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QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers

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Abstract Rice (*Oryza sativa*) cultivar development currently faces the task of overcoming yield plateaus, which is difficult due to the narrow genetic base of breeding programs. *Oryza glumaepatula* is a diploid wild relative of cultivated rice, native to Central and South America, and is therefore a potential source of alleles of agronomic importance to rice breeding programs. We studied 11 agronomic traits in BC_2F_2 families of the interspecific cross *Oryza sativa* × *O. glumaepatula*. Transgressive lines which are almost isogenic to the elite recurrent *O. sativa* parent were identified for most of these traits. Quantitative trait locus (QTL) analysis was performed by single-point and interval mapping using a molecular map based on 157 microsatellite and STS markers. Marker regions accounting for 14.5 to 72.9% of a phenotypic variation trait were identified in 9 of the 12 rice chromosomes. Positive QTL effects from *O. glumaepatula* were observed in chromosomal regions associated with tillering and panicle-number traits.

Keywords Microsatellite markers · Advanced backcross · QTL analysis · *Oryza sativa* · *Oryza glumaepatula*

Introduction

One of the main objectives of rice breeding has been to overcome the average yield plateau reached by irrigated and upland rice during the last few years. Among other factors, the yield plateau appears to be a consequence of the narrow genetic base of modern rice cultivars (Rangel et al. 1996; Tanksley and McCouch 1997). In order to broaden the rice genetic base, which would make it possible to breed for increased crop yield, crosses have been made between elite cultivars and genetically distant relatives such as landraces and varieties from different gene pools (e.g., *indica* × *japonica* crosses) (Wu et al. 1996; Zhuang et al. 1997). Furthermore, despite the overall inferior agronomic phenotypes observed in wild species, they have been a useful source of favorable genes, since the beginning of modern breeding. Their use, however, has usually been restricted to the introgression of major genes controlling qualitative traits, such as disease resistance. Attempts to transfer genes controlling quantitative traits from wild relatives to cultivated elite varieties of rice have, in general, been limited and unsuccessful. Interspecific crosses usually result in progenies with sterility problems, disruption of favorable linkage blocks and gene combinations and, most importantly, linkage drag related problems, making it difficult to select and use superior phenotypes for breeding purposes.

The success of breeding programs based on interspecific crosses depends on a number of factors, including the possibility of obtaining fertile hybrids and the potential to identify and transfer useful genes for agronomic traits. There are several strategies to introgress useful genes from a wild relative to a cultivated variety, usually based on backcrosses, and selection for the trait phenotype. Variations of the backcross method (Wehrhahn and Allard 1965) combined with genetic map information based on molecular markers (Eshed and Zamir 1995; Tanksley and Nelson 1996) have been used to map quantitative trait loci (QTLs) and select families with desired chromosomal regions. The combination of molecular-marker linkage information with breeding methods that exploit the advantages of interspecific crosses provide a unique opportunity to monitor the process of introgression of alien alleles controlling quantitative traits. This has facilitated the use of interspecific crosses to exploit genetic variability for quantitative traits, as has been reported in crops such as tomato (Fulton et al. 1997; Bernacchi et al. 1998) and rice (Doi et al. 1998; Xiao et al. 1998).

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Oryza glumaepatula is a diploid wild species native to the Amazon forest and flooded areas of western Brazil. It is closely related to *Oryza sativa* (Buso et al. 1998) and is considered to be a potential source of useful genes of agronomic importance. We analyzed genetic linkage-map information for yield-related traits of recombinant families derived from an interspecific cross involving an elite *O. sativa* line and a wild accession of *O. glumaepatula*.

Materials and methods

Development of experimental populations

A single plant of *O. glumaepatula* RS-16, selected from a highly inbred population of wild rice collected in the Amazon Region (Buso et al. 1998), was used as a female parent in crosses with the high yielding elite inbred line *O. sativa* BG90-2. Four F1 plants, whose hybrid nature was confirmed with RAPD and microsatellite markers (Cavalheiro et al. 1996), were backcrossed to BG90-2, now used as female parent. Based on their phenotypic similarity to BG90-2, 256 BC_1F_1 plants were selected and backcrossed to BG90-2, used again as the female progenitor. Ninety six BC_2F_1 progenies were selected at random and used in the analysis. The best plant of each progeny, selected for traits such as plant height, lodging and shattering, was selfed to produce BC_2F_2 seeds.

Phenotypic evaluation

The 96 BC_2F_2 families, the two parents and the commercial rice cultivar BR-IRGA 409 (control) were pre-germinated in plastic cups, and transplanted to the field 30 days after germination. The experiment followed a complete randomized block design, with three replications (plots), three rows per plot (3 meters), 20 plants per row. The border rows of each plot were planted with BG90-2 and the middle row with a BC_2F_2 family, which was analyzed. The 576 plots, with a population density of 330,000 plants per hectare, were planted and 5–8 plants per plot were selected for analysis. The experiment was carried out during the summer season of 1999 at the National Rice and Bean Research Center, Goiânia city, Goiás state, Brazil (Location 1, 16°S, 49°W, 749 m), and the Experimental Station of Formoso, Formoso do Araguaia city, Tocantins state, Brazil (location 2, 11°S, 49°W, 240 m). Eight BC_2F_2 plants per plot were examined and evaluated for the following traits: *Days to flowering* (DTF) – number of days from sowing to flowering of 50% of the panicles, *Plant Height* (PHT) – distance (cm) from the plant base to the tip of the panicle of the tallest tiller; *Tiller Number* (TNR) – number of tillers per plant; *Panicle Number* (PNR) – number of panicles per plant; *Panicle Length* (PLH) – average length (cm) of five randomly selected panicles per plant; *Spikelets per Panicle* (SPP) – number of filled and empty spikelets in five panicles per plant; *Percentage of Filled Grains per Panicle* (PFG); *100-Grain Weight* (HGW) – weight (g) of a sample of 100 grains per plant, mean of five plants per plot; *Grain Yield per Plant* (GYP) – total grain weight (g) per plant, mean of five plants per plot; *Filled Grain Number per Panicle* (FGP) – mean of five plants per plot; *Grain Yield per Panicle* (GYPa) – total grain weight (g) per panicle, mean of five plants per plot.

