

Mapping of grain alkali digestion trait using a Cheongcheong/Nagdong doubled haploid population in rice

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Abstract We performed a molecular marker-based analysis of quantitative trait loci for traits that determine the quality of appearance of grains using 120 doubled haploid lines developed by anther culture from the F₁ cross between ‘Cheongcheong’ (*Oryza sativa* L. ssp. *Indica*) and ‘Nagdong’ (*Oryza sativa* L. ssp. *Japonica*). We therefore calculated the alkali digestion value (ADV), used to indirectly measure gelatinization temperature, to evaluate the quality of cooked rice in 2013 and 2014. The ADV score of frequency distribution was higher milled rice than brown rice. In total, nine different quantitative trait loci (QTLs) were found on 5 chromosomes in 2013 and 2014. Also, chromosome 5, 8 were detected over two years. We conclude that selected molecular markers from this QTL analysis could be exploited in future rice quality. In conclusion, we investigated ADV of brown and milled rice in CNDH population. This study found nine QTLs related to the ADV of brown and milled rice. The detected one marker can be used to select lines with desirable eating-quality traits because ADV is closely associated with the eating quality of cooked rice. Therefore, it will be useful to collect resources and distinguishable in many varieties for rice breeding program.

Keywords Alkali digestion value, QTL, Rice, Marker-assisted selection

Introduction

In recent years, rice consumption in the Republic of Korea has seen a decrease in response to economic growth and westernization of dietary habits. To increase rice consumption and prepare for the opening of Korea’s rice market internationally, the production of high-quality rice is necessary. The digestibility and taste of rice is related to rice gelatinization, which is affected by numerous factors, including amylose content (Hu et al. 2004). In Korea, cultivars with relatively low gelatinization temperatures are typically preferred. This characteristic can be measured indirectly using the alkali digestion value (ADV), which also functions to score the taste of cooked rice (Juliano 1985, 2003; Lee et al. 2000; Kwak 2009). Previous research has found that traits related to cooking quality were mainly controlled by genetic effects, but interactions between the genotype and the environment were also present (Shi et al. 1997). Furthermore, a major gene controlling amylose content (AC) was found to be linked with a gene involved in the alkali spreading score (Mckenzie and Rutger 1983). In rice breeding programs, using DNA markers to select promising individuals in early generations can improve efficiency, because the indirect evaluation of eating quality is possible through genotyping. Recently, advances in molecular technology have resulted in the development of DNA markers related to major quantitative trait loci (QTL). If the markers related to a major gene are employed to select for a polygenic trait, selection efficiency in breeding can be improved. Because eating-quality traits in rice are polygenic, it is especially necessary to focus our research on marker assist selection (MAS) associated with QTLs and the accumulation of various DNA markers related to desirable traits (Xiao et al. 1996; Lee et al. 2000). Therefore, we investigated alkali digestion values (ADV) in rice by analyzing QTLs to detect DNA markers associated with alkali digestion. We conducted our experiments one doubled haploid (DH) population, derived from a cross between ‘Cheongcheong’ and ‘Nagdong’ strains.

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Materials and Methods

Plant materials

Through anther culture, we developed 120 DH lines, using an F_1 population derived from a ‘Cheongcheong’ and ‘Nagdong’ cross (hereafter, the CNDH line). ‘Cheongcheong’ is a *indica*-type while ‘Nagdong’ is a *japonica*-type (Fig. 1). In 2013 and 2014, the plants were cultivated and harvested at the experimental paddy field of Kyungpook National University in Gunwi, North Gyeongsang Province, Korea Republic. The population was transplanted at 30×15 cm spacing. They were fertilized with N-P₂O₅-K₂O applied at 9-4.5-5.7 kg/10a, according to the Rural Development Administration (RDA) standard cultivation practice.

