Mapping Quantitative Trait Loci for Palatability of Milled Rice

TANG Shao-qing¹, HU Pei-song¹, JIAO Gui-ai¹, ZHAI Hu-qu², WAN Jian-min², ³ (¹ State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China; ² Chinese Academy of Agricultural Sciences, Beijing 100081, China; ³ State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210015, China)

Abstract: Quantitative trait loci (QTLs) controlling palatability in rice were identified using a set of 98 backcross inbred lines (BILs) population derived from a cross between a japonica variety Nipponbare and an indica variety Kasalath. The palatability scores of the population measured by RQ1/Plus Rice Analyzer, showed a continuous and transgressive segregative distribution with a range from 66 to 92. Four putative QTLs for palatability, qPAL-5, qPAL-7, qPAL-8a and qPAL-8b, were detected on chromosome 5, 7 and 8, and they accounted 7.83, 7.03, 11.58 and 7.19% of the total phenotypic variation, respectively. Three alleles qPAL-5, qPAL-7 and qPAL-8b from Kasalath increased the palatability score, whereas only one Nipponbare allele qPAL-8a increased the score. Eight transgressive lines in palatability were selected to make a comparison between phenotypic and genotypic classes. The result explained the possibility of positive QTLs pyramiding through marker-assisted selection of highly palatable rice.

Key words: rice (Oryza sativa); palatability; quantitative trait loci; backcross inbred lines

Poor rice quality has been currently considered as a major problem in China and many other rice producing regions. Much of this problem arises from the poor cooking and eating quality of many widely grown varieties. Rice cooking qualities are important factors in determining market demand because consumers prefer to good palatable rice increasingly.

The cooking and eating qualities are correlated with three physicochemical properties of the rice endosperm amylose content, gel consistency and gelatinization temperature [1]. The relationships among these physicochemical properties, which determined hardness and adhesiveness of cooked rice, and eating quality have been well documented [2-7]. The development of rice linkage maps with more DNA markers has facilitated the genetic improvement of complex polygenic traits such as grain quality [8,9]. These traits can be dissected into single Mendelian quantitative trait loci (QTLs) [10, 11]. Some QTLs for cooking and eating qualities have been detected [1,12-18]. Therefore, QTL analysis can provide a novel approach to understand the genetic system and mechanism of palatability.

However, the determination of taste quality or palatability is quite complex matter, since it largely relies on sensory basis of a trained panel [19,20] and this testing procedure may lack reproducible palatability score. So far, few genetic dissections have been available on taste quality of cooked rice. Wu et al [21] mapped 3 QTLs controlling palatability, one each on chromosome 2, 4 and 11, using Asominori/IR24 RILs by sensory test method. In this study the palatability score of milled rice was assessed by using an automated RQ1/Plus Rice Analyzer. The objective of this research was to identify QTLs for palatability and to facilitate MAS of this trait by using 98 backcross inbred lines (BILs) derived from a cross between indica and japonica varieties Nipponbare/Kasalath/Nipponbare. The BILs approach has been employed in the identification of individual QTLs conferring several traits such as heading date, seed dormancy, seed longevity, grain quality and resistance to brown planthopper in rice [22-25].

MATERIALS AND METHODS

Plant materials

Ninety-eight BC1F9 lines derived from Nipponbare/Kasalath/Nipponbare [22] were used in this experiment. Of the two parental lines, Nipponbare is a japonica variety, and Kasalath is an indica one. The BILs population was kindly provided by Dr.

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Corresponding author: WAN Jian-min (wanjm@mail.njau.edu.cn)
Field experiment

The 98 BILs and their parents, Nipponbare and Kasalath, were grown in a randomized complete design with two replications at the Experimental Field of China National Rice Research Institute, Hangzhou, China (about latitude 30°N) in 2001. The germinated seeds were sown in a seedling bed on June 10 and seedlings were transplanted to a paddy field on July 2, with a single plant per hill spaced at 16.7 cm × 20.0 cm. Each plot included four rows with twelve plants per line. Plants were grown under normal field management. The heading date of each line was recorded as the day when the first panicle of 50% plants was emerged out.