Linkage map construction

The linkage map was constructed with 157 molecular markers, 150 SSR (Simple Sequence Repeat) markers and seven STS (Sequence Tagged Site) markers, including newly-developed SSR markers from an enriched genomic library from *O. glumaepatula* (Brondani et al. 2001). Fresh leaves of BC_1F_1 plants were collected and used for DNA extraction. The map population consisted of 93 BC_1F_1 plants from the cross *O. sativa* × *O. glumaepatula*. Recombination fractions between pairs of markers and map analysis were performed using the program Mapmaker version 2.0 for Macintosh (Lander et al. 1987). Markers were allocated to linkage groups with a minimum threshold LOD score of 5.0 and a maximum recombination fraction $\theta = 0.25$ using the "group" command. The order of linkage groups was determined using the "compare", "try" and "first order" commands. The use of SSR markers previously mapped on the 12 rice chromosomes (Chen et al. 1997) allowed for direct identification of the linkage groups according to the rice chromosome nomenclature.

Trait analysis

Skewness and kurtosis tests (Snedecor and Cochran 1980) were applied to the distribution of the phenotypic traits. The analysis of variance (ANOVA), genotype by environment (G × E) interaction and correlation tests were performed using the software Genes version 2.0 (Cruz 1997). Trait correlation was evaluated by regressing phenotypic values of one trait on those of another trait, also using the Genes program.

QTL analysis

The analysis of quantitative trait loci was done using both single-point and interval-mapping analyses (flanking-marker regression approach; Haley and Knott 1992), using the software QGene version 2.30 for Macintosh (Nelson 1997). Single-point analysis was performed using simple regression, where the dependent variable is the trait score and the independent one is the allele state at a locus. The statistical threshold for single-point analysis was $P = 0.002$. The multiple-regression model expresses the phenotype as a linear function of the allele states of several marker loci. The proportion of the total phenotypic variation explained by each QTL was calculated as an R^2 value, from the regressions of each marker/phenotype combination. To construct a multiple-regression model, a significance level of $\alpha = 0.002$ for random marker-QTL associations was set and, there after, a marker was included in the model only if it could significantly increase the phenotypic variation. For interval mapping, we used a minimum LOD score of 3.0.

The additivity percentage (Add%) of each significant QTL was calculated as 100-times the additivity (AA–AB) divided by the observed mean of the homozygous recurrent parent (since the homozygous donor parent could not be observed in BC_2F_1 plants), where AA = phenotypic mean for homozygous individuals for *O. sativa* alleles at a specific marker locus, and AB = phenotypic mean of heterozygotes (*O. sativa*/*O. glumaepatula*).

Percentage of recurrent parent genome in the BC_2F_1 plants

Marker genotype-maps from the QGene program were used to determine the percentage of the total genome in each BC_2F_1 plant that came from each parental. Basically, if two consecutive loci have alleles coming from the same progenitor, the marker interval between them was considered to have the genome of that progenitor. If one locus had alleles from one parent and the next consecutive locus had alleles from the other parent, then half of the marker interval between them was considered to have the genome of one progenitor and the other half from the other one (Young and Tanksley 1989).

Results

Trait segregation and field performances

ANOVA indicated that no significant plot to plot variation ($P < 0.001$) was detected for each location (data not

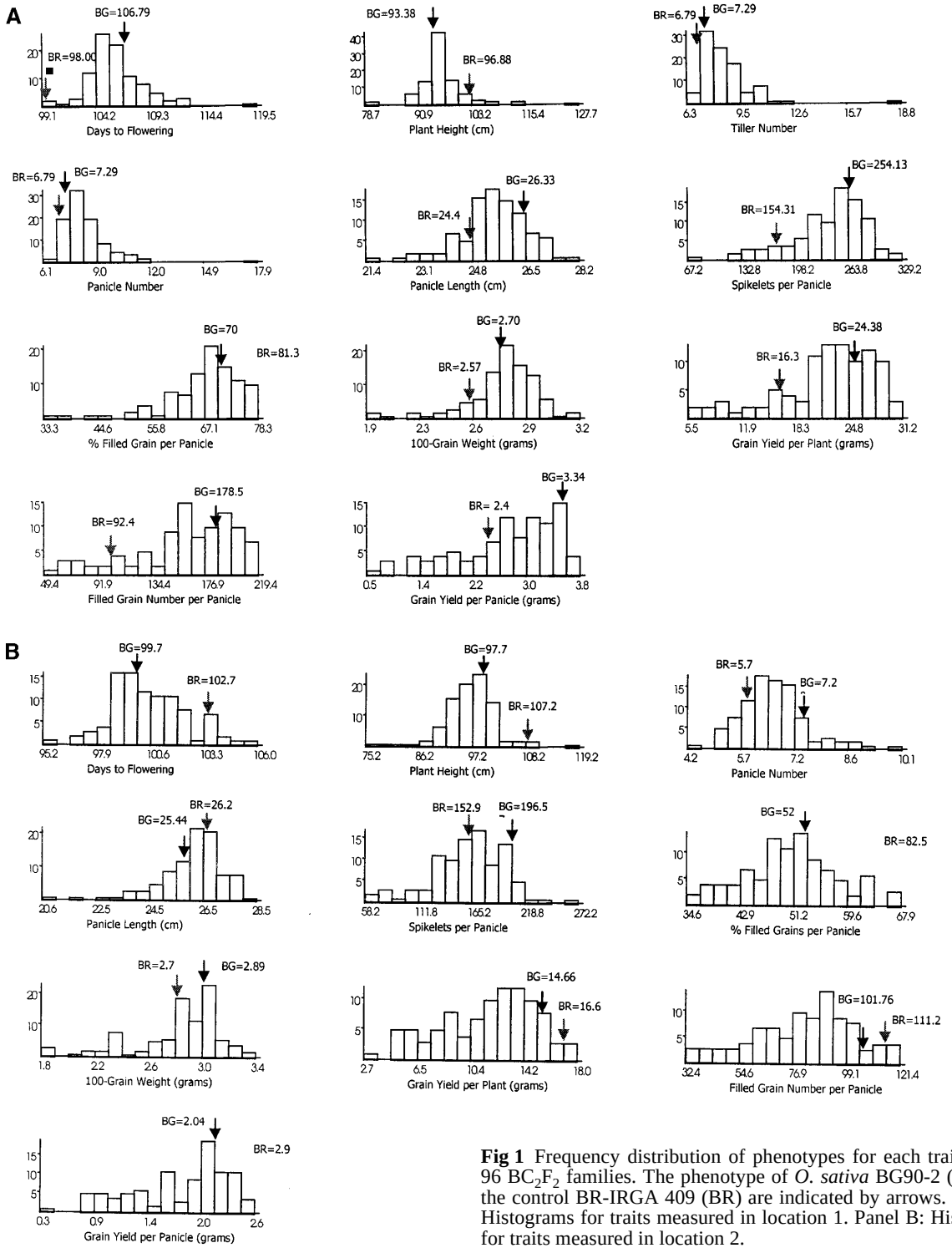

Fig 1 Frequency distribution of phenotypes for each trait for the 96 BC_2F_2 families. The phenotype of *O. sativa* BG90-2 (BG) and the control BR-IRGA 409 (BR) are indicated by arrows. Panel A: Histograms for traits measured in location 1. Panel B: Histograms for traits measured in location 2.

