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ORIGINAL PAPER

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Identification of quantitative trait loci for grain quality in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*

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Abstract The objective of this study was to identify quantitative trait loci (QTLs) associated with grain quality in rice. Two hundred eighty-five BC_2F_2 families developed from an interspecific cross between cv IR64 and *Oryza rufipogon* (IRGC 105491) were evaluated for 14 seed quality traits. A total of 165 markers consisting of 131 single sequence repeats and 34 restriction fragment length polymorphism markers were used to create a genetic linkage map spanning the 12 rice chromosomes. Twenty-three independent QTLs were identified using single point analysis, interval mapping, and composite interval mapping. These loci consisted of one QTL for filled rough/total rough rice ratio, two for grain density, one for percentage of de-husked rice grains, two for percentage of green rice grains, three for percentage of damaged-yellow rice grains, two for percentage of red rice grains, one for milled rice recovery, three for head rice recovery, four for broken rice grains, two for crushed rice grains, one for amylose content, and one for gel consistency. For most of the QTLs identified in this study, the *O. rufipogon*-derived allele contributed an undesirable effect. For amylose content and gel consistency, the *O. rufipogon* allele may be useful in an IR64 background, depending on the cultural preferences of the consumer. Careful selection against the regions associated with negative effects will be required to avoid unwanted grain quality characteristics during the development of improved varieties for yield and yield components using introgressions from *O. rufipogon*.

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Introduction

Grain quality is an important consideration in rice production. Preferences for rice grain performance and cooking quality vary among rice consumers living in different parts of the world or even among people living in different regions of the same country. In Indonesia, for example, Javanese consumers prefer rice with a smooth texture and intermediate amylose content, while West and North Sumatrans favor hard-textured rice with a high amylose content (Damardjati and Oka 1991). When breeding for grain quality, different regional preferences should be considered.

Grain quality is determined by many factors, including milling ratio, head rice recovery, grain shape and size, grain appearance, and cooking and eating quality. After milling, rough rice yield is usually made up of 20–22% (by weight) hulls, 8–10% bran and embryos, and 70% milled rice. Milled rice is separated into broken rice and whole grains with a sieving device, with the proportion of whole grains defined as head rice recovery. Grain appearance is mostly determined by grain shape and the translucent or opaque appearance of the grain, known as the amount of chalkiness. The density of starch granules is lower in chalky grains compared to translucent ones (del Rosario et al. 1968), and because chalky grains are not as hard as translucent ones, they are more prone to breakage during milling.

Key components that determine eating and cooking quality are amylose content, gelatinization temperature, and gel consistency. Amylose is the final product of the granule-bound starch synthase encoded by the *waxy* gene (*wx*) (Sano 1984). This constituent differs widely in rice germplasm, which is classified as waxy (1–2% amylose) or non-waxy (>2% amylose). Furthermore, the non-waxy group is divided into four subgroups: very low amylose (2–10%), low (10–20%), intermediate (20–25%) and high amylose (>25%) (Juliano 1979). Low-amylose cooked rice tends to be moist, tender and cohesive, while high amylose content is more likely to result in dry, fluffy, and separated grains (Juliano 1971). Gelatinization tempera-

ture of starch varies among rice varieties, from about 55°C to 79°C (Juliano 1972), while gel consistency is categorized as hard (26–40 mm), medium (41–60 mm), and soft (61–100 mm) (Perez 1979). Gelatinization temperature and gel consistency can differentiate rice with similar amylose content, especially the waxy and high-amylose rices (Juliano and Villareal 1993).

Advanced backcross quantitative trait locus (AB-QTL) analysis was first introduced to simultaneously identify and transfer valuable alleles from donor lines into the genomes of elite cultivars (Tanksley and Nelson 1996). This analysis can accelerate crop improvement by allowing rapid development of near isogenic lines (NILs) containing the QTLs of interest, derived directly from the advanced backcross population where the useful QTLs were identified (Bernacchi et al. 1998b). Improved lines can be developed from those NILs, where the majority of the genome is the same as the elite recurrent parent. AB-QTL has frequently been employed in QTL studies of tomato (Tanksley et al. 1996; Fulton et al. 1997, 2000; Bernacchi et al. 1998a; Foolad and Chen 1999), and rice (Xiao et al. 1998; McCouch et al. 2001; Moncada et al. 2001; Thomson et al. 2003).