Evaluation of palatability

Bulked seeds from each BIL were harvested at 40 days after heading. The harvested rice seeds were stored at room temperature for 3 months before testing analysis. The paddy rice was de-hulled and milled using Satake machines according to Standard of Ministry of Agriculture of China (NY 147–88). One hundred and eighty grams of milled rice for each line was sampled for palatability measurement. The palatability score of each line was determined using the instrument RQ1/Plus Rice Analyzer made in Japan. The results obtained by this instrument were then validated by the taste panels. The described procedure had been widely used for palatability test of japonica rice in Japan.

QTL mapping

QTL mapping of BILs population was based on the constructed linkage map of 245 RFLP markers distributed among 12 chromosomes, which covered 1179.9 cM with an average distance of 4.8 cM between two markers (http://rgp.dna.affrc.go.jp/publicdata/genotypedata_BILs/genotypedata.html). QTLs affecting palatability were mapped with MAPMAKER/QTL version 1.1 [26, 27]. The possibility (the logarithm 10 of the odds ratio, LOD) of QTL existence was surveyed at regular intervals of 2 cM along chromosomes. A LOD score of 2.0 was considered as a threshold to declare the presence of QTL.

RESULTS

Variations in palatability of BILs

The palatability scores of two parents, Nipponbare and Kasalath, were 75 and 79, respectively. Transgressive segregation occurred on palatability scores of the 98 BILs, which displayed a continuous variation ranging from 66 to 92 (Fig. 1). The palatability scores also showed approximately a normal distribution, indicating polygenic segregation, and thus was reliable for QTL analysis.

QTLs for palatability

Four putative QTLs associated with palatability were detected based on MAPMAKER/QTL analysis using an empirical threshold of LOD = 2 (Fig. 2, Table 1). These putative QTLs were located in the interval R2558-G1458 on chromosome 5 (qPAL-5), C596-C213 on chromosome 7 (qPAL-7), C502-C10122 and R1813-C1121 on chromosome 8 (qPAL-8a, qPAL-8b), respectively. Three alleles qPAL-5, qPAL-7 and qPAL-8b, delivered from variety Kasalath, expressed positive effects on palatability counting for 1.949, 1.947 and 2.077, respectively, whereas the allele qPAL-8a, delivered from Kasalath, showed negative effect on palatability with an additive
The percentages of phenotypic variation explained by each QTL ranged from 7.03 to 11.58% based on MAPMAKER/QTL analysis. The total phenotypic variation governed by the four QTLs was 33.63%.

Genotype analysis of 8 transgressive BILs

Eight transgressive BILs in palatability were selected for comparative analysis. Table 2 showed that the palatability of BIL20, BIL47, BIL49 and BIL61 was poorer than that of parental variety Nipponbare, and these 4 BILs all had 3 alleles from Nipponbare with negative effect on palatability as indicated by genotypic analysis. In contrast, the palatability of BIL13, BIL67, BIL68 and BIL73 was even better than that of high value parent Kasalath, and genotypic analysis showed that these BILs held 2 or 3 positive alleles from Kasalath (Table 2). Transgressive segregation on palatability was due to the recombination of QTLs, and this may contribute to the palatability improvement of rice.

