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ORIGINAL PAPER

G. Aluko · C. Martinez · J. Tohme · C. Castano ·
C. Bergman · J. H. Oard

QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* × *O. glaberrima*

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Abstract International rice export markets are increasing demands for rapid improvements in grain quality characteristics. The African rice *Oryza glaberrima* is a new potential source of genes that will enhance the eating, cooking, and milling properties of the rice grain. The objective of this research was to identify and characterize quantitative trait loci (QTLs) among 312 doubled haploid lines derived from the BC_3F_1 of an interspecific cross of *O. sativa* × *O. glaberrima*. Genetic material was planted in replicated plots and evaluated for ten grain quality traits in 2001 in Colombia. A linkage map was constructed with 100 polymorphic microsatellite markers using the MAPDISTO software program to adjust for segregation distortion. Transgressive segregation was observed for all traits. Interval and composite interval analyses identified 27 QTLs for nine characters located on 11/12 chromosomes. The chromosomal positions of QTLs for percentage amylose, alkali-spreading score, and percentage protein were in agreement with data reported by others, whereas QTL markers for percentage head rice, percentage milled rice, percentage protein, and percentage brown rice were different in our mapping population. Five major QTLs were found to be associated with improved percentage rice bran, percentage amylose, and alkali-spreading score. Seven QTLs for improved percentage rice bran, percentage milled rice, alkali-spreading score, percentage protein, and grain length/width ratio were derived from the *O. glaberrima* accession. Three new QTLs for percentage rice bran are reported here for the first time. Results from this study suggest that the African rice might be a valuable new source for introgression and improvement of several traits that affect quality traits demanded by the different rice export markets.

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G. Aluko · J. H. Oard (✉)
Department of Agronomy, LSU AgCenter, Louisiana State University,
104 Sturgis Hall,
Baton Rouge, LA, 70803, USA
e-mail: joard@agcenter.lsu.edu
Fax: +1-225-5781403

C. Martinez · J. Tohme · C. Castano
Centro Internacional de Agricultura Tropical,
Apdo aereo 6713,
Cali, Columbia

C. Bergman
USDA-ARS Rice Research Unit,
1509 Aggie Drive,
Beaumont, TX, 77713, USA

Introduction

The demand for superior grain quality is increasingly becoming a priority for international export markets in all of the cultivated rice-producing areas worldwide (Juliano et al. 1990; Unnevehr et al. 1992). The primary components of rice grain quality include appearance, eating, cooking, and milling quality, and nutritional qualities, all values that are determined by their physical–chemical properties and other socio-cultural factors. The quality of appearance is determined by grain length, width, width-length ratio, grain size and shape, and translucency of the endosperm (Unnevehr et al. 1992; Juliano and Villareal 1993).

The amylose content of rice, recognized as one of the most important determinants of eating and cooking quality (Bao et al. 2002), has been reported to be governed by the *waxy* (*Wx*) locus and mapped to chromosome 6 (Tan et al. 1999; Septiningsih et al. 2003; Zhou et al. 2003). Other investigators have found amylose content to be specified by a single major gene with modifications by minor genes (McKenzie and Rutger 1983; Kumar and Khush 1988). Three independent studies have indicated that the *Wx* locus is linked to a gene for alkali-spreading score, an indicator of the temperature at which the rice grain becomes gelatinous during cooking (McKenzie and Rutger 1983; Sano 1984).

Milling quality is assessed using three principal characteristics—brown rice percentage, milled rice percentage, and head-milled rice. Brown rice consists of grains from which the bran has not been removed by milling, while milled rice is made up of whole and broken rice grains that have had the bran removed. Head rice, or the proportion of whole kernels, which includes broken kernels that are 75–80% of a whole rice grain, is a major factor determining rice market value and one of the most important criterium of milled rice. Zhou et al. (2003) recently demonstrated the potential for marker-based breeding approaches in modifying milling quality by developing rice grains in hybrid rice with enhanced eating characteristics.

Undomesticated rice harbors useful genes as quantitative trait locus (QTL) analysis has revealed among progeny of *Oryza sativa* × *O. rufipogon* crosses (Xiao et al. 1996, 1998; Moncada et al. 2001). However, QTL mapping studies of grain and milling quality have been focused primarily within the *O. sativa* germplasm (He et al. 1999; Tan et al. 1999, 2000, 2001; Zhou et al. 2003). QTLs associated with various grain quality traits have recently been identified from an interspecific cross between *O. sativa* and a wild relative *O. rufipogon* (Septiningsih et al. 2003). Undesirable effects on the majority of grain quality QTLs were contributed by *O. rufipogon*, but the results may have been influenced by environmental effects and grain quality characteristics of the adapted recurrent parent.

Oryza glaberrima Steud., the native cultivated rice of economic importance in West Africa, is believed to be domesticated from the ancestral *Oryza barthii* A. Chev. (Second 1982). Sterility barriers between *O. sativa* and *O. glaberrima* in early hybrid generations have limited the transfer of useful genes between these species (Jones et al. 1997a,b). The distortion of markers segregating in different mapping populations has been found to be associated with previously characterized gametophyte and sterility genes, particularly in wide crosses (Xu et al. 1997). Recent analyses of *O. glaberrima* × *O. sativa* hybrids have revealed strong marker distortion near the *Wx* and *S1* sterility loci on chromosome 6 (Lorieux et al. 2000; Heuer and Miezan 2003). Despite these occurrences of distorted gene segregation associated with sterility barriers in *O. sativa* × *O. glaberrima* crosses, several useful traits from *O. glaberrima* have been successfully introgressed into adapted *O. sativa* cultivars (Jones et al. 1997a, b; Heuer et al. 2003).