In the present study, we report the application of AB-QTL to identify and map QTLs underlying 14 traits associated with rice grain quality. We used BC_2F_2 plants developed from an interspecific cross between IR64, an internationally recognized, high quality rice cultivar widely planted in the tropics, and *Oryza rufipogon*, a wild accession from Malaysia. The same population was used in a companion QTL study on yield and yield components by Septiningsih et al. (2003). The traits evaluated here were: I) pre-milling quality traits, including (1) moisture content, (2) filled rough/total rough rice ratio, (3) grain density and (4) percentage of de-husked rice grains, II) kernel color traits, including (5) percentage of green rice grains, (6) percentage of damaged-yellow rice grains and (7) percentage of red rice grains, III) milling quality traits, including (8) milling ratio, (9) head rice recovery, (10) percentage of broken rice grains and (11) percentage of crushed rice grain and IV) post-milling quality traits, including (12) percentage of chalky rice grains, (13) amylose content and (14) gel consistency. Ultimately, the information from this study can be integrated into the development of improved lines targeting *O. rufipogon*-derived QTLs for yield and yield components (Septiningsih et al. 2003), where regions associated with negative quality effects can be selected against with the help of molecular markers flanking those regions.

Materials and methods

Population development

The BC_2F_2 population used for trait evaluation and QTL analysis was developed from a cross between IR64 and *O. rufipogon* (IRGC 105491), as described in Septiningsih et al. (2003). Each BC_2F_2 family was represented by 75 plants/plot, with a spacing of 25 cm between each plant, and plots consisted of three rows by 25 plants per row in a randomized complete block design with two replications. BC_2F_3 seeds harvested from different replications were bulked for the seed quality evaluation. A subset of 285 randomly selected BC_2F_2 plants were used for marker genotyping.

Trait evaluation

Fourteen traits were evaluated:

1. Moisture content (MC) was the amount of water in rough rice grains (grains with husks intact), stated as a percentage and evaluated using a moisture tester (Kett, Villa Park, Calif.) (using a sample of 110 g rough rice).
2. Filled rough/total rough rice ratio (FRR) was calculated as the weight of filled rough rice grains divided by the weight of the entire sample of rough rice and taken as a percentage; filled rough rice grains were separated from the empty ones using a slot sieve (using a sample of 300 g total rough rice).
3. Grain density (GD) was a measure of weight per volume, in this case measured as the weight of rough rice grains per liter (using a sample of 1 l rough rice).
4. Percentage of de-husked rice grains (DR) was the percentage of the weight of de-husked grains compared to the weight of the sample of rough rice (using a sample of 300 g rough rice).
5. Percentage of green rice grains (GG) was the proportion of green-colored rice grains, which consisted of whole grains, broken grains, and crushed grains that were green in color, divided by the weight of the de-husked rice grain sample and stated as a percentage. Green grains were visually separated from the rest of the ingredients, using forceps and a magnifying glass (using a sample of 50 g of de-husked rice).
6. Percentage of damaged-yellow rice grains (DYG) was the proportion of the weight of damaged-yellow rice grains, which consisted of whole grains, broken grains, and crushed grains that were yellow in color or contained spots or lesions resulting from physiological factors and/or activities of microorganisms, divided by the weight of de-husked rice sample and stated as a percentage (using a sample of 50 g de-husked rice).
7. Percentage of red rice grains (RG) was provided as the percentage of the weight of red grains, which consisted of whole grains, broken grains, and crushed grains that were red in color due to genetic factors, divided by the weight of the de-husked rice sample (using a sample of 50 g of de-husked rice).
8. Percentage of milled rice grains (MR) was calculated as the percentage of the weight of milled rice grains (following removal of the bran) divided by the weight of the sample of rough rice (using a sample of total de-husked rice out of 300 g sample rough rice).
9. Percentage of head rice (HR) was calculated as the weight of grains that were the same size or larger than 0.6 of the average length of the whole grain, measured with a rice grader, divided by the weight of the milled rice sample and stated as a percentage (using a sample of 100 g milled rice).
10. Percentage of broken rice grains (BR) were grains, smaller in size than 0.60 of the average length of the whole grains but larger than 0.25 of the whole grain, divided by the weight of milled rice sample and stated as a percentage (using a sample of 100 g milled rice).
11. Percentage of crushed rice grains (CR) were the broken grains, which were the same size or smaller than 0.25 part of the whole grain, divided by the weight of milled rice sample, stated as a percent (using a sample of 100 g of milled rice).
12. Percentage of chalky rice grains (CG) were grains that had an opaque, chalky appearance covering half or more of the body of the grain, and were soft in texture as a result of physiological factors, divided by the weight of milled rice sample and stated as a percent (using a sample of 100 g milled rice).
13. Amylose content (AC) was measured as described by Williams et al. (1958) and Juliano (1971), involving a defatting step, where 95% ethanol was added to the milled rice flour prior to

