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Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived from an elite rice hybrid

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Abstract Milling properties, protein content, and flour color are important factors in rice. A marker-based genetic analysis of these traits was carried out in this study using recombinant inbred lines (RILs) derived from an elite hybrid cross 'Shanyou 63', the most-widely grown rice hybrid in production in China. Correlation analysis shows that the traits were inter-correlated, though the coefficients were generally small. Quantitative trait locus (QTL) analysis with both interval mapping (IM) and composite interval mapping (CIM) revealed that the milling properties were controlled by the same few loci that are responsible for grain shape. The QTL located in the interval of *RM42-C734b* was the major locus for brown rice yield, and the QTL located in the interval of *C1087-RZ403* was the major locus for head rice yield. These two QTLs are the loci for grain width and length, respectively. The *Wx* gene plays a major role in determining protein content and flour color, and is modified by several QTLs with minor effect. The implications of the results in rice breeding were discussed.

Keywords Rice quality · Milling characteristics · Protein content · Flour color · Quantitative trait locus (QTL) · Molecular marker

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Introduction

Rice is one of the major staple cereal foods, feeding more than half the world population. Demand for quality rice has always been a major factor in rice marketing and becomes more important in developing countries as the economic status of the people increases (Juliano et al. 1990; Unnevehr et al. 1992).

Although rice quality has many components and is related to preference in different cultures, its major elements include milling properties, grain size, shape and appearance, and cooking and eating characteristics. Among these, protein content, milled rice recovery (especially head rice recovery) and grain color are primary concerns (Khush et al. 1979; Unnevehr et al. 1992; Juliano 1998; Siebenmorgan 1998). Rice is the major protein source in most rice-eating areas and protein can also influence the physicochemical properties of cooked rice (Hamaker and Griffin 1990, 1991; Marshall et al. 1990; Juliano 1993; Hamaker 1994). Head rice yield; which affects market value, is directly related to brown rice yield and milled rice yield which together form the ”milling quality” (Webb 1980; Juliano 1985; Unnevehr et al. 1992). Color is important in consumer acceptance of grain appearance and for end-products such as noodles (Juliano 1985; Collado et al. 1997; Bhattacharya et al. 1999). Few reports on the genetic basis of such traits are available because of the complexity of their inheritance and the effect of environmental and other conditions. For example, protein content can be highly affected by the degree of milling and by environmental conditions, e.g. nitrogen fertilizer and growth duration (Perez et al. 1996). This complexity has led to the failure of breeding efforts to improve the protein content of rice grain (IRRI 1983; Coffman and Juliano 1987). Elucidation of their genetic basis would greatly help to improve the above-mentioned traits.

The recent development of DNA markers and linkage maps of rice have provided new opportunities for the genetic improvement of rice grain quality (Causse et al 1994; Harushima et al. 1998). With linkage maps based on DNA markers, complex polygenic traits can be dissected into single Mendelian quantitative trait loci (QTLs) (Paterson et al. 1988; Tanksley 1993). Many QTLs for traits of agronomic importance, such as yield and quality attributes, have been detected (McKenzie and Rutger 1983; Anh et al. 1993; Yu et al. 1997; Tan et al. 1999, 2000). Information on QTL analysis has accumulated quickly, and will eventually help the manipulation of the complex traits in genetic engineering and rice breeding (Tanksley 1993; Xu 1997; Yano and Sasaki 1997).

In this study we used *Shanyou* 63, the top elite *indica* hybrid rice of China, as the material to investigate the genetic basis of milling characteristics, the protein content and the flour color of milled rice. The objectives were to determine: (1) the relationships among the traits, and (2) the number, positions, and genetic effect of the QTLs responsible for the traits.

Materials and methods

Plant materials

The two parents, Zhenshan 97 (ZB, maternal) and Minghui 63 (MH, paternal), the F_1 hybrid and a population of 238 F_{10} recombinant inbred lines (RILs) derived from the F_2 plants by single-seed descendent (SSD), were planted in a randomized block design with three replications (Yu et al. 1997; Xing 1999) in the summer rice growing season, 1997, at Huazhong Agricultural University, Wuhan. Field management followed the normal agronomic procedures as described, and natural ripening of the grain occurred.

