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Mapping of quantitative trait loci for aroma, amylose content and cooked grain elongation traits in rice

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Abstract

Global demand for high-quality rice has grown substantially in recent years and continues to trend upward due to taste preferences and a greater interest in healthy diets. Most rice quality traits are inherited in a complex way and may be affected by multiple genes and environmental factors. In this study, we conducted a simple sequence repeat (SSR)-based genetic analysis of quantitative trait loci (QTLs) affecting aroma, amylose content (AC) and cooked grain elongation (GE), which are the major quality characteristics in rice. The QTL analysis was performed using an F_2 population of a cross between two *Oryza sativa* ssp. *indica*-type varieties, "Basmati 370" and "MR 84", comprising of 90 plants. A total of 96 polymorphic markers were distributed over 12 rice chromosomes, each containing at least four markers. A total of nine QTLs (LOD>3.0) were detected for the three studied traits, including four for aroma, three for AC and two for GE. The highest number of QTLs was mapped to chromosome 8, with four QTLs for aroma. Two QTLs each for AC (*qAC-3-1* and *qAC-5-1*) and GE (*qGE-2-1* and *qGE-2-2*) are reported here for the first time. These QTL markers could be utilised as indirect selection tools in breeding programmes.

Keywords: F_2 population; *Oryza sativa*; quality traits; SSR markers; (SSR)-based linkage map.
Abbreviations: AC_amylose content; ASA_allele specific amplification; BADH_betaine aldehyde dehydrogenase; CIM_composite interval mapping; GBS_genotyping-by-sequencing; GBSS_granule-bound starch synthase; GE_cooked grain elongation; MAS_ marker assisted selection; QTL_quantitative trait locus; SNP_single nucleotide polymorphism; SSR_simple sequence repeat.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops for more than half of the world's population. In the past, yield potential of rice has somehow compromised grain quality, as increasing the yield potential has always been the priority in research across the world. Nevertheless, developing rice varieties without considering grain quality poses challenges in meeting consumer expectations, particularly those of wealthier consumers living in countries with strong economic growth. Therefore, breeding strategies to attain high grain quality while maintaining high yield are essential to satisfy both consumer needs and preferences (Ni et al., 2011). In recent years, breeders have paid more attention to quality improvement while maintaining the stability of rice production. Publications spanning the past decade indicate that cooking and eating qualities are directly related to three major quality components of rice; aroma, amylose content (AC) and cooked grain elongation (GE) (Amarawathi et al., 2008). One of the most important quality attributes of rice is its typically pleasant aroma. Numerous chemical constituents including different volatile compounds are the major sources of aroma in cooked rice, along with environmental factors. Bergman et al. (2002) established that 2-acetyl-1-pyrroline is the key aroma constituent of fragrant rice. Furthermore, Bradbury et al. (2005) reported that aroma is controlled by a recessive gene (*fgr*) on chromosome 8; that contains of an 8-bp deletion and three single nucleotide polymorphisms (SNPs). Two types of molecular markers, including simple sequence repeats (SSRs) and SNPs, were identified as promising markers for the selection and identification of fragrance in rice (Yeap et al., 2013). A perfect marker system, namely Allele Specific Amplification (ASA) was developed by Bradbury et al. (2005) to genotype and discriminate aromatic and non-aromatic varieties. The AC of rice is generally categorised into five classes; waxy (0-2%), very low (3-9%), low (10-19%), intermediate (20-24%) and high amylose (above 24%). Previous studies have found that AC is governed mainly by an allelic series of genes at one locus and by one or several modifier genes with minor effects. However, the AC inheritance pattern is complex due to cytoplasmic effects, epistasis and environmental effects. Amylose synthesis is catalysed by granule-bound starch synthase (GBSS) encoded by the *Wx* locus (Ayres et al., 1997; Zhang et al., 2005). Ayres et al. (1997) reported two nucleotide polymorphisms that are associated with the *Wx* gene, including a polymorphic microsatellite (CT)*n* and a G–T SNP located at the 5'-leader intron splice site. Thus, the

