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Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment

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Abstract An advanced backcross breeding strategy was used to identify quantitative trait loci (QTLs) associated with eight agronomic traits in a BC_2F_2 population derived from an interspecific cross between Caiapo, an upland *Oryza sativa* subsp. *japonica* rice variety from Brazil, and an accession of *Oryza rufipogon* from Malaysia. Caiapo is one of the most-widely grown dryland cultivars in Latin America and may be planted as a monoculture or in a multicropping system with pastures. The objectives of this study were: (1) to determine whether trait-enhancing QTLs from *O. rufipogon* would be detected in 274 BC_2F_2 families grown under the drought-prone, acid soil conditions to which Caiapo was adapted, (2) to compare the performance with and without pasture competition, and (3) to compare putative QTL-containing regions identified in this study with those previously reported for populations adapted to irrigated, low-land conditions. Based on analyses of 125 SSLP and RFLP markers distributed throughout the genome and using single-point, interval, and composite interval mapping, two putative *O. rufipogon* derived QTLs were detected for yield, 13 for yield components, four for maturity and six for plant height. We conclude that advanced backcross QTL analysis offers a useful germplasm enhancement strategy for the genetic improvement of cultivars adapted to stress-prone environments. Although the phenotypic performance of the wild germplasm would not suggest its value as a breeding parent, it is noteworthy that 56% of the trait-enhancing QTLs identified in this study were derived from *O. rufipogon*. This figure is similar to the 51% of favorable QTLs derived from the same parent in crosses with a high-yielding hybrid rice cultivar evaluated under irrigated conditions in a previous study. In conclusion, parallel studies in rice using AB-QTL analysis provide increasing evidence that certain regions of the rice genome are likely to harbor genes of interest for plant improvement in multiple environments.

Keywords Quantitative trait loci · Upland rice · *Oryza rufipogon* · Yield · Yield components

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Introduction

Cultivated rice (*Oryza sativa* L.) is a genetically diverse species with broad adaptation to a wide range of growing environments. It consists of two major subspecies, *indica* and *japonica*, which represent specialized gene pools that together make it possible to cultivate rice under diverse conditions, including both tropical and temperate climates, and irrigated and rainfed environments (Anonymous, IRRI 1993). Studies based on morphological, physiological and molecular characterization of *Oryza rufipogon* suggest that this species is ancestral to *O. sativa* (Second 1982; Oka 1988; Wang et al. 1992). Consistent with that assumption, *O. rufipogon* consists of accessions that are able to prosper under conditions of both complete water saturation (anaerobic soils) and water deficit (as in upland environments) (Morishima et al. 1962) and is found in both tropical and temperate growing conditions. Some accessions of *O. rufipogon* show a continuum of perennial-annual types, with the presence of annual types depending on the intensity of drought in the dry season (Oka 1988).

While the *indica* and *japonica* subspecies are separated by a partial sterility barrier, both cross readily with most accessions of *O. rufipogon.* The possibility of se-

lectively introgressing useful genes from *O. rufipogon* to elite rice cultivars suggests a way of improving the performance of *O. sativa* while simultaneously broadening the genetic base of cultivated rice (Xiao et al. 1998).

Although upland rice constitutes only 12% of the total area planted to rice on a global basis (Anonymous, IRRI 1993), it is the dominant form of rice culture in many parts of Latin America and most of West Africa, with 69.6% of rice in Brazil (Anonymous, IBGE 1998), 23% in Colombia and 42% of rice in West Africa grown as a dry land crop (Anonymous, IRRI 1993). In Latin America as a whole, about 45% of rice is produced as an upland crop, and two-thirds of it is in Brazil's savannas where large, mechanized farms predominate. Caiapo is one of the most-widely planted varieties in this region of Brazil, covering 32% of the upland acreage in 1996 (Anonymous, EMBRAPA 1997). Grown mostly in low pH soils that are prone to water deficit, yields of Caiapo typically average 2.5 t/ha. Gains in productivity on a global basis have been much greater in irrigated than in upland rice, and have resulted in overall decreases in rice prices around the world. This makes cultivation in the upland ecosystems less competitive at a time when the availability of fresh water for irrigation purposes is reaching a critical level worldwide. Therefore, breeding to increase the productivity of upland rice is of interest for both ecological and economic reasons. It is especially important in Latin America and West Africa where rice is grown on acid soils and irrigation is not an option.

The genetic base of rice in America is narrow due to the fact that a small core of adapted progenitors has been used repeatedly in rice breeding programs in Latin America, the Caribbean and North America (Hargrove et al. 1980; Dilday 1990; Cuevas-Perez et al. 1992; Guimaraes 1993). New resources can provide genetic variation for future advance in plant breeding.

Advanced backcross (AB) QTL analysis (Tanksley and Nelson 1996) can be used to evaluate mapped donor introgressions in the genetic background of an elite recurrent parent. Using this approach, specific regions of the genome derived from either wild or adapted sources of germplasm and tagged with molecular markers can be associated with the performance of segregating off spring. In a previous study, it was demonstrated that quantitative trait loci (QTLs) derived from *O. rufipogon* (accession IRGC #105491) were associated with yield enhancement and earliness in an elite hybrid variety from China evaluated under high-input conditions (Xiao et al. 1996, 1998). In this study, we developed a population using the same accession of *O. rufipogon* as a donor, with Caiapo as the recurrent parent. We evaluated 274 BC_2F_2 families under low-input conditions in an upland site in Colombia, with and without pasture competition. We were interested in determining whether *O. rufipogon*-derived QTL alleles would be associated with positive or negative effects under these cultural conditions and in comparing the putative QTL-containing regions with those previously found in favorable rice-growing environments.

Materials and methods

Plant materials

Caiapo was used as the recurrent parent in this study. It typically yields 2.5 t/ha, is approximately 80-cm tall, has good physical and edible grain quality, tolerance to leaf blast (*Pyricularia oryzae*), moderate resistance to neck blast and tolerance to aluminum toxicity, acidic soil conditions and drought (Anonymous, EPAMIG 1994). *O. rufipogon* (IRGC #105491) is a wild accession from Malaysia. It is approximately 95-cm tall, has resistance to rice blast, small seeds with dark hulls that shatter fairly easily and heads about the same time as Caiapo under the conditions used in this study.