starch dispersion in NaOH, followed by gelatinization in a boiling water bath, and measurement of amylose based on the detection of iodine blue color at pH 4.5 to 4.8 using sodium acetate buffer (using a sample of 100 g 100-mesh sieved-rice flour).

14. Gel consistency (GC) was measured according to the method of Cagampang et al. (1973), where the flow characteristic of milled rice gel was measured in 0.2 N KOH and indexed by the length (in mm) of the cold horizontal gel (using a sample of 100 g 100-mesh-sieved rice flour).

Map construction

The map used in the analysis was the same as the one described in the companion paper by Septiningsih et al. (2003), where a total of 165 markers, consisting of 131 single sequence repeats (SSRs) and 34 restriction fragment length polymorphisms (RFLPs) were used to construct the linkage map using the program MAPMAKER (Lander et al.1987). SSR marker-order along the chromosomes was similar to that described by Chen et al. (1997) and Temnykh et al. (2000), while the order of the RFLP markers followed Causse et al. (1994). The statistical analysis was done using the 154 framework markers (not including the low-LOD markers in parentheses) averaging 14.1 cM for the marker intervals, with genome coverage of 96.8% according to Temnykh et al. (2001).

Statistical analysis

Correlation among traits was calculated using the program Qgene (Nelson1997), with a significance threshold value of 0.05. Qgene was used for the single point analysis (SPA) and interval mapping (IM) (Nelson1997), while QTL Cartographer (Basten et al.1997) was used for the composite interval mapping (CIM) analysis, as described in Septiningsih et al. (2003). However, since the QTL results of SPA were similar to those of IM (data not shown), only the analysis from IM and CIM are presented. The experiment-wise significance threshold corresponded to an average LOD score value of 2.93 and 3.05, which were derived from 10,000 and 1,000 permutation tests, for IM and CIM, respectively. QTLs identified by one or both of the analyses are reported here.

Results and discussion

Trait correlation

GD showed a high positive correlation with hulling, milling, and head rice recoveries, and, as expected, the amount of broken and crushed grains were negatively associated with these three traits (Table 1). This is reasonable because with the increase of hulling, milling, or head rice ratio, the number of grains per volume will be higher. In addition, it might be possible that the denser grains do not break as easily and would weigh more. A similar correlation was also reported by Sarkar et al. (1994). A highly positive correlation was found between chalkiness and green grain (0.288, $P<0.0005$). Consequently, the number of chalky grains appears to increase with increased numbers of green grains. GC had a low but highly significant negative correlation with AC (–0.210, $P<0.0005$), and as a result, families that had high AC tended to have low GC, resulting in the gel having a harder texture.

