F* values were used for testing partial regression coefficients of the selected main-effect markers

drought-tolerance breeding. Likewise, backcrossing based on direct selection for yield under artificially imposed drought can lead to actual gains in such stress tolerance (Lafitte et al. 2006; Venuprasad et al. 2009). Thus, some of the AILs identified in our experiments that were more tolerant than the recurrent parent could be utilized in future crop-improvement programs. The nonsignificant correlation (20%, P=0.05) between *ypp* and *dth* indicated that, despite the terminal stress imposed, late-maturing AILs were not as significantly affected by drought as were earlier lines, and that grain yield under stress was not correlated with heading date. This may have been due to the very wide transgressive segregation in flowering observed among AILs that resulted in some lines heading very early but having low yields because of their poor adaptability. These findings also suggest that genes introgressed from *O. glaberrima* into an elite genetic background can improve key agronomic traits of an elite rice variety, even though the former is phenotypically inferior to the latter. Based on their grain yield performance, we have now identified 30 superior AILs that are suitable for growth under lowland drought conditions.

Allelic Diversity between *O. sativa* and *O. glaberrima*

The polymorphism observed here is similar to results that we reported previously (Bimpong et al. 2004). There, 38% polymorphism was observed between the *O. sativa* and *O. glaberrima* parents. However, those data are low compared with values calculated in other studies with wild crosses and cultivated rice. For example, Causse et al. (1994) have recorded 85% polymorphism in a cross between *O. sativa* and *Oryza longistaminata*, and polymorphism has also ranged between 60% and 90% for *O. sativa* and *O. rufipogon* (Xiao et al. 1998; Septiningsih et al. 2003). The lower percentage of polymorphism that we found here might have been a result of reduced recombination due to the genetic distance between parental lines, as also suggested by Grandillo and Tanksley (2005).

In the second backcross generation (BC_2), the expected segregation ratio for an ideal mapping population would be 75% homozygote (*O. sativa*):25% heterozygote (*O. sativa* × *O. glaberrima*). That would indicate an allele frequency of 87.5% *O. sativa* to 12.5% *O. glaberrima* alleles if no selection were used to develop such a population. However, for our IR64 × *O. glaberrima* AILs, most markers (93.1%) deviated from the expected Mendelian segregation at a probability of 0.001. Distortion was observed in eight chromosomal regions (2, 3, 4, 7, 8, 9, 11, and 12), which contained clusters of skewness and at least one marker per region with extreme skewing. Such movement toward the recurrent parent can be explained by selection pressure imposed at the BC_1 and BC_2 generations during population development. This is a result of the high degree of sterility in F_1 that leads to the bulking of seeds that are rarely fertile. In contrast, the unexpected skewing toward *O. glaberrima* may have arisen from segregation distortion that has also been documented in crosses between *O. sativa and O. glaberrima* and within other species (Causse et al. 1994; Lorieux et al. 2000; Aluko et al. 2004). Sterility loci occur in *O. glaberrima*, including gamete eliminator S_1, and pollen killers S_3, S_{18}, S_{19}, S_{20}, S_{21}, and $S_{29(t)}$, which are located on Chr 6, 11, 10, 3, 7, 7, and 2, respectively (Sano 1986; Doi et al. 1999; Hu et al. 2006). The presence of such loci may have affected pollen fertility, gene segregation, and skewness toward our *O. glaberrima* parent as well (Supplementary Table 1). Li et al. (2008) have identified four pollen sterility QTLs, one each on Chr 1 and 3, and two on Chr 7. In our study, pollen sterility loci *qSS-3* and *qSS-7a* on Chr 3 and 7, respectively, coincided with the previously identified S_{19}, and S_{20}, while loci *qSS-1* and *qSS-7b* (Chr 1 and 7L) appeared to be distinct. An epistatic interaction also controlled the hybrid sterility between *qSS-1* and *qSS-7a*.

The 93.1% skewing in a BC_2 population affects the ability to map markers de novo when based on segregation data for BC lines alone. Thus, we followed the method of Wang et al. (2006) for mapping this high degree of skewing, with markers being assigned to positions on the physical map rather than to the same linkage group. Our constructed map comprised 173 loci, including 86 SSR markers and 87 STS markers (Fig. 3). This entire map covered 371 Mb, averaging 14.42 Mb between adjacent markers.