Evaluation of alkali digestion value

The evaluation of ADV was performed four times, using the following procedure. Prior to evaluation, rice grains were hulled and milled using a rice huller (SYTH-88, Ssangyong Machinery Industry Co., Ltd.) and a rice milling machine

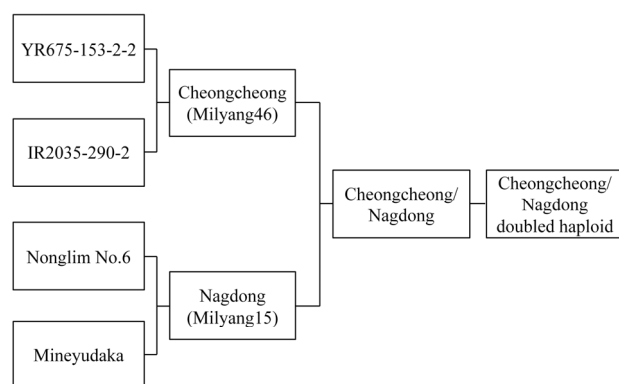


Fig. 1 Genealogical diagram of the Cheongcheong/Nagdong doubled haploid population. Cheongcheong is a Tongil-type rice cultivar from an *indica* and *japonica* cross, while Nagdong is a *japonica*-type rice cultivar. The doubled haploid population was developed using anther culture

Table 1 Criteria for alkali digestion value of rice from the International Rice Research Institute

Score	Alkali digestion value
1	Grain not affected
2	Grain swollen
3	Grain swollen, color incomplete or narrow
4	Grain swollen, color complete and wide
5	Grain split or segmented, color complete and wide
6	Grain dispersed, merging with collar
7	Grain completely dispersed and disintegrated

(Pearlest, Kett Electric Laboratory). Damaged grains, red grains, green grains and broken grains were removed from the brown rice and milled rice before testing. Brown and milled rice were placed on a petri dish (90×15 mm) containing 20 mL of 1.4% KOH solution, in separate rows of four grains each. The KOH solution consisted of 16.47 g of 85% KOH powder dissolved into 1 L water. The grains were left undisturbed for 18 hours at 30°C (Choe and Heu 1975). Brown and milled rice ADVs were graded from 1 to 7, according to the standards set by the International Rice Research Institute (Table 1).

Analysis of QTLs associated with alkali digestion value

The genetic map consisting of 222 SSR markers was constructed by MAPMAKER/EXP Version 3.0 using the SNDH population (Lincoln et al. 1992). The completed genetic map used by the Plant Molecular Breeding Laboratory at the School of Applied Biosciences in Kyungpook National University. WINDOWS QTL Cartographer version 2.5 (WinQTLcart 2.5) was employed to analyze QTLs associated with the ADVs of brown and milled rice. The map was constructed with the Kosambi function, and the QTLs were detected by composite interval mapping (CIM) with a LOD (log of odds ratio) threshold of 3.0 (Zeng 1994). The standard QTL nomenclature was applied (McCouch et al. 1997).

DNA extraction and PCR analysis for MAS

To determine if the markers found from the QTL analysis are related to ADV, DNA extraction and PCR were performed using a NucleoSpin® Plant II Kit (MACHEREY-NAGEL, Cat. 740 770.250). In total, 13 varieties of the *japonica* type and seven varieties of the *indica* type were used for DNA extraction. To prepare the grains for extraction, 100 mg brown rice was frozen with liquid nitrogen and then ground with mortar and pestle. The ground rice was placed into a 1.5 ml tube, and 400 µL P1 solution with 10 µL RNase A were added. The tube was then placed in a 65°C water bath for 10 minutes. Subsequently, the rice solution was transferred into a NucleoSpin® Filter with collection tube and centrifuged at 13,000 rpm for 2 minutes. Upon the end of the centrifuge cycle, 450 µL PC solution was added into the collection tube and the contents were then transferred into a NucleoSpin® Plant II Column with a new collection tube. This was centrifuged at 13,000 rpm for 1 minute. The filtrate (in the spin column) was then removed and 400 µL PW1 solution was added for washing. The sample solution was again centrifuged, at 13,000 rpm for 1 minute. 700 µL PW2 solution was added to

the filtrate, which was centrifuged at 13,000 rpm for 1 minute. To remove the washing solution from the filtrate and dry the silica membrane, 200 μ L PW2 solution was added and the solution centrifuged at 13,000 rpm for 2 minutes. The column containing the final filtrate was placed into a 1.5 ml tube and 50 μ L PE solution was added. The sample solution was incubated at 65°C for 5 minutes and then centrifuged twice, for 1 minute at 13,000 rpm. Lastly, the DNA in the column was eluted by nuclease-free water.

