Development of New CAPS/dCAPS and SNAP Markers for Rice Eating Quality

PUJI LESTARI¹, HEE JONG KOH∗∗∗∗∗

¹Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Jalan Tentara Pelajar No. 3A, Bogor 16111, Indonesia
²College of Agriculture and Life Science, Seoul National University, San56-1, Kwanak-gu, Seoul, South Korea

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Rice eating quality traits are very complex and essential to be evaluated not only through physicochemical analysis and sensory test but also by PCR-based marker approach. To date, simple markers based on single nucleotide polymorphism (SNP) discovery to evaluate eating quality of cooked rice are still limited. Thus, the aims of this study were to develop PCR-based markers, called SNAP (single nucleotide amplified polymorphism) as alternative markers of cleaved amplified polymorphic sequence/derived cleaved amplified polymorphic sequence (CAPS/dCAPS). Four primer pairs specific to targeted alleles (CAPS/dCAPS and SNAP) of four loci were successfully designed based on the discovered SNPs according to the eating quality-QTL and searching genomic database. The primer pairs were able to identify alleles corresponding loci among indica and japonica varieties with diverse palatability (overall eating quality). There was consistent allele pattern produced by SNAP and CAPS/dCAPS for the same base mutation. The SNAP marker for rice eating quality trait could be easily assayed by standard agarose gel electrophoresis, allowing to increase the advantage of genotyping methods. Moreover, the SNAP markers together with our previous developed markers which were recommended as applicable marker set for evaluation of rice eating quality, will facilitate as marker-assisted selection for rice breeding program.

Key words: CAPS, dCAPS, SNAP, SNP, rice eating quality

INTRODUCTION

Evaluation of rice eating quality by the application of sensory test and physicochemical properties has long been performed. Nowadays, we detect palatability as a direct trait of overall eating quality through the glossiness of cooked rice in order to speed up and simplify measurement compared to sensory test. The sensory test requires a large amount of samples and well-trained panelists. Physicochemical traits are an indirect method to estimate the rice eating quality which also depends on the mental condition of panelist. Many researchers have worked on relationships between eating quality and physicochemical properties. However, due to the genetically complicated eating quality traits (Matsue & Ogata 1998), to estimate accurately the eating quality is difficult. A rapid, simple, and more accurate method to evaluate eating quality need to be developed, that will be very useful for both the producer and the consumer of rice.

The complex characters related to genetic and environmental factors influence the difficulty of rice eating quality estimation. QTL analysis has been used as a useful method to detect locigenes controlling complex characters in rice. The use of molecular markers has facilitated the understanding of quantitative trait loci (QTLs) and marker-assisted selection (MAS). Many QTL analyses for grain quality of rice have been reported (Wan et al. 2004; Wada et al. 2008). QTLs related to palatability of cooked rice were identified at different loci in high quality japonica (Li 2004; Wada et al. 2008) and indica rice (Yong et al. 2006; Sabouri 2009). Thus, the allelic effect of QTLs associated with rice eating quality could possibly be used as markers.

Single nucleotide polymorphisms (SNPs), defined as单nucleotide change at a specific nucleotide position, are highly abundant and distributed through out the rice genome (McCouch et al. 2010). These SNPs have recently been developed into genetic markers. The abundance of these polymorphisms leads the SNP marker system to be a more attractive tool than any other marker system, including for developing marker for certain gene of interest or within target gene regions. Since SNPs are highly stable markers that often contribute directly to a phenotype, they can serve as a powerful tool for MAS.