shown). However, significant trait performance differences ($P < 0.01$) between locations were detected, except for PNR trait in location 2 ($P < 0.05$). Trait means over plots were considered for analysis based on data independently collected in locations 1 and 2. The data from *O. glumaepatula* RS-16 was not collected, since it did not flower during the course of the experiment. Significant average trait differences were observed when the data collected in the two locations were compared. Genotype × Environment interactions were significant for the traits DTF, SPP and PFG. The distribution frequency of all traits approximately fit normal distributions (Fig. 1). Kurtosis and Skewness tests, however, were significant for DTF, PHT, TNR, PNR, PLH, SPP, PFG and HGW measured in location 1; and for PNR, PLH, PFG, and HGW measured in location 2. Attempts to normalise

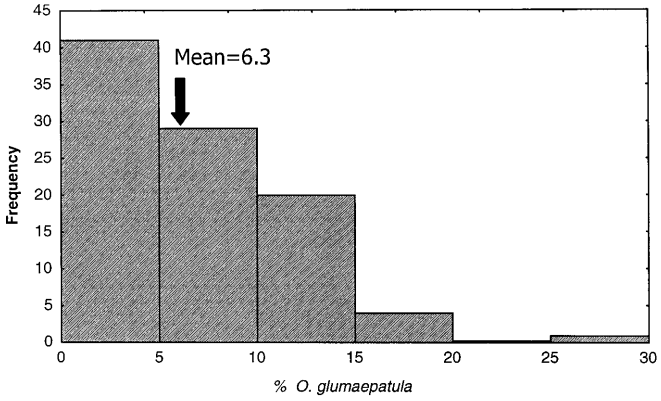

Fig. 2 Frequency distribution of the percentage of *O. glumaepatula* RS-16 genome in the 96 BC_2F_1 plants.

these data were made, but the results were similar to those obtained for the raw data. Transgressive families with superior trait averages to the parental line BG90-2 were observed for traits DTF, PHT, TNR, PNR and HGW in location 1, and DTF, PHT, PNR, PLH, SPP, PFG and HGW in location 2 (Duncan's $P \leq 0.05$).

Percentage of the *O. glumaepatula* genome in BC_2F_1 plants

The percentage of the *O. glumaepatula* genome (in the heterozygous state) based on molecular marker analysis of 96 BC_2F_1 plants varied from 0.0% (plants 35, 14, 63, 71, 47, 76, 96, 1, 64, 43, 52, 9, 72, 8, 33, 42, 87, 32, 12, 21, 83 and 18) to 26% (plant 89), with an average of 6.3% (Fig. 2), lower than the average expected proportion of BC_2F_1 heterozygous plants (25%). The selection of BC_1F_1 plants phenotypically identical to *O. sativa* was probably the cause of such segregation distortion.

Trait correlations

Pairwise trait correlations in the two locations are presented in Table 1. In location 1, the significant, positively correlated traits ($P < 0.0001$) included PNR × TNR (0.993), GYP × SPP (0.842), FGP × SPP (0.902), FGP × GYP (0.946), GYPa × GYP (0.901) and GYPa × FGP (0.835). The negatively correlated traits ($P < 0.0001$) included TNR × PHT (–0.389), PNR × PHT (–0.394), PLH × TNR (–0.447), PLH × PNR (–0.419), PFG × DTF (–0.357), HGW × TNR (–0.351), GYPa × TNR (–0.452) and GYPa × PNR (–0.423). In location 2, the positively correlated traits ($P < 0.0001$) included GYP × SPP (0.793), FGP × SPP (0.823), FGP × GYP (0.975), GYPa × GYP (0.896) and GYPa × FGP (0.847), and the negatively correlated ($P < 0.0001$) were PHT × DTF (–0.444), PLH × PNR (–0.381), PFG × DTF (–0.388), HGW × DTF (–0.431) and GYPa × DTF (–0.358).

Considering just the yield-related traits, some of them were not positively correlated. For example, trait PNR was negatively correlated with the traits PLH, PFG, HGW and GYPa in location 1, and with PLH in location 2. Also, trait PFG was negatively correlated with PLH and SPP in location 2. A positive correlation was found between "HGW" and "FGP", which were negatively correlated in other studies (Xiao et al. 1996, 1998). This was probably due to the allelic complementation between *O. sativa* alleles with the *O. glumaepatula* introgressed alleles; this could be explored by rice breeders.

The mapping data provided information on genomic regions associated with two or more traits (See Tables 4 and 5; and Fig. 3). Significantly correlated traits usually had QTLs located in the same chromosomal regions. All map intervals related to TNR and PNR ($r = 0.993$) were coincident (chromosomes 5, 7, 8 and 11, Fig. 3) in location 1. Also, four markers were common to HGW and GYP ($r = 0.559$) and three between FGP and GYP ($r = 0.975$). In location 2, the traits HGW and GYPa ($r = 0.768$) had in common seven markers and HGW and GYP ($r = 0.709$) had in common four markers.

QTL analysis

Single-point and interval-mapping analyses produced very similar results. When a marker explained the highest proportion of the phenotypic variation for one trait in single-point analysis (Table 2), it was also usually related to this trait in the chromosome region where the LOD score peaked in interval analysis (Fig. 3). Generally, the QTLs detected at location 1 were also detected in location 2 (Table 2 and Fig. 3). The only exception was the trait PHT, where a major QTL detected in location 1 was mapped in chromosome 1, while in location 2 the major QTLs were mapped on chromosomes 3, 5 and 8 (Table 2).