combination of (CT)*n* and G-T SNP identification could be used to discriminate varieties with different classes of amylose content except the classes between low amylose and waxy (Cheng et al., 2012). The cooked rice elongation trait of *indica* rice is governed mainly by genotype, major gene effects and environmental interactions (Bao et al., 2001). In the study conducted by Ahn et al. (1993), a gene for the GE trait was mapped to chromosome 8 of Basmati 370 using restriction fragment length polymorphism markers. In addition, Jain et al. (2006) reported that there is a significant linkage between GE and the aroma traits on chromosome 8. Despite these mapping efforts, only limited information is available on the mapping of GE genes. Aroma, AC and GE are controlled in a complex way and selecting these traits using conventional methods is difficult due to the lack of discrete phenotypic classes in the progeny with tedious quality testing methodologies. Assessment of these qualities is further complicated by the effect of environment (Ge et al., 2005; Cheng et al., 2012). Nevertheless, molecular marker techniques have surpassed conventional methods for the detection of the aforementioned traits and include; the ASA system for the detection of aroma. Among all the available molecular markers, SSRs are among the most favoured and widely used for genetic mapping due to their abundance, high polymorphism and simple assays (Broun and Tanksley, 1996; Cordeiro et al., 2002; Langridge and Chalmers, 2004). To date, effective SSR markers for the detection of aroma, GE and all five different classes of AC have yet to be identified, even though some potential genes for these quality traits have been found. Many QTL analyses for aroma, AC and GE have been reported over the past decade. However, QTLs related to each of these traits were identified at a number of different loci, and to our knowledge no mapping study has concluded the precise chromosomal locations of the potential genes controlling these traits. The objectives of this study were as follows: (1) to construct an (SSR)-based linkage map of rice; (2) to map QTLs controlling aroma, AC and GE; and (3) to confirm linkage with the previously identified QTLs.

Results and Discussion

Frequency distribution in the F_2 mapping population

The frequency distribution in the F_2 population for segregating phenotypic classes of the three major rice quality traits, i.e., aroma, AC and GE, are shown in Fig 1. Aroma was scored as an ordinal trait with arbitrary categories 1-3, whereas AC and GE were measured on a quantitative scale and showed continuous variation with a normal distribution. The normal frequency distribution of AC and GE indicates a quantitative inheritance of these traits, with multiple genes and environmental factors influencing the phenotype.

Basmati rice is known for its distinct and pleasant aroma. The two parents differed in grain aroma; Basmati 370 was highly aromatic, with a sensory score of 3, whereas MR 84 was non-aromatic, with a sensory score of 1. The F_2 individuals were scored as having an additional category of a sensory score of 2 (mildly aromatic). This phenotypic analysis shows that this population segregated according to the expected Mendelian ratio of 1:2:1 ($\chi^2=5.356<\chi_{0.05}^2=5.992$). Only a small proportion of the F_2 individuals (15.6%) were able to reconstitute the original aroma of Basmati 370.

AC is considered to be the key determinant of the cooking and eating characteristics of rice, and it correlates directly with the firmness of cooked rice. Basmati varieties generally have an intermediate AC of 20–25% and their grains remain firm and separated after cooking. In this study, Basmati 370 showed an intermediate AC of 19.6%, whereas the non-basmati parent MR 84 had a high AC of 28%. The AC range for the developed mapping population was 14-26%, and only 15 individuals were found to have an AC lower than 19%. A long slender grain with a delicate curvature is another unique characteristic of Basmati rice. In this study, Basmati 370 was characterised by extra long grains of 2.0 cm, whereas MR 84 had comparatively shorter grains of 1.5 cm. The GE in the F_2 population ranged from 1.1 to 2.0 cm with a population mean of 1.5. According to Nematzadeh et al. (2000), a rice variety is considered to have a good elongation trait when its elongation ratio is 1.6 and above.

