Mapping QTLs for Milling Quality Traits in Japonica Rice

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Abstract: In this study, an F5-generation rice recombinant inbred population (referred to as RILs population) containing of 224 lines derived from cross between Z601 and C14 was selected to analyze the correlation among milling quality traits and associated quantitative trait loci (QTLs). Correlation analysis showed that the brown rice rate, milled rice rate and head milled rice rate all presented positive correlation significantly. Rate of small broken rice was negatively correlated with brown rice rate, milled rice rate and head milled rice rate significantly. A total of 13 QTLs controlling milling quality traits in rice were detected, including two QTLs controlling brown rice rate, two QTLs controlling milled rice rate, three QTLs controlling head milled rice rate and six QTLs controlling rate of small broken rice, which were located on chromosome 2, 6, 7, 8 and 12, respectively. This study would provide a strategy for detection of milling quality in low-generation materials of rice breeding.

1. Introduction

Rice is a staple food for the majority of people in China and half of the world's population. The rice quality is usually evaluated based on the milling quality, appearance quality, eating quality and nutritional quality in all over the world (Birla et al. 2015)[1]. To be specific, milling quality shows a direct influence of rice price, consequent result of reduced income for farmers. Based on these, relevant provisions have been developed in China for paddy and rice quality standards. Various indicators are strictly described and limited by national standards for paddy (GB1350-2009) and rice (GB1354-2009) in China.

Brown rice rate refers to the mass proportion of brown rice (unpolished rice) accounting for the paddy rice samples, and the imperfect grains are calculated by half. The collected brown rice is processed into milled rice, and the milled rice rate refers to the percentage of processed milled rice accounting for the paddy rice samples. Brown rice is processed into milled rice at national standard level 3, head milled rice refers to rice grains with the length of about three-quarter of the average length of complete paddy rice samples, and head milled rice rate refers to the mass proportion of head milled rice accounting for the paddy rice samples. Small broken rice refers to the incomplete rice grains remaining on the round-hole sieve with a diameter of 2.0 mm after sieving through a round-hole sieve 2.0 mm in diameter, and rate of small broken rice refers to the mass proportion of small broken rice accounting for the paddy rice samples.

High-density genome-wide molecular marker linkage maps can be constructed by analysis of appropriate segregating hybrid populations using molecular marker technology, thereby mapping genes controlling agronomic traits and accelerating the breeding process. Specifically, simple sequence repeat (SSR), sequence tagged sites (STS), single nucleotide polymorphism (SNP) and sequence-characterized amplified region (SCAR) are commonly used, which have been widely applied in genetic analysis and gene mapping of rice (Cha et al. 2008; Sardesai et al. 2002; Singh et al. 2006; Yu et al. 2014) [2-4].

A RIL populations of Lemont/Teqing was constructed, and was found no marker intervals with significant effects on the brown rice rate (Mei et al. 2002). In detail, one QTL (QMr12) controlling

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milled rice rate was mapped on chromosome 12. Four QTLs (QHr2, QHr4, QHr6, Qhr7) controlling head milled rice rate were mapped on chromosomes 2, 4, 6 and 7 respectively. By using recombinant inbred line populations (Zhenshan 97B / Minghui 63), a QTL controlling brown rice rate, two QTLs controlling milled rice rate and a QTL head milled rice rate were mapped, respectively (Tan et al. 2001). By using BC2F2 populations, three QTLs controlling brown rice rate, a QTL controlling milled rice rate and three QTLs controlling head milled rice rate, respectively (Septiningsih et al. 2003). Thus, QTLs controlling these traits are located on different chromosome sowing to most quality traits of paddy rice are susceptible to environmental impact, presenting genotype-environment interaction effects (Huang et al. 2015; Kim et al. 2014; Takai et al. 2005; Zhang et al. 2014). Yet the analysis of milling quality dependent on milling accuracy, but the standards for individual experiment are different.

