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# Mapping quantitative trait loci (QTL) associated with cooking quality in rice (*Oryza sativa* L.)

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**Abstract**

A mapping population consisting of 236 $F_{2:3}$ families derived from the cross between two rice varieties, Gharib as female parent (with good cooking quality) and Sepidroud as male parent (with poor cooking quality) was used to analyze the quantitative trait loci (QTLs) associated with amylose content (AC), gelatinisation temperature (GT) and gel consistency (GC). A total of 105 single sequence repeat (SSR) markers were used to construct a genetic linkage map, covering a total length of 1440.7 cM of the genome in rice (*Oryza sativa* L.) with an average distance of 13.72 cM between markers. Twelve independent QTLs were identified using composite interval mapping. These loci consisted of three QTLs for GT, eight QTLs for AC and one QTL for GC, most of which are reported here for the first time. For GT the QTL explaining the largest proportion of variance (18.4%) was located on chromosome 6, the same locus as the alkali degeneration gene (*alk*). For AC, four QTLs were found on chromosome 6, one of which was located at the interval RM586-RM190 explaining 19.3% of the total variation and which should coincide with the waxy region (*wx)* located on the short arm of this chromosome. The results using Iranian rice cultivars, in combination with previous reports further confirmed that *alk* and *wx* regions play a considerable role in determining cooking and eating quality of rice.

**Keywords**: *alk*; Amylose Content (AC), Gelatinisation Temperature (GT), Gel Consistency (GC), SSR, QTL, *wx*.
**Abbreviations:** AC- Amylose Content; GT- Gelatinisation Temperature; GC- Gel Consistency; SSR- Single Sequence Repeat; QTL- Quantitative Trait Loci.

## Introduction

Rice (*Oryza sativa* L.) is one of the major crops feeding more than 50% of the world's population (Brar and Khush, 2002). Grain quality is an important criterion in rice production and a major factor in rice marketing. Grain quality preferences vary among ethnic groups and/or geographical regions (Juliano et al., 1964). Iranian people like less sticky rice with intermediate amylase, therefore rice breeding for cooking and eating quality is an important objective in Iran. The three key components determining cooking and eating quality are amylose content, gelatinisation temperature and gel consistency. Amylose content (AC) is regarded as the most important indicator in classifying rice varieties (Juliano et al., 1964) because it influences texture and retrogradation potential of cooked grains (Champagne et al., 1973). Rice varieties are classified into high (>25%), intermediate (20-25%), low (10-19%), very low (3-9%), or waxy (0-2%) amylase classes (Kumar and Khush, 1987). Gelatinisation temperature (GT) is used in varietal development as an indicator of the cooking time of rice samples. It is an economically important indicator of quality because selecting for shorter cooking times leads to significant potential savings in fuel costs thus; GT is a significant component of the carbon footprint of rice. Three classes of GT are recognized in rice breeding programs: high (>74 °C), intermediate (70-74 °C), and low (<70 °C) (Jennings et al., 1979). Gel consistency (GC) is a measure of firmness of the rice after cooking and is performed to classify rice varieties of the same AC, particularly in the high AC class, into hard, medium, or soft texture. The GC is commonly measured by determining the length of a cooled gel made from flour previously cooked in 0.2 M KOH (Cagampang et al., 1973). GC is a measure of the strength of the gel. The range of GC values to classify rice varieties according to this property is wide. Samples are grouped into arbitrarily set classes based on the length of the gel: hard (length of gel < 40 mm), medium (length of gel 41-60 mm), and soft (length of gel > 61 mm). Weak and rigid gels depend on the association of starch polymers in the aqueous phase (Dea, 1989). Most of these grain quality traits of rice are controlled by quantitative trait loci (QTLs) showing continuous variation in rice progeny (Yano and Sasaki, 1997; He et al., 1999). Molecular marker technology has facilitated the understanding of the genetic basis of complex quantitative traits such as eating quality in rice (McCouch et al., 1988; He et al., 1999). So far, several studies reported the QTLs for rice grain quality by different populations (He et al., 1999; Lanceras et al., 2000; Septiningsih et al., 2003; Aluko et al., 2004; Li et al., 2004; Tian et al., 2005; Takeuchi et al., 2007; Takeuchi et al., 2008; Sabouri 2009). Most of these experiments indicated that a major gene known as *waxy* gene (*wx*) that encodes a granule-