Phenotypic distribution

All of the traits showed a pattern of continuous distribution around the mean, although some traits did not follow a perfect normal distribution (Fig. 1). For the trait red grain, non-red grains were strongly skewed toward the recurrent parent type. Consequently, most of the families in the population had normal color grains, and only a few had either 100% red color or mixed color grains with a greater proportion of red grains. Filled rough/total rough rice ratio and head rice recovery were slightly skewed toward higher values, suggesting that most of the families had good filled rough and head rice counts. In contrast, the green and crushed grain traits were skewed toward lower values, with a narrow range of distribution of small percentages (0%–

Table 1 Trait correlations for grain quality[a]

Trait[b]	WR	GD	FRR	GG	DYG	RG	DR	MR	HR	BR	CR	CG	AC
GD	–												
FRR	–	0.244**											
GG	0.229**	0.170*	–										
DYG	–	–	–	0.143									
RG	–	–0.146	–	–0.139	–0.228**								
DR	–	0.248**	0.233**	–	–0.155*	–							
MR	–	0.270**	0.204**	–	–0.178*	–	0.874**						
HR	0.128	0.176*	–	–	–	–	0.192**	0.471**					
BR	–0.126	–0.154	–	–	–	–	–0.188*	–0.445**	–0.968**				
CR	–	–0.165*	–	–	–	–	–0.120	–0.339**	–0.646**	0.434**			
CG	–	–	–	0.288**	0.126	–	–	–	–	–	0.140		
AC	–0.176*	–	–	0.127	–	–	–	–	–	–	–0.189*	–	
GC	–	–	–	–	–	–	–	–	–	–	–	–0.130	–0.210**

* Significant at $P<0.01$

** Significant at $P<0.001$

[a] All correlations shown are significant at $P<0.05$

[b] Abbreviations: *WR* water ratio, *GD* grain density, *FRR* filled rough/ total rough rice ratio, *GG* green grain, *DYG* damaged-yellow grain, *RG* red grain, *DR* de-husked rice grain, *MR* milled rice grain, *HR* head rice grain, *BR* broken rice grain, *CR* crushed rice grain, *CG* chalky grain, *AC* amylose content, *GC* gel consistency