In this study, high-generation (F5) Z601/C14 recombinant inbred lines (referred to as RILs populations) were analyzed the correlation among various milling quality traits. Furthermore, SSR and InDel marker linkage map was constructed for mapping QTLs for milling quality traits in rice, which provided theoretical basis for the breeding of rice varieties with high milling quality, thus accelerating the variety improvement process and increasing the economic benefits.

2. Materials and Methods

2.1 Materials

In this study, 224 high-generation (F5) recombinant inbred lines (referred to as RILs populations) were used as experimental materials. To be specific, the female parent was Z601, a breeding material of Liaojing 294; the male parent was C14, a progeny material of Jinyuan 45/ Fan 6. The milling quality traits were shown in Table 1.

Table 1 Various milling quality traits in parents

Parent	Brown rice rate (%)	Milled rice rate (%)	Head milled rice rate (%)	Rate of small broken rice (%)
Z601	81.88	74.24	63.39	0.61
C14	78.29	67.75	37.83	10.05

2.2 Population Construction

The construction of genetic populations were performed in Sanya South Breeding Base of Hainan Province and Tianjin Experimental Base in China. In detail, Z601/C14 hybridization was conducted in Sanya in March 2009. F1-generation were collected for inbreeding in accordance with single seed descent method in Tianjin and Sanya to obtain stable F5-generation. A total of 224 F5 recombinant inbred lines were obtained for genetic analysis and phenotyping.

2.3 Traits Analysis

In May 2011, 224 RILs and parents were planted in the experimental base of China National Japonica Rice Research and Development Center in Tianjin. Each line of rice materials was planted in a row, by 30 individuals per row, performing with normal field management. At maturity period, five individuals of each line were randomly selected and dried for determination of brown rice rate, milled rice rate, head milled rice rate and rate of small broken rice successively.

The collected rice samples were processed using JGJ45 brown rice machine (Hangzhou Qianjiang Apparatus & Equipment CO., Ltd.,China) to calculate the brown rice rate. The brown rice samples were processed using Pearlest milled rice machine (Japanese KETT Scientific Research Institute, Japan) to calculate the milled rice rate. Subsequently, head milled rice and small broken rice were distinguished using SC-E seed rice appearance quality detection & analysis system (Hangzhou Wansen Detection Science and Technology Co., Ltd.China) to calculate the head milled rice rate and rate of small broken rice. Each indicator was determined triplicate and averaged.

2.4 Molecular Linkage Map Construction

In the summer of 2011, rice genomic DNA was extracted from leaves of individual plant of parents and various RIL lines in accordance with the method previously reported (Sagi et al. 2009). The total PCR reaction volume was 20 μl, containing 3 μl of genomic DNA template (10 ng/μl), 2 μl of dNTP mix (2.5 μmol/L), 2 μl of 10 × PCR Buffer (25 mmol/L Mg2+), 0.2 μl of Taq DNA polymerase (5 U/μl), 2 μl of forward and reverse primers (2 μmol/L) and 10.8 μl of ddH2O. The PCR amplification was started with initial denaturation at 94 ℃ for 2 min, followed by 40 cycles of denaturation at 94 ℃ for 30 s, annealing at 58 ℃ for 30 s, and extension at 72℃ for 30 s; the amplification was completed by holding the reaction mixture at 72℃ for 10 min to allow complete extension of PCR products. PCR products were detected by using electrophoresis on 4% agarose gel and the amplification results were observed under a UV gel imaging system.

PCR amplification of parent genomic DNA was preformed to analyze the parental polymorphism by using 491 SSR markers and 157 InDel markers which were distributed on all the 12 chromosomes of rice uniformly. In addition, 73 SSR markers and 7 InDel markers with polymorphisms between parents were used for population genotyping. QTLs were named in accordance with the method proposed by McCouch (McCouch et al. 1997; McCouch and Cooperative 2008). Genetic linkage map was constructed using QTL IciMapping V3.0 software (Lander et al. 1987). Various markers were divided into different linkage groups with LOD (log of odds) 3.0 as the threshold. The orders of markers in each linkage group were determined, and the additive effects of each QTL were calculated. The exchange rate between different markers was converted into the genetic distance (cM) using Kosambi function.