PCR molecular markers based on SNPs which are cleaved amplified polymorphic sequences (CAPS;
Konieczny & Ausubel 1993) and derived CAPS (dCAPS; Michaels & Amasino 1998; Neff et al. 1998) are widely used for genotyping. CAPS markers detect polymorphisms that occur in restriction sites and dCAPS makers are created during PCR amplification by introducing a restriction site at an SNP site using specific primers. SNPs can also be detected using allele-specific PCR primers correspond to the site of the SNP (Ugozzoli & Wallace 1991). The other various methods based on allele-specific hybridization, primer extension, oligonucleotide ligation, endonuclease cleavage, or allelic-specific PCR corresponding to the site of the SNP (Gupta et al. 2001) for SNP marker have also been developed. Among many SNP genotyping methods, allele-specific PCR largely satisfies due to its simplicity. The products can be resolved on a standard agarose gel. However, allele-specific PCR has constrain, because a single-base pair change at the 3'-end is often not sufficient to ensure reliable discrimination between the two SNP alleles. To increase specificity, nucleotide-amplified polymorphism (SNAP) markers were developed. It is used the modified allele-specific primers with a mismatched base pair within four bases of the 3' end in addition to the 3'-end base complementary to the SNP site (Drenkard et al. 2000).

To complement sensory test and physicochemical analysis to evaluate rice eating quality, we developed a number of molecular markers for rice eating quality. These comprised microsatellite, indel (insertion/deletion), STS (sequence tagged-site) as well as SNP markers related to rice palatability, amino sugar, amino acid and starch physicochemical properties. Some of the developed markers combined with the validated markers from previous studies have successfully evaluated japonica rice eating quality (Lestari et al. 2009). In this study, we focused on the conversion of SNPs to CAPS/dCAPS and SNAP markers. For this objective, identification of SNP residing in the genes linked to interesting QTLs (Lestari et al. 2009) for rice eating quality traits. Analysis of nucleotide polymorphism of the sequences among japonica varieties was performed to develop primers for eating quality. To design primers, Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3) (Rozen & Skaltsky 2000) was used. PCR amplification of markers was carried out in a PTC-200 Peltier Thermal Cycler (MJ Research, Inc.) in a total volume of 50 μL with the following genotyping PCR reagents: 2 μL of DNA at 20 ng/μL, 2 μL of 10 x buffer containing 25 mM MgCl₂, 1 μL of 2.5 mM dNTPs, 1 unit of ExTaq Polymerase (TaKaRa, Japan), and 1 μL each of forward and reverse primers (10 μM). Amplified PCR products were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide. When the PCR product from each variety showed a single band, each of them was purified using PCR-Purification Kit (Intron Biotechnology, Korea).

### MATERIALS AND METHODS

**Plant Materials and DNA Extraction.** A total of 10 japonica rice varieties, mostly bred in Korea, and 10 indica varieties originated Indonesia were used in this study (Table 1). These varieties were chosen because they represent diverse palatability scores (hedonic score) among japonica and indica rice.

All rice varieties were grown in a light- and temperature-controlled greenhouse until the tillering stage. Only young and healthy leaves were harvested and collected for DNA extraction. Genomic DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) method (Murray & Thompson 1980). The quality of DNA was estimated using NanoDrop2000 on 260/280 and 260/230 wave length ratios. The DNA was migrated on 1% agarose and stained in ethidium bromide, then visualized on UV transilluminator.

**Primer Design and PCR Amplification.** Target regions were selected either close to or within the genes linked to interesting QTLs (Lestari et al. 2009) for rice eating quality traits. Analysis of nucleotide polymorphism of the sequences among japonica varieties was performed to develop primers for eating quality. To design primers, Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3) (Rozen & Skaltsky 2000) was used. PCR amplification of markers was carried out in a PTC-200 Peltier Thermal Cycler (MJ Research, Inc.) in a total volume of 50 μL with the following genotyping PCR reagents: 2 μL of DNA at 20 ng/μL, 2 μL of 10 x buffer containing 25 mM MgCl₂, 1 μL of 2.5 mM dNTPs, 1 unit of ExTaq Polymerase (TaKaRa, Japan), and 1 μL each of forward and reverse primers (10 μM). Amplified PCR products were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide. When the PCR product from each variety showed a single band, each of them was purified using PCR-Purification Kit (Intron Biotechnology, Korea).

