QTL Mapping for Rice RVA Properties Using High-Throughput Re-sequenced Chromosome Segment Substitution Lines

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Abstract: The rapid visco analyser (RVA) profile is an important factor for evaluation of the cooking and eating quality of rice. To improve rice quality, the identification of new quantitative trait loci (QTLs) for RVA profiling is of great significance. We used a japonica rice cultivar Nipponbare as the recipient and indica rice 9311 as the donor to develop a population containing 38 chromosome segment substitution lines (CSSLs) genotyped by a high-throughput re-sequencing strategy. In this study, the population and the parent lines, which contained similar apparent amylose contents, were used to map the QTLs of RVA properties including peak paste viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity (BKV), setback viscosity (SBV), consistency viscosity (CSV), peak time (PeT) and pasting temperature (PaT). QTL analysis was carried out using one-way analysis of variance and Dunnett’s test, and stable QTLs were identified over two years and under two environments. We identified 10 stable QTLs: qPKV2-1, qSBV2-1; qPKV5-1, qHPV5-1, qCPV5-1; qPKV7-1, qHPV7-1, qCPV7-1, qSBV7-1; and qPKV8-1 on chromosomes 2, 5, 7 and 8, respectively, with contributions ranging from -95.6% to 47.1%. Besides, there was pleiotropy in the QTLs on chromosomes 2, 5 and 7.

Key words: rice; chromosome segment substitution line; rapid visco analyzer profile; quantitative trait locus; substitution mapping

Cooking and eating qualities are highly important determinants of rice grain quality, therefore, quality improvement is the ultimate goal of rice breeding. Apparent amylose content (AAC) is considered one of the most important factors affecting cooking and eating qualities. However, AAC is not the sole explanatory factor for all of the variation of cooking and eating qualities, as cultivars with similar AAC possess different cooking and eating properties (Ramesh and Bhattacharya, 1999; Han and Hamaker, 2001; Cai et al, 2011). During heating and cooling on a rapid visco analyser (RVA), the viscometric properties of starch paste simulate the cooking process and serve as an indicator of the cooking and eating characteristics (Shu et al, 1998). Thus, the RVA profile is of value for distinguishing the cooking and eating qualities among rice varieties with similar AAC (Bao et al, 2000; Hu et al, 2004; Kang et al, 2011).

It has been confirmed that rice paste viscosity parameters are mainly controlled by waxy (Wx) gene encoding granule-bound starch synthase on chromosome 6. However, other genes involved in starch biosynthesis and environments are also responsible for the differences in cooking and eating qualities and rice texture (Bao et al, 2000, 2001; Yan et al, 2011). Previous studies on RVA profile mapping are mostly derived from the hybrid combinations of two rice varieties with quite different AAC; therefore, most of the detected quantitative trait loci (QTLs) are located in regions near the Wx gene. For example, Shen et al (2005) used a recombinant inbred line (RIL) population and identified nine QTLs for RVA profiling, and only one QTL was not around the Wx locus. Using an F₂ population, Zhang et al (2007) identified 34 QTLs for RVA profiling and only three stable QTLs were newly detected within the two years. Recently, Zhang et al (2010) identified six stable QTLs for RVA profiling that were all linked to the Wx locus. In addition, several studies have used populations generated from the hybrid combinations of rice varieties with similar AAC. Bao et al (2001) identified 14 QTLs for RVA profiling using a doubled haploid (DH) population in one environment. Using
an RIL population in three environments, Yang et al (2012) identified 57 QTLs for RVA profiling, and only five QTLs were consistently inherited. In these studies, most of the segregating populations are primary mapping populations, which might have resulted in a large mapping interval and poor repeatability of the QTLs. Therefore, it is relatively difficult to obtain reliable markers for marker-assisted selection (MAS).