Broad heritability H (%) of various traits = 100 × VG / VP

Where, VG and VP indicate genotypic variance and phenotypic variance, respectively.

2.5 Data Analysis

According to the sequences of experimental primers, positions of primers were investigated using Markers and BLAST programs on http://www.gramene.org/. Mapping results of various traits were downloaded using QTL program on http://www.gramene.org/ and compared with the experimental results in this study (1 cM = 250 kb). Subsequently, the correlation among various milling quality traits was analyzed using IBM.SPSS.Statistics.v19.0.0 software.

3. Results

3.1 Milling Quality Traits in RIL Populations

The brown rice rate, milled rice rate, head milled rice rate and rate of small broken rice all presented significant continuous distribution with wide distribution range, which exhibited the characteristics of quantitative traits and were in line with the requirements of QTL interval mapping (Fig.1). The brown rice rate greatly varied between parents Z601 and C14, which reached 81.88% and 78.29% respectively, presenting unimodal distribution in populations, and the peak ranged between the two parents. The average brown rice rate was 79.98%, and the maximum and minimum were 82.48% and 76.83%, respectively. No significant bidirectional transgressive phenomenon was observed. The milled rice rate of two parents was 74.24% and 67.75% respectively, which also presented unimodal distribution in populations, and the peak ranged between the two parents. The average milled rice rate was 71.09%, and the maximum and minimum were 73.78% and 67.37%, respectively. No significant bidirectional transgressive phenomenon was observed. The head milled rice rate of two parents was 63.39% and 37.83%, respectively. Most lines in the RIL populations had similar head milled rice rate to high-value parents and the phenotypic average was 63.66%, which was higher than high-value parents. The population distribution was severely biased towards high head milled rice rate. The bidirectional transgressive phenomenon of low-value parents was less significant than that of high-value parents, and the maximum and minimum were 72.98% and 33.46%, respectively. The rate of small broken rice of two parents was 0.61% and 10.05%, respectively. Most lines in populations had similar rate of small broken rice to low-value parents

and the phenotypic average was 0.68%, which was higher than low-value parents. The population distribution was severely biased towards low head milled rice rate, and no lines were found with higher rate of small broken rice than high-value parents, the maximum and minimum were 4.00% and 0.02%, respectively.