PCR was conducted on the extracted DNA, with markers from the analysis of ADV-related QTLs. The 24 μ L PCR mixture consisted of 2 μ L 10–20 ng/ μ L template DNA, 1 μ L 5–10 pM of each primer, 0.1 μ L Taq polymerase (Inclone Biotech Co., IN5001), 0.375 μ L dNTPs mixture, 2.4 μ L 10 \times Ex buffer, and 17.125 μ L nuclease-free water (QIAGEN, Cat. No.129114). PCR was conducted using a GeneAmp PCR System 2700 (Applied Biosystems, USA). The thermos cycling conditions were as follows: initial denaturation for 5 minutes at 96°C, followed by 34 cycles of 30 seconds at 96°C, 30 seconds at 55°C, 1 minute at 72°C, and a final extension for 8 minutes at 72°C. The PCR products were stored at 4°C until needed. Amplicons were examined using gel electrophoresis (QIAGEN, QIAxcel) to determine suitability for subsequent genotyping.

Results

ADV evaluation was conducted in the both the crossed SNDH population and the parental cultivars, ‘Cheongcheong’ and ‘Nagdong’ (Table 2). DH brown rice ADVs were 1.87 ± 1.01 and 2.58 ± 1.23 of ADV in 2013 and 2014, respectively, while DH milled rice ADVs were 4.82 ± 1.53 and 4.93 ± 0.92 in 2013 and 2014, respectively. The broad sense heritability of ADVs score brown rice showed 70% and 83% in 2013 and 2014, respectively. Whereas, the broad sense heritability of milled rice showed 79% and 85% in 2013 and 2014. In the parental cultivars, ‘Cheongcheong’ (indica) and ‘Nagdong’ (japonica)

brown rice ADVs were 1.96 and 1.68 in 2013, and 2.95 and 2.75 in 2014, respectively. In contrast, ‘Cheongcheong’ and ‘Nagdong’ milled rice ADVs were higher, at 5.61 and 4.10 in 2013, and 4.81 and 4.88 in 2014, respectively (Fig. 2).

In 2013, only five ADV-associated QTLs in milled rice were detected, on chromosomes 1, 3, 5 and 8. In 2014, four ADV-associated QTLs in milled rice were detected, on chromosome 5, 6 and only one ADV-associated QTL was found in brown rice, on chromosome 8 (Fig. 3). The milled rice and brown rice QTLs were named qADM and qADB, respectively.

According to the 2013 results of the QTL analysis, QTLs qADM1-1 and qADM1-2 on chromosome 1 had LOD of 3.46 and 4.18, respectively. These two QTLs explained 0.31 and

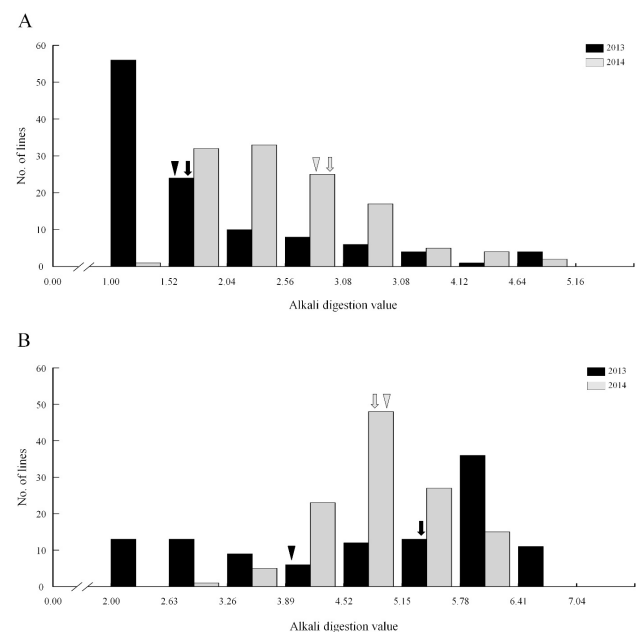


Fig. 2 Frequency distribution of the alkali digestion values in 2013 and 2014. The distribution of brown rice (A) and milled rice (B) are investigated for two years. The black bars and indicators represent 2013 data while the white bars and indicators represent 2014 data. The arrows and triangles indicate Cheongcheong and Nagdong, respectively

Table 2 Alkali digestion values of 120 CNDH (Cheongcheong/Nagdong doubled haploid) population and the parent cultivars