Population development

A single plant of *O. rufipogon* served as female in crosses to several Caiapo individuals. F_1 plants were grown in the greenhouse at the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia and the three most vigorous F_1 hybrid plants were backcrossed to Caiapo (as the female), from which 224 BC_1F_1 seeds were obtained. The resulting BC_1F_1 plants were transplanted under irrigated conditions to ensure survival during population development. Phenotypic selection was performed to eliminate sterility, very late flowering, spreading plant type, excessive shattering, long awns, dark hull color, and excessively tall plants. The best 40 individuals were backcrossed to Caiapo and approximately 30 BC_2F_1 seeds per plant were produced. Twenty BC_2 seeds from each of the 40 BC_1 plants were sown in wooden trays in the screen house and later transplanted to the CIAT nursery in Palmira, Colombia, under irrigated conditions to allow optimal seed production during selfing. The best 300 individuals were selected based on phenotype and harvested individually to generate BC_2F_2 seed. Agronomic traits were measured for all 300 BC_2F_2 families; however, only 274 families were used to collect molecular marker data, because of non-germination of seeds for 26 families.

Field trials

Two different experiments, with two replications each, were performed in the experimental station at Villavicencio, Colombia. In the first experiment, 300 BC_2F_2 families were established as an upland monoculture. The seeds were planted mechanically using a six-row planter. The soil was amended with calcium carbonate before planting. For fertilization, 60 kg of N/ha, 60 kg K_2O/ha and 60 kg of P_2O_5/ha were applied. Weed control was done by applying a post-emergence herbicide, Butaclor 3 l/ha, and Bentazol at 3 l/ha plus manual weeding. The fungicide Bim was applied throughout the vegetative cycle as a preventive disease-control measure.

In the second experiment, the same 300 BC_2F_2 families were established in an adjacent upland field in association with a pasture crop, *Brachiaria brizanta*. In this experiment, rice was planted mechanically using a six-row planter. Three-days later, 17 seeds/row of *Brachiaria* were planted and later thinned to three plants/row which were left to compete with the rice population. Due to the aggressive growth of *Brachiaria*, it was harvested 85 days after sowing to allow the rice to be harvested on a per-plot basis

In both experiments, the plot size was 2.6 m^2, with two rows per plot. The plant density was approximately 100 rice plants per plot. The soils were acidic (pH = 4.0) with a high aluminum content and low fertility. The maximum and minimum temperatures over the course of the experiment, from May 25–Sept 25 1997, were 29.7°C and 21.0°C, respectively. The average relative humidity was 73.4% and rainfall was erratic with 476 mm in June, 371 mm in a 14-day period in July, 289 mm in 13 days in August, and 103 mm in 12 days in September. Thus, there were periods of 15 days or more without rainfall so, for practical purposes, the plants in both experiments experienced drought during the critical periods of panicle development, flowering and grain filling.

Trait evaluations

Ten plants were selected at random from each of the 300 BC_2F_2 families in each experiment and evaluated for eight agronomic traits, including days to heading, plant height, panicle length, number of panicles per plant, percent sterility, grains per plant, 1000-grain weight and yield per plant. Evaluation was similar to that described in Xiao et al. (1998) as follows: (1) *Days to heading* was evaluated as the average number of days from seeding until 10% of the panicles had headed. (2) *Plant height* was measured as the average height of the ten plants in cm from the soil surface to the tip of the tallest panicle (awns excluded). (3) *Panicle length* was measured as the average number of centimeters from the panicle neck to the panicle tip (excluding the awn) based on an evaluation of all panicles from the ten plants. (4) *Panicles per plant* was the average number of panicles on the ten plants (panicles having less than five seeds were not counted). (5) *Percent sterility* was the number of empty spikelets divided by the total number of spikelets per panicle evaluated for each panicle on all ten plants. (6) *Grains per plant* was measured as the average number of filled spikelets per plant calculated for the ten plants. (7) *1000-grain weight* was the average weight of 1000 filled spikelets, measured in grams, averaged over three samples taken from bulk harvested grain from the ten plants. (8) *Yield per plant* was the average weight per plant, measured in grams of bulked harvested grain from the ten plants.

Trait correlations

Correlation among traits was evaluated in Excel using the trait averages from both experiments.

Marker analyses

The population of 274 BC_2F_2 families was analyzed using a total of 125 markers distributed at approximately 10-cM intervals throughout the genome. A total of 200 restriction fragment length polymorphisms (RFLPs), using four restriction enzymes (*Eco*RI, *Eco*RV, *Hind*III and *Dra*I) and 50 simple single-length polymorphisms (SSLPs), were used to survey the parents for polymorphism. The RFLP markers used to do the parental survey were chosen based on genome distribution and previous experiments, including the set of Cornell Anchor Probes widely used for comparative mapping, as described by Van Deynze et al. (1998), and many of the markers previously used for polymorphism analysis (RiceGenes database; http://genome.cornell.edu/rice/). The SSLPs were chosen to close gaps in the linkage map left by the distribution of RFLPs.

Southern analysis was performed as described in Causse et al. (1994). SSLP analysis was done as described in Chen et al. (1997), with the following modifications in the PCR profile: the initial denaturing step was 94°C for 5 min, subsequent denaturing was at 94°C for 15 s, annealing was 55°C for 15 s, extension was 72°C for 15 s; and steps 2 through 4 were repeated for a total of 35 cycles (34 times) with a final extension at 72°C for 5 min.

Linkage map

The order of the RFLP markers was based on the interspecific map of rice described by Causse et al. (1994) and the order of SSLP markers was based on Chen et al. (1997) and Temnykh et al. (1999). Marker integration was done by aligning markers common to both populations and establishing the most-likely order and cM distances using Mapmaker on the BC_2F_2 population. Segregation ratios of individual markers were statistically determined for each marker locus and deviation from the expected Mendelian ratios was determined by χ^2 tests ($P < 0.01$).

QTL analysis

Nomenclature for QTLs was as described in McCouch et al. (1997). The association between phenotype and marker genotype was investigated using single-point analysis (SPA), interval mapping (IM) and composite interval mapping (CIM). For single-point analysis and interval mapping, the QGENE application (Nelson 1997) was used. Composite interval mapping (Liu 1997) was implemented using QTL Cartographer software (Basten et al. 1994, 1997), with a model specifying five cofactors (as recommended by the authors of the software as default) to control for genetic background and a window size of 10 cM that blocked out a region of 5 cM on either side of the markers flanking the test site. The specific cofactors used were obtained by forward-backward stepwise regression with Fin = 0.01 and Fout = 0.01 using the same software.