A larger-than-average gap on Chr 5 may have been due to the presence of a major hybrid sterility locus (S_5). That locus has been cloned and found to encode for an aspartic protease that conditions embryo-sac fertility and which has profoundly influenced efforts in artificial breeding of cultivated rice (Chen et al. 2008). Another wide gap observed on Chr 6 might have been caused by the presence of sex-independent transmission ratio distortion (*siTRD*) near the centromere, thereby effecting an allelic interaction at a specific locus in rice. Koide et al. (2008a) have shown that it is controlled by the S_6 locus through a mechanism by which the S_6 allele acts as a gamete eliminator, such that both male and female gametes possessing the opposite allele (S_6^a) are aborted only in heterozygotes (S_6/S_6^a). Test-cross experiments using near-isogenic lines (NILs) from *O. sativa* × *O. glaberrima* that carry either S_6 or S_6^a have revealed that Asian rice strains frequently harbor an additional allele (S_6^n) that, in heterozygotic states (S_6/S_6^n and S_6^a/S_6^n), does not result in *siTRD* (Koide et al. 2008b). The genetic effects of that S_6 locus on *siTRD* are not altered in either the female or male gametes, even after repeated backcrosses, demonstrating the stability of that phenomenon.

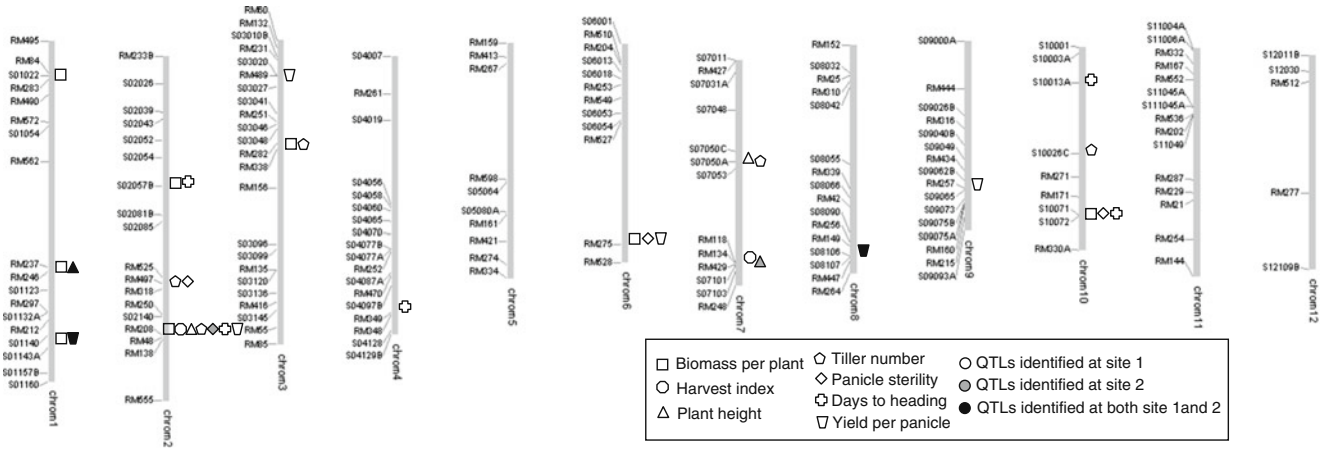

Fig. 3 QTLs identified in IR64 × *O. glaberrima* BC_2F_3 AILs

This ordering of markers on each chromosome is consistent with that from the Nipponbare/Kasalath map (Harushima et al. 1998).

Molecular Characterization of BC_2F_3 AILs and Beneficial Alleles from *O. glaberrima*

After a series of backcrosses, the length of the donor chromosome segments is very important for a plant breeder who is interested in developing stocks with desired genetic traits. Here, our donor segments averaged 8.3 Mbp long per chromosome, which is consistent with results obtained by Zhang et al. (2005) in their work with *japonica* and *indica* rice. Because we used a density of one marker per 6.2 to 11.6 Mb, our estimation of donor genome contents was appropriate. Chromosome 12 was the least representative, with only five marker loci being mapped even though 16 markers had been used during the parental survey. Similar findings have been reported by Heuer et al. (2009), who used SSR markers to identify polymorphism in the *Pup1* region (RM277). There, large-scale polymorphism, such as within the *Indel* regions, may have disabled the design of their co-dominant marker system. The low polymorphism found here on Chr 12 implies that some regions within cultivated and wild genomes may have had a common descent.