**Cloning, Sequencing, and SNP Identification.** The purified PCR products were TA-cloned into pGEM-T Easy vectors and transformed into *Eschericia coli* DH5 naïve competent cells prepared...
according to the protocol of Sambrook and Russell (2001). Plasmids were isolated using the DNA-spin Plasmid DNA Purification Kit (Intron Biotechnology, Korea) and sequenced with an ABI-3700 DNA sequencer following the manufacturer’s instructions (Applied Biosystems, Inc.). To identify SNPs, sequence results were aligned using the CLUSTALW program (Larkin et al. 2007) from EMBL European Bioinformatics Institute (http://www.ebi.ac.uk/tools), with assistance from Codoncode Aligner 2.0.6 (CodonCode Corporation, Dedham, MA) as well as BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

**Designing CAPS/dCAPS Primers.** To detect one bp substitution in a specific fragment, a CAPS/dCAPS primer was designed, facilitated by dCAPS Finder 2.0 (http://helix.wustl.edu/dcaps) (Neff et al. 2002). The primer corresponding to opposite site was generated by Primer3 program. Initially, the resulted sequences corresponding to loci were multiple aligned among rice varieties against reference japonica rice, Nipponbare from database (www.gramene.org) to identify SNPs. The two identical sequences existing an SNP in the middle with approximately 25 nucleotides on each side were entered for CAPS/dCAPS program. No more than 60 nucleotides of reference (SNP existed in Nipponbare) and the alternate SNP should be entered in the box with A, C, G, and T substitution facilitated in dCAPS Finder 2.0 program. In the dCAPS Finder 2.0 box for entered sequence, sequence of a reference allele and sequence of an alternate allele were denoted as wild type and mutant, respectively. A number of mismatches started from zero and increased depending on the generated output. The output from zero mismatches showed whether a CAPS marker was present. If a CAPS marker was not generated, one mismatch was entered to search for a dCAPS marker. The number of mismatches was increased in each run until a potential dCAPS marker has been identified. The dCAPS primer included the necessary mismatches 5’ of the mutation, but not include the SNP being analyzed. One mismatch produced several primer pairs with high stability were selected and tested using optimum PCR reaction and program as SNAP marker.

**Designing SNAP Primers.** In addition to CAPS/dCAPS primer, the identified SNP could be converted to SNAP primer. A primer specific to the identified SNP was designed by entering the segment sequence approximately 500-800 bp containing the SNP site into the Web-available SNAPER program (http://ausubellab.mgh.harvard.edu/) with default option. In SNAPER program, items of PCR product with optimum size range of 325-375 bp and PCR product absolut ranged 300-500 bp were chosen. While other primer criteria followed SNAPER instruction. After submission, optional primers output with reference and alternate alleles could be seen on display. Around 16 output of combination of primer pairs corresponding to the SNP appeared. Candidate of primers pairs with high stability were selected and tested using optimum PCR reaction and program as SNAP marker. Finally, a pair of primer specific to corresponding allele with single band and consistent to the SNP existed in rice varieties could be used as SNAP marker.

PCR was performed in a total volume of 20 μl, which contained 10-100 ng of template DNA, 5 μM of each of the forward and reverse primers, 100 μM of each dNTP, 1.5 mM MgCl2, 1 X-reaction buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), and 1 U of AmpliTaq Gold polymerase (Applied Biosystems). Template DNA was initially denatured at 94°C for 5 min according to Drenkard et al. (2000), followed by 28 or 38 cycles for PCR amplification, using the following conditions: denaturation at 94°C for 30 s and annealing and extension at 64°C for 1 min, and then final extension at 72°C for 10 min on a PTC-225 Peltier Thermal Cycler (MJ Research). Amplified products were separated on a 1.5% agarose gel to estimate each allele in the SNP site as presence or absence of a band.