The significance thresholds for the three different analyses were calculated based on permutation tests at an experiment-wise significance level $P < 0.01$ (Churchill and Doerge 1994). For single-point analysis, the threshold was established by doing 10000 permutations and selecting the experiment-wise threshold of $P < 0.01$ for each trait, which corresponded to an average LOD ≥ 3.6. Subsequently, the experiment-wise threshold for interval mapping was established by carrying out 1000 permutations at $P < 0.01$, corresponding to an average likelihood ratio of 17.28 and a LOD ≥ 3.75. The experimentwise threshold for composite interval mapping was obtained by doing 1000 permutations at $P < 0.01$, with an average likelihood ratio of 17.5, corresponding to a LOD score of 3.80. Confidence limits were calculated for the threshold values corresponding to each individual trait for both IM and CIM (data not shown) and, because the distributions overlap in both analyses, we used the average threshold for all traits (Mood et al. 1974). QTLs identified using these significant thresholds did not always agree for each analytical method. Those reported in this study represent QTLs identified by at least two of the methods described above.

The proportion of observed phenotypic variance attributable to a particular QTL was estimated by the coefficient of determination (R^2) from the corresponding linear model (single-point) analysis, and using maximum likelihood for composite interval mapping (Basten et al. 1997).

Genotype by environment interactions were analyzed using a standard analysis of variance (Proc GLM, SAS 1988), with $P < 0.01$. The rank of the different families in both environments were tested to see if there were shifts in population means using Spearman rank correlation coefficient (r_s) with a $P < 0.01$.

Results

Polymorphism of markers and marker segregation

Sixty percent of the RFLPs and 90% of the SSLPs were polymorphic in the parents, Caiapo and *O. rufipogon*. The BC_2F_2 population was evaluated using 82 RFLPs and 43 SSLPs. Two telomeric regions of the genome, the long arm of chromosomes 4 and 6, showed extensive monomorphism with both types of markers.

In an unselected BC_2 population, the expected segregation ratio would be 75% homozygotes (Caiapo/Caiapo):25% heterozygotes (Caiapo/*O. rufipogon*), resulting in an allele frequency of 87.5 Caiapo:12.5 *O. rufipogon* alleles. The ratio in this population was 89% Caiapo:11% *O. rufipogon*, due to skewed allele frequencies at 47 out of 125 (37.6%) of marker loci. Twenty eight percent of these loci were skewed toward Caiapo while 8.8% were skewed toward *O. rufipogon* ($\chi^2 > 6.635$, $P < 0.01$). Skewing toward the adapted, elite parent can be explained by the selection imposed in the BC_1 and

BC_2 generations during population development, but skewing toward *O. rufipogon* was not expected. This may be the result of reduced recombination due to the genetic distance between the parental lines as suggested by Causse et al. (1994) and Grandillo and Tanksley (1996).

Trait correlations

Correlations among traits were evaluated at $P < 0.05$ and $P < 0.01$. As summarized in Table 1, the strongest correlation was found between yield and grains per plant, with significant correlations also found between yield and the number of panicles per plant, plant height, panicle length and 1000-grain weight. There was a negative correlation between yield and days to heading that can be explained by the fact that drought differentially affected the late-maturing genotypes, depressing yields, while early maturing lines escaped the most serious effects of the drought. As expected, a negative correlation was found between yield and percent sterility.

Phenotypic variation for the different traits

As illustrated in Fig. 1, positive phenotypic transgressive variation in the BC_2F_2 population was observed for all traits except for the number of panicles per plant. For this trait, *O. rufipogon* demonstrated a significantly higher number of panicles per plant than Caiapo or any of the BC_2F_2 families in both experiments. The transgressive variation suggested positive genotype × genotype (G × G) variation where *O. rufipogon* alleles augment performance in a largely Caiapo genetic background. For example, in the case of panicle length (Fig. 1), segregants with panicles 3–4 cm (19–25%) longer than those of Caiapo were observed in the BC_2F_2 population, despite the fact that *O. rufipogon* panicles were consistently shorter than those of Caiapo. Another example is yield per plant, where segregant plants with 2.5–3.7 grains (66–90%) higher than Caiapo were observed.

Genotype by environment interaction

When the experiments with and without pasture association were compared, the means for 6 out of 8 traits were higher under monoculture conditions. G × E interaction was not significant in the two experiments ($P < 0.01$). However, the Spearman correlation coefficient was also significant ($P < 0.01$) for all traits, varying between 0.3 and 0.6 for the different traits; indicating that the rank of the BC_2F_2 families was similar in both experiments. Significant shifts in the mean performance of the parents and in the range of variation of the population between the two experiments were observed for days to heading (both parents headed earlier in Experiment 2 and the range of variation in the population was less than in Experiment 1), grains per plant and yield per plant (parental and population means were significantly lower in Experiment 2) (Fig. 1). Estimates of sterility in the parents were the most erratic, with a percent sterility for *O. rufipogon* almost twice as high in Experiment 2 as in Experiment 1. On the other hand, little difference in performance between the two environments was observed for plant height, panicle length and 1000-grain weight, suggesting that these traits were more heritable.

QTL regions

Significant QTLs were detected for all traits except panicle length as summarized in Table 2 and Fig. 2. Of the 25 QTLs identified, SPA and IM detected 23 (92%) QTLs in common. Seventeen (68%) of the QTLs detected by SPA were also detected by CIM, while 76% of QTLs detected by IM were detected by CIM. The lack of consensus regarding QTL identification was most extreme for plant height, where four QTLs identified by both SPA and IM were not significant using CIM. This is a case where the use of cofactors appears to be particularly important. However, it is interesting to note that when the four plant height QTLs not detected by CIM are removed from the comparison, 17 (81%) and 19 (90%) of the remaining QTLs for all traits detected by SPA and IM, respectively, were also detected by CIM. In several cases, QTLs detected in the monoculture experiment (Experiment 1) were not significant in the experiment with pasture association (Experiment 2) or vice versa. In many of these cases, a sub-threshold peak (indicated by regular font in Table 2) was detected for the alternate experiment. QTLs at or above the empirical threshold are in bold in Table 2.