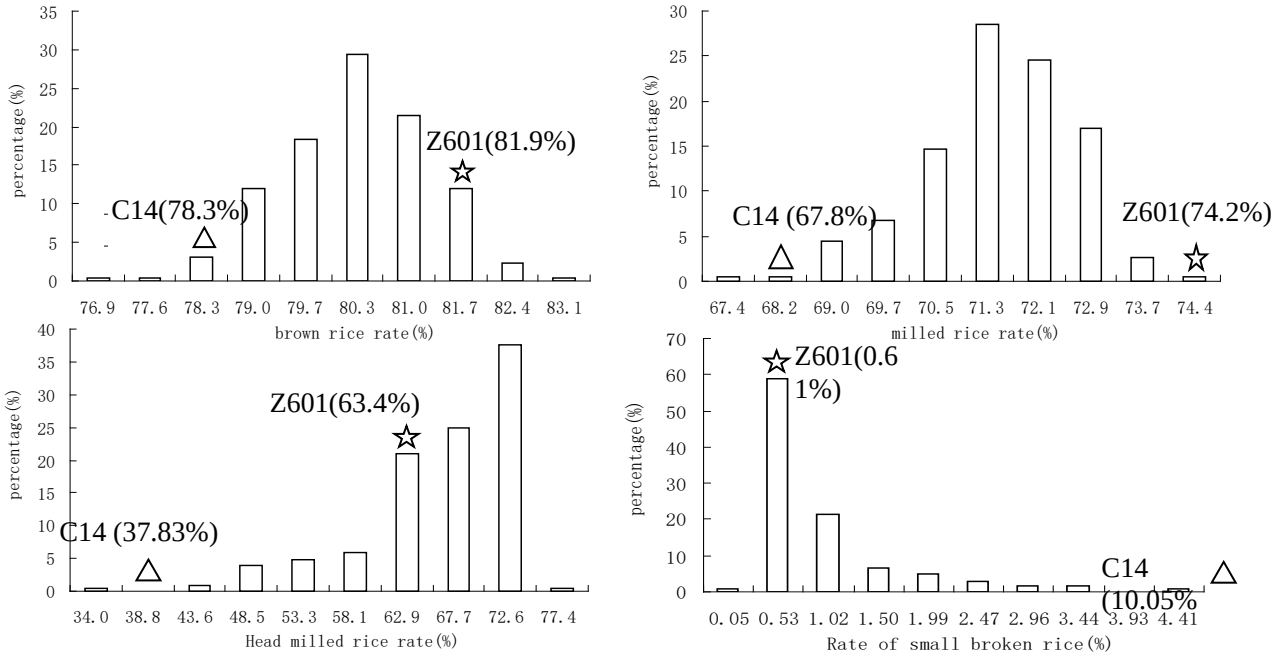

Fig.1 Milling quality traits in two parents and RIL populations

"Triangle" represent the agronomic traits for C14; "Asterisk" represent the agronomic traits for Z604.

Based on the above analysis, the brown rice rate and milled rice rate in progeny of RILs population both ranged between two parents, suggesting that the probability of selecting superior lines with high brown rice rate and milled rice rate by hybridization separation is low, leading to great difficulties in the breeding of superior varieties. However, many hybrid progeny lines have higher head milled rice rate and rate of small broken rice than parents, and superior varieties with high head milled rice rate and rate of small broken rice can be bred by hybridization, separation and selection.

3.2 Correlation Analysis of Milling Quality Traits in RIL Populations

According to the statistical results, all the brown rice rate, milled rice rate and head milled rice rate exhibited positive correlation significantly. The rate of small broken rice and brown rice rate, milled rice rate and head milled rice rate all exhibited negative correlation significantly. Milled rice / brown rice ratio was extremely significantly positively correlated with milled rice rate and head milled rice rate, insignificantly negatively correlated with brown rice rate, and extremely significantly negatively correlated with rate of small broken rice (Table 2).

Table 2 Correlation analysis of milling quality traits in RIL populations

	Brown rice rate (%)	Milled rice rate (%)	Head milled rice rate (%)	Rate of small broken rice
Milled rice rate	0.730**			
Head milled rice rate	0.470**	0.501**		
Small broken rice rate	-0.412**	-0.493**	-0.826**	
Milled rice/brown rice ratio	-0.044	0.651**	0.210**	-0.263**

Note:* and ** indicates significant differences at the 0.05 level and 0.01 level, respectively.

3.3 Analysis of QTLs Controlling Milling Quality Traits in Rice

A total of 13 QTLs controlling milling quality traits in rice were detected (Table3, Fig.2), including two QTLs controlling brown rice rate, two QTLs controlling milled rice rate, three QTLs controlling head milled rice rate and six QTLs controlling rate of small broken rice, which were distributed on chromosomes 2, 6, 7, 8 and 12, respectively.

Table 3 Analysis results of QTLs controlling milling quality traits in rice

Trait	QTL	Chromosome	Interval	LOD value	Contribution rate (%)	EstA
BRR	qBRR-6-1	6	RM5814-RM3827	3.23	5.90	-0.24
	qBRR-8-1	8	Ap004623-46-RM264	4.46	8.01	-0.32
MRR	qMRR-6-1	6	RM20724-ap004329-19011	3.49	7.14	-0.31
	qMRR-7-1	7	RM5426-RM22109	3.78	18.46	0.94
HRR	qHRR-2-1	2	RM3858-RM4702	10.86	55.64	-7.89
	qHRR-7-1	7	RM336-AP003742-48	12.60	54.92	-8.02
	qHRR-12-1	12	RM7102-BX000510-91	4.26	27.80	-9.95
SBRR	qSBRR-2-1	2	RM3858-RM4702	24.40	63.12	0.99
	qSBRR-6-1	6	RM5754-RM1369	10.41	53.50	1.13
	qSBRR-7-1	7	RM336-AP003742-48	25.70	62.94	1.00
	qSBRR-7-2	7	RM5426-RM22109	12.05	65.12	-1.03
	qSBRR-8-1	8	RM23220-RM547	8.78	44.16	-1.25
	qSBRR-12-1	12	RM7102-BX000510-91	13.81	53.92	1.14