**RESULTS**

**SNP Identification.** The analyses of QTL for rice eating quality allow to select candidate genes underlying the QTL regions (Lestari et al. 2009), including *sucrose synthase 3* (S3c, clone AP004988), *UDPN-acetylglucosamine pyrophosphorylase* (AcPh, clone AP003875), and *phosphoserine phosphatase* (PP, clone AP003727). In addition, a candidate gene involved in lipo- polysaccharide biosynthesis, *6-phosphofructokinase-2* (PFruc, clone AP002743) contributing to starch physicochemical properties was selected (Table 2). A basic primer pair designed on the basis on highly
variable region from each locus was used to identify
SNPs (Table 2). Sequence alignment analysis among
japonica rice varieties showed that base changes were
detected in the segment sequences of the genes
positioned in intron and exon. All fragments of each
locus belong to different contig in rice genome.

Point mutation was determined by using japonica
rice genome of Nipponbare as reference and the other
alternate allele on other variety. Finally, base changes
of T/G, T/G, A/G, and A/G on the consensus sequence
of S3c, AcPh, PP, and P-Fruc were identified
respectively, as shown in Figure 1. Deletion of 4 bp

Table 2. Selected candidate genes based on QTL for rice eating quality and searching genomic data base and their designed primers

<table>
<thead>
<tr>
<th>Basic primer name</th>
<th>Candidate gene</th>
<th>Chr</th>
<th>Contig</th>
<th>QTL position</th>
<th>Position of product at genome (bp)</th>
<th>QTL source</th>
<th>Primer sequences</th>
<th>Estimated product size (bp)</th>
<th>SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>S3c</td>
<td>Sucrose synthase 3 (LOC_Os07g42490)</td>
<td>7</td>
<td>AP004988</td>
<td>RM234-RM47</td>
<td>25427790 25428733</td>
<td>Kwon et al. (2007)</td>
<td>F:5'-GCAAACCTG GAAGAGAGAG-3' R5-AAGTGACCAT GCATTGGGA-3'</td>
<td>963</td>
<td>T/G</td>
</tr>
<tr>
<td>AcPh</td>
<td>UDP-N-Acetylglucosamine pyrophosphorylase (LOC_Os08g10600)</td>
<td>8</td>
<td>AP003875</td>
<td>RM547-RM72</td>
<td>6233 6234836</td>
<td>Kwon et al. (2007)</td>
<td>F:5'-GGCCTCTGTTA TTTGAGAGG-3' R5-CTGCTACTGG GGAAGATTGG-3'</td>
<td>849</td>
<td>T/G</td>
</tr>
<tr>
<td>PP2</td>
<td>Phosphoserine phosphatase (LOC_Os12g31820)</td>
<td>12</td>
<td>AP003727</td>
<td>RM1246</td>
<td>19119527 19120418</td>
<td>Wada et al. (2008)</td>
<td>F:5'-TGGTAACTGG CATGGAATTG-3' R5-GCTCTGATTCAT TAGCAAGT-3'</td>
<td>981</td>
<td>A/G</td>
</tr>
<tr>
<td>PFruc</td>
<td>6-Phosphofructokinase 2 (LOC_Os01g09570)</td>
<td>1</td>
<td>AP002743</td>
<td>-</td>
<td>4902825 4903704</td>
<td>Genomic data base</td>
<td>F:5'-CTTCTTCTTCGG GTGTCTCG-3' R5-TGTTAAGTGG GACAGGG-3'</td>
<td>880</td>
<td>A/G</td>
</tr>
</tbody>
</table>

Figure 1. Partial sequence alignments showing exact location of a base substitution among japonica rice varieties at 110, 127, 397, and 176 bp of consensus sequences derived from loci of PP, S3c, AcPh, and P-Fruc, respectively. The reference allele was determined on Nipponbare and the alternate allele was noted by bold letter. The sites of single nucleotide polymorphisms (SNPs) were indicated as bold black asterisks.
at S3c was proved to be potential for indel marker based on the full sequence of this gene (Lestari et al. 2011) and included in the marker set to estimate japonica rice palatability (Lestari et al. 2009). The nucleotide substitutions for four loci were subjected to design SNP primers to evaluate rice eating quality. To validate the markers, rice variety of mapping parent for linkage genetic analysis (Kwon et al. 2007) as source of candidate genes selection, also was used as genetic material for application of these markers developed in our study.