Trait measurement

Milling characteristics

The harvested paddy rice from different replications was combined and stored at room temperature for at least 3 months before testing. The paddy rice was de-hulled and milled as described in Tan et al. (1999). Rough rice (40 g) was de-hulled and milled in duplicate using a mill (Jiading Food and Oil Machinery Factory, Shanghai, China) according to the National Standard NY 147–88. Head rice was manually separated with a set of screens. Broken grains with two-thirds of the whole grain were included in the head rice. Brown rice percentage, milled rice percentage, and head rice percentage were calculated based on the rough rice weight.

The duplicate milled rice grain samples were combined and ground into powder using an Udy Cyclone Sample Mill (Udy, Fort Collins, Colo., USA), through a 100-mesh sieve.

Protein content

Crude protein content was measured using the Kjeldahl method (Kjeltec System 1002, Tecator, Sweden). A nitrogen conversion factor of 5.95 was used to calculate the protein content of the rice flour (AACC 1995).

Flour-color parameters

Flour color was determined with a chromometer (CR-300, Minolta Camera Co., Ltd., Tokyo, Japan) using the CIE 1976 L*a*b* color system (Pomeranz and Meloan 1987). L* is the brightness value ranging from 0 (black) to 100 (white); a* is a function of red-green (positive a* indicates redness and negative indicates greenness); b* is a yellow-blue value (positive value for yellowness and negative for blueness) (Oliver et al. 1992).

Linkage map construction and QTL assays

The linkage map consisted of 162 RFLP (restriction fragment length polymorphism) and 48 SSR (simple sequence repeat) markers covering 12 chromosomes as described in Xing (1999) and Tan et al. (2000). Pearson correlation coefficients among the traits and one-way analysis of variance with the marker genotypes as groups were conducted using the statistical package Statistica (StatSoft, Tusla Okla.). The whole genome was scanned for quantitative trait loci (QTLs) using MAPMAKER/QTL 1.0 (Lander et al. 1987; Lincoln et al. 1992) with a LOD threshold of 2.0 (Lander and Botstein 1989; van Ooijen 1999). If two or more QTLs were detected from the scanning results of interval mapping, the QTL with the largest effect was fixed to re-scan the whole genome. Additionally, QTL Cartographer Version 1.13 was also used for composite interval mapping as the threshold LOD 2.0 is somewhat low (Zeng 1994; Basten et al. 1999; van Ooijen 1999). Only the QTLs detected by both methods were listed and all the QTLs for a specific trait were combined together for the calculation of the total-likelihood and variance contribution.

Results

Distribution and heritability of the traits

The distributions of protein content and the flour color values L*, a*, b* of the RIL population, as well as the parents and the hybrid (F_1) (Table 1, Figure 1), showed

Table 1 Means and standard deviations (in brackets) of traits for parents and the hybrid, and variation of the RIL population and heritability of traits

Source	BR[a]	MR	HR	PRO	L*	a*	b*
MH[b]	77.9 (2.11) a[c]	70.9 (2.75) a	51.0 (6.93) b	7.1 (0.21) b	102.8 (1.06) a	–0.29 (0.03) a	6.76 (0.53) a
ZB	79.6 (1.05) a	71.8 (1.55) a	59.7 (4.58) a	8.6 (0.29) a	102.7 (1.10) a	–0.25 (0.02) a	6.83 (0.62) a
F_1	79.4 (1.01) a	72.1 (1.88) a	59.1 (4.53) a	6.1 (0.28) c	104.0 (0.63) a	–0.28 (0.02) a	5.83 (0.54) b
RIL	79.8 (1.59)	71.5 (2.49)	56.2 (11.10)	7.1 (0.89)	103.3 (1.04)	–0.24 (0.04)	6.34 (0.84)
Range	72.2–85.8	61.2–77.5	24.4–77.5	4.7–9.3	100.4–105.6	–0.40—0.14	4.52–8.84
h^2 [d]	29.8%	30.1%	30.8%	31.5%	39.9%	37.5%	39.6%

[a] BR=brown rice grain percentage (%), MR=milled rice grain percentage (%), HR=head rice grain percentage (%), PRO=protein content of the whole milled flour (%), See Materials and methods for details of parameter calculations

[b] MH=Minghui 63, paternal line of the cross, ZB=Zhenshan 97, maintainer of the sterile line (i.e. maternal line) of the cross

[c] The same letter indicates that the character is not significantly different at P<0.05 by Duncan's multiple range test

[d] Broad-sense heritability calculated as: $h^2=\delta_g^2/(\delta_g^2+\delta_e^2)\times100\%$