bound starch synthase (Wang et al., 1990) itself or the genome region tightly linked to it on chromosome 6 controls AC in rice with some minor QTLs that also influenced AC (He et al., 1999; Bao et al., 2000; Lanceras et al., 2000; Septiningsih et al., 2003; Aluko et al., 2004; Takeuchi et al., 2007). Additionally some researchers reported *wx* gene region is involved in the control of all the three traits for cooking and eating quality of rice (Tan et al., 1999). But several studies found the effect of *alkali* locus (*alk*) on GT, which encodes soluble starch synthase II (SSSII) isoform and was cloned by Gao et al., (2003). On the other hand some minor QTLs were detected for this trait (He et al., 1999; Tian et al., 2005). However, GC is controlled either by the *wx* gene (Tan et al., 1999; Lanceras et al., 2000) or by some QTL with minor effects (He et al., 1999; Bao et al., 2000). Although there are some reports which found no QTL related to *wx* gene (Bao et al., 2000; Sabouri, 2009). According to the gramene database (http://www.gramene.org) for rice cultivars in the present study, to date 51 QTLs for AC, 20 for GT and 22 for GC have been identified. Information about molecular markers found tightly linked to the QTLs that control AC, GT and GC with relatively large phenotypic effects on these traits will facilitate breeding strategies in improving rice grain quality. So far, in terms of marker-assisted selection (MAS), the *wx* gene and eating quality can be applied (Suzuki et al., 2003; Zhou et al., 2003; Tanaka et al., 2006). Zhou et al., (2003) applied the identified QTL-marker associated to rice quality improvement through introgression of *waxy* gene region from Minghui63 to Zhenshan 97. They simultaneously improved four quality traits (AC, GA, GT and opacity) of Zhenshan 97, an elite parent of hybrid rice, by molecular MAS. In Iran, despite the low yields of local varieties (2 to 4 tones/ha) around 70% of the total rice area is still devoted to these varieties because of their excellent quality traits, which are similar to Basmati types (Nematzadeh et al., 2000). Unfortunately, genetic information on Iranian rice germplasm is limited and there are few reports about QTL analysis especially grain quality in Iranian varieties. In order to increase understanding of the genetic basis of the eating quality of Iranian rice germplasm, Gharib (GHB) an elite *indica* traditional rice variety in Iran with good eating quality, was crossed with Sepidroud (SPD) an *indica* improved cultivar with poor quality but known high yielding variety in Iran and 236 $F_{2:3}$ families were developed. In this study, we presented the results obtained from QTL analysis of AC, GT and GC in Iranian rice background and compared them with other genetic backgrounds. This will provide an opportunity to define the QTLs involved in eating and cooking quality in the Iranian cultivar background.

## Results

### P*henotypic evaluations and correlation relationships among traits*

The parents differed significantly in three measured traits. The female parent, GHB, had good grain quality properties according to preferences of Iranian customers with 20.1% AC, soft GC (70mm) and high intermediate GT (3.6). The male parent, SPD, had bad grain quality properties with 27% AC, hard GC (30mm) and low GT (7). Phenotypic values of parents and t-test for grain traits studied are shown in table 1. In the $F_3$ families, all the traits showed continuous variation (Fig 1). It should be pointed out that a large majority of the population fell into the low GC group (around of 30 mm) and showed a skewed distribution. We therefore used logarithmic transformation to normalize before QTL analysis.

Correlation analysis revealed that AC was significantly and positively correlated with GT ($r=0.265$, $p<0.01$), but did not show significant correlation with GC. Additionally GT was significantly and negatively correlated with GC ($r=-0.162$, $p<0.05$).