**Development of CAPS/dCAPS Marker.** Once SNP is identified, it is likely to convert in developing primer of CAPS/dCAPS as demonstrated in the examples of this study. Several optional outputs of primers either forward and/or reverse depending on restriction enzyme site of each sequence, could be used as primer candidates. Importantly, number of mismatch contributed to reveal primer type, CAPS or dCAPS. In case with no mismatch, it seemed to be easy to convert SNP existed in PP locus leading for CAPS primer design. Conversely, the other three loci were preferentially developed as dCAPS primers since at least one mismatch should be entered to produce primer candidate.

Finally, a total of four molecular markers were successfully generated on the basis of SNPs, comprising one CAPS primer for PP locus, and three dCAPS primers for S3c, AcPh and PFruc loci (Table 3). For development of specific CAPS/dCAPS primers in this study, only the commonly used restriction enzymes were selected to be complement with the designed primers. For instance, *MseI*, *EcoRI*, and *TaqI*. The dCAPS method could potentially use any sequences with identified SNP for the development of a PCR-based marker. Application of these markers for genotyping showed polymorphism of the allele belonging to each locus among varieties with diverse palatability (Table 4). This result demonstrates different genetic background of both japonica and indica rice varieties. Loci of PP and S3c revealed dominant alleles of G and T on

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**Table 3. Sequences primers of CAPS/dCAPS and SNAP with the amplification condition for SNP primers**

<table>
<thead>
<tr>
<th>Original primer</th>
<th>Sequence</th>
<th>Primer type</th>
<th>SNAP primer name</th>
<th>Sequence</th>
<th>PCR condition of SNPrimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcPh</td>
<td>F:AGTGGTGTTGTT TAAGCATAAG</td>
<td>dCAPS/Mse</td>
<td>AcPh-T</td>
<td>F:TCAGGTATGCCTGC ATATTTTACCTTT</td>
<td>94°C 5min 94°C 30sec</td>
</tr>
<tr>
<td>R:ATTGTCTTTTCT TAAAGTTTATAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFruc</td>
<td>F:CCCTCTCTTTG GGTGTCTTG</td>
<td>dCAPS/EcoRI</td>
<td>PFruc-A</td>
<td>F:GGGCTGTGTTTGGTG TCGGTTCTCCTA</td>
<td>94°C 5min 94°C 30sec</td>
</tr>
<tr>
<td>R:GTATTAGATCCAG GGCAGAGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3cII</td>
<td>F:TTCCCATGATGTTG CCACTCTC</td>
<td>dCAPS/TaqI</td>
<td>S3cII-T</td>
<td>F:CATCCCAAATTCGTTTTAT TTAATTTAATACCTCT</td>
<td>94°C 5min 94°C 30sec</td>
</tr>
<tr>
<td>R:GGACAAAGTTTTT CAGTGAATAAAAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP2</td>
<td>F:TTGTAAAGGTGC CCCTGTTT</td>
<td>CAPS/Mse</td>
<td>PP2-A</td>
<td>F:AAAGACAAAGGGAAGGT TGGCGGAAGAAATATTTA</td>
<td>94°C 5min 94°C 30sec</td>
</tr>
<tr>
<td>R:CCATGCATCTCA TTAGTCAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Table 4. Allele variation produced by the 4 designed SNP markers observed on japonica and indica rice varieties**