The primary objective of the investigation reported here was to map QTLs for milling, eating, and cooking qualities of rice using a population of doubled-haploid (DH) lines derived from the interspecific cross between African and Asian rice. QTLs for grain quality might facilitate the development of strategies for the improvement of milling and cooking of African rice by providing new genetic sources of enhanced grain quality characteristics.

Materials and methods

Caiapo, an *indica* commercial rice variety developed by the Brazilian national rice program for upland acid soil conditions (Anonymous, EMBRAPA 1997), was used as the recurrent parent in this study. This variety is characterized by good milling and eating characteristics, a long grain type (9–10 mm), early maturity (110–120 days), low tiller number (4–8), a 80-cm height, and typical yields of 2.5 t/ha under upland conditions (Anonymous, EPAMIG 1994). *Oryza glaberrima*, IRGC 103544, originally collected in the wild from Mali, Africa, grows to a height of approximately 95 cm and shows resistance to several biotic and abiotic stresses (Dr. Brar, International Rice Research Institute, personal communication).

IRGC 103544 served as the male parent in crosses to Caiapo. F_1 plants were grown in 1997 in the greenhouse at the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia. A total of 200 F_1 seeds were produced. All F_1 plants were completely sterile, and 20 individuals were selected randomly as females in backcrosses to Caiapo. A total of 154 BC_1F_1 plants were produced and transplanted to irrigated field conditions in 1998 to ensure survival and good plant development. All plants were sterile, and 103 BC_1F_1 plants were randomly selected and backcrossed to Caiapo to generate the BC_2 generation in which a high level of sterility was observed. The final backcrosses to Caiapo were completed in 1999, and these generated 97 BC_3F_1 plants. Anthers were collected from each BC_3F_1 plant and used in anther culture as described by Lentini et al. (1995). A total of 695 DH plants were obtained and grown under irrigated field conditions in 2000. A set of 312 DH lines representing the observed genetic variability was chosen for further agronomic and molecular characterization. Seed from each DH line was grown in one generation to produce sufficient seed for subsequent phenotypic and genetic analyses.

The first field trial was conducted at Palmira, Colombia in August 2001. The 312 DH lines were planted under irrigated conditions in a randomized complete block design, in two-row plots, each row 5-m-long, with three replications. Twenty-five-day-old seedlings were transplanted at a spacing of 30×30 cm. Caiapo and IRGC 103544 were also included as controls. Fertilizer was applied at the rate of 120 kg N ha^{-1}, 73 kg K_2O ha^{-1}, 63 kg P_2O_5 ha^{-1}, and 4 kg $ZnSO_4$ ha^{-1}. A post-emergence application of Butaclor and Bentazol, each at the rate of 3 l ha^{-1}, was used to control weeds supplemented by manual weeding as needed. Experimental plots were harvested in December 2001.

Rice grown at the Colombia location was harvested and stored at room temperature for at least 3 months before processing. The length and width of 20 fully formed paddy rice grains from each DH line were measured using a vernier caliper. Hulls were removed from 50 g of rough rice from each line using a Model TH035A Satake huller (Houston, Tex.) to yield brown rice. The embryo and the bran layer were removed from the brown rice by passing the grains through a McGill miller, model no. 1 (Phillip Rahm). Long grains (length/width ratio of greater than 3) and medium grains (length/width ratio of greater than 2) were milled separately for 30 s each. The whole plus broken kernels obtained after milling were defined as the total milling yield. The hull and bran were collected during milling and weighed. Total milled rice was separated into a batch consisting of whole rice grains plus grains that were at least 75% of the whole rice grain to constitute the head rice, while the remainder was regarded as broken rice. For percentage amylose, well-mixed samples and standards were ground through a 0.40-mm screen using a model no. 3010-018 UDY mill (UDY, Fort Collins, Colo.) and allowed to equilibrate overnight at room temperature in a sample-holding cabinet. Samples (60 mg) of milled ground rice were weighed and transferred to sample culture tubes (item no. T3062-8; Fisher Scientific, Norcross, Ga.), 1 ml of 100% ethanol was added to each sample, and the tubes were shaken gently for 5 min. The samples were covered with plastic wrap and allowed to stand at room temperature overnight in 1 *N* NaOH. Distilled, deionized water (54 ml) was added to each sample, and the mixture was then vortexed for 10–15 s using a Maxi-Mix 1 mixer (type 16700; Barnstead/Thermolyne, Dubuque, Iowa). The samples were held overnight at room temperature, and percentage amylose was

determined on an auto-analyzer 3 (model AA3; Bran and Luebbe, Roselle, Ill.) using automated analyzer control and evaluation software AACE ver. 5.24 (Bran and Luebbe). The method of Little et al. (1958) was used to determine the alkali-spreading score. Ten milled rice grains from each parent or DH line were immersed in 1.7% potassium hydroxide solution at room temperature for 23 h. The grains were carefully separated using forceps, and the spreading value of the grains was scored by visual assessment using the method of Jennings et al. (1979). Protein content was determined by a nitrogen gas analyzer (model 528; LECO). Samples of 1 mg were placed into a quartz combustion tube in an induction furnace at 900°C. Total crude protein was calculated from the nitrogen content of the processed grain where percentage nitrogen × 5.95 = percentage protein.

