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# Mapping QTLs controlling cooking and eating quality indicators of Iranian rice using RILs across three years

**Ali-Akbar Ebadi[1, 2*], EzatollahFarshadfar[2], BabakRabiei[3]**

**[1]Department of Plant Breeding, Rice Research Institute of Iran (RRII), P.O box: 41996-13475, Rasht-Iran**
**[2]Department of Agronomy and Plant Breeding, Faculty of Agricultural Sciences, Razi University, Kermanshah, Iran**
**[3]Department of Agronomy & Plant Breeding, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran**

*** Corresponding author: ebady_al@yahoo.com**

**Abstract**

Recombinant inbred lines (RILs) consisting of 144 lines derived from a cross between Hashemi (an indica rice with high cooking and eating quality growing in North of Iran) and Nemat (an indica rice with low cooking and eating quality) were used to investigate the genetic basis of cooking and eating quality indices of rice. Amylose content (AC), gelatinization temperature (GT) and eight starch pasting viscosity parameters of the lines were evaluated throughout a three years study. A genetic linkage map including 171 simple sequence repeat (SSR) loci was constructed and 54 QTLs were detected for the investigated traits. Results demonstrated that at least three QTLs were involved in monitoring of each individual trait and one QTL detected repeatedly over the three years study. Several QTL clusters were involved in multiple quality traits. Two QTL clusters close to the *Wx* locus on chromosome 6 included seven QTLs: *qAC6a*, *qHPV6b*, *qBDV6a*, *qCSV6b*, *qHPV6d*, *qCPV6b* and *qPeT6b* controlled amylose content (AC), hot past viscosity (HPV), breakdown viscosity (BDV), consistency viscosity (CSV), cool past viscosity (CPV) and peak time (PeT), respectively. Another QTL cluster was detected near the *Alk* locus on chromosome 6 consisted of four QTLs: *qGT6a*, *qCPV6a*, *qCSV6a* and *qSBV6*, which repeated over three years, involved in controlling gelatinization temperature (GT), cool past viscosity (CPV), consistency viscosity (CSV), and setback viscosity (SBV), respectively. At least one new QTL was identified for each trait that was not reported in the previous studies. Our results show that most of the measured cooking and eating quality criteria in this research were controlled by *Wx* and *Alk* regions on chromosome 6, were remarked by the variation from 12.5% up to 84.7% for BDV and GT, respectively. Markers linked to these loci can be used as marker aided selection in breeding programs

**Keywords:** Starch pasting viscosity, Amylose content, Microsatellites markers, QTL cluster.
**Abbreviations:** AC-amylose content; GT- gelatinization temperature; PKV- peak viscosity; HPV- hot past viscosity; CPV- cool past viscosity; BDV- breakdown viscosity; CSV- consistency viscosity; SBV- setback viscosity; PeT- peak time; PaT- pasting temperature.

## Introduction

Cultivated rice (*Oryza sativa* L.) is one of the most important crops around the world. Rice has been consumed beings for more than 5000 years and provides food for more than half of the world's population (Zhou et al., 2002). Providing the high eating quality is the ultimate goal in rice quality improvement. Although, there are many factors affecting the palatability of cooked rice, amylose content (AC) is considered to be the most important factor (Juliano, 1990), which is under the control of the waxy (*Wx*) gene on chromosome 6 (Tan et al., 1999; He et al., 1999; Bao et al., 2000; Aluko et al., 2004). It encodes the enzyme granule bound starch synthase (GBSS) (Wang et al., 1995; Tian et al., 2004). The *Wx* gene alone does not explain all of the variation among rice cultivars and some minor genes might also be involved (Ayres et al., 1997). For example, high AC will lead to the deterioration of viscosity, softness, luster and palatability, but AC does not absolutely determine the texture of cooked rice, because the palatability of cooked rice with similar AC may vary greatly (Liu et al., 2011). Rice starch viscosity profile, also termed RVA profile as being tested on the Rapid Visco Analyzer (RVA) have been applied to evaluate rice eating and cooking quality (Juliano, 1996). The RVA is a pasting curve generated from subjecting rice flour to a standard temperature-programmed heat-hold-cool-hold protocol. Eight characteristics, peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity (BDV), consistence viscosity (CSV), setback viscosity (SBV), peak time (PeT) and pasting temperature (PaT) can be got from the pasting curve (Bao and Xia, 1999). The RVA paste viscosity is measured with a Rapid Visco Analyzer produced by the Newport Scientific Instruments Corporation in Australia. It has been widely used to investigate the viscosity property, because it requires only a small sample size and the procedure is easy to perform (Bao and Xia, 1999). Rice starch comprises 90% of the total dry weight of milled rice which has a great impact on the eating and cooking qualities. It is composed of two classes of polymers: amylose, a lightly branched linear molecule with a degree of polymerization of 1000–5000 glucose units, and amylopectin, a larger polymer unit containing frequent a-1,6 branching linkages (Williams et al., 1958; Jiang et al., 2004).