<table>
<thead>
<tr>
<th>Japonica rice</th>
<th>Hedonic score*</th>
<th>AcPh</th>
<th>S3cII</th>
<th>PP2</th>
<th>PFruc</th>
<th>Indica rice</th>
<th>Hedonic score*</th>
<th>AcPh</th>
<th>S3cII</th>
<th>PP2</th>
<th>PFruc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koshihikari</td>
<td>4.3</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>G</td>
<td>Rojolele</td>
<td>4.1</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>Samgwang</td>
<td>4.5</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>Ciliwung</td>
<td>3.9</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>Hwacheong</td>
<td>3.4</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>G</td>
<td>Cisokan</td>
<td>3.45</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Dobong</td>
<td>2.3</td>
<td>T</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>Cibodas</td>
<td>3.6</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Gopum</td>
<td>4.2</td>
<td>T</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>Jatiluhur</td>
<td>2.2</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Palkong</td>
<td>3.1</td>
<td>T</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>Kalimutu</td>
<td>3.6</td>
<td>T</td>
<td>G</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Dongjin</td>
<td>3.9</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>A</td>
<td>Cirata</td>
<td>3.4</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Samnang</td>
<td>2.9</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>A</td>
<td>Memberamo</td>
<td>4.0</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>Giho</td>
<td>3.5</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>A</td>
<td>Cihang</td>
<td>3.9</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>Chucheong</td>
<td>4.0</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>A</td>
<td>Siantanur</td>
<td>3.1</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

*The higher score represents the higher palatability.*
both japonica and indica, respectively, but not for the other loci. These alleles for each single marker have no significant association with rice palatability either indica or japonica. Consequently, combination of these markers than single marker were preferentially applied. The designed dCAPS markers, both S3cII and AcPh were as components of two marker sets for each comprising 13 and 14 markers to evaluate japonica rice eating quality (Lestari et al. 2009). Establishment of dominant SNP-based markers which included in these marker set detection will give simpler application.

Development of SNAP Marker. A simple and low-cost marker is more prospective and practical especially for selection of early breeding with abundant population. Therefore, another alternative of dCAPS marker that less expensive and simple was generated in this study. All the detected SNPs as modified into CAPS/dCAPS primers were converted into SNAP marker (Table 3). As an example, based on the identified a base change of T to G in AcPh locus, a SNAP marker specific to T allele was developed. SNAP primers were usually developed on specific allele, in which the base change type exhibited. The T-specific primer of AcPh (AcPh-T) showed a band in only variety possessed T allele in AcPh locus. The banding pattern of T/G allele of T-specific primers was the same as that of dCAPS-AcPh primer (Figure 2). In specific case, T allele-specific marker only amplified genomic DNA on varieties that had a T allele at locus AcPh and vice versa for alternate allele.

DISCUSSION

A numerous variation of rice genome sequences has promoted the SNPs search between varieties. Since SNP genotyping is needed for many applications such as map based cloning, marker-assisted breeding, and seed purity test (Komori & Nitta 2005), many new techniques have been established. The techniques were to convert an identified SNP to molecular markers either on the basis of random total genome or emphasizing specific candidate genes related to target traits. This SNPs converted to functional molecular marker might be derived from a functionally characterized sequence motif of the loci studied here, which is superior to random DNA markers owing to its complete linkage with the target gene.

Based on analysis of QTL for rice eating quality (Kwon et al. 2007; Wada et al. 2008), three candidate genes encoding proteins, i.e. sucrose synthase 3 (S3c), UDPN-acetylglucosamine pyrophosphorylase (AcPh), and phosphoserine phosphatase (PP) sequences underlying target QTL were successfully selected as a potential source for markers in this study. Searching gene involved in the rice pathway of lippo-polysaccharide biosynthesis.
which is overlapped its physical position to the QTL region for rice starch physicochemical properties could also be a potential source as functional marker (Lestari et al. 2009). The sucrose synthase 3 functions to synthesize sucrose maximally at filling grain in rice, while UDPN-acetylglucosamine pyrophosphorylase is well known to involve in amino sugar biosynthesis. Phosphoserine phosphatase seems to participate in amino acid metabolism, in contrast, 6-phosphofructokinase-2 focuses on the pathway of lipo-polysaccharides biosynthesis.