### *Population structure and linkage map*

In the present study, of the 501 SSR markers tested, 105 produced polymorphic clear and scorable bands between the two parents and were used for the amplification of $F_2$ population. A linkage map based on $F_2$ population was constructed, which covered a total of 1440.7 cM with an average two loci interval of 13.73 cM (Fig 2).

### QTL mapping

#### *QTLs for amylose content (AC)*

Eight QTLs were mapped for AC. Three QTLs with largest effects at the interval RM586-RM190, RM7434-RM5371 and RM5371-RM340 were located on chromosome 6 designated as *qAC-6a*, *qAC-6c* and *qAC-6d* which explained 19.3%, 16.1%, and 18.3% of the total phenotypic variance, respectively. The additive and dominance effects for these QTLs were negative and positive for decreased and increased AC, respectively and all three QTLs showed positive to incomplete dominance effects for increased AC. Furthermore for all last mentioned QTLs, the alleles from GHB had negative effects for reduced AC. Rest of the QTLs on other chromosomes had small effects on AC (Table 2, Fig 2). Two QTLs had overdominance effects toward reduced AC and others exhibited partial or incomplete dominance effects for increased or decreased AC.

#### *QTLs for gelatinisation temperature (GT)*

Three QTLs were detected for GT located at the interval RM217-RM276, RM549-RM6832 and RM3-RM7434 on chromosome 6 and explaining 18.4%, 12.0% and 7.5% of the total phenotypic variance, respectively. The additive effects from SPD alleles were positive whereas the dominance effects were negative. All QTLs exhibited partial dominance effects for decreased GT (Table 2, Fig 2).

#### *QTL for gel consistency (GC)*

One QTL *qGC-7* mapped for GC at the interval RM3555-RM420 on chromosome 7 also showed minor effects on GC. This QTL explained only 4.5% of the total phenotypic variance showing overdominance effect for increased GT. (Table 2, Fig 2). The additive and dominance effects of this QTL were 2.80 and 3.77 for increased GT and the alleles for increased GC were from GHB.

## Discussion

Recent studies demonstrated that genetic linkage maps constructed with various DNA markers and different mapping populations such as F2, RIL, BC etc are very useful for the analysis and detection of QTLs (Chandel et al., 2011; Sabouri et al., 2011). In spite of the fact that $F_2$ population derived from $F_1$ hybrid is one type of mapping population, its main advantage is that it is easy to conduct and requires only a short time to produce (Collard et al., 2005), but $F_2$ plants do not allow precisely determining the phenotype of complex quantitative traits through replicated experiments because $F_2$

**Table 1.** Phenotypic values of parents and t-test for grain traits studied

| | Parents | | |
|---|---|---|---|
| Trait[a] | GHB[b] | SPD[b] | t-Value (Sig.) |
| AC | 20.11±0.17 | 27.01±0.21 | 24.88 (0.00) |
| GT | 3.60±0.11 | 7.00±0.00 | 29.58 (0.00) |
| GC | 70.00±0.39 | 30.00±0.39 | 71.78 (0.00) |

[a] The bbreviation of traits consists of AC, Amylose content; GT, gelatinisation temperature; and GC, gel consistency. [b] GHB and SPD means Gharib and Sepidroud, respectively.