Beneficial QTL Alleles from *O. glaberrima*

Although *O. glaberrima* accessions are phenotypically inferior to those of *O. sativa*, we found that the former contributed 50% of the beneficial alleles (5 of 10) to QTLs newly identified in the *O. sativa* background. Beneficial QTL alleles from *O. glaberrima* were detected for nearly all traits. The exceptions were for panicle fertility and plant height, where the effect of QTL alleles from *O. glaberrima* could not be determined. Beneficial alleles from *O. glaberrima* and other rice species have been reported in other studies with *O. glaberrima* as the donor parent (Li et al. 2004; Suh et al. 2005). Using approximately 300 BC3F1 hybrids derived from a cross of *O. sativa* and *O. glaberrima*, Li et al. (2004) have identified 11 significant QTLs for seven of 16 grain-related traits, with favorable alleles coming from *O. glaberrima* at eight loci (73%). We also discovered that QTLs for different traits were clustered together within the introgressed segments on Chr 1, 2, 3, 6, 7, and 10. Previous research involving an O. *sativa* × *O. glaberrima* doubled-haploid population has suggested that epistatic interactions on Chr 1, 3, and 6 are responsible for the high performance of some lines (Aluko et al. 2004). Similar findings have been reported when evaluating an *O. sativa* and *O. rufipogon* cross, implying the existence of pleiotropy for three chromosomal regions that are simultaneously associated with 1,000-grain weight and grains per plant (Xiao et al. 1996; Rahman et al. 2007).

Wild-QTL alleles that are favorable for some traits are often associated with negative effects from other traits (Tanksley and Nelson 1996). We also observed this phenomenon. For example, *O. glaberrima* allele *yld2.1* is beneficial to most yield-related traits at locus RM208 on Chr 2. However, the same region is associated with *ps2*.2, a negative QTL from *O. glaberrima* that results in diminished fertile seed set. An *O. glaberrima* allele in the genomic region around locus RM338 (associated with *tn3*) causes greater production of fertile tillers under drought stress and a subsequent increase in yield per plant. Nevertheless, it is also linked to a negative QTL, *bm3*, which results in a rapid decrease in biomass when irrigation is withheld. Such an association of positive and negative QTLs within the same chromosomal regions has been reported from research with *O. glaberrima* and *O. sativa* (Aluko et al. 2004) and *O. rufipogon* and *O. sativa* (Xiao et

al. 1998; Septiningsih et al. 2003; Reddy et al. 2005). Because of this, we recommend careful selection to avoid introducing negative traits during the process of crop improvement.

Epistatic interactions are critical to transgressive segregation in rice (Moncada et al. 2001; Septiningsih et al. 2003). In our study, two significant QTLs associated with yield components under stress (*bm6* and *ps6*) were mapped to the same position for a major yield-enhancing QTL (*ypp6.1*) that is linked to higher grain production from stressed plants. Similarly, the region associated with *tn2.1*, which increases the number of fertile tillers under stress, was mapped with *ypp2*, *bm2.2*, *hi2*, and *ph2*, which control yield per plant, biomass, harvest index, and plant height, respectively. This type of association for multiple traits within a single region has also been reported previously (Septiningsih et al. 2003; Reddy et al. 2005; Rahman et al. 2007).

Comparison of QTLs Across *Oryza* Species

We compared our findings with those from previous evaluations of similar characters within different cross combinations and under various growing environments. The use of a common set of molecular markers makes it possible to determine whether all reported QTLs occur in similar regions of the rice genome. Doing so lends credence as well as caution for those either identified earlier or being reported for the first time. Of the 33 QTLs found here, the locations of 23 were as recorded previously even though different types of mapping populations, ranging from 1 to 105, had been used. Ten new QTLs were also described here, including three for biomass per plant (*bm1.1*, *bm2.2*, and *bm10*), one for harvest index (*hi7*), one for tiller number (*tn3*), two for panicle fertility (*ps2.2* and *ps6*), and two for days to 50% flowering (*dth4* and *dth10.2*). Another novel QTL for yield was located to Chr 9 (*ypp9.1*). No new QTLs were found here for plant height. Only one of these 10 new QTLs was identified at field site S2; the others were found at S1.