The phenotypic variation (PV%) of a single marker varied from 11.34 to 48.37 in location 1, and from 9.85 to 40.76 in location 2. When all markers of the multiple-regression analysis were included in the model, the trait PV% varied from 25.30 to 72.50 in location 1, and from 14.50 to 72.90 in location 2 (Table 2). In single-point analysis chromosome 4 was associated with a higher number of traits in location 1 (nine traits), and chromosomes 3 and 5 were related to a higher number of traits in location 2 (seven traits each). A total of 70 QTLs were detected in location 1, and 66 QTLs in location 2. Eighteen QTLs were identified in the same chromosomal region in both environments. Generally, the positive QTL effect was associated with BG90-2 alleles, except for traits TNR in location 1, PNR in locations 1 and 2, and PHT in location 2 (Table 2). In interval mapping analysis, chromosome 7 was related to a higher number of traits (eight traits) in location 1, and chromosome 5 was related to a higher number of traits in location 2 (seven traits). A total of 41 QTLs were detected in location 1 and 36 QTLs were detected in location 2. Twenty QTLs were identified in the same chromosomal regions in both locations.

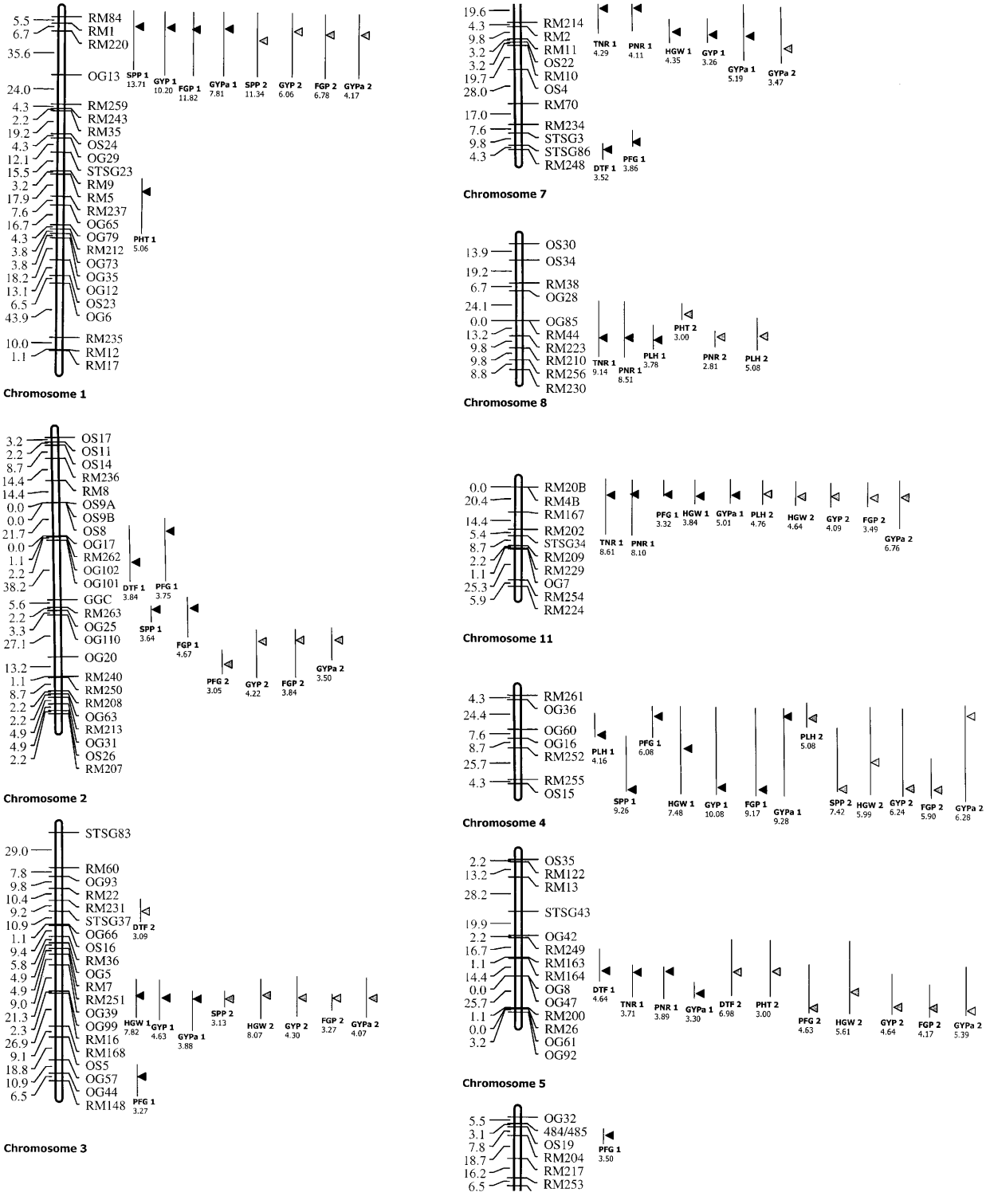

Fig. 3 Linkage map of microsatellite markers used for BC_2F_2 QTL analysis. The order of markers and the distances in cM (Kosambi mapping units) are based on the rice molecular map (Brondani et al., 2001). Solid bar to the right of the chromosomes represent QTL intervals with LOD > 3.0, arrows indicate the position of the peak LOD in the interval, and LOD values are located below the QTL names. The QTL name consists of the trait initials, followed by the trial location in which it was detected (1 = location 1, 2 = location 2).