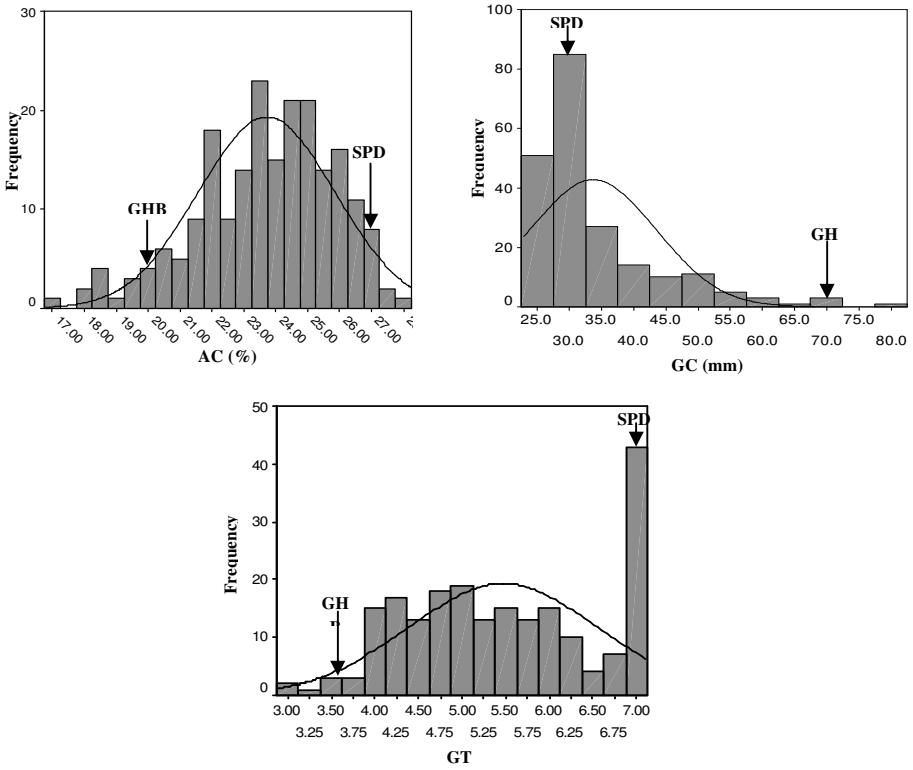


**Fig 1.** Distribution of amylose content (AC), gelatinization temperature (GT) and gel consistency (GC).

population includes individual plants without replication. Therefore if genotyping using $F_2$ plants is combined with $F_3$ or $F_4$ families in order to get phenotypic data from mean of each family for each plant, it will increase the precision of phenotyping in comparison with phenotyping using $F_2$ individual plants. In the present study, we used a $F_{2:3}$ population derived from crosses between Gharib (GHB) and Sepidroud (SPD) as female and male parents respectively to understand the genetic basis of AC, GT and GC. The analysis of the $F_3$ families indicated that AC and GT had normal distribution as illustrated in Fig 1, indicating the polygenic control of traits but GC showed skewed frequency distribution toward the larger values of trait and a large number of $F_3$ lines fell into the hard gel group (26-40 mm). Thus before QTL analysis we used a proper transformation to normalize the data and after logarithmic transformation normal test confirmed normal distribution in data. AC, GT and GC are the three most important traits in determining rice cooking quality and market class (Little et al., 1958; Juliano et al., 1964; Cagampang et al., 1973). A total of 12 QTLs were detected for the three traits as summarized in Table 2, Fig 2. It is notable that chromosome 6 contains QTLs responsible for rice grain qualities. These results are supported by several studies (He et al., 1999; Lanceras et al., 2000; Takeuchi et al., 2007). Out of 12 detected QTLs for three traits in this study, seven QTLs were located on chromosome 6. Eight QTLs were mapped for AC on chromosomes 1, 2, 3, 4 and 6. The largest-effect QTLs were located at the *waxy* locus with another two QTLs on chromosome 6. Amylose content is known to be related with variation at the *waxy* locus on chromosome 6 with several modifiers (He et al., 1999; Lanceras et al., 2000; Septiningsih et al., 2003; Aluko et al., 2004; Takeuchi et al., 2007). However our study indicated that in a population derived from crossed between *Iranian* cultivars (*indica* × *indica*), *waxy* locus is involved in control of AC by *qAC-6a* located at the interval RM586-RM190 at *waxy* gene region, which explains 19.3% of the total phenotypic variance and seven