A total of 125 randomly selected microsatellite markers located across the 12 chromosomes were screened for polymorphism between the Caiapo and IRGC 103544 parents. The population of 312 DH lines was analyzed using a total of 100 polymorphic microsatellite markers located an average distance of every 10.5 cM. PCR protocol was performed as described by Chen et al. (1997) with silver staining carried out following the Promega (Madison, Wis.) technical manual (Silver sequence, DNA sequencing system 1995).

Correlation among traits was evaluated using PROC CORR ver. 8.2 of the SAS Institute (SAS 1998). Mean values for phenotypic traits were separated by Duncan's multiple range test using PROC GLM ver. 9.0 of the SAS Institute (SAS 2003). Mean values for each parent were derived from four to five plants. QTL analyses associated with markers for each trait were performed using MAPMAKER ver. 3.1, F_2 backcross scheme (Lander et al. 1987; Lincoln et al. 1992). Linkage groups were created with a minimum LOD score of 3.0 and a recombination fraction of 0.4 using the "group" command. Marker order within the linkage groups was determined using the "compare," "try," and "ripple" commands.

Table 1 χ^2 values and chromosome location of microsatellite markers showing segregation distortion among 312 DH lines derived from the cross *Oryza sativa* (Caiapo) × *O. glaberrima* (IRGC 103544)

Marker	Chromosome	χ^2	Probability *F*	Skewness[a]	Chromosome position[b]
RM5	1	6.958	0.0083	Caiapo	24.3
RM297	1	8.007	0.0047	Caiapo	60.7
RM315	1	8.889	0.0029	Caiapo	86.5
RM226	1	11.50	0.0007	Caiapo	37.8
RM128	1	14.89	0.0001	Caiapo	35.2
RM236	2	9.188	0.0024	Caiapo	13.9
RM110	2	9.85	0.0017	Caiapo	0.0
RM301	2	12.25	0.0005	Caiapo	56.3
RM71	2	13.14	0.0003	Caiapo	40.1
RM174	2	13.66	0.0002	Caiapo	33.0
RM85	3	28.42	0.0001	IRGC 103544	129.8
RM60	3	193.0	0.0001	IRGC 103544	0.0
RM81B	3	231.0	0.0001	IRGC 103544	14.5
RM280	4	7.23	0.0072	Caiapo	39.0
RM124	4	9.788	0.0018	Caiapo	28.0
RM348	4	11.50	0.0007	Caiapo	8.0
RM349	4	13.7	0.0007	Caiapo	14.8
RM241	4	16.22	0.0001	Caiapo	3.2
RM131	4	16.48	0.0001	Caiapo	23.2
RM317	4	18.2	0.0001	Caiapo	6.7
RM267	5	8.49	0.0036	Caiapo	24.0
RM31	5	12.4	0.0001	Caiapo	75.0
RM274	5	12.57	0.0001	Caiapo	84.0
RM194	5	14.76	0.0001	Caiapo	27.0
RM169	5	14.89	0.0001	Caiapo	30.0
RM103	6	8.54	0.0035	Caiapo	225.1
RM253	6	81.81	0.0001	IRGC 103544	115.9
RM190	6	32.21	0.0001	IRGC 103544	0.0
RM10	7	14.89	0.0001	Caiapo	15.0
RM308	8	7.23	0.0072	Caiapo	86.0
RM42	8	8.47	0.0036	Caiapo	46.0
RM25	8	9.97	0.0016	Caiapo	12.0
RM149	8	10.32	0.0013	Caiapo	78.0
RM256	8	18.06	0.0001	Caiapo	67.0
RM316	9	14.70	0.0001	Caiapo	5.0
RM184	10	7.23	0.0072	Caiapo	22.0
RM239	10	9.60	0.0019	Caiapo	0.0
RM229	11	18.72	0.0001	Caiapo	46.0
RM277	12	12.76	0.0004	Caiapo	26.0

[a]Skewed marker segregation towards the Caiapo or IRGC 103544 parent

[b]Linkage map location of marker in centiMorgans

Map distances were calculated by utilizing the Kosambi function. Due to distorted marker segregation detected on all chromosomes, the MAPISTO software program by Lorieux et al. (2000) was used with the "doubled haploid population model" to adjust for non-Mendelian inheritance of certain microsatelllite markers. The transformation of data for normality of each grain quality trait was performed using inverse or log transformations in MICROSOFT EXCEL ver. 2002), and normality was checked using PROC UNIVARIATE ver. 8.2 of the SAS Institute (SAS 1998). QTLs were detected by interval (Lander and Botstein 1989) and composite interval mapping (Zeng 1994) procedures. Default LOD threshold values of 2.5 from MAPMAKER ver. 3.1 and the QTL CARTOGRAPHER software program (Basten et al. 1994) were used to declare the presence of a QTL. A model using five co-factors was selected in QTL CARTOGRAPHER to control for genetic background and applied to the composite interval mapping procedure. Only QTLs detected both by interval and composite interval mapping were used in the analysis. Epistatic interactions were determined using EPISTAT SOFTWARE (Chase et al. 1997).

Results and discussion

Correlations among the majority of traits in this study were consistent with those obtained from previous investigations using both inter-sub-specific and *O. sativa* × *O. rufipogon* crosses (McKenzie and Rutger 1983; Tan et al. 2000). Significant negative correlations were detected in our study between protein content and the length-width ratio (−0.33, $P \leq 0.01$**) and between rice bran percentage and milled rice percentage (−0.84, $P \leq 0.01$**).