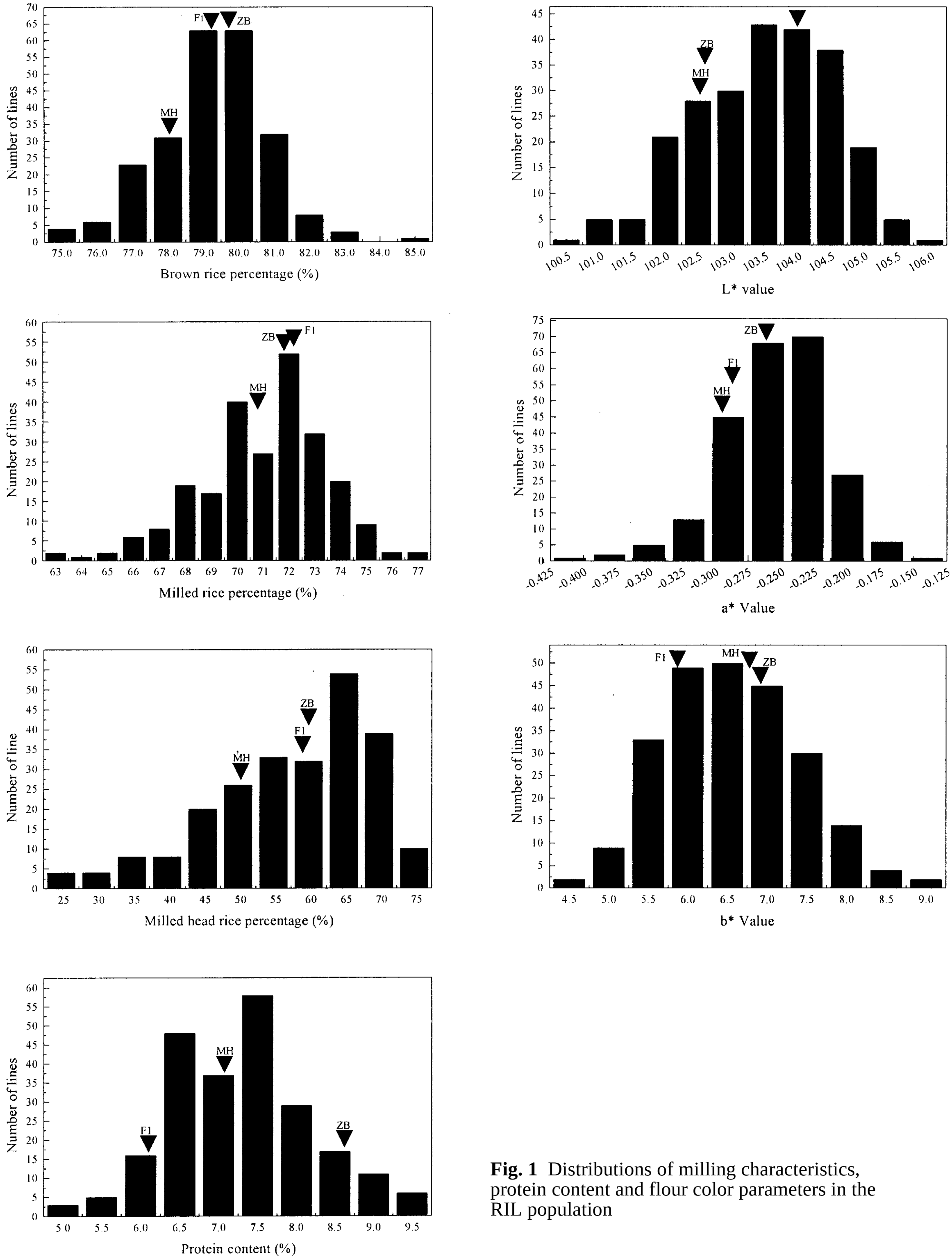

Fig. 1 Distributions of milling characteristics, protein content and flour color parameters in the RIL population

that the differences in all traits between the two parents were small. The bell-shaped phenotypic distributions and the wide range of variation of the investigated parameters indicated transgressive segregations, suggesting the polygenic inheritance of the traits.

Though the differences of the traits between the two parents were generally small, significant differences were still observed for head rice percentage (HR), protein content (PRO), and the b* value of the flour color (B) (Table 1). The values of the hybrid are close to that of ZB, indicating the possibility of a maternal effect and/or dominance in the cross.