**Table 2.** Putative QTLs for quality traits in $F_{2:3}$ rice population derived from GHB and SPD cross.

| Trait | QTL[a] | Chr. | Flanking markers | LOD | a[b] | d[c] | d/\|a\| | PEV[d] | Dpe[e] |
|---|---|---|---|---|---|---|---|---|---|
| AC | | | | | | | | | |
| | *qAC-1* | 1 | RM8132-RM237 | 3.10 | -3.530 | 0.719 | 0.203 | 3.8 | GHB |
| | *qAC-2* | 2 | RM424-RM262 | 2.60 | 3.950 | -0.693 | -0.175 | 3.7 | SPD |
| | *qAC-3* | 3 | RM416-RM7389 | 2.52 | -0.342 | -0.556 | -1.624 | 3.5 | GHB |
| | *qAC-4* | 4 | RM273-RM5473 | 2.54 | 1.125 | -0.942 | -0.837 | 3.6 | SPD |
| | *qAC-6a* | 6 | RM586-RM190 | 4.08 | -0.382 | 0.282 | 0.736 | 19.3 | GHB |
| | *qAC-6b* | 6 | RM276-RM402 | 3.67 | 0.546 | -1.015 | -1.858 | 16.1 | SPD |
| | *qAC-6c* | 6 | RM7434-RM5371 | 3.22 | -4.340 | 0.846 | 0.190 | 5.8 | GHB |
| | *qAC-6d* | 6 | RM5371-RM340 | 3.79 | -3.910 | 1.570 | 0.400 | 18.3 | GHB |
| GT | | | | | | | | | |
| | *qGT-6a* | 6 | RM217-RM276 | 4.02 | 0.66 | -0.072 | -0.109 | 18.4 | SPD |
| | *qGT-6b* | 6 | RM549-RM6832 | 3.14 | 0.543 | -0.183 | -0.337 | 12.0 | SPD |
| | *qGT-6c* | 6 | RM3-RM7434 | 2.52 | 0.480 | -0.131 | -0.270 | 7.5 | SPD |
| GC | | | | | | | | | |
| | *qGC-7* | 7 | RM3555-RM420 | 3.47 | 2.800 | 3.770 | 1.340 | 4.5 | GHB |

[a] QTLs are named by abbreviations plus chromosomal number. [b] Additive effect. [c] Dominance effect. [d] Percentage of explained variance or Percentage of total phenotypic variance explained by the QTL. [e] Direction of phenotypic effect, GHB and SPD indicate Gharib and Sepidroud, respectively. Amylose content (AC), gelatinisation temperature (GT) and gel consistency (GC).