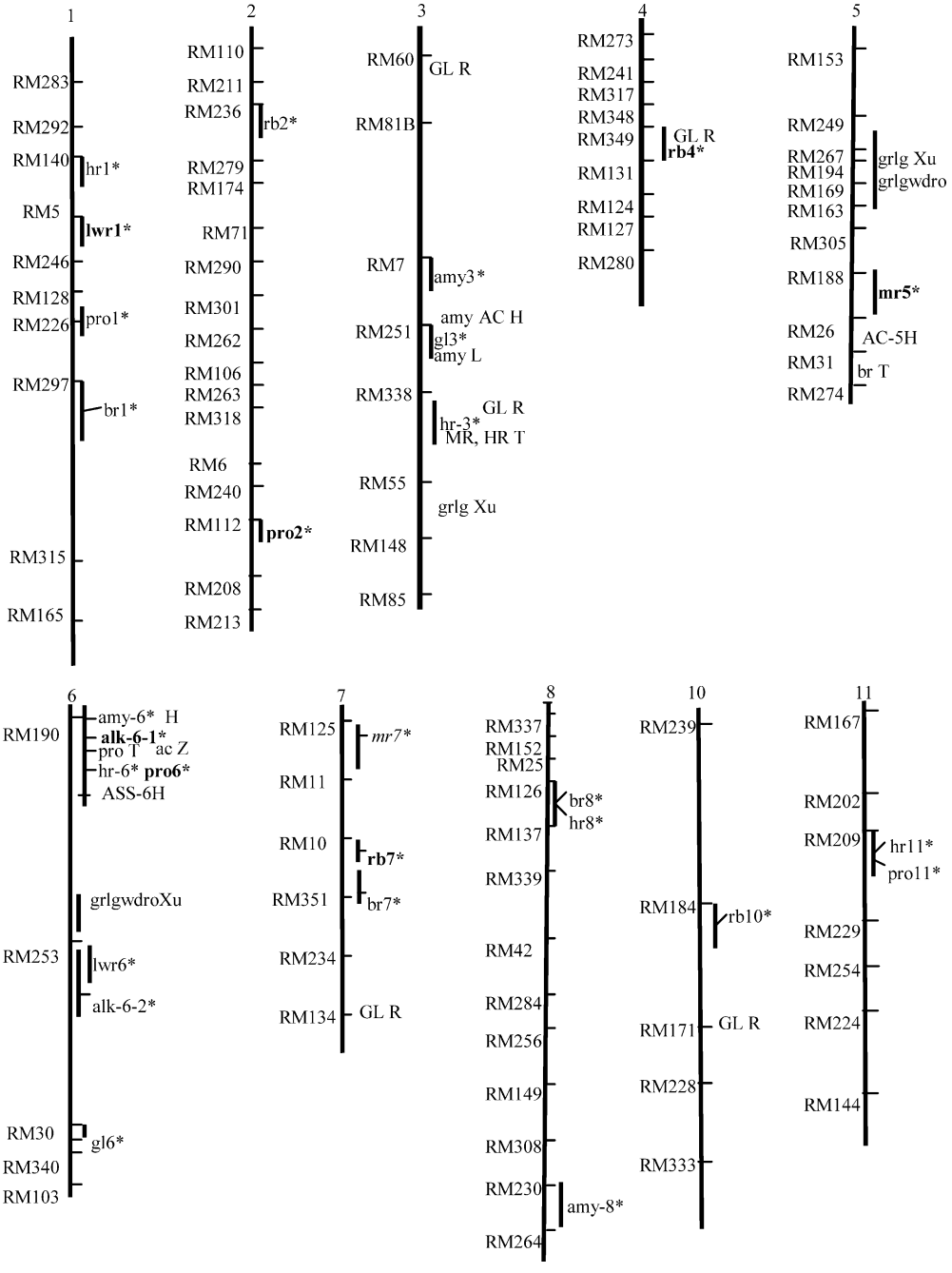

Fig. 1 Assignment of 27 QTLs for nine grain and milling traits on the rice linkage map adjusted by the MAPDISTO program among 312 DH lines derived from *Oryza sativa* (Caiapo) × *O. glaberrima* (IRGC 103544) cross. Confidence intervals for each QTL are indicated as a *bar* to the *right* of each chromosome. QTLs in *bold* indicate positive allelic effects from IRGC 103544. *H* QTL reported by He et al. (1999), *T* QTL reported by Tan et al. (2001), *L* QTL reported by Lanceras et al. (2000), *Z* QTL reported by Zhou et al. (2003), *AC* QTL for amylose content reported by Zhou et al. (2003) and He et al. (1999), *GL R* QTL for grain length reported by Redoña and Mackill (1998), *grlgwdro Xu* QTL for grain length/width (*l/w*) ratio reported by Xu et al. (2000), *grlg Xu* QTL for grain length reported by Xu et al. (2000), *alk* alkali-spreading score, *amy* percentage amylose, *ASS* alkali-spreading score by He et al. (1999), *br* percentage brown rice, *gl* grain length, *gw* grain width, *hr* percentage head rice, *lwr* length/width ratio, *mr* percentage milled rice, *pro* protein content, *rb* percentage rice bran. * QTL detected in this study

Table 2 Quantitative trait loci for grain quality traits among 312 DH lines derived from the cross *O. sativa* (Caiapo) × *O. glaberrima* (IRGC 103544)