Chromosome segment substitution lines (CSSLs) are a series of near-isogenic lines in which the substituted segments of the population contain the entire information of the donor. Each CSSL carries one or several donor chromosome segments under the genetic background of the recipient, which is very useful for the precise mapping of QTLs and dissection of the genetic basis of complex traits (Xu et al, 2010). Therefore, phenotype evaluation can be performed repeatedly and stably in different environments, which can improve the accuracy of QTL detection. Liu et al (2011) carried out extensive QTL mapping for 16 rice quality traits across eight environments by using a set of CSSLs developed from two rice varieties with similar AAC, and detected 10 stable RVA profiles from the QTLs of four environments. However, the number of molecular markers used during the construction of their CSSLs was limited, and there were double-crossovers in the population, therefore, the possibility of missing small introgression segments was high, decreasing the accuracy of the QTL detection.

Previously, we developed a set of CSSLs derived from the indica rice cultivar 9311 (donor) and the japonica rice Nipponbare (recipient), and identified their genotypes using a high-throughput re-sequencing strategy (Zhang et al, 2011). The genetic background and the exact length of each substituted segment in the CSSLs were clear, and thus could improve the accuracy of QTL detection. In the present study, we detected 10 stable RVA profiles for RVA profiling in two environments over the span of two years using these CSSLs, which could be applied for the fine mapping of QTLs and improving the cooking and eating quality of rice using MAS.

MATERIALS AND METHODS

Rice materials
The CSSL population, consisting of 38 lines, was derived from the indica rice cultivar 9311 (donor) and the japonica Nipponbare (recipient). Their genotypes are identified using a high-throughput re-sequencing strategy and the substituted segments cover about 87.4% of the rice whole genome (Zhang et al, 2011). The location and length of each substituted segment are shown in Fig. 1.

All the materials were grown in experimental farms in the campus farm of Yangzhou University or in Hangji town (Hangji farm), Yangzhou, China, during the growing seasons in 2009 and 2010, respectively. Field management was carried out according to normal practice. The plots were arranged in a randomized block design, with two replications in two experimental farms, and each plot consisted of six rows with 10 plants per row. The seeds of all the materials were harvested from three plants in the middle of each plot after maturity, dried, and stored at room temperature for about three months in approximately 14.0% relative humidity. The harvested seeds were then dehusked with a rice huller (Model SY88-TH, Korea) and polished with a grain polisher (Model Kett, Tokyo, Japan). The polished grains were ground by a mill (FOSS 1093 Cyclotec Sample Mill, Sweden) with a 0.50-mm screen.

RVA profiling
The pasting properties of the rice flour were investigated with a rapid visco analyser (RVA) (Tech Master, Newport Scientific, Warriewood, Australia) and analyzed with the Thermal Cycle for Windows software. Flour (3 g) from each sample was placed in an aluminum canister containing 25 mL of distilled water. The sample was dispersed by rotating the paddle at 960 r/min for 10 s before testing, and the viscosity was determined using a constant paddle at 160 r/min. The protocol was to hold at 50 °C for 1.0 min, increase the temperature to 95 °C in 3.8 min, hold at 95 °C for 12.5 min, decrease the temperature to 50 °C in 3.8 min, hold at 50 °C for 12.5 min. The following rice viscosity parameters were obtained from the pasting curve: peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity (BDV), consistency viscosity (CSV), setback viscosity (SBV), peak time (PeT) and pasting temperature (PaT). All the viscosity parameters were expressed in centipoise (cP).

QTL mapping
QTL analysis was conducted based on the 38 CSSLs and their two parents. The QTL nomenclature was adapted according to McCouch et al (1997). The significance of each QTL was determined by comparing the mean values of individuals to the recipient parent Nipponbare according to the stated method of Eshed et al (1995) with some modifications. The QTL was considered as present when the chromosomal segment had significant
effects in the two environments in the two years. For the lines used in both 2009 and 2010, we performed analysis of variance (ANOVA) and the Dunnett’s test by SPSS to detect the QTLs with a corresponding probability value of $P < 0.01$, which was considered statistically significant. To fine-map and estimate the effect of each QTL within the CSSLs, the QTL was considered present when a CSSL line exhibited a significant difference compared with the recipient parent. If several CSSLs showed differences simultaneously, the QTL was then estimated as being located within the chromosomal region shared by these CSSLs. The QTL effects were estimated according to Eshed et al (1995).