Table 1 Correlation coefficients among traits in a *O. rufipogon*-derived BC_2 population (*=$p<0.05$; **=$p<0.01$)

Item	Days head	Plant height	Pan. length	Pan./pl.	Percent sterility	Gr/pl	1000-gw	Plant yield
Days to heading	1							
Plant height	–0.31**	1						
Panicle length	–0.08	0.50**	1					
Number pan./pl.	–0.22**	0.09	0.03	1				
Percent sterility	0.31**	–0.13*	–0.09	–0.13*	1			
Grains/plant	–0.39**	0.47**	0.47**	0.60**	–0.43**	1		
1000-grain weight	–0.12*	0.22	0.09	0.25**	–0.04	0.23**	1	
Plant yield	–0.40**	0.48**	0.44**	0.61**	–0.41**	0.97**	0.43**	1

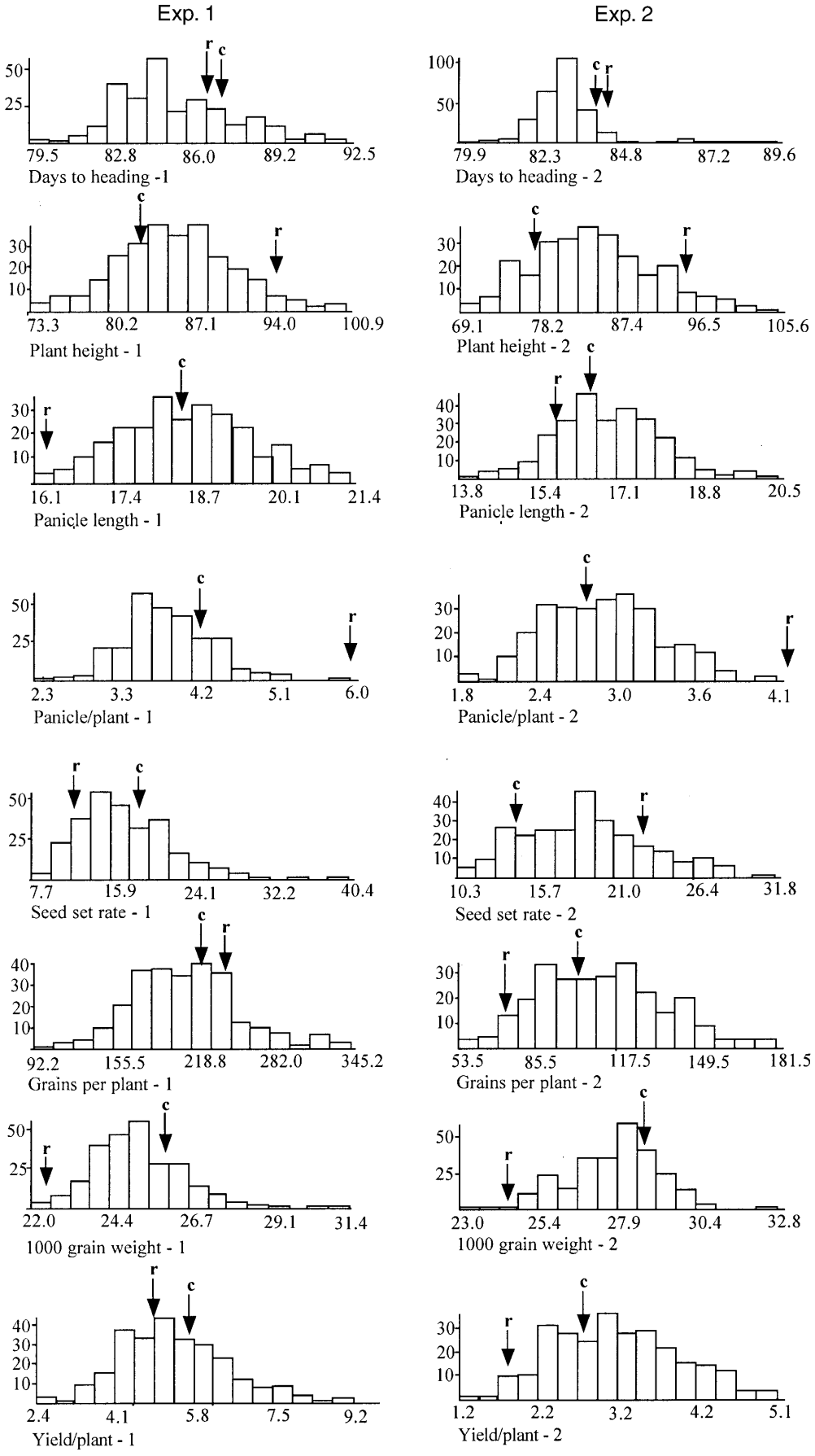

Fig. 1 Frequency distribution of the means of the 300 F_2BC_2 families for each trait. The parental means of *O. rufipogon* (*r*) and Caiapo (*c*) are indicated by arrows. Exp 1=Rice Monoculture; Exp 2=Rice-Pasture Association

Table 2 Putative QTLs detected in a BC_2 population from a Caiapo X *O. rufipogon* cross