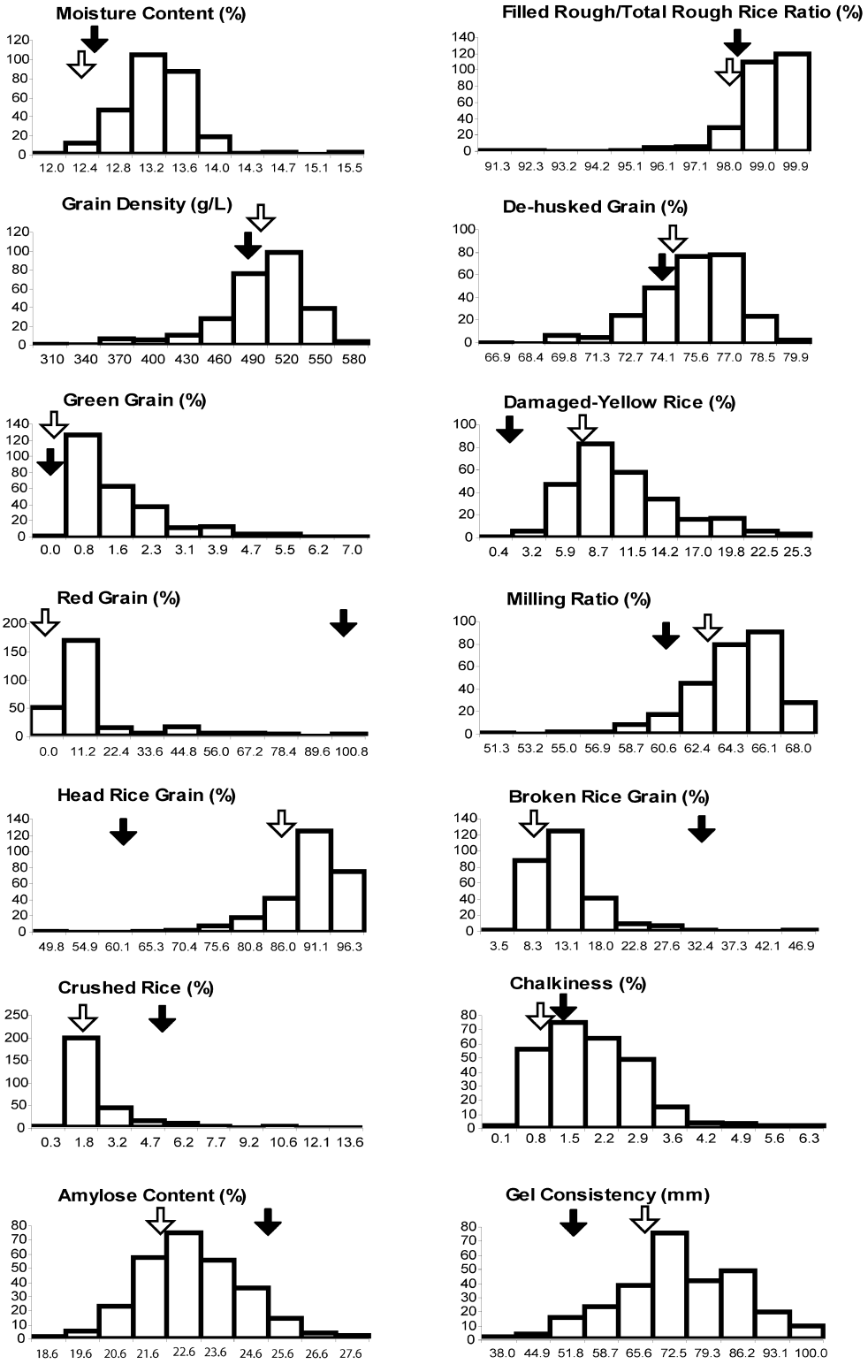

Fig. 1 Phenotypic distribution of the 14 rice grain quality traits of a BC_2F_2 population derived from a cross between *Oryza rufipogon* (IRGC 105491) and IR64. *Filled arrows* represent *O. rufipogon* parental values, while *empty arrows* represent IR64 parental values

7.02% and 0.3%–13.6%, respectively). This indicated that most of the famlies had only a small amount of crushed and green grains, as expected. The remaining nine traits had approximately normal distributions.

QTL analysis

QTLs were identified for 12 out of the 14 traits measured (no QTL were detected for moisture content or percent chalky grains). Twenty-three QTLs were identified, with 52.2% (12 loci) of them identified by both QTL analysis