RESULTS

Variation of RVA profiles among CSSLs

The two parents Nipponbare and 9311 exhibited the similar AAC, 15.1% ± 0.2% and 16.0% ± 0.2%, respectively. However, the variation of the RVA profiles revealed a continuous distribution among the CSSLs (Table 1), and there were significant differences in all of the RVA traits between the two parents in both years. For example, Nipponbare had higher PKV and HPV than 9311 in the two environments during the two years. The BDV and SBV phenotypic values among the CSSLs exhibited greater distribution than the other traits in the two environments during the two years, whereas PeT and PaT had stable and little variation. These results imply that except for the $Wx$ locus located on chromosome 6, there might be other QTLs exerting minor effects on the viscosity parameters as well as the interaction effects from the environment.

QTL analysis

To elucidate the genetic basis for the variation of starch viscosity among the CSSLs, we mapped the QTLs controlling the RVA profile characteristics in two environments over the two years. Ten stable QTLs for all the RVA characteristics except BDV, PeT and PaT were detected (Table 2). Among them, four, two, two and two constant QTLs, controlling PKV, HPV, CSV and SBV, respectively, were identified, which were located on chromosomes 2, 5, 7 and 8, respectively. Only one QTL, $qSBV2-1$, exerted a positive effect,
while the other nine QTLs exerted the opposite effect, which suggests that the QTLs derived from 9311 mainly had negative effects on the rice RVA profile characteristics under the Nipponbare background.

For the variation of PKV property among the CSSLs, four constant QTLs, \( qPKV2-1 \), \( qPKV5-1 \), \( qPKV7-1 \) and \( qPKV8-1 \), were detected on chromosomes 2, 5, 7 and 8, respectively. In 2009, the additive effects of these four QTLs in campus farm and Hangji farm ranged from -259.3 to -109.3 cp and -206.5 to -107.5 cp, and the additive effect contributions ranged from -7.3% to -3.1% and -6.0% to -3.1%, respectively (Table 2). In 2010, these QTLs exhibited similar trends for the additive effects (-543.8 to -114.3 cp and -563.8 to -155.8 cp) or the additive effect contributions (-23.4% to -3.6% and -23.0% to -4.8%) in campus farm and Hangji farm, respectively. These results imply that though the QTLs exhibited large variation in different years, the directions of the effect value were the same.

We detected two HPV property QTLs, \( qHPV5-1 \) and \( qHPV7-1 \), located on chromosomes 5 and 7, respectively. In 2009, their corresponding additive effects were -334.0 and -285.0 cp, and the additive effect contributions were -19.5% and -15.8%, respectively, in the campus farm environment, whereas at the Hangji experimental farm, they were -251.0 and -308.5 cp, and -12.5% and -16.3%, respectively (Table 2). However, in 2010, the additive effects and their contributions were -151.0 and -421.0 cp, and -7.7% and -29.4%, at the campus farm, and -200.0 and -449.3 cp, and -9.9% and -29.7% in the Hangji environment, respectively. These QTLs were detected in both environments, but their effect values differed over the two years. The effect value of \( qHPV5-1 \) was higher than that of \( qHPV7-1 \) in 2009 but lower in 2010, which might be due to the temperature difference between the two years: it was much hotter in the summer of 2010.