Note: BRR:brown rice rate;MRR:milled rice rate;HRR:head milled rice rate;SBRR:small broken rice rate

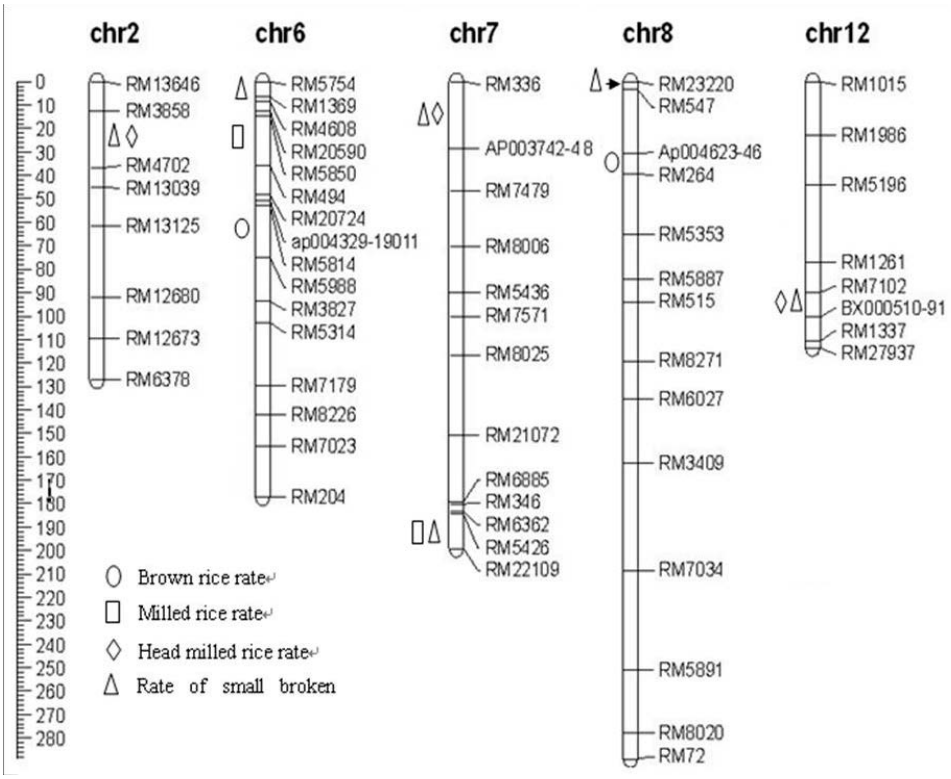

Fig. 2 Distribution of QTLs controlling milling quality traits in rice genome

QTLs controlling brown rice rate and milled rice rate showed relatively low contribution rate, while only the contribution rate of RM5426-RM22109 was higher than 10% and reached to 18.46%, and this locus produced great effects on the rate of small broken rice. There were many QTLs that controlling head milled rice rate and rate of small broken rice, with contribution rate higher than 50%

mostly. Particularly, RM3858-RM4702 and RM336-AP003742-48 had simultaneously great effects on head milled rice rate and rate of small broken rice. Therefore, the two loci should be highly used to improve the directivity of breeding.