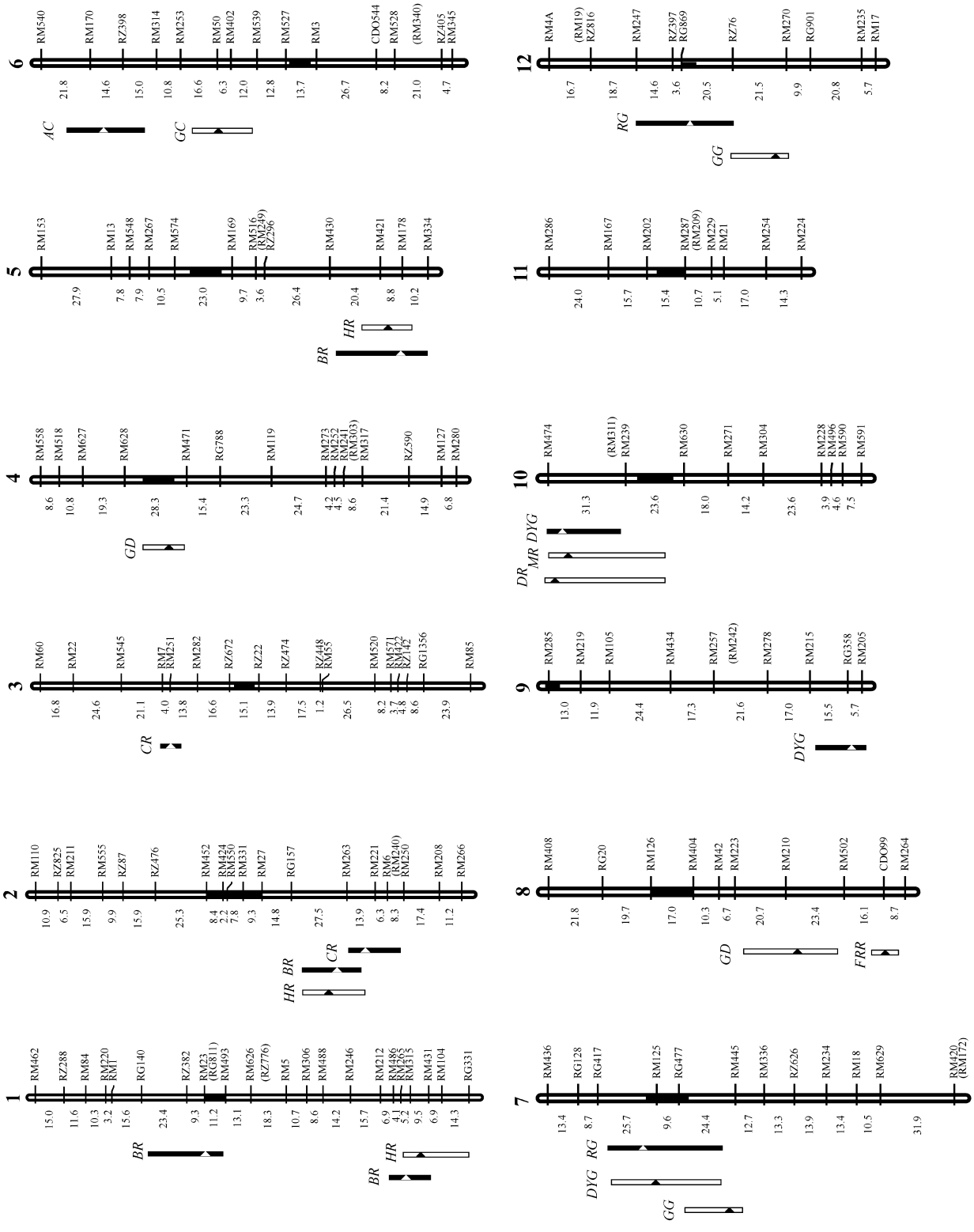

Fig. 2 Molecular linkage map of a BC_2F_2 population derived from a cross between IR64 and *O. rufipogon* (IRGC 105491) along with the positions of QTLs for 12 grain quality traits of rice. Centromeres are indicated by *black bars* within each chromosome. *Filled black boxes* indicate increased effects from *O. rufipogon* and *white boxes* indicate decreased effects from *O. rufipogon*. The peak of the QTL interval plot is indicated by a *triangle*. The QTL boundaries are defined by the closest flanking markers that reach the empirically-determined significance threshold for each trait (P<0.05)

Table 2 Grain quality quantitative trait loci (QTLs) detected in an IR64/*Oryza rufipogon* BC_2F_2 population[a]

QTL	Chr	Peak marker	Increased effect	IM			CIM		
				LOD	R^2(%)	A	LOD	R^2(%)	A
Filled/total rough rice ratio (FRR)	8	CDO99	IR64	3.08	5.1	0.43	3.50	10.7	1.17
Grain density (GD)	4	RM471	IR64	6.23[b]	10.1	19.01	3.12	8.3	30.66
	8	RM210	IR64	7.01	11.2	19.81	6.40	14.2	45.56
De-husked rice grain (DR)	10	RM474	IR64	3.95	6.5	0.96	5.59	26.8	2.85
Green grain (GG)	7	RM445	IR64	3.05	5.1	0.38	–	–	–
	12	RM270	IR64	3.22	5.3	0.40	–	–	–
Damaged-yellow rice grain (DYG)	7	RM125	IR64	2.98	4.9	1.41	4.12	6.3	3.26
	9	RG358	*O. rufipogon*	–	–	–	4.31	6.2	−3.50
	10	RM474	*O. rufipogon*	–	–	–	3.71	28.3	−5.68
Red grain (RG)	7	RM125	*O. rufipogon*	33.20	43.0	−16.73	50.89	70.4	−47.09
	12	RG869	*O. rufipogon*	3.35	5.5	−6.50	–	–	–
Milled rice grain (MR)	10	RM474	IR64	3.55	5.8	1.13	6.08	20.5	2.73
Head rice grain (HR)	1	RM431	IR64	3.22	5.3	1.78	–	–	–
	2	RM263	IR64	3.17	5.2	1.84	8.86	42.6	11.56
	5	RM178	IR64	3.36	5.5	1.63	-	–	–
Broken rice grain (BR)	1	RM23	*O. rufipogon*	3.30	5.4	−1.38	3.61	5.5	−1.90
	1	RM265	*O. rufipogon*	3.88	6.4	−1.65	3.20	4.8	−1.44
	2	RM263	*O. rufipogon*	–	–	–	4.45	16.1	−4.72
	5	RM178	*O. rufipogon*	3.77	6.2	−1.46	–	–	–
Crushed rice grain (CR)	2	RM263	*O. rufipogon*	3.17	5.2	−0.52	–	–	–
	3	RM251	*O. rufipogon*	2.99	4.9	−0.42	–	–	–
Amylose content (AC)	6	RM170	*O. rufipogon*	14.63	21.9	−0.88	15.78	28.2	−1.74
Gel consistency (GC)	6	RM50	IR64	4.02	6.6	3.57	5.06	9.0	7.79