Discussion

Transgressive Segregation

One of the main advantages of broad crosses is the real possibility to introgress genetic variability. This transgressive segregation is important evidence of the favorable effect of such introgressions. Even though trans-

Table 1 Partial correlation matrix of the traits analysed. The first value of each column is the correlation coefficient, and the second is the *P* value

Location 1																				
Trait	DTF		PHT		TNR		PNR		PLH		SPP		PFG		HGW		GYP		FGP	
PHT	–0.210	0.040																		
TNR	0.376	0.000	–0.389	0.000																
PNR	0.377	0.000	–0.394	0.000	0.993	0.000														
PLH	–0.304	0.002	0.149	0.146	–0.447	0.000	–0.419	0.000												
SPP	0.037	0.720	–0.189	0.065	0.133	0.197	0.176	0.086	0.304	0.003										
PFG	–0.357	0.000	–0.079	0.442	–0.284	0.005	–0.267	0.008	0.273	0.007	0.276	0.007								
HGW	–0.283	0.005	0.055	0.593	–0.351	0.000	–0.339	0.001	0.335	0.001	0.297	0.003	0.452	0.000						
GYP	–0.166	0.106	0.152	0.140	0.060	0.559	–0.022	0.831	0.390	0.000	0.842	0.000	0.648	0.000	0.559	0.000				
FGP	–0.146	0.157	–0.173	0.091	–0.020	0.848	0.020	0.849	0.352	0.000	0.902	0.000	0.654	0.000	0.416	0.000	0.946	0.000		
GYPa	–0.287	0.005	0.026	0.801	–0.452	0.000	–0.423	0.000	0.488	0.000	0.677	0.000	0.686	0.000	0.628	0.000	0.901	0.000	0.835	0.000

Location 2																				
Trait	DTF		PHT		PNR		PLH		SPP		PFG		HGW		GYP		FGP			
PHT	–0.444	0.000																		
PNR	0.088	0.392	–0.217	0.034																
PLH	–0.084	0.417	0.395	0.000	–0.381	0.000														
SPP	–0.047	0.648	0.005	0.963	0.393	0.000	0.337	0.001												
PFG	–0.388	0.000	0.350	0.000	–0.022	0.830	0.138	0.181	–0.038	0.715										
HGW	0.431	0.000	0.270	0.008	–0.059	0.565	0.355	0.000	0.479	0.000	0.344	0.001								
GYP	0.300	0.003	0.246	0.016	0.282	0.005	0.284	0.005	0.793	0.000	0.512	0.000	0.709	0.000						
FGP	–0.240	0.019	0.185	0.071	0.331	0.001	0.235	0.021	0.823	0.000	0.507	0.000	0.604	0.000	0.975	0.000				
GYPa	0.358	0.000	0.297	0.003	–0.140	0.173	0.403	0.000	0.619	0.000	0.564	0.000	0.768	0.000	0.896	0.000	0.847	0.000		

Table 2 Marker loci associated with 11 yield-related traits in two locations. Percentage of phenotypical variation explained (PV%) in bold indicates the result of the multiple marker regression on trait phenotype. *P* is the probability that the marker genotype had no effect on the trait. AA correspond to the genotypic class of BG90-2, Aa correspond to the genotypic class of the interspecific heterozygote. Chr is the marker chromosomal location. Add% is the additivity percentage

Location 1									Location 2							
Trait	Marker	Chr.	Source	PV(%)	*P*	AA	Aa	Add%	Marker	Chr.	Source	PV(%)	*P*	AA	Aa	Add%
DTF	RM163	5	RS-16	21.00	0.0000	105.55	109.20	–3.46	OG47	5	RS-16	26.31	0.0000	100.15	103.00	–2.85
	OG17	2	RS-16	15.48	0.0001	105.91	110.80	–4.62	RM163	5	RS-16	26.05	0.0000	100.17	102.97	–2.80
	OG101	2	RS-16	15.35	0.0001	105.86	110.80	–4.67	RM240	2	RS-16	15.19	0.0002	100.39	104.06	–3.66
	RM8	2	RS-16	13.97	0.0002	105.90	111.14	–4.95	STSG37	3	RS-16	13.93	0.0002	100.25	102.26	–2.01
	OG60	4	RS-16	12.39	0.0004	105.60	107.98	–2.26	OG61	5	RS-16	12.30	0.0005	100.21	101.88	–1.67
	STSG86	7	RS-16	11.75	0.0006	105.69	108.27	–2.44				**35.10**				
				38.20												
PHT	RM237	1	RS-16	20.30	0.0000	95.55	105.31	–10.22	OG47	5	BG90-2	21.10	0.0000	97.78	89.88	8.08
	OG79	1	RS-16	16.70	0.0000	95.72	105.16	–9.86	RM163	5	BG90-2	20.19	0.0000	97.76	90.03	7.91
	OG65	1	RS-16	16.18	0.0000	95.73	105.02	–9.71	STSG37	3	BG90-2	13.67	0.0002	97.56	91.42	6.30
				25.30					RM200	5	BG90-2	12.02	0.0005	97.72	92.61	5.23
												25.80				
TNR	RM223	8	RS-16	32.57	0.0000	8.39	13.42	–59.95	–	–	–	–	–	–	–	
	RM4B	11	RS-16	27.25	0.0000	8.30	11.04	–33.01								
	RM2	7	RS-16	15.97	0.0003	8.40	10.65	–26.79								
	RM163	5	RS-16	15.80	0.0001	8.30	10.05	–21.09								
	STSG34	11	RS-16	14.76	0.0001	8.35	10.11	–21.08								
	OG60	4	RS-16	12.05	0.0005	8.27	9.56	–15.60								
				49.00												
PNR	RM223	8	RS-16	30.82	0.0000	8.29	12.91	–55.73	RM223	8	RS-16	10.98	0.0010	6.53	8.35	–27.87
	RM4B	11	RS-16	25.92	0.0000	8.20	10.73	–30.86	RM4B	11	RS-16	9.85	0.0020	6.49	7.52	–15.87
	RM167	11	RS-16	19.31	0.0000	8.21	10.20	–24.24				**14.50**				
	RM210	8	RS-16	16.94	0.0000	8.30	10.98	–32.29								
	RM163	5	RS-16	16.60	0.0000	8.20	9.88	–20.49								
				47.20												
PLH	OG60	4	BG90-2	17.06	0.0000	25.74	24.62	4.35	RM223	8	BG90-2	19.22	0.0000	26.11	22.92	12.22
	RM11	7	BG90-2	15.60	0.0001	25.63	24.11	5.93	RM4B	11	BG90-2	16.93	0.0000	26.17	24.39	6.80
	RM223	8	BG90-2	14.63	0.0001	25.57	23.10	9.66	OG60	4	BG90-2	12.83	0.0003	26.25	25.16	4.15
	OG42	5	BG90-2	11.61	0.0007	25.64	24.48	4.53	RM224	11	BG90-2	12.17	0.0005	26.16	24.40	6.73
				34.70					OG85	8	BG90-2	11.78	0.0006	26.12	24.16	7.51
												39.80				
SPP	RM1	1	BG90-2	48.37	0.0000	262.68	192.81	26.60	RM220	1	BG90-2	40.76	0.0000	177.46	125.97	29.02
	OS15	4	BG90-2	35.87	0.0000	247.66	144.50	41.66	OS15	4	BG90-2	29.96	0.0000	168.01	94.69	43.64
	OG25	2	BG90-2	15.22	0.0001	245.74	187.43	23.73	RM16	3	BG90-2	13.21	0.0003	166.26	120.06	27.79
	OG110	2	BG90-2	11.39	0.0008	244.54	191.95	21.51				**55.50**				
				65.10												