Identification of polymorphic markers for linkage map construction

One of the main objectives of the present study was to construct an (SSR)-based linkage map of rice. A total of 212 markers were used for parental screening between Basmati 370 and MR 84, and 102 (48.1%) of these showed polymorphism on 3% MetaPhor agarose gels. All of the 90 F_2 individuals were genotyped for these 102 marker loci. Deviation of the observed frequencies of the two segregating alleles of individual markers from the expected 1:1 Mendelian ratio has been defined as segregation distortion, which can seriously affect QTL mapping results (Xu et al., 1997). Segregation distortion was analysed for all 102 loci using the χ^2 test, and 15 markers showing distorted segregation ($\chi^2>10.5$, $P<0.05$) were distributed over seven different chromosomes: 2, 3, 6, 8, 9, 10 and 12. Hence, the distortion was random and not restricted to any specific part of the genome. Such distorted segregation in mapping populations has been previously reported (Xu et al., 1997; Harushima et al., 2002). Nevertheless, six of the 15 polymorphic markers, i.e., RM282, RM16, RM190, RM314, RM219 and RM330, showing extreme segregation distortion ($\chi^2>10.5$, $P<0.001$) were eliminated from the analysis and only 96 markers showing normal segregation were utilised for the construction of a molecular linkage map using MAPMAKER 3.0b (Lander et al., 1987). These 96 SSR markers were distributed over all 12 rice chromosomes (Fig 2). The average genetic distance between the markers for 96 intervals on the 12 chromosomes was 21.7 cM, but there were five large genetic gaps of 74-114 cM on chromosomes 2, 3, 5 and 8. There were another four large genetic gaps of 56-66 cM; two on chromosome 1; and one each on chromosomes 4 and 6. Excluding these nine loci, the average distance of the remaining 85 intervals was only 16.5 cM, providing a fairly dense molecular map for QTL interval mapping.

Mapping of QTLs for quality traits

QTL mapping has become a routine strategy for the discovery of genes for many important quality traits of rice. Numerous mapping studies have been conducted in the past decade to identify QTLs for aroma, AC and GE traits (Ahn et al., 1992; Lorieux et al., 1996; McCouch et al., 1997; Tian et al., 2005; Amarawathi et al., 2008; Ahamadi et al., 2008). However, only limited information is available to date on the association between these traits. Furthermore, the published results on the locations of the major QTLs for each trait vary from one source to another and this does not allow accurate and repeated mapping analyses. The present mapping study identified a total of nine QTLs affecting aroma, AC and GE (Table 1; Fig 2). These QTLs were distributed on chromosomes 2, 3, 5, 6 and 8. It was important to compare

Table 1. Identification of QTLs for aroma, amylose content and grain elongation traits using 90 individuals derived from Basmati 370 and MR 84 using composite interval mapping function (CIM).

Trait	Name	Chr.	QTL	QTL interval	LOD	Peak (cM)	Addictive	Varians (R^2) %
Aroma	AR	8	qAR-8-1	RM42 – RM223	23.28	99.2	-0.85	24
		8	qAR-8-2	RM223– AROE	24.17	107.3	-0.94	28
		8	qAR-8-3	AROE– RM342	13.37	122.1	0.05	24
		8	qAR-8-4	RM515–RM210	6.48	168.6	-0.40	20
Amylose content	AC	3	qAC-3-1	RM36 – RM7	3.20	0.0	0.47	5
		5	qAC-5-1	RM440–RM421	3.19	123.0	-0.18	2
		6	qAC-6-1	O485 - Wx	25.95	26.7	4.51	64
Grain elongation	GE	2	qGE-2-1	RM53 – RM174	3.80	42.4	-0.07	23
			qGE-2-2	RM525 – RM6	3.04	246.1	-0.01	10

Chr- Chromosome; LOD- log10 (probability of linkage/probability of no linkage); Peak- QTL peak; Additive- additive effect expressed in terms of estimated change in the phenotype expected from introgression of Basmati 370 alleles; R^2- proportion of variation explained by the QTL