The existence of SNP generating a restriction site difference between rice varieties on the PP locus is critical for CAPS marker development. However, the SNP generating no restriction site differences on AcPh, S3c and PFuc could be converted to dCAPS marker, as proved in Arabidopsis thaliana as well (Hayashi et al. 2004; Komori & Nitta 2005). Thus, by introducing 1-2 mismatches into forward or reverse primer could disrupt the redundant restriction sites (Komori & Nitta 2005) for dCAPS. The dCAPS is different from CAPS in using specific primers, which are designed with one or two mismatches (Drenkard et al. 2000). Through these mismatches, primers bring mutations into the target sequences during amplification and in conjunction with the identified SNP resulting in a unique restriction site. Moreover, the output of this program provides a set of suitable primer sequences along with appropriate restriction endonucleases (Hruba 2007). The primary application of CAPS/dCAPS method allows to advance application with restriction enzyme and was successful to detect the distinction of rice varieties based on the alleles corresponding to their loci as shown in our study. The simple PCR, treatment of restriction enzyme and observation on agarose gel of this marker bring it to be still preferentially applied in any laboratories (Michaels & Amasino 1998).

The SNP markers mainly developed are CAPS/ dCAPS that need a large amount of restriction enzyme (Ayres et al. 1997; Liu et al. 2004; He et al. 2006). In some cases, a high cost of restriction enzyme might be a problem, especially for selection process using a large number of breeding population. Thus, the other allele specific primer, called SNAP could be an alternatively promising solution. When observed in both japonica and indica varieties, we found the same DNA banding patterns between CAPS/CAPS and SNAP markers corresponding to all locus. SNAP markers on the basis of SNP with easy and low cost will complement to the previous established markers for rice eating quality (Bao et al. 2006; He et al. 2006).

Several functional markers for targeted eating quality traits of rice with high association have been developed to be powerful markers as single marker (Bao et al. 2006; He et al. 2006; Wang et al. 2010; Ni et al. 2011) or marker set for marker assisted selection (Nakamura et al. 2004). These markers themselves may not have direct functions, but they can still relate to the variations in phenotypic traits, between the marker site and the functional domain of the target genes (Bao et al. 2006). These SNP-based primers investigated in this study virtually which were components of the marker sets as multiple interaction showed highly significant association to rice eating quality both on japonica and indica (P < 0.001) (Lestari et al. 2009). Relevant to this study, several markers for rice eating quality also have been developed based on universal primers, RAPD (random amplified polymorphic DNA) converted to SCAR (sequence characterized amplified regions) and made in marker set showing powerfully to evaluate rice premium with high palatability in Japan (Ohtsubo et al. 2002, 2003). In addition, this result was supported by other study that markers for palatability developed on the basis of japonica genome was able to identify Indonesian indica rice with high palatability (Lestari et al. 2012). No doubt that even the SNPs were discovered based on the japonica rice genome, those identified SNPs were also detected and could be applied in indica rice because of the wide range of genetic diversity between japonica and indica rice varieties. Wider diversities of landraces and breeding lines could be included for genotyping study to assure the allele variation patterns.

Due to the genetical complexity of rice eating traits, by combining with other functional markers that we developed, these SNAP markers are recommended to be used as marker set than single marker. These dominant markers will simplify the marker set performed for evaluation of rice eating quality (Lestari et al. 2009) and are useful for selection of rice eating quality with large samples. These SNP-based markers investigated in this study could be complement the other functional markers (STS, indel, and microsatellite) that we developed on the basis of loci genotypes controlling rice starch physicochemical properties determining eating quality. These all SNP-based markers together with the other developed markers (Lestari et al. 2009) will expectedly use to establish more marker sets for evaluation of other physiochemical properties of both japonica and indica rice.
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