Table 2 (continued)

Location 1									Location 2							
Trait	Marker	Chr.	Source	PV(%)	*P*	AA	Aa	Add%	Marker	Chr.	Source	PV(%)	*P*	AA	Aa	Add%
PFG	OG60	4	BG90-2	23.86	0.0000	68.83	59.01	14.27	OG92	5	BG90-2	17.96	0.0000	52.52	45.51	13.35
	OG44	3	BG90-2	16.64	0.0000	67.79	56.71	16.35	RM200	5	BG90-2	16.84	0.0000	52.18	44.70	14.34
	STSG86	7	BG90-2	16.44	0.0000	68.19	59.15	13.26	RM217	6	BG90-2	11.44	0.0013	51.03	40.91	19.83
	OG32	6	BG90-2	14.59	0.0001	67.83	57.86	14.70	RM240	2	BG90-2	10.83	0.0017	51.52	39.81	22.73
	OS17	2	BG90-2	14.22	0.0003	68.02	59.27	12.87				**29.30**				
	STSG3	7	BG90-2	13.74	0.0002	67.88	58.88	13.26								
	RM4B	11	BG90-2	13.54	0.0002	67.66	57.17	15.51								
	RM9	1	BG90-2	11.75	0.0006	67.96	60.31	11.26								
				46.80												
HGW	RM16	3	BG90-2	30.30	0.0000	2.80	2.36	15.72	RM16	3	BG90-2	32.75	0.0000	2.86	2.21	22.73
	RM252	4	BG90-2	25.18	0.0000	2.80	2.46	12.15	RM252	4	BG90-2	24.08	0.0000	2.85	2.37	16.84
	OS15	4	BG90-2	23.26	0.0000	2.79	2.38	14.70	OG60	4	BG90-2	21.93	0.0000	2.87	2.49	13.24
	RM11	7	BG90-2	17.94	0.0000	2.79	2.45	12.19	OG47	5	BG90-2	20.14	0.0000	2.85	2.41	15.44
	OG16	4	BG90-2	16.18	0.0001	2.79	2.51	10.04	OS15	4	BG90-2	19.65	0.0000	2.83	2.30	18.73
	RM4B	11	BG90-2	13.25	0.0003	2.78	2.49	10.43	OG92	5	BG90-2	16.27	0.0000	2.87	2.56	10.80
	RM10	7	BG90-2	12.95	0.0005	2.79	2.53	9.32	RM4B	11	BG90-2	15.53	0.0001	2.83	2.38	15.90
	RM261	4	BG90-2	12.71	0.0005	2.79	2.56	8.25	RM254	11	BG90-2	13.21	0.0003	2.83	2.41	14.84
	RM223	8	BG90-2	12.70	0.0004	2.77	2.29	17.33	OS17	2	BG90-2	12.55	0.0007	2.83	2.50	11.66
	STSG34	11	BG90-2	12.60	0.0004	2.79	2.54	8.96				**65.50**				
	STSG43	5	BG90-2	11.52	0.0007	2.79	2.54	8.96								
				57.60												
GYP	RM1	1	BG90-2	39.11	0.0000	24.58	16.80	31.65	OS15	4	BG90-2	23.83	0.0000	12.12	6.13	49.42
	OS15	4	BG90-2	38.34	0.0000	23.09	10.11	56.22	RM1	1	BG90-2	23.59	0.0000	12.83	9.31	27.44
	RM252	4	BG90-2	27.75	0.0000	23.22	14.30	38.42	RM220	1	BG90-2	23.36	0.0000	12.70	9.13	28.11
	RM16	3	BG90-2	19.68	0.0000	22.84	14.01	38.66	RM16	3	BG90-2	18.65	0.0000	12.13	7.12	41.31
	OG36	4	BG90-2	14.96	0.0001	23.04	17.02	26.13	OG20	2	BG90-2	17.81	0.0000	12.11	7.43	38.65
	RM11	7	BG90-2	13.88	0.0002	22.71	15.30	32.63	OG61	5	BG90-2	17.30	0.0000	12.32	8.78	28.74
	STSG86	7	BG90-2	12.90	0.0003	22.94	17.36	24.33	RM4B	11	BG90-2	13.63	0.0002	12.02	7.72	35.78
				69.80					OG63	2	BG90-2	12.82	0.0005	12.13	8.38	30.92
									OS17	2	BG90-2	12.58	0.0006	12.08	8.69	28.07
									OS19	6	BG90-2	12.53	0.0005	12.09	8.24	31.85
									OG32	6	BG90-2	12.29	0.0005	12.05	8.32	30.96
												66.50				
FGP	RM1	1	BG90-2	42.39	0.0000	179.67	123.46	31.29	RM220	1	BG90-2	26.11	0.0000	88.55	65.14	26.44
	OS15	4	BG90-2	35.60	0.0000	167.87	80.17	52.25	OS15	4	BG90-2	23.18	0.0000	84.53	47.89	43.35
	OG60	4	BG90-2	20.28	0.0000	170.25	125.99	26.00	OG20	2	BG90-2	16.29	0.0000	84.37	56.58	32.94
	RM263	2	BG90-2	18.23	0.0000	166.48	109.70	34.11	OG61	5	BG90-2	15.85	0.0001	85.64	64.62	24.55
	STSG86	7	BG90-2	12.74	0.0004	167.05	128.14	23.29	RM16	3	BG90-2	13.86	0.0002	84.20	57.42	31.81
	RM231	3	BG90-2	12.71	0.0004	167.04	128.18	23.27	RM10	7	BG90-2	12.42	0.0007	85.09	62.72	26.29
	RM259	1	BG90-2	12.37	0.0006	169.62	136.23	19.69	RM208	2	BG90-2	12.38	0.0004	84.14	60.90	27.62
	OS22	7	BG90-2	12.30	0.0005	165.15	116.26	29.61	RM4B	11	BG90-2	12.02	0.0006	83.89	58.85	29.85
				70.90								**57.40**				