**Table 1.** Values of cooking and eating quality parameters for the parents and RILs lines.

| Traits[a] | Parent (Mean ± SD) | | RIL population | | $h^2$ |
|---|---|---|---|---|---|
| | Hashemi | Nemat | Mean ± SD | Range | |
| AC(%) | 18.40 ± 0.20 | 27.67 ± 0.35 | 22.98 ± 3.46 | 14 – 28.4 | 83.4 |
| | 20.33 ± 0.62 | 27.37 ± 0.60 | 23.98 ± 2.18 | 17 – 28.7 | |
| | 19.52 ± 0.57 | 27.5 ± 0.46 | 23.82 ± 1.84 | 17.5 – 27.5 | |
| GT | 4.47 ± 0.11 | 7 ± 0 | 5.81 ± 1.13 | 2.6 – 7 | 91.3 |
| | 4.43 ± 0.17 | 7 ± 0 | 6.12 ± 0.99 | 3.2 – 7 | |
| | 4.30 ± 0.20 | 7 ± 0 | 6.06 ± 0.98 | 3.6 – 7 | |
| PKV | 269.5 ± 4.7 | 344.8 ± 11.1 | 306.4 ± 32.2 | 225.2 – 376.7 | 69.8 |
| | 275.4 ± 4.9 | 332.1 ± 7.6 | 277.8 ± 47.1 | 131.3 – 361.3 | |
| | 277.9 ± 3.7 | 339.5 ± 5.5 | 275.5 ± 33.8 | 184 – 368.9 | |
| HPV | 190.8 ± 9.2 | 310.2 ± 8.1 | 257.4 ± 30.3 | 193.7 – 327.5 | 77.3 |
| | 193.1 ± 6.7 | 301.9 ± 6.2 | 237.7 ± 46.4 | 110.6 – 328.8 | |
| | 180.5 ± 5 | 316.4 ± 5.6 | 234.8 ± 35.7 | 153.4 – 337.9 | |
| BDV | 78.7 ± 8.3 | 34.7 ± 6.1 | 49.1 ± 23.5 | 15 – 125 | 83.7 |
| | 82.3 ± 2.7 | 30.2 ± 4.4 | 40.1 ± 21.9 | 9 – 103.3 | |
| | 97.3 ± 5.8 | 23.2 ± 4 | 40.7 ± 17.3 | 10.5 – 101.3 | |
| CPV | 321.8 ± 4.8 | 431.1 ± 10.1 | 360.7 ± 48.3 | 255 – 485.8 | 72.2 |
| | 325.8 ± 5.1 | 428.9 ± 10.3 | 362.8 ± 63.6 | 180.1 – 491.2 | |
| | 317 ± 7.6 | 443 ± 8.8 | 363.1± 48.1 | 221.1 – 472.8 | |
| CSV | 131.0 ± 6.8 | 120.9 ± 6.4 | 103.3 ± 35.2 | 43 – 196.6 | 72.2 |
| | 132.7 ± 4.4 | 126.9 ± 4.2 | 124.8 ± 46.8 | 21 – 271.8 | |
| | 136.4 ± 5.9 | 126.6 ± 5.8 | 128.3 ± 35.5 | 53.8 – 256.6 | |
| SBV | 52.3 ± 4.5 | 86.2 ± 5.2 | 54.1 ± 36.8 | -18 to 164.7 | 68.5 |
| | 50.3 ± 3.5 | 102.8 ± 6.9 | 84.9 ± 45.7 | -17.1 to 214 | |
| | 39.11 ± 4.3 | 103.5 ± 6.8 | 87.6 ± 35.1 | -9.9 to 200.7 | |
| PeT | 6.0 ± 0.12 | 6.32 ± 0.18 | 6.64 ± 0.32 | 5.87 – 7 | 86.3 |
| | 6.02 ± 0.13 | 6.26 ± 0.16 | 6.53 ± 0.36 | 5.63 -7 | |
| | 5.98 ± 0.10 | 6.30 ± 0.16 | 6.47 ± 0.29 | 5.73-7 | |
| PaT | 85.2 ± 0.2 | 75.1 ± 1.8 | 81.7 ± 3.4 | 73.6 – 90.1 | 73.1 |
| | 83.1 ± 2.7 | 76.4 ± 1.7 | 82.62 ± 4.8 | 69 – 93.2 | |
| | 82.6 ± 1.0 | 76.2 ± 1.7 | 82.1 ± 3.9 | 72.4 – 90.1 | |

[a]amylose content (AC), gelatinization temperature (GT), peak viscosity (PKV), hot past viscosity (HPV), breakdown viscosity (BDV), cool past viscosity (CPV), consistency viscosity (CSV), setback viscosity (SBV), peak time (PeT), pasting temperature (PaT)

The fact that the cooking and eating quality varied among cultivars with similar AC suggests that the structure of amylopectin also has an effect in determining the physical and chemical properties (Juliano, 1985). Quantitative trait locus (QTL) analysis has demonstrated that apparent amylose content and paste viscosity parameters are mainly controlled by the *Wx* gene on chromosome 6, which encodes the granule bound starch synthase (GBSS) (Bao and Xia, 1999; Zhang et al., 2008; Traore et al., 2011; Teng et al., 2012). However, other researchers reported that these traits controlled by other genes on chromosomes 1, 2 and 8 (Li et al., 2011; Liu et al., 2011). As for the gelatinization consistency (GC), some researchers reported that the *Wx* gene was the major determinant (Tan et al., 1999; Tian et al., 2004; He et al., 2006; Mallikarjuna Swamy et al., 2012), and some believed that the other QTLs with minor effects were responsible for the GC (He et al., 1999; Sabouri et al., 2012). The *alk* gene was reported to determine the GT, and the gene was mapped at the same locus as *SssIIa* on chromosome 6 that encodes soluble starch synthase IIa (Umemoto et al., 2002; He et al., 2006; Sabori et al., 2012). In short, it has not been fully understood that how many genes are involved and how they interact with each other in controlling the GC and GT traits. The objectives of this study were to (i) investigate the genetic control mechanism of grain quality traits (ii) detect and map QTLs controlling cooking and eating quality criteria of rice across three years using a recombinant inbred lines (RILs) population and (iii) identify the potential target of stable QTLs that could be used for marker aided selection in rice breeding programs.

## Results

### *Phenotypic variation in parents and population*

Simple and combined analysis of variance (ANOVA) revealed significant variation between parents, RILs lines and parents versus RILs lines for each year and over three years for all the investigated traits. The F value of genotypes ranged from 12.1 ($P \leq 0.001$) to 89.7 ($P \leq 0.001$) for PaT and PKV, respectively. The effect of year was significant for all studied traits except for CPV. Genotype × year interaction exhibited a significant effect for all studied traits (data not shown). Nemat showed higher AC and GT in during the three years of study compared with Hashemi. All viscosity parameters in Nemat except BDV, CSV and PeT were much larger than Hashemi. Normal phenotypic distribution observed for all traits indicating the quantitative inheritance of the investigated traits (data not shown). The means of RILs population for all traits were located within the range of the parental values over three years. The RILs population displayed transgressive segregation in both directions for all traits, indicating that both parents transmitted favorable alleles for each trait. Broad-sense heritability ($h^2$) of the cooking and eating traits were relatively high ranging from 68.5% to 91% for SBV and GT, respectively, expressing that genetic components accounted for most of the phenotypic variations (Table 1). Pairwise correlation coefficients between traits are listed in Table 2. AC revealed a negative