The estimates of broad-sense heritability of the traits did not vary much from trait to trait. BR, MR, HR and

Table 2 Correlation coefficients among the parameters from 238 RILs derived from the cross Zhenshan 97×Minghui 63

Item	BR[a]	MR	HR	PRO	L*	a*	b*
BR	1.000						
MR	0.273** [a]	1.000					
HR	–0.061	0.570**	1.000				
PRO	–0.064	0.090	0.123	1.000			
L*	0.312**	–0.326**	–0.352**	–0.396**	1.000		
a*	–0.035	–0.172**	–0.168**	–0.104	0.000	1.000	
b*	–0.286**	0.333**	0.392**	0.462**	–0.928**	–0.207**	1.000

[a] Abbreviations are the same as in Table 1
[b] Significance at $P<0.05$ * or $P<0.01$ **

Table 3 QTL information for rice flour color parameters and protein contents by interval mapping with the RIL population of the cross Zhenshan 97×Minghui 63. All intervals with a QTL peak with LOD >2.0 are listed. The positive additive value indicated a contribution from the allele of Zhenshan 97 (ZB) whereas the negative value is from Minghui 63 (MH)

Trait	Chrom	Interval	Position (cM)[a]	a[b]	LOD	R^2 (%)[c]
Brown rice percentage (%)	5	*RM42-C734b*	8.0	1.00	4.0	10.0
				Total	**4.0**	**10.0**
Milled rice percentage (%)	3	*C1087-RZ403*	6.1	1.10	2.2	4.8
	5	*C246-C1447*	10.9	–1.32	2.8	7.0
				Total	**4.9**	**11.3**
Head rice percentage (%)	3	*C1087-RZ403*	6.2	6.00	5.2	10.1
				Total	**5.2**	**10.1**
Protein (%)	6	*C952-Wx*	1.5	–0.61	6.8	13.0
	7	*R1245-RM234*	1.4	–0.43	3.2	6.0
				Total	**9.2**	**17.7**
L*	5	*R3166-RG360*	7.5	0.46	2.1	4.5
	6	*C952-Wx*	4.6	0.84	8.6	15.7
	8	*L363A-RZ66*	26.0	0.50	2.4	10.2
				Total	**11.4**	**21.9**
a*	4	*G102-G235*	8.7	0.02	3.3	6.9
	7	*C1023-R1440*	5.6	0.03	5.2	10.5
				Total	**8.3**	**16.6**
b*	1	*G359-RG532*	7.9	–0.33	2.5	5.9
	3	*C1087-RZ403*	6.9	0.27	2.3	4.3
	6	*C952-Wx*	1.7	–0.79	13.7	25.4
	8	*RM223-L363 A*	2.8	–0.33	3.0	5.6
				Total	**20.5**	**35.8**

[a] Distance by Haldane function (Haldane 1919) from the left marker of the interval
[b] Additive effect computed as: (ZB–MH)/2
[c] Phenotypic variance explained by the QTL(s)

PRO were estimated to have heritabilities of around 30%, whereas those of the color parameters were around 40%. The heritabilities of the investigated traits were much lower (30%–40%), compared with those of yield (67%) and yield components (62%–87%) (Yu et al. 1998), a result which is consistent with the correlation coefficients of the traits (see below).

Correlation of the traits

The phenotypic correlation coefficients among the investigated parameters (Table 2) were generally small, indicating the complexity of the relationship among the traits. A significantly positive correlation was observed between BR and MR (r=0.273), and between MR and HR (r=0.570). This is easy to understand as the milling degree was limited to bran-removal of 8–10% of the brown rice weight. Both parameters are calculated based on the rough rice weight. More brown rice would yield more milled rice and thus give more head rice. PRO was not significantly correlated with the milling parameters, indicating good control of the milling.

For the flour color parameters, the L* value was positively related to BR (r=0.312), and negatively correlated to MR (r=–0.326), HR (r=–0.352) and PRO (r=–0.396), respectively. The correlation among the milling parameters and PRO is also consistent with the former result, i.e. the milling process can influence PRO (Juliano 1985). The a* value was negatively correlated with MR (r=–0.172) and HR (r=–0.168), respectively. The b* value was negatively correlated to BR (r=–0.286), and positively to MR (r=0.333), HR (r=0.392) and PRO (r=0.462), respectively. These results were consistent with the high correlation between L* and b* values (r=–0.928).