QTLs dispersed on five chromosomes explained rest of the total phenotypic variance. Nevertheless, our results differed from Sabouri (2009) who detected no QTL on chromosome 6 for AC in his study using another Iranian population derived from Tarommahalli × Khazar cross. However, several factors influenced results of mapping genetic projects such as type of population, trait value differences between two parents, method of phenotyping and interaction between QTL by QTL and QTL by environment (Liu 1998). In addition, observing the different levels of partial, incomplete and overdominace effects could explain the complexity of AC. To our knowledge as mentioned before except *qAC-6a* and *qAC-6b* which was in agreement with He et al., (1999), Lanceras et al. (2000), Septiningsih et al. (2003), Aluko et al. (2004) and Takeuchi et al. (2007), the rest of the identified QTLs on chromosomes 1, 2, 3, 4 and 6 in the present study are being reported here for the first time. Gelatinisation temperature (GT) is controlled by a major QTL on chromosome 6 at a location coinciding with the *alk* locus (He et al., 1999; Lanceras et al., 2000; Umemoto et al., 2002) and/or *waxy* gene region (Tan et al., 1999). Also some minor QTLs were reported for GT (He et al., 1999; Tian et al., 2005). Our results on Iranian population GHB × SPD are in agreement with researchers who concluded that GT is controlled by *alk* gene region and are also in agreement with others who mapped some minor QTLs for GT. We detected three QTLs for GT which are *qGT-6a* located at the interval RM217-RM276, in the vicinity of *alk* locus, *qGT-6b and qGT-6c* that explained 18.4%, 12.0% and 7.5% of the total phenotypic variance of GT, respectively. We found only one minor QTL for GC on chromosome 7 that explained 4.5% of the total phenotypic variance. However we detected no QTL in the *waxy* gene region which was in agreement with Bao et al. (2000), Takeuchi et al. (20080 and Sabouri (2009). Our results revealed that there are regions on chromosome 6 that comprise QTLs in the vicinity of each other such as *qGT-6a* and *qGT-6b* adjacent to *qAC-6b* and *qAC-6c* respectively, affecting related traits GT and AC which could provide a reason for the phenotypic correlation observed between AC and GT ($r=0.265$, $p<0.01$). However according to our results we found no reason related to QTL analysis for significant correlation between GC and GT. Significant correlation can be the result of linkage, pleiotropy or environmental effects. However the possibility that these factors were responsible for this correlation should be considered. Most of the QTLs found here showed a range of partial to overdominance effects, indicating complexity of the traits under consideration (table 2). In most of the cases, degree of dominance (d/|a|) was low or near to 0, suggesting the importance of additive or partial dominance effects for the respective QTLs. Furthermore, there were some cases of overdominance toward increased or decreased in a trait of interest which can be associated with observed heterosis in $F_1$ hybrids (data not shown). Although, additive and dominance genetic effects could result from the present cross, one should take into consideration that epistatic effects and linkage may upwardly bias the dominance, and even partial dominance estimation to become pseudo-overdominance (Falconer and Mackay, 1996). Grain quality is an economically important character in rice varieties and any knowledge of genetic mechanism or major and minor genes affecting it will be beneficial for rice breeders and accelerate the process of breeding new rice varieties with both a higher yield and a better quality (He et al., 1999). As mentioned before most Iranian rice varieties are well-known because of their excellent quality but potentially are low yielding, tall (>130 cm) with fragile stem and very susceptible to lodging. So we have to explore the quality controlling gene from popular cultivars and integrate them into high yielding varieties. In fact, our main objective was to breed these complex traits and gather favorite traits into suitable background. Thus we selected GHB and SPD as parents for the present population because they differed significantly in yield and yield component besides differences in quality traits as GHB is a tall, low tillering and low yielding but good quality, while SPD is dwarf, heavy tillering and a known high yielding but poor quality. We are going to accomplish QTL analysis of yield and component yield using the present population. Certainly a genotype with high yield and good quality can be obtained by pyramiding the positive alleles for important traits using MAS after finding stable QTLs and their conformation.