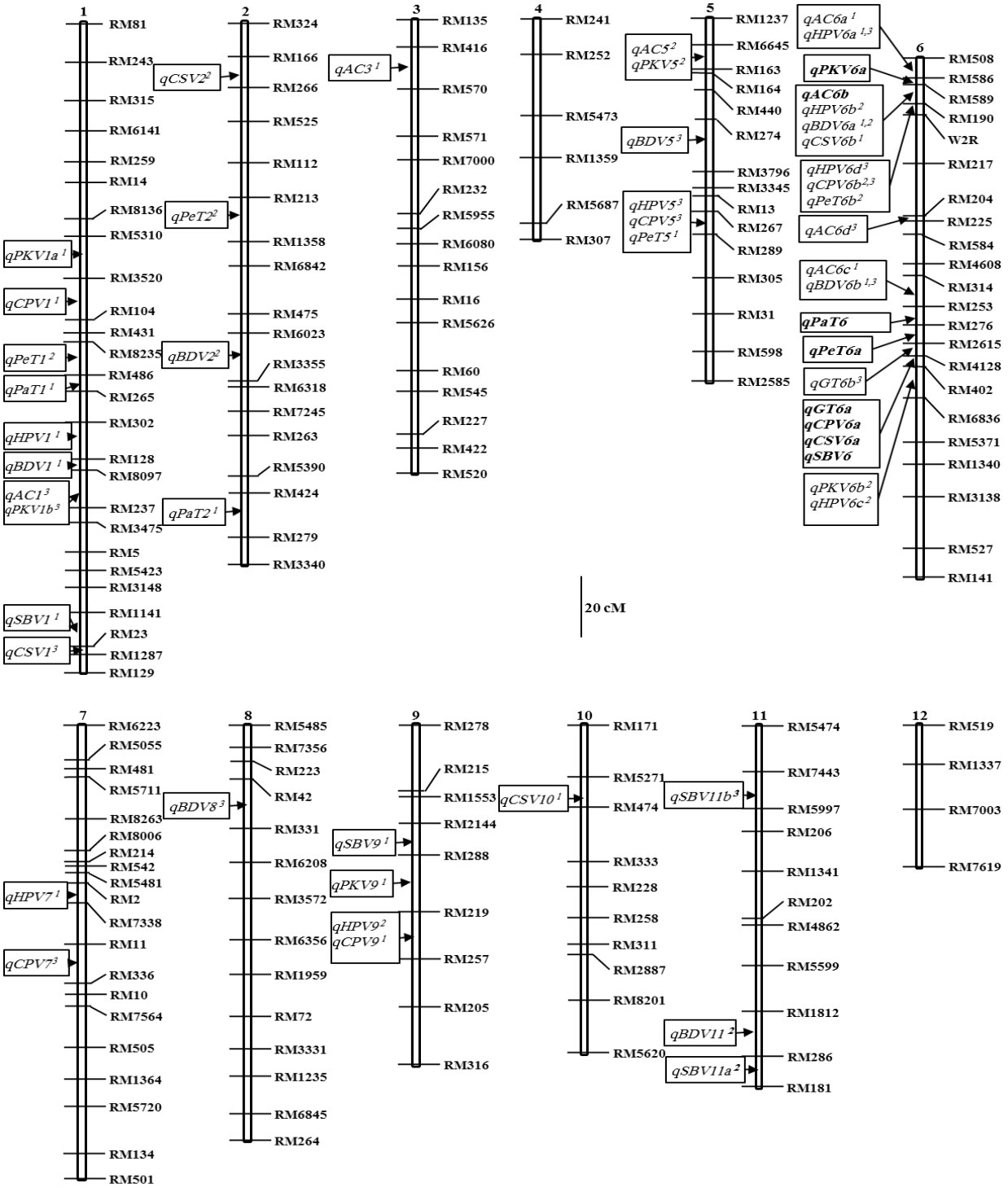


**Fig 1.** Distribution of the QTLs for eating and cooking quality traits in the linkage map. The marker name is shown on the right of the chromosome. The superscript 9, 10 or 11 indicates the year (2009, 2010 or 2011) in which the QTL is detected; the letters in bold indicate that the QTLs are detected in all three years. See Table 1 for abbreviations and descriptions of the traits and parameters.

significant correlation with BDV and a positive significant association with GT, HPV, CSV, SBV and PeT. GT showed positive significant correlation with PeT and negative significant relation with PKV, BDV, CPV and CSV. The results also indicated that the RVA parameters were strongly correlated with each other, except for PKVvs CSV, PeT, and SBV; HPV vs CSV, PaT, and SBV; BDV vs CPV; CPV vs PeT and PaT; and CSV vsPaT (Table 2).

**QTL analysis**

***Amylose content and gelatinization temperature***

Seven QTLs were detected for AC collectively in 2009, 2010 and 2011, accounting for 46.7%, 30.6% and 22% of the phenotypic variation, respectively (Table 3; Fig. 1). The largest QTL found over the three years was associated to *Wx* locus (the locus RM190 tightly linked with the *Wx* gene)

**Table 2.** Pairwise correlation coefficients between cooking and eating quality parameters.

| Traits | AC | GT | PKV | HPV | BDV | CPV | CSV | PeT | PaT |
|---|---|---|---|---|---|---|---|---|---|
| GT | 0.34** | | | | | | | | |
| PKV | 0.04 | -0.30** | | | | | | | |
| HPV | 0.34** | 0.03 | 0.83** | | | | | | |
| BDV | -0.51** | -0.40** | 0.31** | -0.26* | | | | | |
| CPV | 0.03 | -0.23** | 0.65** | 0.66** | 0.07 | | | | |
| CSV | 0.31** | -0.35** | 0.03 | -0.12 | 0.26* | 0.66** | | | |
| PeT | 0.28** | 0.46** | 0.03 | 0.35** | -0.55** | -0.17 | -0.59** | | |
| PaT | -0.08 | 0.14 | -0.31** | -0.17 | -0.25* | -0.05 | 0.11 | 0.33** | |
| SBV | 0.36** | -0.10 | -0.14 | 0.04 | -0.31** | 0.66** | 0.83** | -0.26* | 0.23* |

*; ** significant at 5% and 1% level of probability, respectively.