QTL mapping of the traits

Milling characteristics

One QTL was detected for BR in the interval *RM42-C734b* of chromosome 5 (Table 3). The QTL could ex-

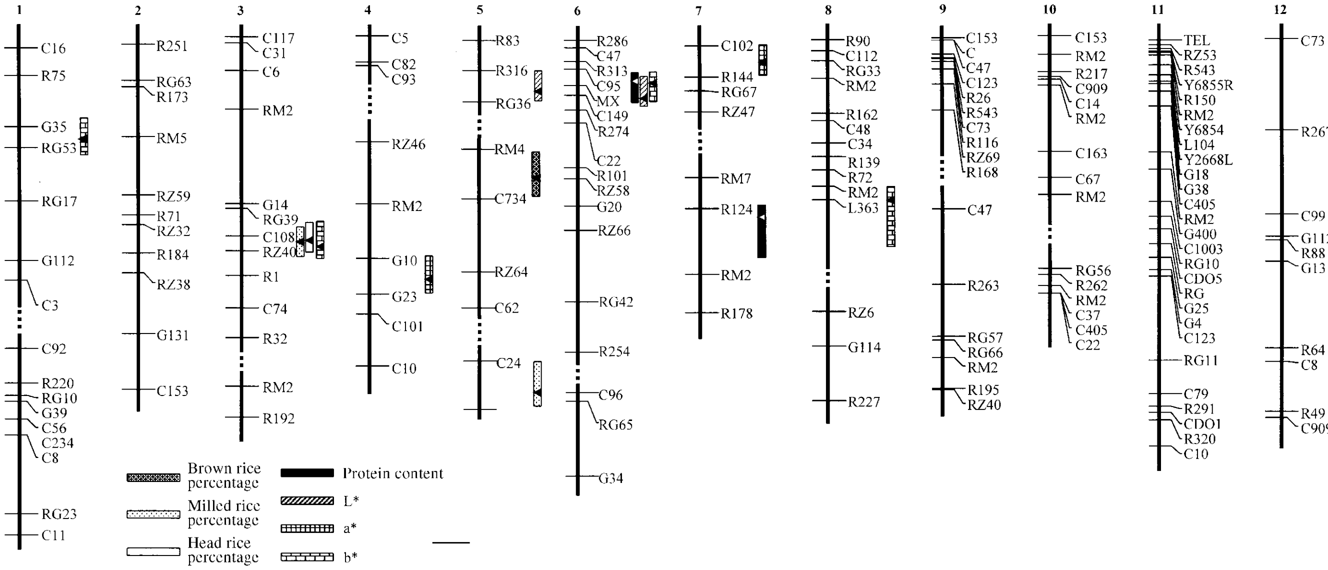

Fig. 2 Locations of the QTLs for milling parameters, protein content and color parameters of rice grain and flour. The *numbers* on the top indicate chromosome order. *Dashed lines* show linkage gaps or regions with a distance >50 cM. The *bars* indicate the 1-LOD support intervals of the QTLs identified. *Small triangles* indicate peaks of the LOD contours

plain 10.0% of the phenotypic variance with LOD=4.0. At this QTL the ZB allele increased the BR by 1.00%. We found that the QTL for grain width also maps in this region (Tan et al. 2000). This is also consistent with the significant positive correlation between BR and grain width (data not shown).

Two QTLs were detected for MR with opposite effects in the intervals *C1087-RZ403* and *C246-C1447* of chromosomes 3 and 5, respectively (Table 3). The QTL on chromosome 3 explained 4.8% of the phenotypic variance with an increasing additive effect of 1.07% from the ZB allele, whereas the one on chromosome 5 explained 7.0% of the phenotypic variance with an increasing additive effect of 1.28% from the MH allele. In total, the two QTLs could explain 11.3% of the variance with LOD=4.9.

One QTL was mapped on *C1087-RZ403* of chromosome 3 to have an effect on HR, where a QTL for grain length was also detected (Tan et al. 2000). The QTL explained 10.1% of the variance, and the ZB allele at this locus increased head rice percentage by 6.0%.

To avoid ‘‘false” QTLs from close linkage, re-scanning of the chromosome was carried out by alternately fixing one of the two QTLs (Lander and Botstein 1989; Lincoln et al. 1992). Also one-way analysis of variance (ANOVA) and composite interval mapping (CIM) (Zeng 1994) were performed to scan the chromosome region. The QTLs were also detected by ANOVA and CIM, thus confirming the presence of the QTLs in this chromosome.

It was interesting to note that a QTL for grain length was mapped in the interval *C1087-RZ403* (Tan et al. 2000), which is again consistent with a negative correlation of the traits (data not shown). Generally, longer grains can be easier to break during abrasive milling than short grains under the same conditions.