Trait	QTL	Chromosome	Marker interval	Marker position[a]	Additive effect	LOD	R^2	Allelic source
Brown rice (%)	*br1*	1	RM297–RM315	60.7	−0.74	3.4	2.8	Caiapo
	br7	7	RM10–RM351	21.0	−2.73	3.4	4.9	Caiapo
	br8	8	RM126–RM137	21.0	−1.38	3.3	3.6	Caiapo
Head rice (%)	*hr1*	1	RM140–RM5	24.0	−24.11	3.0	17.8	Caiapo
	hr3	3	RM81B–RM7	41.0	−12.51	4.0	12.0	Caiapo
	hr6	6	RM190–RM253	10.0	−2.41	11.9	54.1	Caiapo
	hr8	8	RM126–RM137	21.0	−11.68	3.2	7.6	Caiapo
	hr11	11	RM209–RM229	43.0	−20.60	4.1	17.4	Caiapo
Rice bran (%)	*rb2*	2	RM236–RM279	19.0	−18.25	55.6	32.7	Caiapo
	rb4	4	RM349–RM131	22.8	34.49	28.4	39.7	IRGC 103544
	rb7	7	RM10–RM351	21.0	1.02	3.2	4.0	IRGC 103544
	rb10	10	RM184–RM171	26.0	−18.23	52.9	32.8	Caiapo
Milled rice (%)	*mr5*	5	RM188–RM26	61.0	35.17	3.2	6.1	IRGC 103544
	mr7	7	RM125–RM11	0.0	−3.62	3.7	5.3	Caiapo
Amylose (%)	*amy3*	3	RM7–RM251	60.0	−2.73	3.7	21.5	Caiapo
	amy6	6	RM190–RM253	36.0	−2.60	19.3	73.7	Caiapo
	amy8	8	RM230–RM264	104.0	−1.85	3.1	10.9	Caiapo
Alkali-spread.	*alk6-1*	6	RM190–RM253	6.0	0.87	32.5	50.1	IRGC 103544
score	*alk6-2*	6	RM253–RM162	156.0	−0.87	10.0	44.0	Caiapo
Protein (%)	*pro1*	1	RM226–RM297	55.0	−1.01	5.8	15.0	Caiapo
	pro2	2	RM6–RM112	98.0	1.06	3.0	7.4	IRGC 103544
	pro6	6	RM190–RM253	10.0	1.41	3.6	8.8	IRGC 103544
	pro11	11	RM209–RM229	35.0	−0.67	3.3	4.8	Caiapo
Grain length	*gl3*	3	RM251–RM338	72.2	−0.67	3.8	12.5	Caiapo
	gl6	6	RM162–RM30	204.0	−0.61	3.3	4.7	Caiapo
Grain *l*/*w* ratio	*lwr1*	1	RM5–RM246	24.3	0.71	2.8	4.0	IRGC 103544
	lwr6	6	RM253–RM162	118.0	−0.74	13.4	14.0	Caiapo

[a]Position of peak marker in centMorgans within the interval

Of the 100 markers used in this study 39 showed varying degrees of segregation distortion on all 12 chromosomes (Table 1). The majority of markers (34/39) were skewed toward Caiapo, which may be explained by the three backcrosses that were carried out and subsequent selection against sterile plants during population development. However, five of the most distorted markers—detected on chromosomes 3 (RM85, RM60, RM81B) and 6 (RM190, RM253)—were unexpectedly skewed toward the *O. glaberrima* parent. RM60 and RM81B, showing the highest level of distortion among all markers, mapped near the top of chromosome 3 (Fig. 1). A different pattern was found with distorted markers RM103, RM253, and RM190, which mapped to different positions on chromosome 6, where the QTL for percentage amylose (*amy-6*) detected in this study and the previously reported *waxy* (*Wx*) QTL (He et al. 1999) also map. The hybrid sterility of QTL SA1 has been previously mapped to this same