(Ayres et al., 1997) on chromosome 6. It was designated as *qAC6a* explained 21.5%, 13.2% and 13.8% of the phenotypic variance in 2009, 2010 and 2011, respectively. The allele of Nemat increased AC at this locus. The other 6 minor QTLs were only detected in one year and mapped on chromosomes 1, 3, 5 and 6 (3 QTLs). A major and one minor QTL were identified for GT on chromosome 6. The major QTL mapped at the interval RM4128-RM402 (the locus linked with the gene *Alk*) explained 49%, 65.1% and 85.7% of the phenotypic variation in 2009, 2010 and 2011, respectively. The additive effects of Nemat alleles increased GT at this locus. The minor QTL was only detected in 2011 which explained 8.68% of the phenotypic variation (Table 3; Fig. 1).

***The parameters of the viscosity profile***

Six QTLs were detected for peak viscosity (PKV) collectively over three years, 2009, 2010 and 2011, justified 57.5%, 60.4% and 35.3% of the phenotypic variation, respectively. A major QTL was mapped at interval RM586-RM589 on chromosome 6 (close to *Wx* locus) revealed 34.7%, 33.6% and 27.2% of the phenotypic variation in 2009, 2010 and 2011, respectively (Table 3; Fig. 1). The additive effect of this QTL was positive and Nemat alleles increased PKV at this locus. Five other QTLs were mapped on chromosomes 1 (containing 2 QTLs), 5, 6, and 9. Two QTLs (*qPKV1a* and *qPKV9*) were detected in 2009 at interval RM5310-RM3520 on chromosome 1 and interval RM288-RM19 on chromosome 9, described 11.9% and 10.7% of the phenotypic variation, respectively. The additive effect of these QTLs was negative and Hashemi alleles increased PKV at these loci (Table 3; Fig. 1). The other two QTLs were detected on chromosomes 6 and 5 in 2010 explained 14% and 12.7% of the phenotypic variation, respectively. Alleles of Hashemi increased the PKV at these loci. One QTL was detected on chromosome 1 in 2011, explained 8.1% of the phenotypic variation. Hashemi alleles increased PKV at this locus. A reach QTL was identified for hot past viscosity (HPV) in 2009, 2010 and 2011, explained 37.5%, 64.8% and 33.9% of the phenotypic variation, respectively. One QTL was detected near to *Wx* locus on chromosome 6 in 2009 and 2011 explained 14.9% and 10% of the phenotypic variation, respectively. One major QTL was detected at position of *Wx* locus in 2010 justified 52.1% of the phenotypic variation. Another QTL was identified in 2011 located at interval RM190-W2R on chromosome 6 explained 12.7% of the phenotypic variation. Nemat alleles increased HPV at this locus. Additionally, five QTLs were mapped on chromosome 1 (in 2009), 7(in 2009), 6 (in 2010), 9 (in 2010) and 5 (in 2011), explaining 12.7%, 9.9%, 6.8 5.9%, and 11.1% of the phenotypic variation, respectively (Table 3; Fig. 1). Seven QTLs were identified for breakdown viscosity (BDV) in 2009, 2010 and 2011, explained 33.4%, 32.7% and 37.5% of the phenotypic variation, respectively (Table 3; Fig. 1). One QTL corresponded to the *Wx* locus on chromosome 6 was acquainted in 2009 and 2010 explained 13.5% and 12.7% of the phenotypic variation, respectively. Another QTL was detected at interval RM314-RM253 on chromosome 6 in 2009 and 2011, which justified 13.2% and 13.3% of the phenotypic variation, respectively. Alleles from Nemat at these two loci decreased the trait value. The other minor QTLs were identified on chromosome 1 in 2009, chromosomes 2 and 11 in 2010 and chromosomes 8 and 5 in 2011 (Table 3; Fig. 1). For cool paste viscosity(CPV) three QTLs were detected in 2009, two in 2010 and four in 2011, explained 39.8%, 34.3% and 47.9% of the phenotypic variation, respectively (Table 3; Fig. 1). One major QTL was recognized at interval RM4128-RM402 (close to the *Alk* locus) on chromosome 6 in 2009, 2010 and 2011 explained 18%, 22.6% and 9.3% of the phenotypic variation, respectively. The alleles from Nemat at this QTL decreased CPV, while the other detected QTLs in 2010 and 2011 corresponded to the *Wx* locus where the alleles of Nemat increased the CPV. The other QTLs were mapped on chromosomes 1 and 9 in 2009 and chromosomes 5 and 7 in 2011 (Table 3; Fig. 1). Three QTLs were identified for consistency viscosity (CSV) in 2009, two in 2010 and two in 2011, explained 36, 29.6 and 28.8% of the phenotypic variation, respectively (Table 3; Fig. 1). One major QTL was detected at interval RM4128-RM402 (close to the *Alk* locus) on chromosome 6 in 2009, 2010 and 2011 explained 14.7, 19.9 and15.1% of the phenotypic variation, respectively. The alleles from Nemat decreased the trait value at this locus. Another QTL was found at interval RM589-RM190 (tightly linked with *Wx* locus) in 2009 where the alleles of Nemat decreased the trait value. The other three QTLs were detected on chromosomes 10, 2 and 1 in 2009, 2010 and 2011, respectively (Table 3; Fig. 1). For setback viscosity (SBV) three QTL were mapped in 2009, two in 2010 and two in 2011, described 34.2%, 23.6% and 23.2% of the phenotypic variation, respectively (Table 3; Fig. 1). One QTL was recognized at interval RM4128-RM402 (close to the *Alk* locus) on chromosome 6 explained 11.4%, 12.7% and 13.3% of variability in 2009, 2010 and 2011, respectively. The alleles of Nemat decreased the trait value at this QTL. The other four QTLs were identified on chromosomes 1 and 9 in 2009 and chromosome 11 in 2010 and 2011 at interval RM288-RM181 and RM7443-RM5997, respectively. (Table 3; Fig. 1). Two QTLs were found for peak time (PeT) in 2009, four in 2010 and one in 2011, justified 38.5%, 44.7% and 21.3% of the phenotypic variation, respectively (Table 3; Fig. 1). One QTL was detected at interval RM276-RM2615 on chromosome 6 explained 28.6%, 19.3% and21% of the phenotypic variation in 2009, 2010 and 2011, respectively. The alleles of Nemat increased the trait value at this QTL with an average effect of 0.15℃. Another QTL was mapped at interval RM190-W2R (tightly linked with *Wx* locus) in 2010,