Protein content

Two QTLs were detected to have an effect on protein content. One mapped in the interval of *C952-Wx* on chromosome 6, with the larger effect explaining 13.0% of the phenotypic variance and LOD=6.8. In this locus the MH allele increased the protein content by 0.61%. The other, with a smaller effect, mapped on chromosome 7 in the interval *R1245-RM234* (Table 2). In total, the two QTLs explained 17.7% of the phenotypic variance with LOD=9.2.

Color parameters

Three QTLs were detected to have an effect on L*, i.e. the brightness of the whole milled flour, on chromosomes 5, 6 and 8. One mapped on chromosome 6 and explained 15.7% of the phenotypic variance with LOD=8.6. The other two had relatively small effects. In total, the three QTLs explained 21.9% of the phenotypic variance.

Two QTLs were detected for the flour a* value, on chromosomes 4 and 7. The QTL on chromosome 7 had a larger effect, explaining 10.5% of the variance with a LOD score of 5.2, whereas the other QTL accounted for 5.6% of the variance. In both cases alleles from ZB increased the a* value (i.e. decreased the greenness of the flour). In total, they explained 14.8% of the phenotypic variance.

Four QTLs on chromosomes 1, 3, 6 and 8 influenced the b* value of the flour. The QTL in the interval *C952-Wx* of chromosome 6 had the largest effect with LOD=13.7 and explained 25.4% of the phenotypic variance. This is consistent with the negative correlation of

Table 4 List of significant two-way interactions between different loci covering the genome

Trait	Marker 1		Marker 2		*F*-Test[a] *p*	MC-Test[b] 1-(1-*p*)[n]
	Name	Chrom.	Name	Chrom.		
Brown rice percentage (%)	G1128b	1	RM200	3	0.004	0.047
	RZ599	2	C148	10	0.01	0.114
	C624	5*[c]	G359	1	0.000 [d]	0.000
	C1447	5	C732	12	0.0002	0.002
	R1789	7	C87	12	0.004	0.047
	R1629	8	C472	9	0.002	0.024
	RM258	10	R496	12	0.002	0.024
Milled rice percentage (%)	RG173	1	RZ404	9	0.008	0.092
	R321	3	RG360	5	0.002 [d]	0.024
	R3166	5	RG333	8	0.001	0.010
Head rice Percentage (%)	RG173	1	RM42	5	0.002	0.024
	C63	3*	C962	6	0.0001	0.001
	C1087	3*	C1003B	11	0.000 [d]	0.000
	RZ467	4	C1232	9	0.01	0.114
	C1016	4	L1044	11	0.004	0.047
	C1447	5	TEL3	11	0.002	0.024
Protein (%)	C922	1	R19	3	0.003	0.035
	C1016	4	C909B	12	0.006	0.070
	R1629	8	TEL3	11	0.0002 [d]	0.002
	RM228	10	R496	12	0.007	0.081
L*	G1128b	1	RM53	2	0.002	0.024
	RG173	1	C734b	5	0.004	0.047
	R2510	2	RG653	6	0.001	0.012
	R1014	6*	RG653	6	0.000 [d]	0.000
	R2749	6*	C483	8	0.000	0.000
	RZ471	7	R2174	6	0.0004	0.005
	RM70	7	RM239	10	0.0001	0.001
	RG561	10	C1237	11	0.0001	0.001
a*	G393	1	RZ599	2	0.0001 [d]	0.001
	RZ599	2	RG393	3	0.0001	0.001
	C746	3	C56	4	0.003	0.035
	RZ599	3	RZ667	6	0.000	0.000
	R712	3	L1044	11	0.001	0.012
	C746	3	R887	12	0.002	0.024
	RG360	5	R265	9	0.0004	0.005
	RG360	5	C1003B	11	0.0006	0.007
	RM234	7	R543a	11	0.004	0.047
b*	RG101	1	C112	1	0.001	0.012
	G1128b	1	RM53	2	0.004	0.047
	R2510	2	RG653	6	0.001	0.012
	C952	6*	C153B	2	0.000	0.000
	R2869	6*	C483	8	0.000 [d]	0.000
	C952	6*	C2	9	0.000	0.000
	C474	6*	Y6854L	11	0.000	0.000
	RM70	7	RM222	10	0.0002	0.002

[a] *F* test for the four sub-groups of the two marker alleles
[b] Monte Carlo simulation using EPISTAT program (Lark et al. 1995)
[c] * Significant effect (QTL) was also detected in the region
[d] The mean value comparison within the four groups of the combinations were listed in Table 5

L* and b* (Table 2) and the mapping result of L* on chromosome 6 (Table 3 and Fig. 2). The MH allele at this locus increased b* by 0.79. The other three QTLs had smaller effects on this trait. The four QTLs explained 35.8% of the phenotypic variance of b* with a LOD score of 15.8.