QTL	Chrom.	Marker	Exp.	Increased effect	F-SPA	P-SPA	LOD-SPA[a]	Var-SPA	LOD-IM[a]	Var-IM	LOD-CIM[a]	Var-CIM
Days to heading												
dth2.1	2	RM266-RM207	1	*rufipogon*	14.51	0.0002	3.08	0.054	3.49	0.125	**4.65**	0.140
	2	RM266-RM207	2	*rufipogon*	17.42	0.0000	**3.68**[b]	0.064	**3.86**	0.096	**3.94**	0.101
dth3.1	3	RG104-RZ329	1	*rufipogon*	25.01	0.0000	**5.23**	0.085	**5.18**	0.088	**5.94**	0.097
	3	RG104-RZ329	2	*rufipogon*	14.55	0.0002	3.09	0.052	**3.69**	0.092	**4.95**	0.106
dth3.2	3	RZ576-RZ22	1	Caiapo	15.37	0.0001	3.26	0.055	3.31	0.053	**3.88**	0.064
	3	RZ22-RZ576	2	Caiapo	18.51	0.0000	**3.91**	0.064	**3.96**	0.064	**3.80**	0.062
dth7.1	7	RG30-RM125	1	*rufipogon*	21.40	0.0000	**4.50**	0.073	**4.88**	0.116	**4.12**	0.078
	7	RG30-RM125	2	*rufipogon*	14.82	0.0001	3.15	0.052	3.36	0.071	3.12	
Plant height												
ph1.1	1	RG462-RZ613	1	*rufipogon*	11.86	0.0007	2.54	0.042	**3.74**			
	1	RZ613-RZ513	1	*rufipogon*	5.51	0.0196	2.65	0.020	3.65	0.095		
	1	RZ613-RZ513	2	*rufipogon*	13.21	0.0003	**6.46**	0.046				
ph1.2	1	RM104-RZ801	1	*rufipogon*	16.99	0.0001	**3.60**	0.063	**4.18**	0.118	**3.76**	
	1	RM104-RZ801	2	*rufipogon*	67.10	0.0000	**13.02**	0.210	**13.28**	0.215	**10.03**	0.176
ph2.1	2	RG256b-RM207	1	*rufipogon*	8.03	0.0050	1.73	0.029	2.06		2.02	
	2	RG256b-RM207	2	*rufipogon*	25.41	0.0000	**5.31**	0.087	**5.84**	0.138	**4.41**	0.094
ph2.2	2	RM266-RM207	1	Caiapo	9.89	0.0019	1.62	0.038	2.03			
	2	RM266-RM207	2	Caiapo	26.28	0.0000	**3.66**	0.094	**4.96**			
ph4.1	4	RG169-CDO244	1	*rufipogon*	11.96	0.0006	2.56	0.042	**4.57**			
	4	CDO244-RZ740	1	*rufipogon*	10.81	0.0011	2.31	0.039	2.59			
	4	CDO244-RZ740	2	*rufipogon*	30.09	0.0000	**6.23**	0.102	**6.83**	0.101		
ph5.1	5	CDO202-RZ925	1	*rufipogon*	14.61	0.0002	3.11	0.052	3.01	0.059		
	5	CDO202-RZ925	2	*rufipogon*	37.49	0.0000	**7.67**	0.124	**7.80**	0.144		
Panicles per plant												
ppl6.1	6	RM3-CDO78	1	*rufipogon*	20.79	0.0000	**4.36**	0.079	**4.54**	0.076	**5.25**	0.183
ppl11.1	11	RZ537-RZ900	2	*rufipogon*	18.63	0.0000	**3.94**	0.064	**4.11**	0.083	**4.29**	0.085
Percentage of sterility												
ste10.1	10	CDO98-RM304	1	*rufipogon*	21.07	0.0000	**3.55**	0.076	**4.29**	0.090		
	10	CDO98-RM304	2	*rufipogon*	10.62	0.0013	2.27	0.040	2.63			
ste10.2	10	RM147-RZ500	1	*rufipogon*	30.89	0.0000	**6.37**	0.108	**6.22**	0.128	**6.56**	0.131
Grains per plant												
gpl1.1	1	RZ513-RZ613	1	*rufipogon*	15.42	0.0001		0.054	3.49	0.083	**4.87**	0.112
	1	RZ513-RZ613	2	*rufipogon*	17.68	0.0000	**3.74**	0.061	**3.80**	0.061	**5.17**	0.084
gpl2.1	2	RG256b-RM207	1	*rufipogon*	13.82	0.0002	2.95	0.049	3.44	0.074	**4.08**	0.078
	2	RG256b-RM207	2	*rufipogon*	14.22	0.0002	3.03	0.050	**3.83**	0.094	**5.91**	0.119
gpl6.1	6	waxy-RZ1002	1	Caiapo	16.85	0.0001	**3.57**	0.062	**3.73**	0.067	**7.03**	0.118
	6	waxy-RZ1002	2	Caiapo	4.50	0.0349	0.97	0.018				
gpl11.1	11	RZ537-RZ900	1	*rufipogon*	7.50	0.0066	1.62	0.027				
	11	RZ537-RZ900	2	*rufipogon*	15.21	0.0001	3.24	0.053	**3.66**	0.073	**4.72**	0.114

Table 2 continued

QTL	Chrom.	Marker	Exp.	Increased effect	F-SPA	P-SPA	LOD-SPA[a]	Var-SPA	LOD-IM[a]	Var-IM	LOD-CIM[a]	Var-CIM
1000-grain weight												
gw1.1	1	RZ613-RZ513	1	*rufipogon*	18.59	0.0000	2.03	0.064	**4.43**	0.214	**6.27**	0.174
	1	RZ613-RZ513	2	*rufipogon*	16.95	0.0001	**3.60**	0.059	**4.28**	0.080	2.60	
gw1.2	1	RG462-RZ613	1	*rufipogon*	9.47	0.0023	**3.93**	0.034	**6.02**	0.220	**5.45**	0.199
gw3.1	3	RZ996-RM227	1	*rufipogon*	18.21	0.0000	**3.85**	0.063	**4.63**	0.116		
	3	RZ996-RM227	2	*rufipogon*	16.83	0.0001	**3.56**	0.062	3.20	0.053		
gw11.1	11	RZ537-RZ900	1	*rufipogon*	31.41	0.0000	**6.50**	0.104	**7.19**	0.118	**7.21**	0.121
	11	RZ537-RZ900	2	*rufipogon*	19.11	0.0000	**4.03**	0.066	**4.11**	0.076	**4.80**	0.078
gw11.2	11	RM254-RM224	2	*rufipogon*	4.07	0.0447	3.35	0.016	**3.89**	0.139	**4.03**	0.085
	11	RM254-RM224	1	*rufipogon*	17.00	0.0001	**3.60**	0.063			**6.95**	0.092
Yield per plant												
yld1.1	1	RZ513-RZ613	1	*rufipogon*	18.39	0.0000	**3.89**	0.064	**4.09**	0.139	**5.01**	0.143
	1	RZ513-RZ613	2	*rufipogon*	18.18	0.0000	**3.85**	0.063	**4.36**	0.106	**4.66**	0.115
yld11.1	11	RZ537-RZ900	1	*rufipogon*	15.42	0.0001	3.28	0.054	3.44	0.070	**3.71**	
	11	RZ537-RZ900	2	*rufipogon*	21.47	0.0000	**4.52**	0.073	**4.96**	0.098	**5.95**	0.139

[a] Significance thresholds levels (SPA = 3.6, IM = 3.75, CIM = 3.8) were determined by permutation tests
[b] Bold numbers indicate QTLs detected with LOD scores greater than the significance thresholds levels. A normal font indicates QTL peaks in the same regions that were detected in the alternate experiment or alternate analyses with LOD scores below the significance thresholds levels

Days to heading

Four QTLs were significantly associated with days to heading. *O. rufipogon* alleles contributed earliness at one of them, *dth 3.2*, located on chromosome 3, while for the other three, *O rufipogon* alleles increased the number of days to heading. The variation explained by these individual QTLs ranged from 6% to 14% as determined by CIM and was similar based on maximum-likelihood estimates for SPA and IM (Table 2).