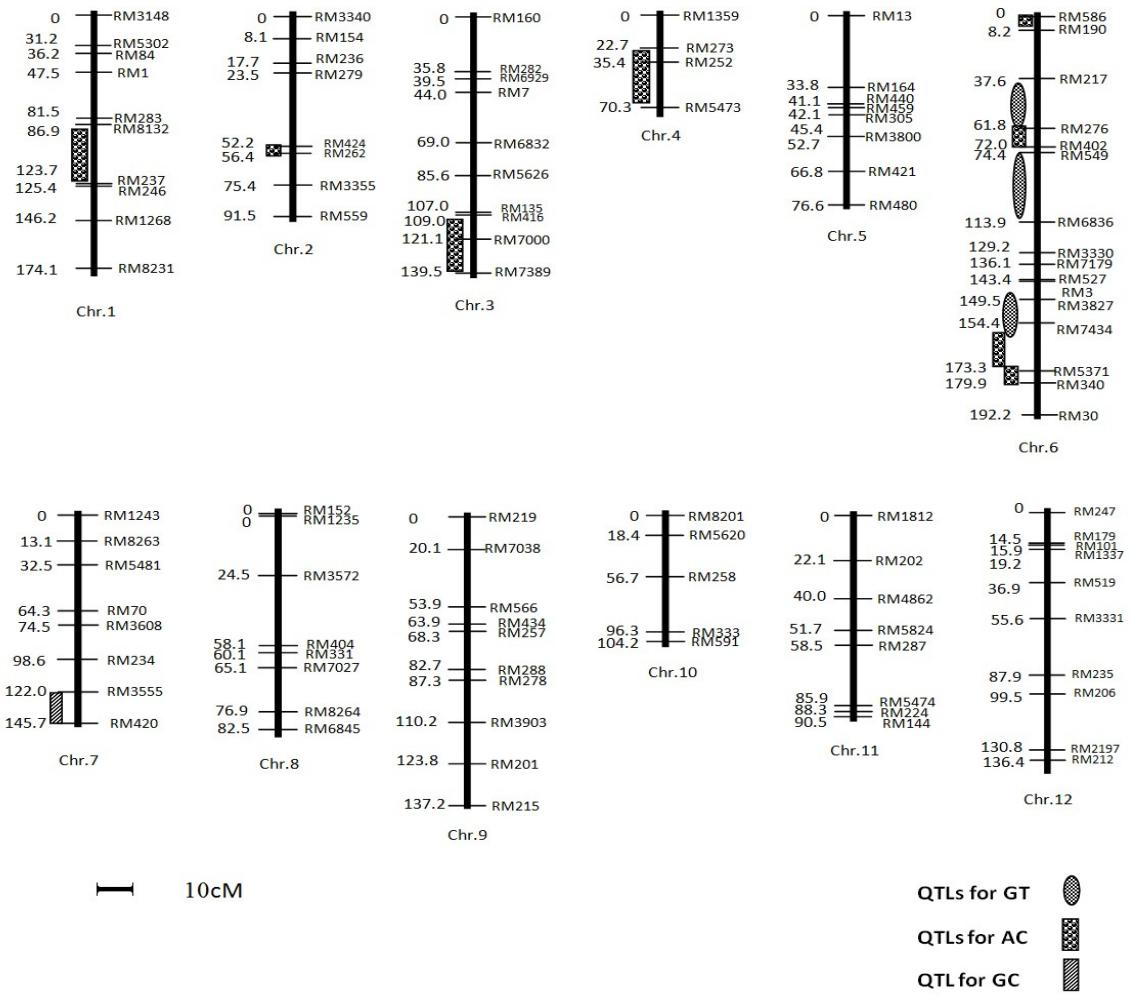


**Fig 2.** Linkage map from 236 $F_2$ from Gharib and Sepidroud with 105 SSRs markers were used to locate QTLs for Amylose content (AC), gelatinisation temperature (GT) and gel consistency (GC). Significance threshold for composite interval mapping determined at LOD=2.5.

## Materials and methods

### *Plant materials*

The genetic material involved 236 $F_3$ families, each derived from bagged seeds of a single $F_2$ plant from a cross between two indica rice varieties, Gharib (GHB) and Sepidroud (SPD) as female and male parents, respectively. All the $F_3$ families and their parental lines were grown in the experimental farm of the Rice Research Institute of Iran (RRII), Rasht, Iran, in 2010. All seeds were stored at room temperature for a period of at least three months after harvesting, before quality characteristics were measured. All the traits for each F2 plant were recorded as the average data from at least 17 F3 progenies.

### *Collection of phenotypic data*

### *Amylose content*

Amylose content (AC) was measured as described by Williams et al., (1958), Perez and Juliano, (1978) and Li et al., (2004). Two 100-mg samples of milled rice flour were placed in 100-mL volumetric flasks and 1mL of 95% *v/v* ethanol was added. Then the volumetric flasks were shaken quietly to disperse the powder in ethanol and 9 mL of 1M NaOH was added to the samples. The samples were boiled for 10 min in a boiling water bath (100°C), followed by cooling to room temperature and were then diluted to 100mL with distilled water. A 5-mL sample suspension was removed and added to 70mL distilled water in a 100-mL volumetric flask. Then 1mL of 1M acetic acid and 2mL iodine (0.2% *w/v* $I_2$ in 2% KI) solution were added to each sample and distilled water was added to a volume of 100mL, mixed well and kept for 20min. The optical density of the amylose-iodine blue color was measured at 620 nm using a spectrophotometer.