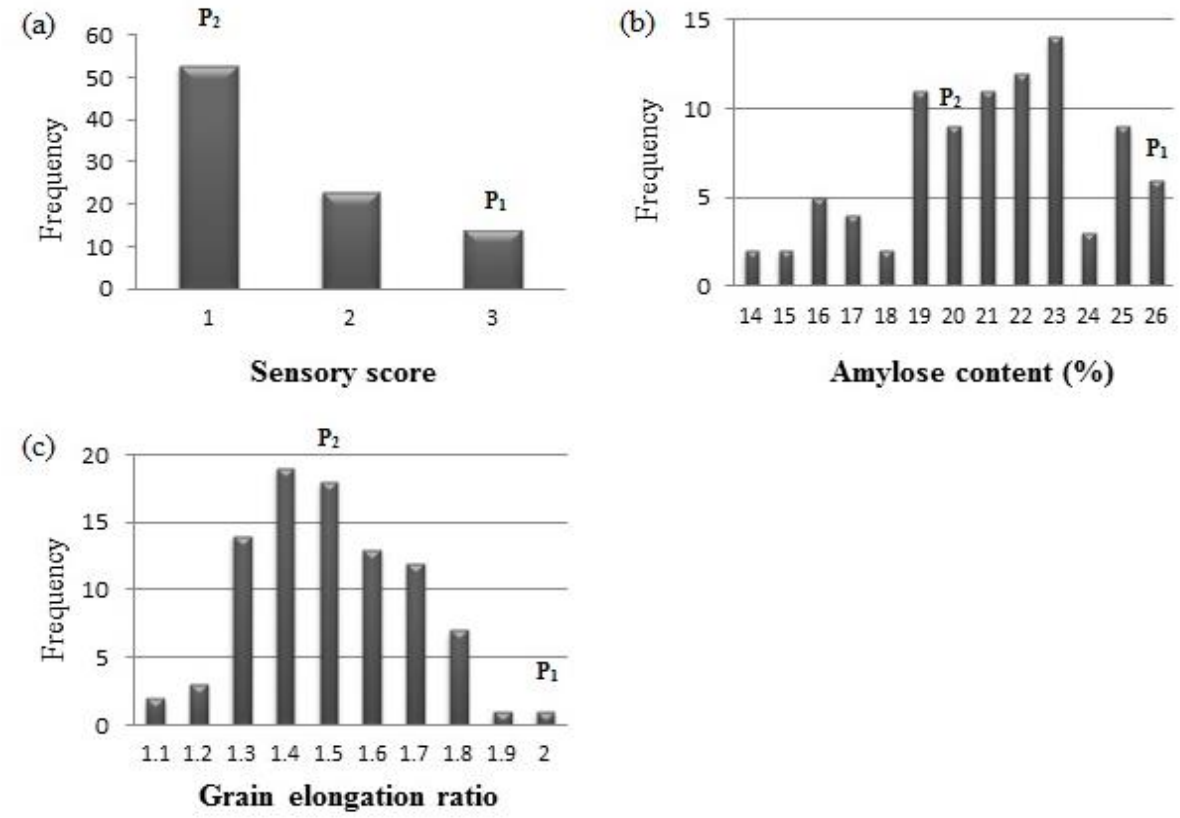

Fig 1. Frequency distribution of phenotypic variation for (a) aroma, (b) amylose content and (c) cooked grain elongation traits among 90 F_2 individuals derived from a cross between (P_1) Basmati 370 and (P_2) MR 84.

the location of these QTLs in relation to previous reports, both for validation of the earlier results and to identify new QTLs specific to the present mapping population, if any. A total of four QTLs were identified for aroma by QTL Cartographer, and all of them were all mapped to chromosome 8. The most effective QTL for aroma was *qAR-8-2*, with a LOD score of 24.17, which was located between markers RM223 and AROE and explained 28% of the phenotypic variation for aroma. There were two other significant QTLs for aroma with high LOD scores, explaining approximately 24% of the phenotypic variance; *qAR-8-1*, *qAR-8-3*. The *qAR-8-1* locus mapped in the interval RM42-RM223, with a LOD score 23.28, whereas *qAR-8-3* was located in the marker interval AROE-RM342, with a LOD score of 13.37. One of the most important quality traits that characterises well-known high-quality Basmati rice is the pleasant aroma. As expected, the positive alleles for aroma QTLs in this study were contributed by Basmati 370. However, only a small proportion of the F_2 individuals were able to reconstitute the original aroma of Basmati 370, suggesting the involvement of multiple genes for aroma which was also observed in the studies conducted by Pinson (1994) and Loriuex et al. (1996). However, many published reports indicate the involvement of only one recessive gene (*fgr*) for aroma located on chromosome 8 (Ahn et al., 1992; Bradbury et al., 2005; Wanchana et al., 2005). According to the sequence analysis of 17 genes in the *fgr* region by Bradbury et al. (2005), *badh*2 gene which encodes the putative betaine aldehyde dehydrogenase (BADH2) enzyme is most likely to be the *fgr* gene, due to its sequence divergence