Table 2 (continued)

Location 1								Location 2						
Trait	Marker	Chr.	Source	PV(%)	*P*	AA	Aa	Add%	Marker	Chr.	Source	PV(%)	*P*	AA	Aa	Add%
GYPa	OG60	4	BG90-2	31.72	0.0000	2.91	1.86	36.08	OG60	4	BG90-2	23.31	0.0000	1.91	1.33	30.37
	RM1	1	BG90-2	31.17	0.0000	3.00	2.08	30.67	RM4B	11	BG90-2	22.98	0.0000	1.86	1.05	43.55
	OS15	4	BG90-2	29.39	0.0000	2.81	1.30	53.74	RM252	4	BG90-2	21.62	0.0000	1.88	1.20	36.17
	OG36	4	BG90-2	21.67	0.0000	2.84	1.88	33.81	OS15	4	BG90-2	21.33	0.0000	1.86	1.03	44.63
	RM4B	11	BG90-2	20.82	0.0000	2.80	1.59	43.22	OG92	5	BG90-2	21.22	0.0000	1.92	1.40	27.09
	RM214	7	BG90-2	12.94	0.0003	2.79	1.86	33.34	RM16	3	BG90-2	18.23	0.0000	1.86	1.15	38.17
	STSG43	5	BG90-2	11.74	0.0006	2.78	1.95	29.86	RM1	1	BG90-2	17.08	0.0000	1.94	1.51	22.17
	RM200	5	BG90-2	11.34	0.0008	2.81	2.16	23.13	RM10	7	BG90-2	16.96	0.0001	1.88	1.25	33.51
				72.50					RM220	1	BG90-2	16.47	0.0000	1.92	1.48	22.92
									RM167	11	BG90-2	16.45	0.0000	1.86	1.23	33.87
									OS17	2	BG90-2	14.88	0.0002	1.85	1.32	28.65
									OS14	2	BG90-2	13.81	0.0002	1.87	1.36	27.28
									RM263	2	BG90-2	12.46	0.0004	1.85	1.27	31.35
									RM164	5	BG90-2	12.28	0.0005	1.86	1.35	27.42
									OS19	6	BG90-2	11.24	0.0010	1.85	1.32	28.65
												72.90				

gressive segregation was observed in yield-related traits, the *O. glumaepatula* inserts were not identified, probably due to a low degree of saturation with molecular markers in the map. As a result the fragment between two alleles from *O. sativa* would not be detected, or it could be situated in a terminal position on the chromosome, and therefore not mapped. In both cases, additional molecular markers could be employed to saturate the current *O. glumaepatula* × *O. sativa* map. Even though there was no yield-improving identified allele from *O. glumaepatula*, the finding of transgressive BC_2F_2 families in those traits may mean that they were a consequence of introgressed genes that were not detected by QTL analysis. The BC_2F_2 families with average trait performances significantly superior to the elite cultivar BG90-2 were DTF, PHT, PNR and HGW in location 1, and DTF, PHT, PNR, PLH, SPP, PFG, HGW and GYPa in location 2 (Duncan's $P \leq 0.05$). Except for DTF and PHT, the transgressive families found were related to favorable traits. The *O. glumaepatula* alleles contributed to an increase in TNR and PNR trait values. Both traits are known to substantially affect plant architecture. If a panicle is produced for every new tiller, plant yield can be positively increased by the combined action of the genes controlling these traits. Thirteen families had a significantly higher PNR than BG90-2 at location 1 (Duncan's $P \leq 0.05$). In family 84, for example, there were 145.8% more panicles per plant than in the elite recurrent progenitor. In this family 12.6% of the genome is from *O. glumaepatula*, and it outperformed BG90-2 for the traits PHT and TNR at location 1. It also had the shortest panicles among all 96 families tested (21.41 cm in location 1 and 20.59 cm in location 2). PNR and PLH are negatively correlated (Table 1), and therefore selection for higher PNR may result in PLH reduction. The marker locus RM223 accounted for 30.82% of the PNR phenotypic variation in location 1, and 10.98% in location 2, which means that this is a chromosomal region with a significant effect on PNR. Research on rice ideotypes has concentrated on plant types with a decreased tiller number. However, this type of plant has fewer filled grains per panicle due to the reduced dry matter (Peng et al., 1999). A competitive rice variety would have high yields due to a high tiller/panicles per plant, and an increased number of filled grains per panicle. Marker-assisted selection for both traits could help the identification of chromosomal regions with favorable alleles, facilitating the selection of best performing NILs.

G × E interaction

In the analysis of BC_2F_2 families based on phenotypic data there were significant G × E interactions for three of the ten traits measured (DTF, SPP and PFG). This means that, for these traits, there are families specifically adapted for the locations 1 or 2, and the loci controlling these traits are significantly influenced by the environment. However, 13% of the QTLs mapped by single-point

analysis were detected in both locations (Table 2), while the interval-mapping analysis indicated that 26% of the chromosome segments were related to the same trait at both locations (Table 2). Only PHT did not have a major QTL detected in both locations. For this trait, the alleles from RS-16 in chromosome 1 increased the plant height in location 1, and those from BG90-2 in chromosomes 3 and 5 increased the plant height in location 2. This indicates that different chromosomal regions may be involved in this trait expression, as a response to different environmental conditions. However, based on QTL analysis, most of the traits had QTLs identified in both locations. Of course, additional experiments have to be conducted in both locations, but for QTL analysis, just one location could be used.