4. Discussions

Correlation analysis shows that brown rice rate, milled rice rate, head milled rice rate and rate of small broken rice all exhibit positive or negative correlation significantly, which might be related with the hardness of the rice grains, and rice materials can be screened based on 1-2 traits in the breeding process. The trait milled rice / brown rice ratio can be used to reflect the thickness of aleurone layer: higher milled rice / brown rice ratio indicates thinner aleurone layer. Milled rice / brown rice ratio is positively correlated with milled rice rate and head milled rice rate significantly, suggesting that the milled rice rate and head milled rice rate increase with the reducing thickness of aleurone layer. Milled rice / brown rice ratio is negatively correlated with rate of small broken rice significantly, suggesting that the amount of small broken rice increases with the reducing thickness of aleurone layer. Milled rice / brown rice ratio is negatively correlated with brown rice rate, suggesting that the brown rice rate and thickness of rice hull decrease with the reducing thickness of aleurone layer. Therefore, in the breeding process, the thickness of aleurone layer and rice hull should be coordinated appropriately to achieve high milled rice rate.

Brown rice rate is an important indicator reflecting the glume thickness of rice grains. Generally, higher brown rice rate indicates thinner glume of rice grains. In this study, two QTLs controlling brown rice rate were detected on chromosomes 6 and 8, with the contribution rate lower than 10%. Eight QTLs controlling brown rice rate on chromosomes 6 and 8 were mapped, but the loci are not coincided (Mei et al. 2012). Some other locuses were also mapped on chromosomes 2,3,4,5,6,8,11 and 12 under different environment using different materials (Jiang et al. 2005; Weng et al. 2007; Tan et al. 2001). However, no loci coincided with QTLs detected in this study have been found. Therefore, the loci detected in this study may be new QTLs controlling brown rice rate.

Milled rice rate is an important indicator for determining rice quality. Higher milled rice rate indicates better commodity. In this study, two QTLs controlling milled rice rate were detected on chromosomes 6 and 7, respectively. Interestingly, the contribution rate of RM5426-RM22109 on chromosome 7 reaches 18.46%, and the contribution rate of the other locus is lower than 10%. In addition to our results, others milled rice rate locus were detected on chromosome1, 3,4,5,6,7 and 10, respectively (Tan et al. 2001; Weng et al. 2007; Jiang et al. 2005; Mei et al. 2012).However, no loci coincided with QTLs detected in this study have been detected. In particular, RM528–RM340 on chromosome 6 is basically coincided with RM20724-ap004329-19011 on chromosome 6 that was detected in this study, indicating that this locus exists in many materials. Therefore, RM5426-RM22109 on chromosome 7 that was scaned may be a new QTL in our study.

Head milled rice rate is the best indicator reflecting the commodity of rice varieties, which is directly related to the economic benefits of rice farmers. Three QTLs controlling head milled rice rate were detected on chromosomes 2, 7 and 12, with relatively high contribution rate. Specifically, the contribution rate of RM3858-RM4702 on chromosome 2 and RM336-AP003742-48 on chromosome 7 is higher than 50%. Up to seventeen QTLs were detected on chromosomes 1,2,3,5,6,7,8,9,10 and 12 respectively (Tan et al. 2001; Weng et al. 2007; Jiang et al. 2005; Mei et al. 2012). The density of the QTLs on each chromosomes is different. However, no loci coincided with QTLs detected in this study have been detected. In our research, G1340-XNbp132 on chromosome 2 is slightly coincided with RM3858-RM4702 on chromosome 2 that was detected.

Rate of small broken rice is an unspecified indicator in the national standards, which has not been studied previously. However, small broken rice gradually reflects the commercial value in the current rice production. In the present study, six QTLs controlling rate of small broken rice were detected on chromosomes 2, 6, 7, 8 and 12, and the contribution rate of most QTLs is higher than 50%. So it is meaningful to pay some attention in the rate of small broken rice research to improve the quality of rice

Many of these detected QTLs simultaneously control different traits. RM5426-RM22109 on

chromosome 7 not only controls the milled rice rate but also has high contribution rate to rate of small broken rice. The contribution rate of RM3858-RM4702 on chromosome 2 and RM336-AP003742-48 on chromosome 7 to head milled rice rate and rate of small broken rice is higher than 50%. The contribution rate of RM7102-BX000510-91 on chromosome 12 to head milled rice rate is lower than 50%, but its contribution rate to rate of small broken rice is higher than 50%. These would facilite the detection of milling quality in low-generation materials.

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