Plant height

Of the six QTLs associated with plant height that were detected by SPA and IM, only *ph1.2* and *ph2.1* were detected by CIM; *ph1.2* on chromosome 1 had the highest level of significance of any QTL detected in this experiment and alone accounted for 17–21% of the variation for plant height in experiment 2 where rice was grown in association with pastures, while in monoculture the variation ranged from 6 to 12%. Significance levels were consistently higher for plant-height QTLs associated with Experiment 2, and only those associated with Experiment 2 were detected by CIM. The QTL with the second largest R2 value was *ph5.1* which explained 12–14% of the phenotypic variance in experiment 2. This QTL was not detected by CIM in either experiment. *ph2.1*, which was detected by CIM, IM and SPA, explained 9–14% of the phenotypic variance. Alleles from *O. rufipogon* increased stature at all but locus *ph2.2*.

Panicle length

No QTLs were detected for panicle length in this study.

Panicles per plant

Two QTLs, *ppl6.1* and *ppl11.1*, affected the number of panicles per plant and they were identified as significant by all three analytical procedures. In both cases, the wild allele conferred a positive effect, increasing the number of panicles per plant, as would be predicted from the phenotype of *O. rufipogon*. The QTL *ppl6.1* was significant only in Experiment 1, explaining 18.3% of the phenotypic variation as determined by CIM. The QTL *ppl 11.1* was significant only in Experiment 2 and had an R^2 value of 8.5% as determined by CIM.

Percent sterility

Two QTLs, both located on chromosome 10, were associated with plant sterility and in both cases, increased sterility was associated with the *O. rufipogon* allele. Evidence for these QTLs was strongest in Experiment 1. Only one of the QTLs, *ste10.2*, was detected by CIM and it explained 13% of the phenotypic variation.

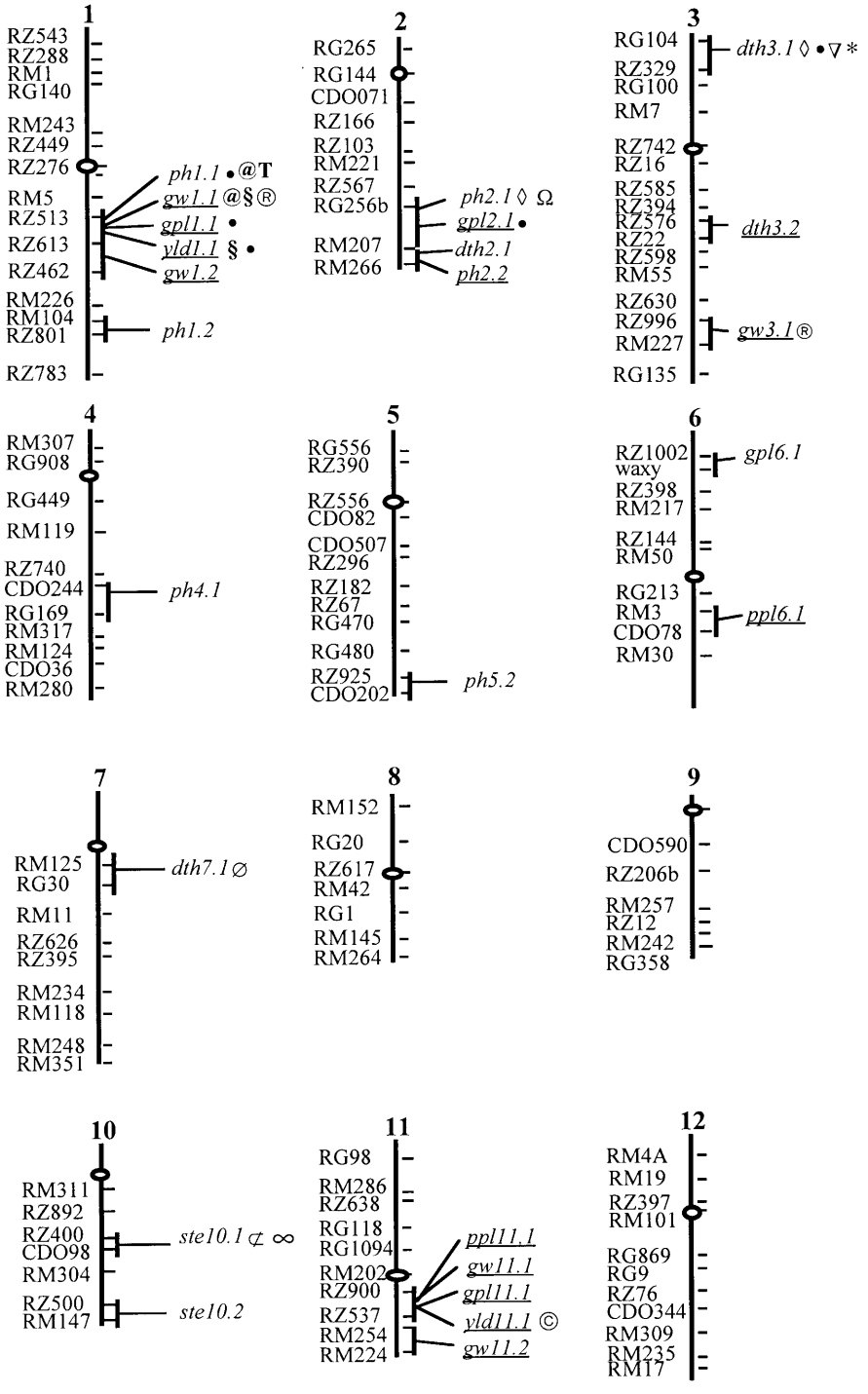

Fig. 2 Map locations of the putative QTLs detected in this study. RFLP and SSLP marker order is based on the rice molecular genetic map (Causse et al. 1994; Temnykh et al. 1999). Centromeres are indicated by a hollow oval along chromosomes, as first described by Singh et al. (1996). *Solid bars* to the right of the chromosomes indicate QTLs defined by the marker interval with the most significant LOD score when all three analytical methods were compared. *Underlined* QTLs signify those for which the wild species, *O. rufipogon*, confers a positive effect from a breeding perspective. Symbols to the right of QTL designations indicate that a QTL for the same trait was identified in a similar position as reported previously by: •Xiao et al. (1998), ◊ Li et al. (1995), ∇ Price et al. (1997), * Lin et al. (1995), ∅ Yano et al. (1997), @ Wu et al. (1996), t Cho et al. (1994), Ω Kinoshita (1995), ∝ Yao et al. (1997), ∞ Tan et al. (1998), § Zhuang et al. (1997), ® Lu et al. (1997), £ Lin et al. (1996), and © Yu et al. (1997)

Grains per plant

Four regions were associated with the number of grains per plant. All four were detected by both IM and CIM, and two were also detected by SPA. Three of the QTLs, *gpl1.1*, *gpl2.1* and *gpl11.1*, showed the positive effect coming from the *O. rufipogon* introgression. The phenotypic variance explained by any of the loci associated with this trait ranged from 6 to 12% (IM and CIM).