**Table 3.** QTLs identified for the rice eating and cooking quality traits in RILs population.

| Trait | QTL | Chr | Marker interval | LOD score | | | Additive effect | | | PVE(%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 |
| AC | *qAC6a* | 6 | RM508-RM586 | 4.91 | | | 1.03 | | | 8.80 | | |
| | *qAC6b*** | 6 | RM589-RM190 | 11.24 | 4.37 | 5.26 | 1.61 | 0.79 | 0.68 | 21.50 | 13.21 | 13.76 |
| | *qAC6c* | 6 | RM314-RM253 | 4.62 | | | 1.00 | | | 8.51 | | |
| | *qAC3* | 3 | RM416-RM570 | 2.96 | | | -0.69 | | | 7.87 | | |
| | *qAC6d* | 6 | RM204-RM225 | | 4.09 | | | 0.59 | | | 9.96 | |
| | *qAC5* | 5 | RM6645-RM163 | | 2.85 | | | 0.89 | | | 7.69 | |
| | *qAC1* | 1 | RM8097-RM237 | | | 2.58 | | | -0.71 | | | 8.21 |
| GT | *qGT6a*** | 6 | RM4128-RM402 | 20.41 | 27.27 | 38.92 | 0.81 | 0.79 | 0.90 | 48.98 | 65.12 | 84.69 |
| | *qGT6b* | 6 | RM2615-RM4128 | | | 6.42 | | | 0.21 | | | 8.68 |
| PKV | *qPKV6a*** | 6 | RM586-RM589 | 13.87 | 12.96 | 10.36 | 25.54 | 27.44 | 17.07 | 34.69 | 33.64 | 27.17 |
| | *qPKV1a* | 1 | RM5310-RM3520 | 3.98 | | | -16.86 | | | 11.87 | | |
| | *qPKV9* | 9 | RM288-RM19 | 3.67 | | | -15.75 | | | 10.68 | | |
| | *qPKV6b* | 6 | RM402-RM6836 | | 5.31 | | | -17.54 | | | 14.0 | |
| | *qPKV5* | 5 | RM6645-RM163 | | 3.73 | | | -16.86 | | | 12.74 | |
| | *qPKV1b* | 1 | RM8097-RM237 | | | 3.23 | | | -9.64 | | | 8.14 |
| HPV | *qHPV7* | 7 | RM2-RM7338 | 3.64 | | | -11.54 | | | 12.68 | | |
| | *qHPV1* | 1 | RM302-RM128 | 2.83 | | | -10.76 | | | 9.86 | | |
| | *qHPV6a** | 6 | RM508-RM586 | 4.59 | | 4.61 | 11.71 | | 11.36 | 14.92 | | 10.05 |
| | *qHPV6b* | 6 | RM589-RM190 | | 18.9 | | | 33.46 | | | 52.12 | |
| | *qHPV6c* | 6 | RM402-RM6836 | | 3.29 | | | -12.10 | | | 6.85 | |
| | *qHPV9* | 9 | RM219-RM257 | | 2.89 | | | 10.73 | | | 5.86 | |
| | *qHPV6d* | 6 | RM190-W2R | | | 5.84 | | | 12.71 | | | 12.70 |
| | *qHPV5* | 5 | RM267-RM289 | | | 4.54 | | | -11.94 | | | 11.14 |
| BDV | *qBDV1* | 1 | RM128-RM8097 | 2.95 | | | 6.11 | | | 6.68 | | |
| | *qBDV6a** | 6 | RM589-RM190 | 4.77 | 4.59 | | -8.64 | -7.8 | | 13.46 | 12.65 | |
| | *qBDV6b** | 6 | RM314-RM253 | 5.24 | | 4.41 | -8.55 | | -6.23 | 13.23 | | 13.34 |
| | *qBDV2* | 2 | RM6023-RM3355 | | 3.23 | | | 8.63 | | | 10.23 | |
| | *qBDV11* | 11 | RM1812-RM286 | | 2.86 | | | 7.82 | | | 9.85 | |
| | *qBDV8* | 8 | RM42-RM331 | | | 3.42 | | | 7.84 | | | 12.43 |
| | *qBDV5* | 5 | RM274-RM3796 | | | 3.64 | | | 6.84 | | | 11.69 |
| CPV | *qCPV1* | 1 | RM3520-RM104 | 3.23 | | | 14.86 | | | 12.53 | | |
| | *qCPV9* | 9 | RM219-RM257 | 2.89 | | | 13.68 | | | 9.21 | | |
| | *qCPV6a*** | 6 | RM4128-RM402 | 5.6 | 8.44 | 3.40 | -23.66 | -30.61 | -14.62 | 18.04 | 22.64 | 9.26 |
| | *qCPV6b** | 6 | RM190-W2R | | 4.60 | 5.81 | | 22.01 | 20.09 | | 11.67 | 17.42 |
| | *qCPV5* | 5 | RM267-RM289 | | | 2.96 | | | 12.86 | | | 10.32 |
| | *qCPV7* | 7 | RM11-RM336 | | | 2.89 | | | -13.94 | | | 10.86 |
| CSV | *qCSV10* | 10 | RM5271-RM474 | 3.65 | | | 11.80 | | | 12.65 | | |
| | *qCSV6a*** | 6 | RM4128-RM402 | 4.55 | 6.88 | 5.08 | -12.40 | -20.84 | -13.77 | 14.73 | 19.95 | 15.12 |
| | *qCSV6b* | 6 | RM589-RM190 | 2.87 | | | -10.38 | | | 8.66 | | |
| | *qCSV2* | 2 | RM166-RM266 | | 2.89 | | | 10.24 | | | 9.64 | |
| | *qCSV1* | 1 | RM23-RM1287 | | | 3.12 | | | 12.34 | | | 13.64 |
| SBV | *qSBV1* | 1 | RM1141-RM23 | 2.92 | | | 14.32 | | | 10.36 | | |
| | *qSBV9* | 9 | RM2144-RM288 | 3.32 | | | 15.37 | | | 12.53 | | |
| | *qSBV6*** | 6 | RM4128-RM402 | 4.11 | 4.21 | 4.24 | -15.89 | -16.36 | -17.12 | 11.36 | 12.70 | 13.32 |
| | *qSBV11a* | 11 | RM286-RM181 | | 3.45 | | | 13.86 | | | 10.87 | |
| | *qSBV11b* | 11 | RM7443-RM5997 | | | 2.84 | | | 10.65 | | | 9.89 |
| PeT | *qPeT6a*** | 6 | RM276-RM2615 | 9.14 | 8.65 | 6.68 | 0.17 | 0.16 | 0.13 | 28.63 | 19.34 | 21.03 |
| | *qPeT5* | 5 | RM267-RM289 | 2.87 | | | -0.10 | | | 9.90 | | |
| | *qPeT6b* | 6 | RM190-W2R | | 5.97 | | | 0.12 | | | 11.94 | |
| | *qPeT1* | 1 | RM8235-RM486 | | 2.83 | | | -0.08 | | | 5.41 | |
| | *qPeT2* | 2 | RM213-RM1358 | | 3.33 | | | -0.10 | | | 8.05 | |
| PaT | *qPaT1* | 1 | RM486-RM265 | 2.81 | | | -0.99 | | | 8.15 | | |
| | *qPaT2* | 2 | RM424-RM279 | 3.19 | | | -1.05 | | | 9.39 | | |
| | *qPaT6*** | 6 | RM253-RM276 | 4.46 | 5.36 | 6.66 | 1.61 | 1.94 | 2.19 | 14.46 | 17.36 | 20.21 |