### Gelatinisation temperature

To determine gelatinisation temperature (GT), milled rice derived from six-grain samples were put into small plastic petri dishes, and 5 mL of 1.7% *w/v* KOH solution was added to each petri dish. The petri dishes were incubated at 30°C for 23 hr. The degree of spreading and dissolving out of the

grains was evaluated using a 7-point scale as described by Little et al., (1958) via visual observation in seven categories from one (unaffected) to seven (completely dissolved). Each sample was tested in two replications. Grains with scale of 1-3 are recorded as high (>74 °C), with 4 or 5 as intermediate (70-74 °C) and a score of 6 or 7 is recorded as low (<70 °C) gelatinisation temperature (Jennings et al., 1979).

***Gel consistency***

Two samples of 100 mg of milled rice flour were placed in a 10mm × 110mm culture tube with 0.2 mL of 95% *v/v* ethanol containing 0.025% thymol blue (Cagampang et al., 1973). Then 2 mL of 0.2 M KOH solution was added to each sample and mixed and the tubes were placed in a boiling water bath (100°C) for eight min. Next the tubes were removed and were mixed again and cooled for 5 min at room temperature, followed by 20 min in an ice water bath. The tubes were then laid horizontally on a table surface and gel length (mm) from the bottom of the tube to the top of the gel was measured after 1hr as gel consistency value. Samples were grouped into arbitrarily set classes based on the length of the gel: hard (length of gel < 40 mm), medium (length of gel 41 – 60 mm), and soft (length of gel > 61 mm) (Jennings et al., 1979).

***Construction of SSR linkage map***

Fresh and young leaf tissue was sampled from each $F_2$ plant and genomic DNA was extracted using the CTAB method (Saghai Maroof et al., 1994). Parental survey was conducted to identify polymorphism between the parents using simple sequence repeat (SSR) markers. Five hundred and one SSR primer pairs distributed evenly on 12 rice chromosomes (Chen et al., 1997; Temnykh et al., 2000; McCouch et al., 2002), were tested for polymorphic survey. The primers exhibiting polymorphism were used to amplify the DNA of each $F_2$ plant. Polymerase chain reaction (PCR) was carried out in a total volume of 10 µl per reaction containing 2 µl of template DNA, 0.4 µl of forward and reverse primers each of 10 pmol concentration, 0.6 µl dNTPs (2mM), 0.12 µl *Taq DNA polymerase* (5U/µl), 0.48 µl of $MgCl_2$ (50mM), 1 µl 10x PCR buffer and 5 µl steril nanopure $H_2O$. PCR amplification was performed on a thermal cycler (Applied Biosystems, Germany) in the biotechnology laboratory of the Agricultural Biotechnology Research Institute of North of Iran with thermal cycle profile of 94°C for 5 min (initial denaturation), followed by 35 cycles of 94°C for 30s (denaturation), 55°C for 30s (primer annealing with most of the primers while some were adjusted), 72°C for 2min (extension) and at least 72°C for 5min (final extension) and stored at 4°C. PCR products were separated on 6% polyacrylamide gels (19:1 acrylamide: bisacrylamide) as described by Bassam et al., (1991) and Creste et al., (2001). A SSR linkage map of the $F_2$ population was constructed using QTXb17 Mapmanager (Manly and Olson 1999), and genetic distances (cM) were calculated from recombination values between markers using the Kosambi function (Kosambi 1944).

**QTL analysis**

QTL identification for the studied characteristics was sought with interval mapping using QTL Cartographer v. 2.5 (Basten et al., 1997), keeping a threshold of LOD>2.5 for testing the hypothesis for the presence of QTL. Parameters, such as additive, dominance effects and phenotypic variance explained were also estimated. To identify additional QTLs that may have been masked by the larger QTLs, CIM was employed. An automatic cofactor selection using a forward/backward regression was also performed with QTL Cartographer v 2.5. Pearson phenotypic correlation coefficients among the traits were calculated by SAS PROC CORR (SAS Institute Inc, 1996).

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