Of the five QTLs for days to heading, *dth2.1* and *dth10.1* are in the same or similar regions as for previously identified QTLs. All six QTLs for plant height have genomic locations nearly identical to those previously reported, including *ph1.1* (Wu et al. 1996; Xiao et al. 1998) and *ph2* (Li et al. 2004). Moreover, QTL *ph7.1* in this study shares the same position as Ph7, at the tip of Chr 7 (Xiao et al. 1996). QTL *ph1.1* may represent the semi-dwarf locus *sd-1*, which is located in a similar region on Chr 1 (Cho et al. 2003). The same can be said for our QTL *ph2* and known dwarfing mutants *d-30* (waisei-shirasasa dwarf) and *d-5* (bunketsu-waito) (Kinoshita 1995). Of the 53 semi-dwarf genetic stocks reported in rice, nine are allelic to the highly mutable *sd-1* locus, while the others appear to be independent. It will be interesting to determine how many of them define QTLs associated with plant height. Future examinations might also focus on whether *O. glaberrima* harbors new alleles that differ in structure and function from any of those widely used to modify plant stature, harvest index, and other important agronomic traits in programs for plant improvement.

Three of our four QTLs affecting panicle fertility do not coincide with any previously published QTLs in rice. Two of these from our study, as well as *O. glaberrima* alleles, are associated with decreased fertility at both loci. QTL *ps10* occupies the same location as ste10.1 (Yao et al. 1997; Tan et al. 1998). Likewise, a known fertility restoration locus, *Rf-1*, is located on Chr 10, in a position similar to that of ste10.1. *O. glaberrima* alleles associated with increased yield have been found with four of the six QTLs identified for this trait. On Chr 6 and 8, *ypp6* and *ypp8* are at the same locations as for the yield QTLs reported by Suh et al. (2005) and Li et al. (2004). There, *O. glaberrima* alleles have a beneficial phenotypic effect on yield at loci RM204 and RM275. Our yield QTL *ypp2* at locus RM208 on chromosome 2 has previously been reported by Xiao et al. (1998) to be associated with a 17% rise in grain yield per plant, without delaying maturity or increasing plant height. There, they used a BC_2 population derived from *O. sativa* × *O. rufipogon*. This discovery suggests that the innovative application of molecular maps and markers can alter the way researchers utilize wild species.

Selective Genotyping of AILs

We genotyped plants through a selective approach, using 173 DNA markers. Briefly, the highest-yielding and lowest-yielding 10% of AILs were chosen, based on the means from stress and nonstress trials at our two field sites. Because grain yield is negatively correlated with the number of days to flowering, we compensated by choosing AILs after stratifying them into three categories: lines that flowered less than 10, 10–14, or 14 days post-stress (i.e., 100 DAS). In all, 200 AILs were genotyped—50 each from the highest- and lowest-yielding tails of the stress treatment, 50 from the highest-yielding tails of the nonstress treatment, and 50 that were randomly selected. We found nine overlaps with three AILs between the low-yielding/stress and the high-yielding/nonstress group as well as between the random and the low-yielding groups. Another two overlaps were observed between the highest-yielding/stress and random groups, plus one overlap between the low-yielding and random groups. This selective genotyping approach has been successfully utilized to identify several other major QTLs (Bernier et al. 2007; Navabi et al. 2009).

Conclusions

Our results indicate that, despite its overall inferior quality, *O. glaberrima* contains QTL alleles that are likely to provide substantially improved, agronomically important traits, including yield. Of the 29 QTLs identified here, 19 correspond to previously reported QTLs, thereby demonstrating that they are stable across genetic backgrounds. An additional 10 QTLs that control yield and yield components are potentially novel. Their introgression from *O. glaberrima* could serve as a new source of variation for genetic improvement toward drought tolerance, with the locus near RM208 appearing to be an especially good candidate for such enhancement. Those novel QTLs are suitable for studies of fine mapping and positional cloning, whereas QTLs that we mapped to regions consistent with others previously reported can be useful in marker-assisted transfers. Future research can focus on developing NILs by using different recipient *sativa* varieties or evaluating QTL functioning under different growing environments.

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