Variety	Year	Brown rice ^a	Variance	H ^b (%)	Milled rice ^a	Variance	H ^b (%)
Cheongcheong	2013	1.96 \pm 0.76	0.66	-	5.61 \pm 1.30	0.25	-
	2014	2.95 \pm 0.14	0.46	-	4.81 \pm 0.64	0.04	-
Nagdong	2013	1.68 \pm 0.81	0.39	-	4.10 \pm 1.31	2.41	-
	2014	2.75 \pm 0.15	0.59	-	4.88 \pm 0.58	0.16	-
CNDH	2013	1.87 \pm 1.01	1.00	65	4.82 \pm 1.53	0.51	86
	2014	2.58 \pm 1.23	2.42	74	4.93 \pm 0.92	0.40	83

^aMean \pm SD, ^bBroad sense heritability

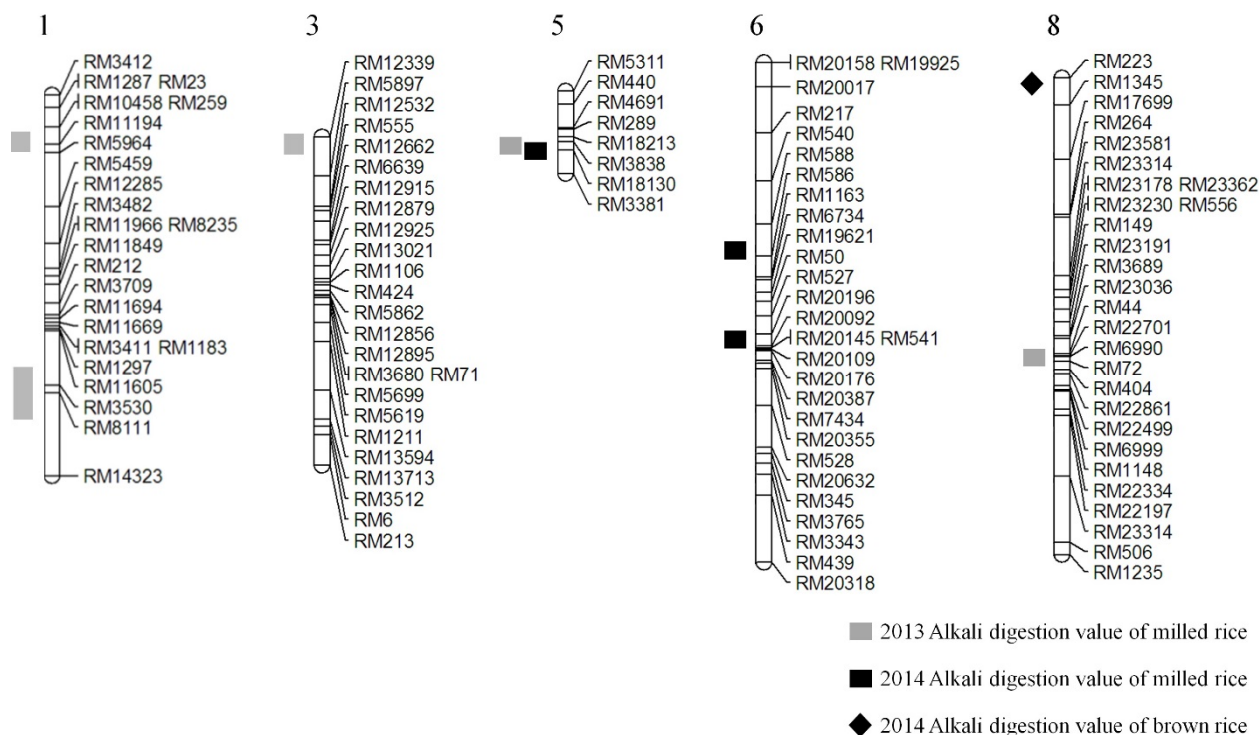


Fig. 3 Genetic locations of QTLs related to the alkali digestion of brown and milled rice. The gray quadrangles are QTLs for milled rice in 2013, while the back quadrangle and diamond are QTLs for milled rice and brown rice, respectively, in 2014

Table 3 QTL analysis for the alkali digestion value of brown and milled rice in DH population for two years