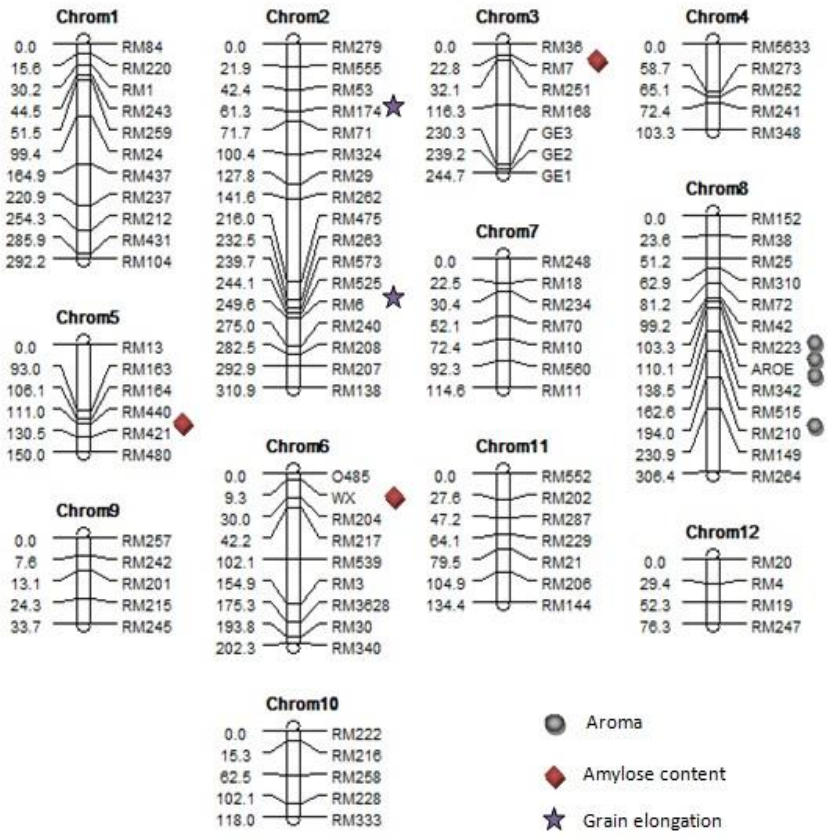

Fig 2. Molecular genetic maps of the 12 rice chromosomes based on 96 polymorphic marker loci segregating in the derived F_2 mapping population (Basmati 370 x MR 84). Genetic distances between markers (in cM) are shown on the left side of the chromosome bars. Significant QTLs for Basmati quality traits are shown on the left side using different symbols.

between fragrant and non-fragrant rice varieties. However, it was later shown by Amarawathi et al. (2008) that *badh*2 gene alone is not sufficient to explain the aroma of rice, supporting the results obtained in the present study. To date, the exact role of the BADH2 enzyme in aroma development is yet to be validated and there have been no conclusions made with regard to whether this trait is controlled by a single gene or multiple genes. For AC, QTL Cartographer detected three QTLs, one each on chromosomes 3, 5 and 6. The most effective QTL for AC, qAC-6-1, was found to be located in the interval O485-Wx on chromosome 6, with a high LOD score of 25.95, and explained 64% of the phenotypic variation for AC. This QTL is located in the *Wx* gene (GBSS1) region, as reported in the study of Tian et al. (2005). Ayres et al. (1997) suggested that the wide variation for AC in non-waxy *indica* rice varieties might be due to the presence of a series of alleles at the *Wx* locus. However, the wide variation observed for AC in this study (64%) may be due to the influence of modifier genes or environmental factors, as only two alleles were found to be segregating at the qAC-6-1 locus. There were two other significant QTLs detected for AC, qAC-3-1 and qAC-5-1, which are believed to have never been reported in previous studies. The qAC-3-1 mapped to the interval RM36-RM7, with a LOD score of 3.20, and explained 5% of the phenotypic variance, whereas qAR-5-1 mapped to the marker interval RM440-RM421 with a LOD score of 3.19 and explained 2% of the phenotypic variance. The identification and addition of polymorphic SSR markers between the intervals (RM36-RM7 and RM440-RM421) can be further carried out to identify the exact position of these QTLs. Numerous studies have been conducted to characterise the genetic basis of AC in the endosperm. Several published results have indicated that AC is controlled by a single major locus, with the modification of some minor genes. However, other studies have suggested that AC had a complex genetic basis due to the triploid nature of the endosperm, cytoplasmic effects and epistasis (Ayres et al., 1997; Zhang et al., 2005). Thus far, questions still remain as to whether *Wx* is the only major gene controlling AC and whether AC is closely correlated to other cooking and eating qualities such as cooked grain elongation and gelatinisation temperature. Furthermore, there have been no reported functional markers that can discriminate all five classes of AC, though a number of SNPs have been developed (Cheng et al., 2012). The parental varieties used in the present study did not differ highly in their GE, but the progeny showed a much larger range (Fig 1c). Grain elongation upon cooking is an essential quality attribute of the basmati rice varieties that can be measured in terms of the ratio of the grain length after and before cooking. Two QTLs were identified for GE on chromosome 2, designated *qGE-2-1* and *qGE-2-2*. The *qGE-2-1* mapped to the interval RM53-RM174, with a LOD score of 3.80, and explained 23% of the phenotypic variance, whereas *qGE-2-2* was mapped to the marker interval RM525-RM6, with a LOD score of 3.04 and explained 10% of the phenotypic variance. One QTL for GE was previously reported on chromosome 2 in the marker interval R2510-RM211 in the study conducted by Ge et al. (2005). However, marker RM211 in the present study did not show polymorphism in the parental screening. Ahn et al. (1993) reported that one major QTL is located on chromosome 8. The evolution of chromosome number 8 in rice is of particular interest because genes for both aroma and GE have been mapped on to this chromosome (Jain et al., 2006).