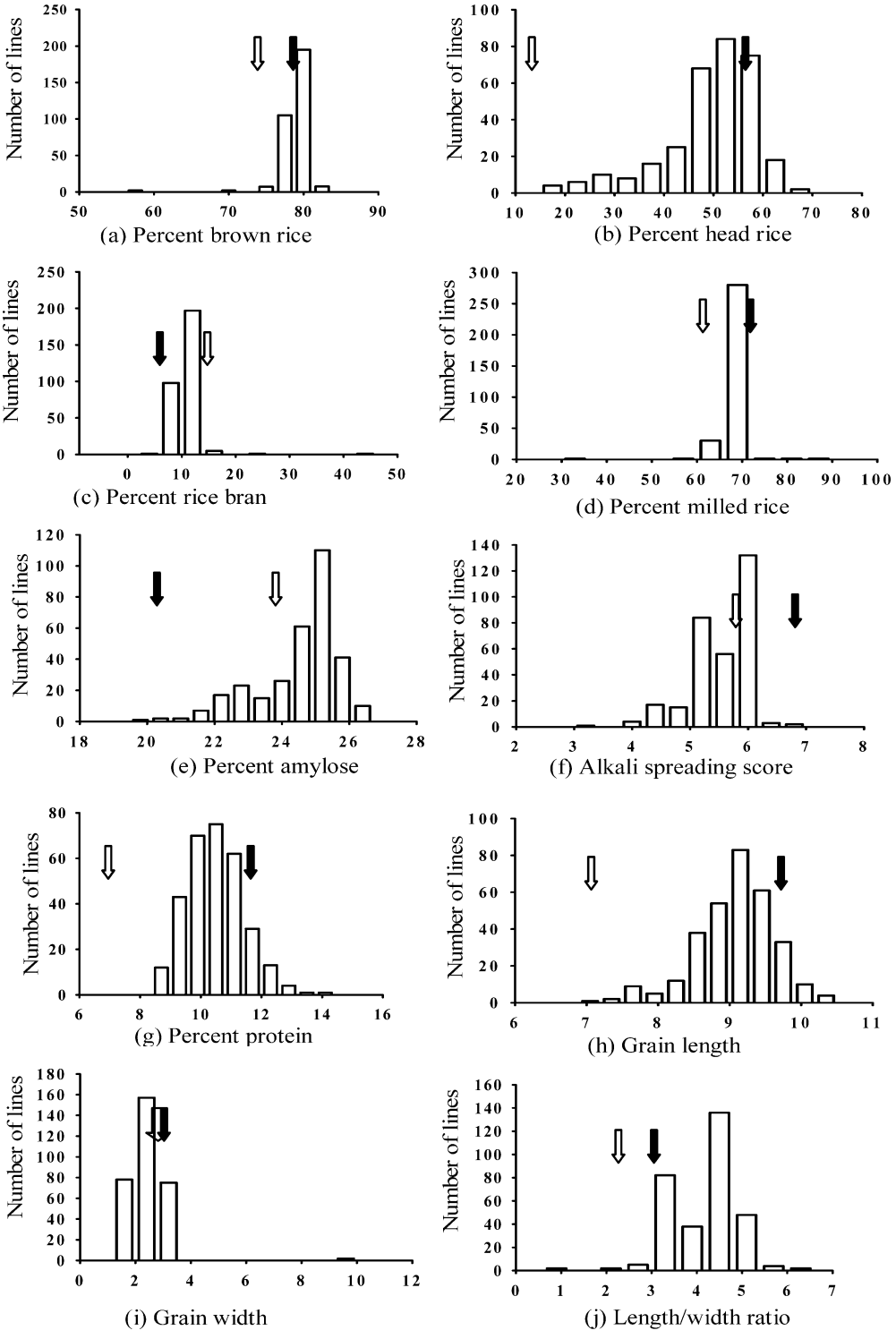

Fig. 2 Distribution of milling and grain quality characteristics among 312 DH rice lines evaluated at CIAT, Colombia, 2001. *Filled arrows* represent mean values for the Caiapo parent, *empty arrows* represent mean values for the *O. glaberrima* parent IRGC 103544

region on chromosome 6 (Gramene website: http://www.gramene.org/db/cmap/map_details?ref_map_set_aid=morph-2000;ref_map_aid=morph-2000-6). The order and position of the distorted markers on our linkage map were unclear or switched in position when compared to a different *O. sativa* × *O. glaberrima* population (Lorieux et al. (2000) or previously published maps (data not shown). The MAPDISTO program used in the present study successfully restored marker order relative to the map of Lorieux et al. (2000). The adjusted map in our study showed an average interval length of 10.5 cM between markers, with a total length of 1,050 cM (Fig. 1), which is 1.3–fold shorter than the lengths of previous maps constructed from other populations (Causse et al. 1994; Panaud et al. 1996; Chen et al. 1997; Lorieux et al. 2000; Temnykh et al. 2000). Nevertheless, subsequent QTL analysis of the DH lines (see below) showed similar mapping results for different grain quality traits as previously reported (Redoña and Mackill 1998; Tan et al. 1999, 2001; Lanceras et al. 2000; Xu et al. 2000).

We detected three QTLs—*br1, br7*, and *br8*—for percentage brown rice on chromosomes 1, 7, and 8 in the intervals RM297–RM315, RM10–RM351, and RM126–RM13 that explained 3%, 5%, and 4% of the phenotypic variance, respectively (Table 2). Alleles from the Caiapo parent were associated with increased percentage brown rice at all three loci. In contrast to these results, Tan et al. (2001) detected one QTL for brown rice on chromosome 5 in an *O. sativa* × *O. sativa* cross that explained 10% of the phenotypic variation. The frequency distribution of percentage brown rice among the 312 DH lines shown in Fig. 2a suggests simple inheritance of this trait, but the distribution was skewed toward Caiapo, most likely due to the three backcrosses made to this parent. The large variation in percentage brown rice among the DH lines indicates that there is ample opportunity for improvement of this trait. Of the DH lines 6% showed transgressive segregation over the high parent for percentage brown rice based on Duncan's multiple range test. Three QTLs were detected on chromosomes 1, 7, and 8 that represent potentially new chromosomal regions associated with percentage brown rice, but additional experiments are needed to confirm these results.

As was the case for percentage brown rice, the frequency distribution of percentage head rice was skewed towards Caiapo (Fig. 2b). The continuous pattern of the distribution suggests quantitative inheritance, a result similar to that of Tan et al. (2001) who observed a wide range among the recombinant inbred lines (RILs) tested. Five chromosomal regions were associated with QTLs for percent head rice (Table 2). The Caiapo parent contributed all five QTLs for this trait. QTL *hr1* on chromosome 1 was detected in the interval RM140–RM5 that explained 17.8% of phenotypic variation with a LOD score of 3.4. The QTL *hr3* in the interval RM338–RM55 accounted for 12% of the variation and mapped approximately 8 cM from the head rice QTL interval C1087–RZ403 as reported by Tan et al. (2001). A major QTL, *hr6*, with a LOD value of 11.9, was located on chromosome 6 in the interval RM190–RM253 and accounted for 54.1% of the total variation. On chromosome 8, *hr8* was detected with a LOD score of 3.2 that explained 7.6% of the variation. QTL *hr11* mapped to chromosome 11 in the interval RM209–RM229 with a LOD of 4.0 and explained 17.4% of the phenotypic variation. The high performance of some of the DH lines could have resulted from epistatic interactions found on chromosomes 1, 3, and 6 associated with head rice (Table 3). Non-additive interactions have been shown to be correlated with transgressive segregation in rice (Moncada et al. 2001; Tan et al. 2001). The head rice QTL *hr3* in our study mapped to the same chromosomal region as in intraspecific crosses with *O. sativa* (Tan et al. 2001), suggesting that these QTLs may be common among other rice species. Finally, one QTL for percentage head rice and brown rice mapped to the same interval on chromosome 8, a result that may be due to linkage or pleiotropy, even though these two traits were not highly correlated.