QTL interaction for the traits

Because of the transgression of the traits and the significantly lower-PRO and higher-B value of the hybrid as compared with the parents (Table 1 and Fig. 1), the QTLs detected were of small magnitude and accounted for a small proportion of the variation (Table 3). Therefore, we carried out a two-way analysis to detect epistatic interactions of all two-marker combinations across the whole genome. In total 21945 combinations were assayed and 136 combinations showed a significant interaction, covering all the chromosomes (data not shown). Two kinds of interaction were detected: one was between loci that did not have significant effects on the traits (non-effect locus), and the other was between QTLs and

Table 5 Comparison of selected two-locus combinations from Table 4 within the RIL population and the hybrid indicating significant epistasis between two markers

Marker combination[a] Marker 1	Marker 2	BR[b] (C624/G359)		MR (R321/RG360)		HR (C1087/C1003B)		PRO (R1629/TEL3)		L* (R1014/RG653)		a* (G393/RZ599)		b* (R2869/C483)	
AA[c]	AA	78.8	c B[d]	70.8	b A	55.0	b A	7.3	a A	102.9	b B	–0.23	a A	6.97	a A
AA	BB	79.8	b A	72.1	a A	48.3	c B	6.7	b B	103.3	b B	–0.26	b B	6.35	b B
BB	AA	80.4	a A	72.3	a A	57.5	ab A	6.8	b B	103.9	a A	–0.25	b B	5.97	c B
BB	BB	79.8	b A	71.2	b A	61.6	a A	7.2	a AB	103.2	b B	–0.24	a A	6.04	c B

[a] Marker order the same as in Table 4
[b] Abbreviations the same as in Table 1
[c] AA represents alleles from Minghui 63, and BB from Zhenshan 97
[d] The same letter indicates that the character is not significantly different at $P_{0.05}$ (lower case) or $P_{0.01}$ (upper case) by Duncan's multiple range test

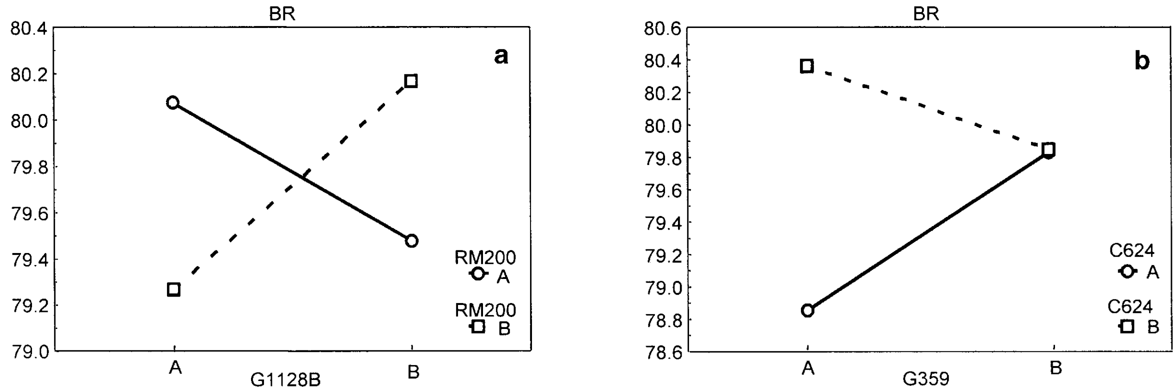

Fig. 3 Schematic representation of two-way interactions for BR indicating epistasis between loci

the non-effect locus (Table 4). No interaction was detected between QTLs. The significant combinations with the highest likelihood [if the markers are linked (<50 cM)] are listed in Table 4, and mean-value comparisons of the four groups are listed in Table 5.

Taking BR as an example, when *C624* (for simplicity using the marker name to denote the linked QTL) had the allele from Zhenshan 97 (BB) and *G359* had the allele from Minghui 63 (AA), the combination (BBAA) had a significantly higher value than the other three groups (P<0.05). The difference between the BBAA and BBBB genotypes should be due to the epistasis of the QTL linked to the two loci. Another example is the combination *R321*/*RG360* for MR: when both alleles were from Minghui 63 (AAAA) or Zhenshan 97 (BBBB) MR had significantly lower values (P<0.05) and vice versa. Similar situations were observed in other traits (Table 5).