DISCUSSION

The components of eating quality are related to stickiness, flavor, gloss of cooked rice. Generally, eating quality of rice varieties was evaluated by a taste panel. The taste scores may vary significantly due to the differences in ages, sexes, and areas where the panel members come from. Moreover, evaluation span...
for such a taste trial usually lasts for several weeks or even a few months [28, 29]. Therefore, eating quality was excluded from standards GB/T17891-1999 and NY122-86 for grading of high quality rice in China [30, 31]. As mentioned above, palatability is an important character that should be incorporated in the quality assessment, if rapid and reliable analysis is available. Some instruments have been developed for taste measurement by companies such as in Japan and so on. The majority of these taste analyzers had been calibrated by the taste panel scores and the physiochemical properties of japonica rice. We had compared the palatability scores by the RQ1/Plus Rice Analyzer with those of sensory test, and found that the correlation coefficient of japonica rice was relatively higher than that of indica rice (0.883 and 0.667, respectively) [32]. Liu et al [7] reported that correlation coefficient between the sensory test and the taste Analyzers (milled rice) ranged from 0.56 to 0.72 in indica hybrid rice. Thus, the taste Analyzer is a suitable instrument for the palatability evaluation of indica rice after being calibrated by taste panel scores.

In this study, each lines of the BIL population with introgressed segments of Kasalath chromosomes comprise about 75% of the japonica genome and 25% of the indica genome. The palatability of the BILs, measured by RQ1/Plus Rice Analyzer, was theoretically superior to that of indica rice varieties.

It has been well established that the palatability is correlated to amino acids and protein content in rice. Huang et al [33] reported that palatability was significantly linked to the component and content of free amino acids especially glutamic acid. Positive relationship between palatability and free amino acids was reported [34]. In our previous study, similar result was reproduced [32]; multiple regression analysis between palatability and 17 amino acids showed that there was significant correlation between them, with a correlation coefficient of 0.918**. Comparison of their QTL loci showed that all the 4 amino acids Gly, Val, Thr and Phe displayed significant correlation with palatability also shared same QTL interval of \( q_{PAL-5} \) and \( q_{PAL-8b} \).

Marker-assisted selection (MAS) offers some obvious advantages in the improvement of qualitative traits. But MAS in palatability progressed at a slower pace owing to the complex nature of this trait. In this study four QTLs controlling palatability of milled rice were detected. QTL mapping for palatability showed that Kasalath carried 3 alleles with positive effects and one allele with negative effect for the trait, even though Nipponbare and Kasalath had almost the same palatability score. The BILs population expressed a continuous variation in palatability scores, which meant that a number of QTLs were involved in the inheritance of palatability. Xiao et al [35] also reported that transgressive segregants occurred in BILs, even though no significant difference existed between the

### Table 2. Genotypes of 8 transgressive BILs and related palatability scores.

<table>
<thead>
<tr>
<th>Material</th>
<th>Palatability score</th>
<th>Genotype for QTLs *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R2558-G1458 (( q_{PAL-5} ))</td>
</tr>
<tr>
<td>Nipponbare</td>
<td>75</td>
<td>N</td>
</tr>
<tr>
<td>Kasalath</td>
<td>79</td>
<td>K</td>
</tr>
<tr>
<td>BIL 13</td>
<td>90</td>
<td>K</td>
</tr>
<tr>
<td>BIL 67</td>
<td>90</td>
<td>K</td>
</tr>
<tr>
<td>BIL 68</td>
<td>92</td>
<td>K</td>
</tr>
<tr>
<td>BIL 73</td>
<td>88</td>
<td>N</td>
</tr>
<tr>
<td>BIL 20</td>
<td>72</td>
<td>N</td>
</tr>
<tr>
<td>BIL 47</td>
<td>69</td>
<td>N</td>
</tr>
<tr>
<td>BIL 49</td>
<td>70</td>
<td>N</td>
</tr>
<tr>
<td>BIL 61</td>
<td>67</td>
<td>N</td>
</tr>
</tbody>
</table>

* K= Kasalath; N= Nipponbare.
parents. Prioul et al.\textsuperscript{36} reported that transgressive segregants could be stemmed from different alleles which may be dispersed in different parents. The palatability of some BILs in the present study are superior to that of the parent varieties, and these BILs all held 3 or 4 alleles with positive effect on palatability, indicating that the QTLs detected in this study can be potentially utilized in rice quality improvement through MAS.

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