1000-grain weight (grams)

Five QTLs were associated with 1000-grain weight. In contrast to what would be predicted based on the phenotypes of the parents, alleles derived from *O. rufipogon* increased 1000-grain weight at all five loci. The phenotypic variation explained by individual QTLs ranged from 5 to 22% (IM and CIM). The largest QTLs (*gw1.1* and *gw1.2*, located on chromosome 1) individually ex-

plained 17–22% of the variation (IM and CIM) under monoculture conditions (Exp. 1).

Yield per plant

Two genomic regions were significantly associated with yield per plant. The *O. rufipogon* allele was responsible for increasing yield in both cases. Individual loci explained 7–14% (IM and CIM) of the variance. Both QTLs were detected by all three analytical procedures.

Discussion

A comparison of results obtained from SPA, IM and CIM in this study demonstrated that these three methods of associating quantitative phenotypic variation with molecular marker-defined regions of the genome identified the same QTL about 80% of the time. It was important to harmonize the *F*-values from SPA with LOD scores of IM and likelihood ratios of CIM by converting them to LOD scores [see method described by Doerge in the Appendix to Champoux et al. (1995)], in order to compare results obtained from these three analytical approaches. Interestingly, some QTLs with *P* values lower than 0.001 were found to correspond to LOD scores lower than 2.0 while, in general, only those corresponding to LOD scores greater than 2.5 were detected by IM and/or CIM. In view of these findings, the common practice of reporting QTLs detected by SPA at $P \leq 0.05$ is likely to detect numerous false positives.

The largest number of QTLs was identified using IM and the results of SPA agreed most closely with those of IM. CIM estimates the position of the QTL differently than SPA or IM, and by identifying multiple QTLs that simultaneously affect a trait and extracting the variance associated with them, this analysis eliminates some of the loci that meet the significance criteria with the other analyses. Therefore, some of the QTLs that appear significant in SPA and IM fall below the assigned significance threshold with CIM. To confirm which of these putative QTLs are associated with real genetic effects, near-isogenic lines carrying single introgressions in the target regions will be developed and evaluated phenotypically.

When SPA, IM, and CIM disagree about a QTL detection, it suggests that at least one analysis is giving a false positive or a false negative. However, with any statistical procedure, the exact diagnosis for individual cases is uncertain. Beavis et al. (1994) demonstrated by simulations that QTL effects are often overestimated when using interval mapping, and Southey and Fernando (1998) reported that the rate of false positives among QTL detections can be much larger than the experiments' nominal Type-I error rate. Thus while permutation tests give a threshold that controls the rate of false positives when testing the null hypothesis of ''no QTL'' at each marker or interval in the genome, the error rate of greater relevance to plant breeders is the rate of false positives *among QTLs detected* when these statistics are used. Hopefully, in the future, methods will be developed to better quantify the rate of false positives and false negatives among QTL detections. Meanwhile, the evidence for a putative QTL can be increased by comparing a given experiment with other, related experiments, as has been done here. The most conservative approach is to focus on those QTLs detected by at least two of the three analyses or in multiple experiments. Finally, especially strong evidence can be sought by subsequent confirmation of a QTL effect in an NIL, preferably tested in several locations within the target environment, as planned here.

QTL studies are plagued by G × G and G × E interactions that make it difficult to predict which putative QTLs are likely to be most stable when transferred to a new genetic background and/or evaluated under different environmental conditions. Veldboom and Lee (1996) addressed this question and suggested that the best approach to QTL detection was to use an average of randomly occurring environments, including the rarer stress-prone environments. Identifying QTLs in this ''mean environment'' seems to provide the best strategy for detecting QTLs that will be broadly useful in a marker-assisted selection program. Fulton et al. (1997) similarly addressed this issue by suggesting that QTLs that were most consistent over environments were more likely to be useful than those that showed an extremely high significance value but were detected in only one year or under one set of conditions.

In this study, we compared the findings of this set of experiments with those previously reported by other researchers evaluating similar characters in different cross combinations and different environments. The use of a common set of molecular markers made it possible to determine whether QTLs from all the different studies were in similar regions of the rice genome. By doing so, newly reported QTLs could be compared to previously reported QTLs, lending legitimacy to those with a prior history and suggesting caution for those flagged for the first time.

Of the four QTLs identified for days to heading, *dth3.1* and *dth7.1* are in the same or similar regions as previously identified QTLs (as summarized in Fig. 2). The QTL *dth3.1* in this study is in a similar location as *dth3* (Xiao et al. 1996), QHd3a (Li et al. 1995), DH 3 (Price et al. 1997) and hd3 (Lin et al. 1995), and *dth7.1* coincides with Hd-4 (Yano et al. 1997). Future work aimed at cloning and characterizing these alleles will confirm whether these previously reported QTLs correspond to the same or tightly linked QTLs and whether the alleles coming from *O. rufipogon* are the same as those found in the cultivated *O. sativa* gene pool.

Plant-height QTLs in the same region as *ph1.1* have been previously reported by Xiao et al. (1998) and Wu et al. (1996), and a QTL similar to *ph2.1* was reported by Li et al. (1995). QTL *ph1.1* may represent the semidwarf locus, *sd-1*, which is located in a similar region on

chromosome 1 (Cho et al. 1994). The QTL *ph2.1* is in similar position to the known dwarfing mutants *d-30* (waisei-shirasasa dwarf) and *d-5* (bunketsu-waito) (Kinoshita 1995).