Analysis of quantitative trait loci

QTL analysis of agronomic traits in rice is well documented, which facilitates comprehension and permits comparisons of the results from different research groups. The present QTL analysis was done using both single-point and interval mapping. The high values of %PV was probably due to a lack of independent action between individual markers in the model. Nevertheless, interval mapping produced very similar results, which could mean that the QTL location is real. Along this line, when individual markers are used in marker-assisted selection programs, one should consider the markers with the highest PV% values, since the associated QTLs are probably real. It is difficult to make comparisons among results obtained by studies performed with different types of markers, populations, and environments. The number of QTLs detected in each study varies, as does the segregating population size and the number of markers tested. However, since it is possible to identify each of the rice chromosomes using molecular markers (Fukui and Iijima 1991; Chen et al. 1997), some of the major chromosome regions associated with yield-related traits found here and in several other studies were identified, despite the use of different sets of markers. This means that the identified region can be associated with QTLs with larger effects. The interspecific cross between *O. glumaepatula* and *O. sativa* was useful to detect such regions, after chromosome pairing and crossing-over, in the resultant *O. glumaepatula* fragment introgressions. For example, a region of chromosome 3 was significantly associated with HGW in this study and several others (Xiao et al. 1995; Lin et al. 1996; Xiao et al. 1996; Li et al. 1997; Lu et al. 1997; Zhuang et al. 1997; Xiao et al. 1998). The same was observed for SPP in chromosome 1 (Zhuang et al. 1997; Xiao et al. 1998; Xiong 1999) and for FGP also in chromosome 1 (Li et al. 1997; Zhuang et al. 1997; Xiao et al. 1998). The marker locus RM223, that we found to be significantly associated with PLH, was mapped in chromosome 8, the same marker region associated with this trait in another interspecific cross (Xiao et al. 1998). Similarly, the marker locus RM20B mapped in chromosome 10 was significantly associated with the trait HGW in our study and with "thousand-grain weight" in the interspecific cross studied by Xiao et al. (1998).

Specific marker regions strongly associated with more than one trait were observed in: RM1 (chromosome 1) for the traits SPP, GYP, FGP and GYPa; RM 16 (chromosome 3) for the traits SPP, GYP, FGP, GYPa and HGW; OS15 (chromosome 4) for the traits SPP, GYP and FGP; and RM4B (chromosome 11) for the traits PNR, PFG, HGW, GYPa, PLH, GYP and FGP. As the majority of these traits are based on grain counting, we would expect this high QTL correlation. Although it is not possible to make conclusions about pleiotropy or gene linkage in these QTL regions, they represent interesting "hot spots" for major loci controlling such traits. As the rice genome sequencing is almost complete, one could concentrate on such regions to identify and to clone putative genes that control those traits. However, some caution is necessary since the lack of a simple correspondence between genotype and phenotype for complex traits limits the usefulness of QTL cloning.

Genome coverage and QTL mapping

QTLs associated with the 11 traits we evaluated were detected in all rice chromosomes, except for chromosomes 9, 10 and 12. Yield-related QTLs were detected in these chromosomes in other studies (Xiao et al. 1996; Li et al. 1997; Lu et al. 1997; Xiong et al. 1999; Yan et al. 1998). Chromosomes 9 and 10 had relatively good marker coverage based on the comparison of their physical lengths in pro-metaphase and on the total number of markers mapped (Brondani et al. 2001). Probably the *O. glumaepatula* and *O. sativa* alleles located in these chromosomes had similar effects for the traits we analyzed. In chromosome 12, fewer markers were mapped and this would certainly restrict the power to detect significant associations. On the other hand, significant marker/trait associations were detected in chromosome 4, even with limited marker coverage. Additional markers will be tested, in order to increase the probability to saturate this chromosome and to proceed with efficient marker-assisted selection for yield-related trait QTLs.

O. glumaepatula QTL alleles

We investigated the potential of *O. glumaepatula* as a source of useful genes for rice breeding programs. Xiao et al. (1998) studied the introgression from *Oryza rufipogon* to cultivated rice, and found that 51% of the trait-enhancing alleles came from the wild parent. We found that only a fraction of the detected QTLs had the positive alleles contributed by *O. glumaepatula* (15.7% in location 1 and 9.1% in location 2). Although the percentage contribution of trait-enhancing alleles by *O. glumaepatula* was low, it was, nevertheless, relevant for some

traits that can effectively change plant architecture and affect grain yield, such as TNR and PNR. In this case, marker-assisted selection can be very useful to speed up the process of fragmenting introgressions, the rest of the genome can also be monitored for the presence of *O. sativa* alleles in the near-isogenic lines (NILs) development process. Additionaly a genomic region introgressed from *O. glumaepatula* to *O. sativa* had different effects for two traits. For example, *O. glumaepatula* alleles in marker locus RM223 of chromosome 8 increased panicle number. However, the effect of an *O. sativa* allele substitution by an *O. glumaepatula* allele at this locus was also associated with a decrease in PLH and HGW (Table 2). This could be caused by linkage between loci affecting two negatively correlated traits, or by pleiotropy. If two or more loci controlling different traits are closely linked, then larger segregating populations and continuous marker saturation of such regions can be used to break the linkage, allowing selection for different traits. By reducing the introgressed fragment size by means of selection of the best NILs and crossing them with *O. sativa*, it will be possible to distinguish the linkage and pleiotropic effects on QTLs, increasing the probability to generate better lines for rice breeding. In addition, the superiority of the identified BC_2F_2 families due to the *O. glumaepatula* alleles will be confirmed by analyzing the NILs, in order to determine whether the allele effects were real or were due to heterosis at the introgressed loci. At this stage, the NILs will be genotyped, and the homozygous families for *O. sativa* and *O. glumaepatula* alleles will be related to their respective phenotypes. If no PNR-favorable *O. glumaepatula* allele is detected, the effect on generation BC_2F_2 would be due to dominance or overdominance. However, if the *O. glumaepatula* allele has a positive effect on PNR, it could be used to improve this trait in other rice varieties by marker-assisted selection. For the yield-related agronomic traits that mapped at the same chromosome region, the NILs with a high average yield can be crossed again to BG90-2, in order to analyze the sub-NILs for pleiotropy or gene linkage of these traits.

The combined use of molecular-marker linkage information with breeding methods that exploit the advantages of wide crosses provide a unique opportunity to determine the extent and to monitor the process of introgression of alien alleles controlling quantitative traits. Furthermore, this will allow us to exploit the genetic variability available in accessions conserved in germplasm banks, which normally are of limited use for breeding programs. The introgressed segments from wild species can be mapped and estimates of the number, effect and interaction of identified chromosomal regions related to phenotypic trait variation can be performed by means of QTL analysis. Therefore, genetic linkage maps based on molecular markers can be used to minimize linkage drag, allowing for the selection of individuals containing the desired introgressed DNA regions from the donor parent, thus reducing the time required to recover advanced recombinant inbred lines.

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