Four QTLs—*rb2, rb4, rb7*, and *rb10*—were significantly associated with percentage rice bran (Table 2). Positive alleles *rb4* and *rb7* were contributed by IRGC 103544 while *rb2* and *rb10* were derived from the Caiapo parent. QTLs identified for percentage rice bran in

Table 3 Significant two-way interactions detected between microsatellite loci associated with grain quality traits as determined using the EPISTAT program

Trait	Marker 1		Marker 2		*F*-test (*P* value)[a]	MC-test[b]
	Name	Chromosome	Name	Chromosome		
Head rice (%)	RM297	1	RM253	6	0.002	0.024
	RM148	3	RM253	6	0.000	0.000
Amylose (%)	RM283	1	RM253	6	0.005	0.048
	RM81B	3	RM333	10	0.001	0.012
	RM148	3	RM85	3	0.015	0.015
Grain width	RM297	1	RM81B	3	0.028	0.028
	RM297	1	RM167	11	0.024	0.024
	RM297	1	RM209	11	0.002	0.002
	RM81B	3	RM288	9	0.011	0.011
Bran (%)	RM224	11	RM144	11	0.006	0.072
Milled rice (%)	RM224	11	RM144	11	0.023	0.021

[a] *F*-test for the four genotypic subgroups of the two marker alleles

[b] Monte Carlo simulation using the EPISTAT program (Lark et al. 1995) to evaluate significance of interactions

this study are the first to be reported for this trait. A small proportion (7%) of the DH lines showed transgressive segregation for high percentage bran while 10% displayed values lower than that of Caiapo.

The majority of the 312 DH lines produced approximately 6% more milled rice than the IRGC 103544 parent (62%) and approximately 2% less than the Caiapo parent (70%). The percentage milled rice trait of the DH lines exhibited a wide range of values—from 33% to 89%—which was similar to the range of values found in the RILs of Tan et al. (2001) and Septiningsih et al. (2003). Two regions were found to be associated with the QTLs for percentage milled rice on chromosomes 5 and 7 (Table 2). The QTL *mr5* on chromosome 5 was located a distant 47 cM from a milled rice locus reported by Tan et al. (2001). The second QTL, *mr7* from Caiapo, was detected on chromosome 7. Tan et al. (2000) detected minor QTLs on chromosomes 3 and 5, respectively.

Fifty of the the DH lines (16%) developed in the current study produced a higher percentage of amylose than the high-amylose-producing IRGC 103544 parent (Fig. 2e). One major QTL, *amy6*, was detected on chromosome 6 in the interval RM190–RM253 with a LOD score of 19.3; this QTL accounted for 73.7% of the phenotypic variation. Other researchers have also detected a QTL for amylose content at the same region near the *waxy* gene on chromosome 6 (He et al. 1999; Tan et al. 1999; Lanceras et al. 2000; Septiningsih et al. 2003). Two additional QTLs have also been detected on chromosomes 3 and 8—*amy3* and *amy8*, respectively. Earlier studies based on mapping populations derived from *O. sativa* × *O. sativa* crosses identified QTLs controlling amylose content on chromosomes other than chromosome 6 (He et al. 1999; Lanceras et al. 2000). These results support the idea that both the major *waxy* gene and modifying genes control amylose content, as previously reported by McKenzie and Rutger (1983).

A low alkali-spreading score was predominant as 60% of the DH lines produced scores between 3.2 and 5.7, although 1% of the DH lines showed values greater than that of IRGC 103544. In contrast to most of the other traits, the distribution of alkali-spreading scores was skewed towards IRGC 103544. The two QTLs detected for alkali-spreading score mapped to chromosome 6 (*alk6-1* and *alk6-2)* (Table 2). The alleles of *alk6-1* from IRGC 103544 were associated with an increased trait value at this locus. He et al. (1999) also detected a QTL for alkali-spreading score in this region. Alleles associated with an increase in alkali-spreading scores were contributed by both IRGC 103544 and Caiapo. Lanceras et al. (2000) detected two QTLs on chromosomes 6 and 7 for alkali-spreading score that accounted for 57% of the total variation. QTL *alk6-1* in our study was detected in the same region as the major QTL controlling amylose content. Similar results by other researchers (Tan et al. 1999; He et al. 1999) showed a single QTL for amylose content and alkali-spreading score at the same locus on chromosome 6. The same observation was made by Bao et al. (2002). These results may be due to linkage or pleiotropic effects of a single gene.

Four QTLs were identified for percentage protein content. Tan et al. (2001) detected a QTL in the interval C962–Wx for protein content on chromosome 6 near the *waxy* locus, and the QTL *pro6* mapped at this position in this study.