Year	Chromosome	QTL	Region ^a	LOD ^b	Variation	Additive effect	Allele
2013	1	qADM1-1	RM10458-RM5964	3.46	0.31	0.86	Cheongcheong
	1	qADM1-2	RM3530-RM8111	4.18	0.27	-0.77	Nagdong
	3	qADM3	RM14330- ^c	3.69	0.27	0.67	Cheongcheong
	5	qADM5	RM3838-18130	3.71	0.24	0.49	Cheongcheong
	8	qADM8	RM44	3.15	0.34	0.44	Cheongcheong
2014	5	qADM5	RM18130-	3.75	0.29	0.22	Cheongcheong
	6	qADM6-1	RM586-	3.43	0.30	0.30	Cheongcheong
	6	qADM6-2	RM20196-20092	3.25	0.36	-0.23	Nagdong
	8	qADB8	RM223-	3.75	0.27	0.28	Cheongcheong

^aThe region of the QTLs interval between markers, ^bLogarithm of the odds, ^cOnly one marker detected.

0.27 of the phenotypic variation with additive effects of 0.86 and -0.77, respectively. In other words, the qADM1-1 allele was from the 'Cheongcheong', while the qADM1-2 allele was from the 'Nagdong'. The QTL qADM3 was detected on chromosome 3 with an LOD of 3.69 and an additive effect of 0.67. This QTL explained 0.27 of the phenotypic variation and the qADM3 allele was from the 'Cheongcheong'. The QTL qADM5 was detected on chromosome 5 with an LOD of 3.71 and an additive effect of 0.49. This QTL explained 0.24 of the phenotypic variation and the qADM5 allele was from the 'Cheongcheong'. The QTL qADM8 was detected on chromosome 8 with an LOD of 3.15 and an additive effect of

0.44. This QTL explained 0.34 of the phenotypic variation and the qADM8 allele was from the 'Cheongcheong', too.

In the 2014 results, this QTL qADM5 was found on chromosome 5, with an LOD of 3.75 and an additive effect of 0.22. The QTL explained 0.29 of the phenotypic variation and the qADM5 allele was from the 'Cheongcheong'. The QTL qADM6-1 and qADM6-2 on chromosome 6 had LOD of 3.43 and 3.75. Both an additive effect and the phenotypic variation explained 0.29, 0.22, 0.30 and 0.30, respectively. However, the qADM6-1 allele was from the 'Cheongcheong', the qADM6-2 allele was from the 'Nagdong'. Finally, the QTL qADB8 was detected on chromosome 8 with an LOD of 3.75,

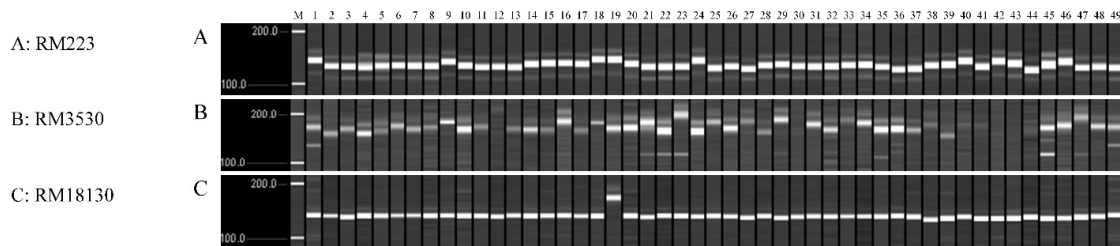


Fig. 4 The PCR results of 49 cultivars using three markers detected from the analysis of QTL related to alkali digestion value. A: RM223, B: RM3530, C: RM18130, 1: Cheongcheong, 2: Nagdong, 3: Jogwang, 4: Dongjin 1, 5: Dunnae, 6: Keumo 3, 7: Goun, 8: Geuroo, 9: Haepyeong, 10: Hanareum, 11: Hopum, 12: Hwayoung, 13: Jeoktomi, 14: Jinbu, 15: Jinmi, 16: Joan, 17: Joun, 18: Junam, 19: Namcheon, 20: Namil, 21: Nampyeong, 22: Obong, 23: Odae, 24: Samdeog, 25: Samgang, 26: Sangju, 27: Seolgang, 28: Wangchal, 29: Yangjo, 30: U-2, 31: Akenohgshi, 32: Fukunohana, 33: Hokuriku 130, 34: Ishikari, 35: Kitaake, 36: Koganenilcari, 37: Milk Princess, 38: Milky queen, 39: Princess sari, 40: Silewah, 41: Cakmak, 42: Demir, 43: Ece, 44: Efe, 45: Gonen, 46: Karadeniz, 47: Kizilmak, 48: Ilmi, 49: Baekjinju.

an additive effect of 0.28 and the phenotypic variation of 0.27, respectively. And the qADB8 allele was from the ‘Cheongcheong’, too (Table 3).