[a] QTLs detected at an experiment-wise $P<0.05$ (IM LOD>2.93, CIM LOD>3.05)
[b] Underlined LOD values were QTLs detected at an experiment-wise $P<0.01$ (IM LOD>3.76, CIM LOD>3.99)

methods, while 34.8% (8 loci) were only detected by IM, and 13.0% (3 loci) were only identified by CIM (Fig. 2; Table 2). Differences between QTLs detected by IM and CIM have been previously reported (Moncada et al. 2001). Despite discrepancies between the two methods, together they provide valuable hypotheses about QTL location that can be tested in future genetic experiments. For all marker loci, interactions between loci were also tested for each trait, with a small number of interactions detected (Table 3).

Two QTLs were detected for green rice grains. They were located on chromosomes 7 and 12, and alleles from *O. rufipogon* decreased the number of rice grains having green color. Unexpectedly, the effect of the wild alleles was beneficial, suggesting that they are associated with more synchronous grain ripening. A high percentage of green-kernelled rice grain has a negative impact on grain quality, especially under conditions of delayed seeding (Hwang et al.1998). Moreover, there was a highly positive correlation between GG and chalkiness (0.288, $P<0.0005$). Consequently, the number of chalky grains increases with increased numbers of green grains, and chalky grains are more prone to breakage during milling.

QTLs for grain density were identified on chromosomes 4 and 8, and the allele originating from IR64 increased the weight of the grains per volume. A similar effect was seen at the QTL for the ratio of filled and total rough rice detected at the bottom of chromosome 8, where the allele from IR64 also increased the ratio. It has been proposed that grain yield and milling yield can be improved by increasing GD (Venkateswarlu et al.1987). QTLs controlling damaged-yellow rice grains were detected on the bottom of chromosomes 4 and 9, with alleles from *O. rufipogon* associated with deleterious effects at both loci. However, there was also a QTL associated with this trait on chromosome 7, and in this case, the allele originating from *O. rufipogon* contributed a beneficial effect by decreasing the amount of damaged-yellow rice grains.

Table 3 Digenic interactions detected at $P<0.001$ by two-way interaction analysis based on marker genotypes

Trait	Locus 1[a]	Chr	Locus 2	Chr	F
Red grain (RG)	RM125	7	RM104	1	26.25
			RM250	2	17.94
			RZ476	2	24.33
			RM555	2	21.79
			RM628	4	21.26
			RM430	5	24.19
			RM540	6	32.28
			RM16	11	17.72
Head rice grain (HR)	RM265	1	RM3	6	19.49
Broken rice grain (BR)	RM265	1	RM3	6	21.27
	RM421	5	RM528	6	24.82
			RM496	10	18.69

[a] If several closely linked markers were significant, the marker with the highest level of significance is shown

QTLs for the head rice recovery trait were identified on chromosomes 1, 2 and 5, with the beneficial alleles coming from IR64 in all cases. All of these QTLs were in regions similar to those associated with the percent of broken rice. The QTL on chromosome 2 overlapped with the crushed rice QTL and *O. rufipogon* alleles were associated with deleterious effects in those cases. These clusters of QTLs can be readily explained, since an increase in the percentage of head rice recovery (associated with the IR64 allele) is negatively correlated with the amount of broken and crushed grains (associated with *O. rufipogon* introgressions in the same region). A study by Tan et al. (2001) identified QTLs for many similar traits but none of them overlapped with the current study.