The mean grain length of the DH lines—9.0 mm—was intermediate between both parents. Grain length showed a normal distribution, indicating quantitative inheritance with a positive skewness observed toward Caiapo, the long-grained parent. Of all the DH lines, 6% showed transgressive segregation for longer grains. Both QTLs reported for grain length were contributed by alleles from the Caiapo parent. The QTL *gl3* for grain length detected in our study has been found in the same region on chromosome 3 in other populations (Redoña and Mackill 1998; Xu et al. 2000). The Fusayoshi long-grain gene *Lk-f* was mapped to chromosome 3 by Takeda and Saito (1980) and Takamure and Kinoshita (1991). It is possible that *gl3* might be linked to the *Lk-f* gene or represents it.

No statistical difference for grain width was observed between the two parents (Fig. 2I). Mean grain width of the DH lines was 0.6 mm less than that of either parents, with approximately 80% of the DH lines exhibiting grain widths between 1.4 mm and 2.8 mm. The frequency distribution was unimodal with 2% of the lines showing transgressive segregation for wider grains. No QTLs were detected for grain width, a result most likely associated with the similar phenotypic values for both parents. In contrast, Tan et al. (2000) detected a major QTL for grain width on chromosome 5 and a minor locus on chromosome 6 in both $F_{2,3}$ and RIL populations.

The mean grain size of the DH lines, as indicated by their length/width ratio of 4.1, was approximately 25% greater than that of Caiapo and 40% greater than that of the IRGC 103544 parent (Fig. 2j) The smallest grains among the DH lines were similar in size to those of IRGC 103544. The frequency distribution of this trait indicates a quantitative inheritance with 36% of the lines showing transgressive segregation for high length/width ratio. Two chromosomal regions were significantly associated in our study with grain length/width ratio.

In this study, 27 QTLs for nine economically important grain quality traits were detected on all linkage groups except chromosome 9. It is important to note that even with segregation distortion detected among the DH lines, marker order was restored by the MAPDISTO software, and 18% (5/28) of the QTLs detected in this study (*hr3*, *amy6, alk6-2, pro6*, and *gl3*) mapped to positions within 10 cM of the positions of these grain quality traits evaluated in other populations (He et al. 1999; Xu et al. 2000; Tan et al. 2001). The majority of the positive alleles for these QTLs (21/28; 75%) were detected as coming from the adapted *indica* variety Caiapo, which exhibited high grain quality in our study. On the basis of LOD scores of 19.3–55.6, we detected five major QTLs for quality—*rb2*, *rb4*, *rb10*, *amy6*, and *alk6-1*—with the three QTLs associated with percentage rice bran being reported here for the first time.

Two of the major QTLs that contributed to enhanced percentage rice bran and alkali-spreading score (*rb4, alk6-1*, respectively) were derived from the *O. glaberrima* accession. Minor QTLs from the African rice accession also contributed to greater protein content and grain dimension. Multiple location trials are required to confirm the map positions and relative effects of the QTLs reported here.

Segregation distortion has been documented in crosses between rice sub-species (Xu et al. 1997; Xu and Shen 1992) and species (Causse et al. 1994; Xu et al. 1995; Yamagishi et al. 1996; McCouch et al. 1998; Lorieux et al. 2000; Heuer and Miezan 2003; Septiningsih et al. 2003). The presence of the putative *ga-2* gametophyte gene (Nakagahra et al. 1972) on chromosome 3 and the *S* locus on chromosome 6 in *O. glaberrima* (Heuer and Miezan 2003) may have affected pollen fertility, gene segregation, and the observed skewness toward the IRGC 103544 parent (Table 1). Distortion detected in our study with markers RM315, RM 280, and RM281 on chromosomes 1 and 4 were interestingly the same marker (RM315) or were linked to another (RZ590) displaying non-Mendelian segregation in the *O. sativa* × *O. rufipogon* backcross population (Septiningsih et al. 2003).

A test of epistatic effects was carried out to identify chromosomal regions that by themselves expressed no discernible effects but interacted with other loci to produce an observable phenotype. Table 3 shows the list of digenic interactions we obtained using the EPISTAT software (Chase et al. 1997). A total of 12 markers were detected on six chromosomes that produced 11 two-way interactions. Eight of the 11 interactions consisted of markers that mapped to six different chromosomes. An interaction between RM253 and RM297 was detected that may affect the variation detected for percentage head rice. RM297 also interacted with RM81B, RM167, and RM209 on chromosomes 3 and 11 for grain width. This interaction may bias the variation explained by the QTL in the interval containing RM253. It is interesting to note that a two-way interaction for markers RM297 and RM253 associated with percentage head rice was detected on chromosomes 1 and 6 in our study (Table 3) and that a digenic interaction between RM265 and RM3 on the same chromosomes associated with percentage head rice was detected among BC_2F_2 families derived from an *O. sativa* × *O. rufipogon* cross (Septiningsih et al. 2003).

The results presented here suggest that hybridization of *O. sativa* and *O. glaberrima* can be successfully exploited to improve the milling, cooking, and eating properties of rice for international export markets in Africa, Asia, South America, and other rice-growing regions. High levels of transgressive segregation for most of the characters examined in our study provide the potential for improvement of these economically important traits. Moreover, the detection of new QTLs from *O. glaberrima* that enhanced grain quality underscores the potential value of African rice as a useful source for germplasm improvement. Certain QTLs, especially *hr3*, *amy6*, and *alk6-1* that mapped to regions consistent with map locations in other studies, may be useful in marker-assisted selection experiments. Finally, the new QTLs detected in this study for milling quality that are derived from *O. glaberrima* could serve as candidates for future fine-mapping and positional cloning projects.

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