However, no QTL for GE was detected on chromosome 8 in the present study, even though the chromosome is fairly dense with evenly distributed markers. This might be because although Basmati 370 exhibits an extremely high cooked kernel length, with an elongation ratio of 2.0, this value was not much higher than 1.5 for MR 84. Another mapping population developed from a cross between parental varieties showing extreme values for kernel elongation ratio will be more suitable for identifying the QTLs controlling this trait (Ahn et al., 1993). Previous findings on the positions of QTLs as well as the major gene(s) controlling aroma, AC and GE have been inconsistent and contradictory. In the present study, five of the nine locations of the detected QTLs are consistent with the locations found in previous studies and four new QTLs (two QTLs each for AC and GE) have been identified. These QTLs could possibly be used for further fine mapping and identification of the specific genes to develop functional markers for these traits. The findings in this study indicate that further analysis is required to exemplify the complete genetic basis of the large variation among the three studied traits in nature. A reasonable approach to tackle this issue could be to use the latest ultra-high throughput genotyping technology namely genotyping-by-sequencing (GBS). According to Deschamp et al. (2012), GBS is a simple highly-multiplexed system due to its ability to combine the detection of sequence differences in large populations together with the scoring of markers, thus allowing a rapid and direct study of its diversity targeted towards the mapping of a trait of interest. In addition to that, no preliminary sequence information is required for this system and no ascertainment bias towards a particular population will occur as it uses data directly from the population being genotyped. Thus, it is one of the most promising approaches for further investigation of the three studied traits.

Materials and Methods

Plant materials

The plant material consisted of 90 F_2 individuals derived from a cross between two *Oryza sativa* spp. *indica*-type varieties, Basmati 370 and MR 84, which differ in all three studied traits. Basmati 370 is a high-quality aromatic variety with a long grain (elongation ratio = 2.0) and intermediate AC (19.6%). It was crossed as the female to MR 84, a non-aromatic variety with short grain (elongation ratio = 1.5 cm) and high AC (28.0%). The F_1 plants were selfed to produce F_2 progeny. The 90 F_2 individuals were planted in the normal rice growing season of 2011 with the parental lines and were harvested upon maturity. All the plants were evaluated individually for the QTL analysis.

Phenotyping for quality traits

The presence and absence of aroma was determined by sensory testing using the procedure of Sood and Siddiq (1978) with minor modifications. Approximately 100 g of young rice leaves were placed in a ventilation vial containing 10 ml of 1.7% KOH and incubated at room temperature for 10 min. The samples were then smelled and rated for aroma by a panel of three experts on scale of 1-3, where 1 was non-aromatic and 3 was highly aromatic. The test was repeated three times for each of the samples. Estimation of AC was determined by the method of Juliano (1971) with minor modifications. A set of 30 polished grains were ground to a fine powder and sieved through a 0.40 mm screen. Rice flour (40 mg) was extracted overnight in an amylose-iodine solution. The pH of the solution was stabilised with a corresponding amount of acetic acid. The absorbance was recorded at 620 nm using a spectrophotometer calibrated with a control solution containing no rice flour. The AC was estimated using a standard curve developed from known quantities of purified potato amylose. A set of five unbroken polished rice grains was used for the determination of the GE ratio according to the method of Dela Cruz et al. (2000) with minor modifications. The ratio was calculated by dividing the mean GE after cooking with the mean GE before cooking. The measurements of grains before and after cooking were repeated with another two sets of grains.