A total of 53 semidwarf genetic stocks have been reported in rice, and while nine of them are known to be allelic to the highly mutable *sd-1* locus (Rutger 1992), many others appear to be independent. It will be interesting to determine how many of them define QTLs associated with plant height and to determine whether *O. rufipogon* and other wild species may harbor novel alleles that differ in structure and function from any of the alleles that have been so widely used to alter plant stature, harvest index and other important agronomic characteristics in plant improvement to-date.

Neither of the two QTLs affecting the number of panicles per plant in this study coincided with any previously published QTLs in rice. Despite the lack of historical support for the existence of these QTLs, this is the only trait where the putative QTLs were supported by all three analytical procedures.

Two QTLs were associated with plant sterility in this study and, in both cases, the *O. rufipogon* alleles were associated with increased sterility. A known fertility restoration locus, *Rf*-1, resides on chromosome 10 in a similar position to *ste10.1* on chromosome 10 (Yao et al. 1997; Tan et al. 1998).

O. rufipogon alleles were associated with an increase in the number of grains per plant for three of the four QTLs identified for this trait. Two of these loci, *gpl1.1* and *gpl2.1*, are in the same locations on chromosomes 1 and 2 as the yield QTLs reported by Xiao et al. (1998), where the *O. rufipogon* alleles increased yield in a hybrid variety grown under high-input conditions. This suggests that *O. rufipogon* introgressions at these loci may be advantageous in divergent genetic backgrounds and growing environments. The QTL on chromosome 1, *gpl1.1* was also located in the same region as the height QTL *ph1.1*, possibly corresponding to the *sd-1* gene. It has long been observed that the *sd-1* gene not only dwarfed plant stature, but was associated with increased harvest index and yield. It will be worth investigating further to understand the molecular structure and function of *O. rufipogon*-derived QTL in this region, to better interpret the relationship between tall stature and grains per plant. It should be kept in mind that the optimal plant height varies with the ecosystem in which the cultivars are grown, with intermediate to tall stature being preferred under upland conditions.

For all grain-weight QTLs, *O. rufipogon* alleles were associated with heavier grains despite the fact that *O. rufipogon* itself has small, light grains. *gw1.1* appears to be located in a similar position as 1000G (Wu et al. 1996), gwt1 (Zhuang et al. 1997), and g370 (Lu et al. 1997), while *gw3.1* in this study appears to correspond with the position of g1318 (Lu et al. 1997).

Alleles from *O. rufipogon* were associated with a positive effect on yield at both putative QTL loci *yld1.1* and *yld11.1*. Both of these QTLs are in similar positions to the yield-QTLs reported previously by Lin et al. (1996) and Yu et al. (1997). The QTL on chromosome 1, *yld1.1*, is in a similar position to the one reported by Xiao et al. (1998) using the same donor species in a Chinese hybrid background grown under irrigated conditions. Both of the other studies used completely different genetic material but both also evaluated yield under height input, irrigated conditions. Thus, it will be of interest to determine whether the *O. rufipogon* alleles associated with improved yields of Caiapo under dryland conditions are the same as or different from alleles from this and other sources described previously.

A total of 25 QTLs were detected for the eight traits evaluated in this study. For 14 (56%) of them, alleles from *O. rufipogon* were associated with a positive effect on plant performance. So although the *O. rufipogon* parent was phenotypically inferior for seven of the eight traits studied, transgressive segregants that out-performed the elite Caiapo parent were obtained. No strongly deleterious characters appear to be associated with any of the favorable *O. rufipogon*-derived QTLs described above. In some cases, loci associated with yield and yield components were located in the same regions as QTLs for plant height. However, intermediate to tall stature is considered advantageous in upland cultivars like Caiapo, so until the relationship between these two phenotypes is better understood and the interaction among genes in a given genetic background can be verified, no conclusions about the agronomic value of these plant height loci can be reached.

Several genetic regions were associated with more than one trait, indicating linkage and/or pleiotropic effects. For example, on chromosome 1 there are QTLs for grain weight, the number of grains per plant and plant yield, for which the flanking markers were the same. By developing further generations of near-isogenic lines containing finely mapped QTLs, we aim to develop the basis for better characterizing these loci.

The establishment and evaluation of the BC_2F_2 population under conditions of monoculture (Exp. 1) and pasture (*Brachiaria* sp.) competition (Exp. 2) provided an opportunity to evaluate the impact of genotype by environment interaction due to plant competition. It was surprising that, in these experiments, the rank of the genotypes was very similar in both environments and, in general, the same QTLs were detected in both experiments. In some cases, the level of significance was different, and as a result some QTLs fell just beneath the threshold in one of the experiments (Table 2). It will be of interest to evaluate selected families in other environments to determine what kind of $G \times E$ interaction might be observed.

Intercropping upland rice with pastures, maize, cassava and/or beans is a common practice in Latin America**.** Few, if any, QTL studies have been undertaken to examine whether the same or different QTLs contribute to phenotypic success in a monocropping versus an intercropping situation. Of equal interest is whether the same QTL allele may have a positive effect in one environment but be detrimental in another. Specifically, we were interested to determine whether any of the yield or yield

component QTLs demonstrated a differential effect when plants were grown as a monoculture or in the presence of a second crop in close association with rice. The fact that the same QTLs (position and parental allele) were generally detected in both experiments suggested that the drought may have been more severe than the stress caused by the pasture competition, masking the effect of the intercropping. It is possible that the expression of the QTLs detected in this study was truly unaffected by pasture competition, but confirmation of this question would require further experimentation. It might be expected that *O. rufipogon* would display strong competitive ability in the face of weed or pasture competition, given its wild habit. On the other hand, Caiapo was bred to be productive under the stressful conditions typical of upland farming and it is likely that an important characteristic would be good competitive ability. If QTLs having detectable effects on competitive ability in this context are rare, or if similar QTLs were shared between Caiapo and *O. rufipogon*, they would go undetected in this population.

It should be kept in mind that upland rice environments vary widely in terms of climate and edaphic factors, making it difficult to use genetic material developed for one location in other locations. Therefore, the results obtained in this work should not be applied directly to different upland areas. It is proposed that near-isogenic lines containing individual introgressions associated with positive QTLs from *O. rufipogon* be developed from this population and evaluated in a wide range of upland environments, so that QTL × environment interaction can be assessed and ''mega-environments'' can be identified for future testing. Of particular interest to upland rice breeders will be the evaluation of the performance of NILs containing *yld1.1* and *yld11.1* in the Caiapo background.

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