*represents QTLs detected in two years and ** represents QTLs detected in three years

Where, the alleles of Nemat increased the PeT. The other QTLs were detected on chromosome 5 in 2009, and 1 and 2 in 2010, where the alleles of Hashemi increased PeT. One major QTL was recognized for pasting temperature (PaT) on chromosome 6 explained 14.5%, 17.4% and 20.2% of the phenotypic variation in 2009, 2010 and 2011, respectively. The alleles of Nemat increased the PaT at this locus. Two minor QTLs were detected on chromosomes 1 and 2 in 2009, where the alleles of Nemat decreased the trait value (Table 3; Fig. 1).

***QTLs co-localization***

Comparison of the identified QTLs exhibited 10 QTL clusters for the studied traits distributed on four chromosomes (Fig. 1). Six QTL clusters were detected on chromosome 6. The QTL clusters linked to *Wx* locus simultaneously controlled AC and most of the viscosity parameters, but had no effect on PKV, PaT, SBV and GT. The QTL clusters linked to *Alk* locus played an important role on GT and most of the viscosity parameters but had no effect on AC, HPV, PeT and PaT. In one QTL cluster (composed of *qGT6a, qCPV6a, qCSV6a* and *qSBV6*) linked with *Alk* locus, were common across three years. The other QTL clusters were found on chromosome 1 for AC and PKV, on chromosome 5 (one for AC and PKV and the other for HPV, CPV and PeT) and on chromosome 9 for HPV and CPV.

**Discussion**

Hashemi is the most popular Iranian local variety that is used in rice breeding programs. Thus, it is necessary to investigate the genetic factor controlling its cooking and eating quality. In this study, a permanent mapping population, the recombinant inbred lines (RILs) population (obtained by a cross between Hashemi and Nemat), was used to investigate the main effects of QTLs controlling the eating and cooking quality in rice. The relationships between AC, GT and viscosity parameters PKV, HPV, BDV, CSV, SBV and CPV were different from the results of another RILs population which derived from parents with different AC, suggesting that the relationship between AC, GT and RVA profile parameters is genotype-dependent (Wang et al., 2007; Zhang et al., 2008). Overall 54 QTLs were controlled cooking and eating quality, were found on all chromosomes except chromosomes 4 and 12 (Table 3, Fig. 1). Our results show that cooking and eating quality is a complex trait which is monitored by many regions in the rice genome. Twenty two QTLs were detected on chromosome 6 in which trait at least one major QTL was mapped for each trait on this chromosome. Thus, chromosome 6 played an important role in controlling cooking and eating quality. Our results are supported by several studies (He et al., 1999; Wang et al., 2007; Sabouri et al., 2012). Several studies have investigated the genetic basis of cooking and eating quality of rice. AC is mainly controlled by the *Wx* region on chromosome 6 (Tan et al., 199; Aluko et al., 2004; Sabouri et al., 2012). However, our results derived from Iranian RILs population (indica/indica crosses) detected one major QTL for AC (*qAC6b*) located at interval RM586-RM190 (tightly linked to *Wx* locus) in all three years. Sabouri et al. (2012) reported this QTL exactly at the same location for AC. Several minor QTLs identified on chromosomes 1, 3 and 5 for AC are reported for the first time in this study. Gelatinization temperature (GT) is controlled by a major QTL on chromosome 6 at a location coincided with the *Alk* locus (He et al., 1999; Umemoto et al., 2002; Wang et al., 2007). In this study one major QTL was detected on chromosome 6 at interval RM4128-RM402 near to *Alk* locus in all three years. Our results for AC and GT were in consistent with Sabouri (2009) who used F2:3 populations derived from cross between two Iranian rice variety (Tarommahalli/Khazar). However they did not report any QTLs for AC and GT on chromosome 6. The evidence from QTL co-localization indicated that the quality of cooked rice can be directly evaluated by viscosity parameters. AC and GT have been long used as indices of rice cooking and eating quality (Juliano 1985; Webb 1991). Our study showed that most of identified QTLs for viscosity parameters were co-localized with the QTL of AC or GT. However, chromosomal regions or genes that control AC and GT traits are involved in monitoring most of the viscosity parameters. Results from this study, demonstrated that all viscosity parameters were typically quantitative traits controlled by a major and several minor genes. We identified one major QTL for PKV on chromosome 6 (*qPKV6a*) close to *Wx* locus which repeated over three years, but was in consistent with other studies (Bao et al., 2003; Wang et al., 2007; Zhang et al., 2008). However Bao et al. (2002) reported a QTL on chromosome 6 for PKV which had different location compared to this study. For HPV, a major QTL on *Wx* locus (*qHPV6b*) was identified in 2010 and another major QTL near to the *Wx* locus (*qHPV6a*) was repeated over two years (2009 and 2011) and was in consistent with other studies (Bao et al., 2002 and 2003; Wang et al., 2007; Zhang et al., 2008; Liu et al., 2011). Several studies have also reported minor QTLs for HPV on chromosomes 1, 5, 7 and 9 but in different location of these chromosomes (Wang et al., 2007; Liu et al., 2011). For BDV, a QTL was detected at *Wx* locus on chromosome 6 over two years. Bao et al. (1999) and Wang et al. (2007) also reported a major QTL for BDV on this location. The other minor QTLs were identified on chromosomes 1, 2, 5 and 8 for BDV in this study were previously reported by other researchers (Bao et al., 1999; Liu et al., 2011), except a minor QTL detected on chromosome 11. Cool paste viscosity (CPV) was controlled by *Wx* and *Alk* genes, because we found one QTL at *Wx* locus and another QTL near to the *Alk* locus on chromosome 6, which was the same as in the previous studies (Wang et al., 2007; Zhang et al., 2008). Several minor QTLs recognized for CPV on chromosomes 1 and 7 had reported in the previous studies (Wang et al., 2007; Liu et al., 2011), whereas some of them on chromosomes 5 and 9 are reported for the first time in this study. The parameter CSV is derived from CPV and HPV, and one major QTL for this trait (*qCSV6a* detected over three years repeatedly) was co-localized with the QTL for CPV (*qCPV6a*). One minor QTL for CSV (*qCSV6b*) was co-localized with QTL of HPV (*qHPV6b*). The other detected QTLs for CSV on chromosomes 1, 2 and 10 had previously reported by others (Zhang et al., 2008; Liu et al., 2011). The parameter SBV is also a secondary viscosity parameter (similar to BDV and CSV) and derived from CPV and PKV. We found one QTL for SBV (*qSBV6* near to the *Alk* locus on chromosome 6 and repeated in three years) which was co-localized with QTL of CPV (*qCPV6a*). In this study, we were not able to investigate any co-localized QTL between SBV and PKV which is in consistent with the result of Wang et al. (2007). Two detected QTLs on chromosomes 1 and 9 (*qSBV1* and *qSBV9*) had previously reported for SBV (Liu et al., 2011; Wang et al., 2007), and two minor QTLs on chromosome 11 (*qSBV11a* and *qSBV11b*), are reported for the first time. In this study, two clusters harboring 7 QTLs at the *Wx* and adjacent to the *Wx* locus, were found to control