Figure 3 shows schematically the interactions between loci. Although neither of the loci (*G1128B*/*RM200*) had a significant effect on BR, the four subgroups had different values showing the dependence of the two alleles (Fig. 3a). When the *G359* alleles were from Minghui 63 (AA), *C624* had significantly different BR between the two alleles (Fig. 3b), i.e. the phase of *G359* could significantly influence the effect of *C624*.

Discussion

Generally, the QTLs all had a low magnitude which is consistent with the low heritability (30%–40%) of the traits. Another reason may be the limited difference between the two parents, with most of the traits not being significantly different (Table 1). However, we still detected QTLs which could not be found using traditional methods, by using interval mapping, and confirmed their occurrence with the composite interval-mapping algorithm.

It is not surprising that the QTLs for the milling parameters are located in the regions for grain shape (Table 2 and Tan et al. 2000). The most important milling parameter is head rice percentage which shares the QTL for grain length (Tan et al. 2000). This result is easily understandable, i.e. the longer rice tends to break more easily during milling, and implies that medium-long and slender rice is preferable in breeding practice. Alternatively, the control of the milling method is also very important to obtain a higher yield of head rice for the long-grain type (van Ruiten 1985).

We detected a QTL in the *Wx* gene region, responsible for the protein content, that had a large effect. This result is consistent with the previous reports that starch synthetase, which is correlated with amylose content and is embedded in the starch granule, is one of the milled rice proteins (Sano 1984; Villareal and Juliano 1986, 1989). Although it is generally considered that protein content is influenced largely by environmental conditions and the level of nitrogen fertilization (Nanda and

Coffman 1979; Perez et al. 1996), our results strengthen the recognition of a genetic component for protein content.

It is worth emphasizing that as rice is unique among cereals by having a storage protein primarily made of glutelin [which has a more balanced amino-acid profile than the prolamin-rich storage proteins (Juliano 1985)], increasing the protein content will increase and balance the protein intake people whose staple food is rice. The recent report of “golden rice” promotes the potential of genetic engineering to enhance the nutritional quality of rice (Ye et al. 2000), and hence improve the nutritional state of people in poor areas where rice is the staple food. The availability of the *Wx* gene sequence provides the possibility of improving the protein content via *Wx* gene modification (Wang et al. 1995).

Also, one of the major QTLs for color parameters happens to be located in the *Wx* gene region, which is also the major one responsible for protein content. The two QTLs on chromosome 5 (*R3136-RG360*) and chromosome 3 (*C1087-RZ403*) are also responsible for grain width and length (Table 3; Tan et al. 2000). This result is consistent with the fact that milling removes the outer parts of the brown rice grain, i.e. the bran, which contains more of the pericarp, seed coat, aleurone layer, and the embryo and, hence, has a higher protein content. These components are all darker than the starchy endosperm (Juliano 1985). The wider and longer the grain, the more bran will be removed, and therefore the color of the grain is lighter. Overall a compromise for long grain, a better color of the grain/flour and high head rice yield, seems to have been reached. Genetic manipulation can be focused on the corresponding regions of chromosomes 3, 5 and the *Wx* gene when marker-assisted selection is carried out.

The results show strongly the importance of epistasis between different loci in accounting for transgressive segregation of the traits, consistent with previous reports (Lark et al. 1995; Li et al. 1997; Yu et al. 1997). First, the numbers of loci involved in the interactions are much higher than those of the QTLs detected. For example, only one QTL was detected on chromosome 5 for BR. However, seven pairs of loci covering ten chromosomes were detected as having significant effects on this trait by two-way interaction analysis (Table 4). If the higher levels of interactions are taken into consideration, the involved loci should be much higher. Second, although the variance explained by the QTLs was relatively small (most of which are <10%), that explained by the interaction should be very large. Effective methods need to be developed to extract all of the variance. Finally, the epistasis has an important impact on breeding practice. Because of the interaction between different loci, QTL-linked C624 would have significant effects on BR only when the allele of G359 is from Minghui 63 (Fig. 3). This means that the offspring phenotype will be largely influenced by the genetic background of the receptor line when marker-directed selection is carried out.

With the use of DNA markers, improvement for these traits can be quickly achieved by focusing on the target QTLs, without sacrificing important agronomic traits. Meanwhile, the interaction between different loci should be carefully considered. These results should facilitate the improvement of hybrid rice quality in future breeding programs.

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