Genotyping of F_2 individuals

A total of 212 PCR-based markers were used for the parental polymorphism screening (see Supplementary Table 1). PCR amplification was carried out in 10 µl reaction mixtures, containing the respective 0.25 µg of template DNA, 1x Green GoTaq flexi buffer, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotides, 0.5 µM of forward primer, 0.5 µM of reverse primer and 2.5 units of GoTaq DNA polymerase (Promega, USA). The PCR cycle was 94°C for 5 min, followed by 35 cycles of 60 s denaturation at 94°C, 60 s annealing at 55°C, and 120 s extension at 72°C, plus a final extension of 5 min at 72°C. The cycles were performed using a gradient thermocycler (Eppendorf, Germany). The PCR products were separated by electrophoresis for 3 h in 3% MetaPhor agarose gels in 1 X TAE buffer. The resolved PCR bands were detected by staining the gels for 30 s with ethidium bromide (EtBr), followed by destaining for 30 min in distilled water and visualisation with a UV gel imager (Alpha Innotech, USA).

Marker assay and linkage analysis

For the marker assay, the primer pairs that showed polymorphism between the parental DNAs, Basmati 370 and MR 84 were analysed for the population. The data generated after genotyping 90 F_2 individuals by polymorphic markers were tested using the χ^2 test against the 1:1 segregation ratio. Linkage groups and the order of markers were determined using MAPMAKER/EXP version 3.0b (Lander et al., 1987). The marker order within a linkage group was determined using the ''compare'', ''try'' and ''ripple'' commands. The map distances were based on the Kosambi function, which transformed the recombination frequencies into genetic distances (Kosambi, 1944). To avoid detecting pseudo-linkage of markers, a relatively high threshold level of LOD 3.0 was employed to establish the linkage groups.

QTL mapping

A whole-genome scan was performed using QTL Cartographer version 2.5 (Wang et al., 2002). The composite interval mapping (CIM) function, which combines interval mapping with multiple regressions, was employed to identify QTLs. The level of significance for the QTLs in this study was determined as LOD $\geq$ 3.0 at $P < 0.001$, and the proportion of observed phenotypic variance explained by each QTL was estimated by the coefficient of determination, R^2 (McCouch et al., 1997).

Conclusion

This study achieved a considerable enhancement of the available detection of three major quality traits of rice in an F_2 population. The current findings add to a growing body of

literature on the locations of genes/QTLs controlling aroma, AC and GE. Although some of the detected QTLs are likely present at the same locus as in previous studies, two new QTLs each for AC and GE are reported here for the first time. These QTLs could be used for further fine mapping and validation of specific genes that are important in the development of functional markers for improving existing breeding schemes.

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References

Ahamadi J, Fotokian MH, Fabriki-Orang S (2008) Detection of QTLs influencing panicle length, panicle grain number and panicle grain sterility in rice (*Oryza sativa* L.). J Crop Sci Biotech. 11(3):163-170.

Ahn SN, Bollich CN, Tanksley SD (1992) RFLP tagging of a gene for aroma in rice. Theor Appl Genet. 84:825-828.

Ahn SN, Bollich CN, McClung AM, Tanksley SD (1993) RFLP analysis of genomic regions associated with cooked kernel elongation in rice. Theor Appl Genet. 87:27-32.

Amarawathi Y, Singh R, Singh AK, Singh VP, Mohapatra T, Sharma TR, Singh NK (2008) Mapping of quantitative trait loci for basmati quality traits in rice (*Oryza sativa* L.). Mol Breed. 21:49-65.

Ayres NM, McClung AM, Larkin PD, Bligh HFJ, Jones CA, Park, WD (1997) Microsatellites and a single nucleotide polymorphism differentiate apparent amylose classes in an extended pedigree of US rice germplasms. Theor Appl Genet. 94:773-781.

Bao JS, Xie JK., Xia YW (2001) Genetic analysis of cooked rice elongation in *indica* rice (*Oryza sativa* L). Acta Agronomica Sinica. 27: 489-492 (in chinese)

Bergman CJ, McClung AM, Pinson SR, Fjellstrom RG (2002) Development of PCR-based markers associated with cooked rice kernel elongation and aroma. Proceedings of rice technical working group meeting, Arkansas, 2002.

Bradbury LMT, Henry RJ, Jin Q, Reinke RF, Waters DLE (2005) A perfect marker for fragrance genotyping in rice. Mol Breed. 16:279-283.

Broun P, Tanksley SD (1996) Characterization and genetic mapping of simple repeat sequences in the tomato genome. Mol Gen Genet. 250:39–49.