the 6 rice quality traits simultaneously (Fig. 1). One of the seven QTLs was found in all three years, but two of them were found in two years. Results suggested that the *Wx* gene region had a main effect for controlling cooking and eating quality of rice. Wang et al. (2007) found one cluster at *Wx* locus harboring 9 QTLs related to rice quality traits and suggested that *Wx* gene controls most of the rice cooking and eating quality traits. Another cluster harboring 4 QTLs were found on chromosome 6 close to the *Alk* locus which controls 4 rice quality traits simultaneously in this study (Fig. 1). All four QTLs detected repeatedly over three years, which subjected their high stability and repeatability. Wang et al. (2007) found one cluster harboring 8 QTLs in RM276-RM549 close to the *Alk* locus (like this study), which seven QTLs were repeated over two years. They also suggested that *Alk* gene beside the *Wx* gene controls rice cooking and eating quality. Taken our observation together, the identified QTLs close to *Wx* and *Alk* genes exhibit a significant effect on controlling cooking and eating quality in rice. Further studies are needed for fine mapping of these QTLs and to investigate the sequence variation at both *Wx* and *Alk* loci and their association with the traits characterizing eating and cooking quality of rice thus facilitate the breeding of these traits.

## Materials and methods

### Mapping population and field experiment

The mapping population consisted of 144 rice recombinant inbred lines (RILs; F9, F10 and F11) was developed from a cross between Hashemi (indica)/Nemat (indica) by the single seed descent method (Supplementary Table 1). Hashemi is a local cultivar that is adapted to the northern region of Iran. Which is widely cultivated by farmers in this area because of its good cooking quality and. However, Nemat is an improved Iranian variety derived from IR24/Hasansaraiee// Sangetarom crosses. The field experiment was conducted in the rice growing season in the experimental farm at Rice Research Institute of Iran (RRII), Rasht, Iran over three years (2009-2011). Recombinant inbred lines (RILs) were sown in April and transplanted in May 8 in each year (F9, F10 and F11 were used in 2009, 2010 and 2011, respectively). Sixty four plants from each parent and RILs were transplanted in 2m × 2m plots with 8 rows in an augmented design. The spacing was 25 cm either between or within the rows. Only six middle rows in each plot were used for measuring traits. Field management essentially followed the normal agricultural practices.

### *Trait measurement*

Approximately 40 days after heading, the plants were harvested in bulk and the hulled grain was air-dried and stored at room temperature for 30 days before milling. The grains were dried to a moisture content of 13.0%, dehulled and milled to white rice using a Satake Rice Machine (Satake Corporation, Japan). They were grounded to flour in a Cyclone Sample Mill (UDY Corporation, Fort Collins, Colorado, USA), and sieved through a 100-mesh sieve. Samples were refrigerated until analysis.

### *Evaluation of AC and GT*

Amylose content (AC) was estimated following procedure described in Juliano (1971). Rice flour weighting 100 mg was extracted in a solution of 1 ml 95% ethanol and 10 ml 1 N NaOH. The samples were kept 10 min in water bath at 100 °C followed by cooling at room temperature. The volume of the extract to 50 ml was adjusted with distilled water, and 5 ml was transfered into a fresh 100-ml volumetric flask and mixed with 92 ml of distilled water, 1 ml 1M acetic acid and 2 ml iodine (0.2% w/v $I_2$ in 2%KI), and kept 20 min at room temperature. The optical density of the amylose-iodine blue color was measured at 620 nm using a Spectrophotometer (Model 3000 series, Cecil, Italy). The gelatinization temperature (GT) was determined using the method by Little et al. (1958) with a minor modification. Two sets of six random polished rice grains from each line in two replication was immersed in a freshly prepared 1.7% KOH solution and incubated at 30° C for 23 h. The spreading of the rice grains was recorded by visual observation in seven categories from 1 (unaffected) to 7 (completely dissolved).