Bres-Patry et al. (2001) identified two QTLs that controlled the color of the rice pericarp, located on chromosomes 1 and 7. Genes identified as classical mutants, such as *rc* on chromosome 7 and *rd* on chromosome 1 reside within the QTL-containing regions and are potentially responsible for producing the red pigments in the pericarp tissue of wild rice (Kinoshita 1995). In this study, the QTL with the largest effect (R^2=70.4%) was detected in a similar region to the *rc* mutant on chromosome 7. In addition, a minor QTL for red grain was identified on chromosome 12 which has not been previously reported. This QTL may be an enhancer or modifier gene that influences the intensity of the red color.

Amylose content is one of the most important components of rice eating-quality. Sano et al. (1986) identified two alleles of the *wx* locus using RFLP markers, which roughly corresponded to the *indica* and *japonica* subspecies alleles. Employing SSR markers known to reside in the splice site of the *wx* gene, Ayres et al. (1997) identified seven different alleles that explained 82.9% of the variation for AC existing in 89 non-glutinous rice cultivars tested. In other studies, AC was strongly controlled by an allelic series at one locus in the *wx* region, and accompanied by one or several modifying genes with minor effects (McKenzie and Rutger 1983; He et al.1999). In the current study, the QTL region associated with AC, where the allele from *O. rufipogon* increased the percent amylose, was in a region previously reported to contain AC-associated genes and QTLs (Li et al.1994; He et al.1999; Tan et al.1999), and was in the vicinity of the *wx* locus. The *wx* gene encodes the granule-bound starch synthase, which controls the production of amylose, and is one obvious candidate gene underlying the QTL in the current study. However, this QTL explained less of the phenotypic variation than in previous studies. This would be expected if more than one gene affecting the AC phenotype were segregating in this population. Modifying gene(s) may either be linked to the *wx* locus, as described by McKenzie and Rutger (1983) and He et al. (1999), or may go undetected as independent QTL(s) due to the small size of their individual effect(s) on phenotype.

Using a doubled-haploid population derived from ZYQ8 (*indica*) and JX17 (*japonica*), He et al. (1999) detected QTLs for gel consistency (GC) on chromosomes 2 and 7, while Tan et al. (1999) based their study on 'Shanyou 63', the most widely grown hybrid in China, and reported QTLs for AC, GC and gelatinization temperature in the *wx* region. In the current study, the only QTL detected for GC was located in the middle of chromosome 6, below the *wx* region, and although the QTLs for AC and GC were on the same chromosome arm, the QTL regions did not overlap. The highly significant, negative correlation between AC and GC observed in this study, as well as in a study by Hsieh (1991), may be the result of frequent co-inheritance where alleles from IR64 were associated with lower AC but higher GC and alleles from *O. rufipogon* conferred the opposite phenotypes. The range of AC was from 18.6 (low) to 27.6 (high), with a mean of 22.3 (medium), and the GC ranged from 38 (hard) to 100 (soft), with a mean of 71.2 (soft). The relative amounts of AC and GC for certain lines developed from the population used in this study could be adjusted to suit the different preferences that exist among different rice-consuming regions by manipulating the QTLs associated with these traits.

For most of the grain quality traits tested, the alleles coming from *O. rufipogon* were inferior in the 'IR64' background. While other aspects of grain quality remain to be tested, such as protein content, gelatinization temperature, grain length and grain width, it is likely that the reknowned grain quality of IR64 will largely defy efforts to improve it. Thus, the information provided by the current study is critical for understanding which loci contribute positively to grain quality in an elite cultivar such as IR64 and for efficiently selecting against those introgressions that would diminish it. It is worth noting that all but one of the positive *O. rufipogon*-derived yield and yield components QTLs reported in Septiningsih et al. (2003) were not linked to the negative grain quality QTLs detected in this study. This suggests that there is not likely to be a large amount of linkage drag associated with grain quality if markers are used to selectively introgress positive yield QTLs from *O. rufipogon* into an IR64 background. Further research aimed at fine mapping and cloning these agriculturally important QTLs will accelerate efforts to improve the resolution and accuracy of marker assisted plant improvement as well as provide insights into the molecular mechanisms that govern critical aspects of crop performance.

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