Two constant QTLs controlling CPV, \( qCPV5-1 \) and \( qCPV7-1 \), were identified on chromosomes 5 and 7, respectively. In 2009, \( qCPV5-1 \) and \( qCPV7-1 \) exhibited a similar direction of effect value with additive effects of -163.0 and -193.0 cp, and additive effect contributions of -5.1% and -6.1% in the campus environment, whereas the values were -110.8 and -163.5 cp, and -3.4% and -5.2%, respectively, at the Hangji farm (Table 2). Similar effects were observed in both environments in 2010, indicating that the environment did not influence the effects of these QTLs.

Only one positive QTL, \( qSBV2-1 \), which controlled SBV and located on chromosome 2, was detected, and it exerted a stable effect, with additive effects of 165.3
and 118.8 cp, and 230.3 and 71.5 cp, and additive effect contributions of 46.5% and 32.2%, and 47.1% and 31.6% at the campus and Hangji farms, respectively, over the two years (Table 2). Another SBV property QTL, qSBV7-1, was also detected, but it exerted a negative effect, with additive effects of -207.8 and -137.3 cp, and -266.8 and -119.3 cp, and additive effect contributions of -53.2% and -95.6%, and -52.8% and -76.9% at the two farms, respectively, in 2009 and 2010.

### Substitute mapping and pleiotropic effects of mapped QTLs

Since the detailed lengths of the introgressed segments in each CSSL have been detected through a high-throughput re-sequencing strategy (Zhang et al, 2011), the accurate length of each mapped QTL can be refined by a substitution mapping approach. The QTL qPKV2-1 was detected in the CSSL line N010, which contains a long segment with an overlap by the introgressed segment in another line N006 (Fig. 2-A). As the N006 line showed no significant difference for the PKV when compared with its recipient parent, it indicated that qPKV2-1 was located in the 15.8-Mb segment. Similarly, the QTL qSBV2-1 was mapped in the same region, which suggested that the co-localization of the two QTLs might be the result of their pleiotropic effects or close linkage.

Three QTLs, qPKV5-1, qHPV5-1 and qCPV5-1, were detected within the same line N022, which contains a small introgressed segment of about 6.0 Mb in length (Fig. 2-B). The co-localization of these QTLs might also be the result of pleiotropic effects or close linkage. Based on the substitute mapping method, the QTL qSBV7-1 was mapped on chromosome 7 in the N026 line with a length of about 22.2 Mb. The other three QTLs, qPKV7-1, qHPV7-1 and qCPV7-1, were also observed within the same N026 line and were about 5.1-Mb long (Fig. 2-C). Though both CSSLs N033 and N046 contain a large introgressed segment on chromosome 8, an overlap was present between the two segments. Thus, with the exception of the segment in the N046 line that had no significant effect on PKV, qPKV8-1 was then mapped in a small region spanning approximately 4.1-Mb (Fig. 2-D).

### DISCUSSION

In this study, a CSSL population derived from two parents containing similar AAC was employed to identify genetic factors that might influence paste viscosity. As the parents of the CSSLs possessed the same Wx allele, which is a major QTL controlling the RVA profile characteristics, the mapping population used in this study could avoid the influence of the Wx gene on the RVA profile. Thus, we could identify new QTLs, which would be particularly helpful in marker-aided manipulation of the cooking and eating quality of rice.

Previous studies used several types of mapping populations for genetic analysis, such as F2:3, BC1, DH...
and RILs, and a number of QTLs have been mapped for rice RVA profiles. However, most of the populations are difficult to repeat or have complicated noise under their genetic backgrounds. Therefore, it is difficult to generate convincing mapping results. The CSSLs are powerful populations for mapping complex traits. Moreover, the CSSLs previously reported are selected via MAS and their genotypes are identified using limited markers, therefore, the detected QTLs might be inaccurate. Since 2012, two rice CSSLs have been accurately genotyped using the whole-genome resequencing method, and ultra high-density linkage maps thereof have been constructed for QTL mapping (Xu et al, 2010; Zhang et al, 2011). The CSSLs used in this study covered about 87.4% of the rice whole-genome and thus might have omitted some QTLs. We are now extending this population, and have generated some backcross lines using the lines containing the detected QTLs to fine-map the corresponding QTLs detected in this study.