Chen S, Wu J, Yang Y, Shi W, Xu M (2006) The *fgr* gene responsible for fragrance was restricted within 69 kb. Plant Sci. 171:505-514.

Cheng A, Ismanizan I, Mohamad O, Habibuddin H (2012) Simple and rapid molecular techniques for identification of amylose levels in rice varieties. Int J Mol Sci. 13:6156-6166.

Cordeiro GM, Christopher MJ, Henry RJ, Reinke RF (2002) Identification of microsatellite markers for fragrance in rice by analysis of the rice genome sequence. Mol Breed. 9:245-250.

Dela Cruz NM, Khush GS (2000) Rice grain quality evaluation procedures. In: Singh RK, Singh US, Khush GS (eds) Aromatic Rices, IRRI, p 16-27.

Deschamps S, Llaca V, May GD (2012) Genotyping-by-sequencing in plants. Biol. 1:460-483.

Ge XJ, Xing YZ, Xu CG, He YQ (2005) QTL analysis of cooked rice grain elongation, volume expansion, and water absorption using a recombinant inbred population. Plant Breed. 123:121-126.

Harushima Y, Nakagahra M, Yano M, Sasaki T, Kurata N (2002) Diverse variation of reproductive barriers in three intraspecific rice crosses. Gen. 160:313-322.

Jain N, Jain S, Saini N, Jain RK (2006) SSR analysis of chromosome 8 regions associated with aroma and cooked kernel elongation in Basmati rice. Euphytica. 152:259-273.

Juliano BO (1971) A simplified assay for milled-rice amylose. Cereal Sci Today. 16:334-360.

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eug. 12:172-175.

Lander ES, Green P, Abrahamson J, Barlow A, Daley, M (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics. 1:174-181.

Langridge P, Chalmers K (2004) The principle: identification and application of molecular markers. Biotech Agriculture Forestry. 55:1-2.

Lorieux M, Petrov M, Huang N, Guiderdoni E, Ghesquiere A (1996) Aroma in rice: genetic analysis of a quantitative trait. Theor Appl Genet. 93:1145-1151.

McCouch SR, Chen X, Panaud O, Temnykh S, Xu Y, Cho TG, Huang N, Ishii T, Blair M (1997) Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Mol Biol. 35:89-99.

Nematzadeh GA, Karbalaie MT, Farrokhzad F, Ghareyazie B (2000). Aromatic rices of Iran. In: Singh RK, Singh US, Khush GS (eds). Aromatic rices, IRRI, p 191-193.

Ni DH, Zhang SL, Chen S, Xu Y, Li L, Li H, Wang ZY, Cai XL, Li ZF, Yang JB (2011) Improving cooking and eating quality of Xieyou57, an elite *indica* hybrid rice, by marker-assisted selection of the *Wx* locus. Euphytica. 179:355-362.

Pinson SRM (1994) Inheritance of aroma in six rice cultivars. Crop Sci. 34:1151-1157.

Sood BC, Siddiq EA (1978) A rapid technique for scent determination in rice. Indian J Genet Plant Breed. 38:268–271.

Tian R, Jiang GH, Shen LH, Wang LQ, He YQ (2005) Mapping quantitative trait loci underlying the cooking and eating quality of rice using a DH population. Mol Breed. 15:117-124.

Wanchana S, Kamolsukyunyong W, Ruengphayak S, Toojinda T, Tragoonrung S, Vanavichit A (2005) A rapid construction of a physical contig across a 4.5 cM region for rice grain aroma facilitates marker enrichment for positional cloning. Sci Asia. 31:299–306.

Wang S, Basten CJ, Zeng Z-B (2002) Windows QTL cartographer, WinQTLCart V 2.0 program in statistical genetics. North Carolina State University, Raleigh.

Xu Y, Zhu L, Xiao J, Huang N, McCouch S (1997) Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol General Genet. 253:535-545.

Yeap HY, Faruq Q, Zakaria HP, Harikrishna JA (2013) The efficacy of molecular markers analysis with integration of sensory methods in detection of aroma in rice. Sci World J. 2013:1-6.

Zhang SL, Ni DH, Yi CX, Li L, Wang XF, Wang ZY, Yang JB (2005) Lowering grain amylose content in backcross off-springs of *indica* rice variety 057 by molecular marker-assisted selection. Rice Sci. 12:157–162.