The polymorphisms found 49 varieties of *japonica* and *indica* types, including the ‘Cheongcheong’ and ‘Nagdong’ parent cultivars, were analyzed using three markers from the detected QTLs (Fig. 4). Nine cultivars (9, 18, 19, 24, 40, 42, 43, 45, 46) shown such as *indica* type. The results show that genetic polymorphisms in these varieties can be detected using the markers. Therefore, we considered the two markers, RM223, to be suitable for marker-assisted selection. But RM3530 and RM18130 were not distinguish in electrophoresis and more need study for marker in this study.

Discussion

A previous study examining heritability values were high in ADV treatment for eating quality (Kim et al. 1988). QTLs related to rice ADVs found that the phenotypic variation explained by the QTL on chromosome 8 was 48% (Lee et al. 2000). Thus, the QTLs associated with CNDH brown and milled rice found in this study are minor, because the total phenotypic variation explained by all QTLs in this study is close to the phenotypic variation explained by two QTLs found on chromosome 3 and 11 by Lee et al. (2000), which is only half of the QTLs they detected. The fact that so many major and minor QTLs are present is unsurprising, as previous studies have demonstrated that ADV is clearly a very polygenic trait. For example, Heu and Park (1979) reported that the major genes affecting ADV variation is influenced by several modifying genes, and differing expression of governing genes also affect their influence on ADV. Furthermore, a lower ADV was found to be the dominant trait.

Our results, showing ADV-related QTLs on chromosomes

1, 3, 5, 6, and 8, corroborate previous research. For instance, Shin et al. (1995) found five such QTLs on chromosome 1, 5, 7, 8, and 12. Moreover, QTLs associated with two similar traits, the alkali spreading value and the alkali-spreading score (related to gelatinization temperature), were mapped to chromosomes 2, 3, and 6 (Yan et al. 2001) and chromosome 6 (Sabouri et al. 2012, Aluko et al. 2004), respectively. In a large-scale study comparing grain quality characters that spanned multiple rice strains, the amylose content of *indica* rice was positively correlated to alkali spreading value and negatively correlated to gel consistency (Wang et al. 2005). However, no significant correlations were found between amylose content, alkali spreading value, and gel consistency in *japonica* rice (Wang et al. 2005). Additionally, a gene influencing the alkali-spreading score (*alk*) was found to be linked to the *Wx* locus (McKenzie and Rutger 1983; Sano 1984). Rice grain gelatinization temperature is one of the most important determinants of cooking quality. Generally, this trait is tested indirectly using the alkali spreading value test (Little et al. 1958), which is related to the alkali digestion value used in this study (Table 1). The ‘Cheongcheong’ and ‘Nagdong’ ADVs exhibited only small differences, but greater differences were observed between brown rice and milled rice. This is because the KOH solution can more easily reach amylose in milled rice compared to brown rice, which is shielded by a bran layer. Thermal properties closely linked to *alk* identified as a major gene in a research and the QTLs on chromosome 1 and 7 showed different effects to the *alk* gene. In this study, we detected three ADV-related QTLs across two years. As stated earlier, our results supported findings from prior studies: two QTLs (qADM) on chromosome 1 of milled rice had been detected previously (Bao et al. 2004), and three QTLs on chromosome 5, 6 and 8 had been reported by Kang et al. (1996). We point out, however, that although the QTLs reported here are similar to those reported in previous research

in terms of chromosomal position, the precise gene locations remain unknown. Thus, future research should focus on the fine mapping of QTLs detected with a single marker.

Differing outcomes across studies may be due to variation in study materials and study environment (Xiao et al. 1996). We analyzed polymorphisms in 49 rice varieties, including *indica*- and *japonica*-type cultivars, based on three markers detected from the QTL analysis. We consider this marker useful for marker-assisted selection in breeding programs to improve the eating quality of cooked rice.

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