### *RVA profile measurement*

The RVA paste viscosity was determined using a Rapid Visco Analyzer (RVA) (Model No. RVA-3D, Newport Scientific, Sydney, Australia), according to the standard method AACC61-02 given by the American Association of Cereal Chemists (2000). The RVA was running with Thermocline Windows Control and Analysis Software, Version 1.2 (Newport Scientific, Sydney, Australia). Approximately 3 gr rice flour was mixed with 25 ml water; a paddle was placed in the canister and rotated at 960 rpm for 10 s to disperse the rice sample. The viscosity was evaluated using a constant paddle rotation of 160 rpm. The sequential temperature curves for a 12.5 min test were determined as: (1) incubate at 50° C for 1.0 min; (2) increase to 95° C; (3) keep at 95° C for 1.4 min; (4) cool down to 50° C; and (5) hold at 50° C for 1.4 min. Viscosity values were recorded in Rapid Visco Units (RVU). The starch viscosity characteristics including the following original components: Peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), break down viscosity (BDV), setback viscosity (SBV) and consistency viscosity (CSV) were calculated based on the original data: BDV = PKV – HPV; SBV = CPV - PKV and CSV = CPV - HPV. In addition, pasting temperature (temperature of the initial viscosity increase, PaT) and peak time (time of the initial viscosity increase, PeT) were also recorded. Some of the important Japanese varieties (like Todorokiwase, Owarihatamochi and Kinuhikari) had good cooking and eating quality for Japanese taste with low AC and CSV, high PKV and BDV. In contrast, the Iranian cultivar (like Hashemi and Local Sadri) with good cooking and eating quality for Iranian taste had intermediate levels of AC and CSV while recording high PKV and BDV (Allahgholipour et al., 2006).

### *DNA markers and assays*

Genomic DNA was extracted from fresh leaves of each line by cetyltrimethyl-ammonium bromide (CTAB) method (Murray and Thompson, 1980). PCR amplification was carried out in a total volume of 10 µl containing 2 µl of genomic DNA (5 ng/µl), 0.25 µl forward and reverse primers, 0.12 mM dNTPs, 0.9 U Taq polymerase, 0.24 µM of MgCl2 and 1 µl 10X PCR buffer. PCR amplification was performed on a thermal cycler (Model T-Gradiant, Bio-Rad, USA) at an initial temperature of 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s (primer annealing occurred with most of the primers while some were adjusted), 72° C for 2 min and a final cycle of 72° C for 5 min and then stored at 4° C. PCR products were separated by electrophoresis in 10% polyacrylamide gel (PAGE) with

0.8% cross-linker (ratio of bis-acrylamide to acrylamide) in 0.5 × tris-borate EDTA (TBE) buffer. The resolved PCR bands were detected by staining with ethidium bromide (EtBr). In total, 500 SSR markers covering all 12 chromosomes with known sequences and chromosome locations, RM-series (Temnykh et al., 2000; McCouch et al., 2002) were tested for parental polymorphisms in the analysis of the population. About 178 (177 SSR marker and one STS marker) exhibited polymorphisms between parental lines (Supplementary Table 2). The polymorphic markers were associated with all rice chromosomes ranged from 4 to 26 markers per chromosome. Data generated after genotyping of 144 RILs by polymorphic SSR markers were tested for 1:1 segregation ratio using the $\chi^2$ goodness of fit test. Seven loci showed deviation from 1:1 ratio and thus were excluded from further analysis. Mapmaker 3.0 was used to construct a genetic linkage map (Lincoln et al., 1992). The total genetic map lengths were 1851.1 cM with an average interval of 10.8 between adjacent markers.

***Data and QTL analysis***

Some statistical analysis such as orthogonal comparison of the parental genotypes, parents versus population lines and simple and combined analysis of variance (ANOVA) were done by using PROC GLM ver.9.0 of SAS software (SAS, 2003). Inclusive composite interval mapping (ICIM) (Wang, 2009) was used to map the QTLs and to estimate the phenotypic variation explained (PVE), as implemented in the integrated software QTL Ici Mapping for mapping quantitative trait loci (available from http://www.isbreeding.net). Marker variables were considered in a linear model in ICIM for additive mapping. Two steps were included in ICIM. In the first step, stepwise multiple linear regression was applied to identify the most significant regression variables with different probability levels of entering and removing variables. In the second step, a one-dimensional scanning or composite interval mapping was conducted for mapping additive effect. Composite interval analysis was undertaken using a stepwise multiple linear regression with a probability of 0.001 in and out of the model and the window size set at 1 cM. Significant thresholds for QTL detection were calculated for each data set using 1000 permutations and a genome-wide error rate at 0.01. Broad-sense heritability (%) percentage was calculated as $h^2 = \sigma^2_g / ( \sigma^2_g + \sigma^2_e / n)$, where $\sigma^2_g$ is the genotypic variance, $\sigma^2_e$ is the error variance and n is the number of environments (Wang et al., 2007). Correlation coefficients among measured traits were calculated using PROC CORR in SAS 9.0 software (SAS, 2003).

**Conclusion**

In conclusion, based on the comparison of chromosomal location, the result showed that some QTLs are likely in the same locus as in previous study. However, it is difficult to determine whether QTLs are in the same locus or are tightly linked. Therefore, further analysis, including fine mapping of some QTLs using common markers, cloning and sequence comparison of these QTLs, will be required to answer these questions. Based on these results, we conclude that the *Wx* locus on chromosome 6 controlled AC, PKV, HPV and BDV and *Alk* locus on this chromosome controlled GT, CPV, CSV and SBV. All detected QTL on *Alk* locus are major QTL and were detected in all three years. In addition to the major genes, attention should also be directed to the effects of minor QTLs. Pyramiding of all favorable alleles into a recipient background could be useful for improvement of grain cooking and eating quality. The information obtained in this study could be useful in marker-assisted fast track improvement of grain cooking and eating quality of rice cultivars.

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