Starch comprises approximately 90% of the rice grain and its structure is widely recognized as one of the most important determinants of eating, cooking and processing qualities. Starch biosynthesis is a complex system composed of multiple genes and transcription factors (Isshiki et al, 2000; Tian et al, 2009; Fu et al, 2010). Among these genes, several genes, such as SBEs (encoding of starch branching enzymes), have been reported to be sensitive to high temperature and thus alter grain quality under high temperatures (Jin et al, 2005; Wei et al, 2009). The detection of stable and non-environment-specific QTLs

Fig. 2. Physical mapping of QTL for rapid visco analyser (RVA) properties with developed chromosome segment substitute lines (CSSLs).

The genotypes of the CSSLs were constructed based on the whole-genome resequencing data. Black colour indicates substituted segments from 9311 and grey colour indicates chromosomal regions of Nipponbare.

Compared with previous studies, the QTLs qPKV2-1 and qSBV2-1 might be linked to the locus reported by Zhang et al (2007). Liu et al (2011) have detected only one QTL controlling SBV but not PKV in the same locus, which might be attributed to the different populations used among different studies. In the present study, one locus with pleiotropic effects was mapped on chromosome 5, but Yang et al (2012) detected one unstable QTL controlling only PKV in this locus, which might be due to the influence of background noise from the primary population. The QTLs qSBV7-1 and qPKV8-1 were consistent with that of several previous reports (Liu et al, 2011; Yang et al, 2012). In addition, the stable QTLs that contained qPKV7-1, qHPV7-1 and qCPV7-1 are new, and might be fine mapped in future.

Amylose content is considered one of the most important factors affecting the cooking and sensory properties of rice. However, the cooking and eating properties can differ greatly among different rice cultivars, even those with similar amylose content. Therefore, the RVA profile is of value for distinguishing the cooking and eating quality among rice varieties with similar AAC (Han and Hamaker, 2001; Li et al, 2001; Chung et al, 2011). Several studies have shown that rice varieties with higher BDV and lower SBV are preferred for good cooking and eating quality (Shu et al, 1998; Wu et al, 2001). The cooking and eating quality of indica rice 9311 is not as good as japonica rice Nipponbare, and the variation of RVA profile characteristics between these two cultivars is lower than that among the CSSL population, which is consistent with the result of a recent study (Yang et al, 2012). With the exception of BDV, PeT and PaT, the other pasting parameters exhibited higher variation in the two environments over the two years, which might be due to the interactions of the multiple genes in rice (Tian et al, 2009). Previous studies have shown that temperature plays an important role in controlling grain quality and high temperature will cause poor grain quality (Jin et al, 2005; Wei et al, 2009). The temperature of the two years in the summer differs greatly, so the non-environment-specific QTLs identified in this study might be very useful for the improvement of rice grain quality (Wan et al, 2005).
would be immensely useful for the improvement of rice quality through MAS. In this study, most of the detected QTLs exerted negative effects and might lead to poor grain quality. On the contrary, the QTLs from the japonica rice cultivar Nipponbare might have a positive effect and thus be useful for the improvement of cultivars without these loci.

In summary, the present findings imply that using a convenient genetic population for QTL mapping could avoid the influence of major QTLs and thus identify new QTLs that exert minor effects. Therefore, the use of this CSSL population, combined with the next-generation sequencing technology, forms the foundation for the fine mapping and subsequent cloning of these QTLs and the molecular breeding research aiming at improving rice quality.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China (Grant No. 31071383), the Ministry of Science and Technology of China (Grant Nos. 2012AA10A302-7 and 2013ZX080 09003-004) and the Jiangsu Government of China (Grant Nos. BK2012010 and CX(12)1003).

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