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DNA markers for eating quality of indica rice in Indonesia

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Abstract

Rice eating quality is considered to be one of the top priorities in determining the agronomical value of rice; thus, the rapid evaluation of eating quality at early breeding generations in breeding programmes for better eating quality is of great importance. In an attempt to develop DNA markers associated with eating quality of indica rice, we used multiple regression analysis to test 54 markers, which were preselected for their possible association with eating quality, using 24 indica varieties with different palatability scores. Of these markers, eighteen markers were found to be significantly associated with palatability according to sensory evaluation. Accordingly, a marker set in the model regression equation with a high R^2 (0.997) was formulated to estimate indica rice palatability. Validation suggests that markers and the statistical parameters formulated by the equation could be a potential tool to predict the palatability of cooked Indonesian *indica* rice and could be reliable in developing country-dependent model equations for eating quality.

Key words: eating quality — indica rice — regression analysis

Eating quality is the most important end-use trait of cooked rice. Consequently, taste preferences become increasingly important for utilizing and selecting rice varieties especially for consumers in rice-producing countries. Rice grain features diverse physicochemical properties, such as starch quality, which is major determinant of the eating and cooking properties for different varieties (Allahgholipour et al. 2006). Among such properties, amylose content (AC), which also contributes significantly to the pasting property that can be measured with such instrumentation as RVA (Rapid Visco Analyser), is an important determinant of the eating, cooking and processing quality of rice. Total protein content in grain also affects the palatability of cooked rice (Yu et al. 2008), reflecting the complex interplay of numerous traits defining rice eating quality, which is a critical component of breeding programmes. In addition to genetic factors involved in the synthesis of starch and protein, rice eating quality is also largely affected by environmental factors, cultural practices and postharvest practices such as air temperature during ripening, the amount of fertilizer, irrigation management, grain-drying after harvest and cooking methods (Izumi et al. 2007).

While japonica rice is mostly grown and consumed in north-eastern Asian countries, indica rice is cultivated worldwide, including south-eastern Asian countries. Indica rice is generally characterized by its higher amylose content and long grains; it is bland, flaky, firm and fluffy when cooked (Kim and Rhee 2004). The textural difference between indica and japonica rice cannot be explained only by the relative amylose content and gelatinization temperature. The stickiness of cooked rice is largely correlated with the sucrose content and the amount of short-chain amylopectin fraction, which, in turn, is inversely proportional to the amount of amylose in the surface layer of cooked rice extract. Once eluted from the rice during cooking, these components are then absorbed into the surface layer, giving it a sticky quality (Hatae et al. 2004).

The sensory test, the fundamental test of eating quality, provides information on appearance, aroma, taste, hardness, stickiness and overall quality. Estimation of rice palatability has been mainly accomplished by utilizing sensory evaluation as well as chemical analysis, even though these methods have some pitfalls. The chemical method is costly and time-consuming, whereas the sensory evaluation can be hampered by high variability resulting from the subjective judgment of the panel members (Azuma et al. 1994). Physicochemical properties determining eating quality of rice can be measured with a smaller amount of rice than that required for the sensory test. Some studies on mathematical models for predicting eating quality of rice from physicochemical properties have also been reported. However, it is still difficult to predict eating quality of rice by these models. Moreover, the taste instrument to determine eating quality of cooked indica rice is less precise. Thus, it is essential to develop a method that can estimate indica rice eating quality with low cost and improved speed, simplicity and precision.

In a breeding programme, molecular genetics approaches can be integrated with conventional breeding to facilitate the development of new varieties with better eating quality. For instance, marker-assisted selection (MAS) could be an effective alternative to conventional evaluation and selection for the desirable varieties at early generations, thereby providing faster results than conventional approaches (Bao et al. 2008). To assess eating quality using MAS, identification of DNA markers closely linked to the genes controlling the quality is essential, and accordingly, previous QTL analyses for rice eating quality in several types of different populations derived from crosses within japonica cultivars (Suh et al. 2004, Tanaka et al. 2006, Wada et al. 2008, Kwon

et al. 2011) as well as between japonica and indica cultivars (Li et al. 2003, Takeuchi et al. 2007, Wan et al. 2007) have been excellent sources for marker development.

Progress in the development of molecular markers related to rice eating quality has been achieved through genetic and molecular marker-based QTL analyses, and genomic database searches. Some SSR (simple sequence repeat), SNP (single nucleotide polymorphism) and STS (sequence-tagged site) markers for starch-synthesizing genes have been developed in this way (He et al. 2006, Zeng et al. 2007, Bao et al. 2008). Other STS markers that were obtained by transformation of random amplification of polymorphic DNA (RAPD) markers have been used to differentiate japonica varieties, particularly the superior Japanese variety, Koshihikari (Ohtsubo et al. 2002, 2003, Ohtsubo and Nakamura 2007). These markers were also useful for evaluating the palatability of japonica varieties, so several studies have used them to predict eating quality (Ohtsubo et al. 2003, Nakamura et al. 2004, Lestari et al. 2009). However, DNA-marker-based evaluation for eating quality in indica rice has not been reported yet.

In this study, the eating quality of indica rice originating from Indonesia was estimated by sensory test and physicochemical property assays. New molecular markers associated with the eating quality of indica rice were then identified and compiled with previously reported markers to be used for a genotyping test. Thus, by defining physicochemical properties and molecular markers correlated with the palatability of *indica* rice, this study aimed at initially establish a formulated set of methods to evaluate overall eating quality of cooked rice in indica varieties.

Materials and Methods

Plant materials and DNA extraction: In this study, we used a total of 24 indica rice varieties and 23 local accessions, originating from Indonesia and had diverse palatability. All varieties were grown using national recommended cultural practices at the experimental farm of Indonesian Center for Rice Research, Sukamandi, Indonesia, during the dry season from March to October. Rice grains used for physicochemical analysis were dried to 15% moisture content. For DNA extraction, all rice varieties were grown in a well-controlled greenhouse until the tillering stage. The healthy and young leaf tissues were harvested and collected. Genomic DNA was extracted using cetyl trimethylammonium bromide (CTAB).

Development of molecular marker: Analysis of nucleotide polymorphisms in the sequences among japonica and indica cultivar groups was performed to develop primers for eating quality. Additionally, we searched genomic databases to identify potential candidate genes from which to develop markers in this study (Table 1). PRIMER3 software facilitated by http://www.biotools.umassmed.edu/bioapps/primer3_www.cgi was used for primer design. The amplified bands were purified, TA-cloned and sequenced using an ABI 3700 DNA sequencer following the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). To identify SNPs, indels (insertions and deletions) and/or microsatellites among rice varieties, the sequence results were aligned using EMBL-European Bioinformatics Institute Clustal W program (Thompson et al. 1994) (http://www.ebi.ac.uk/tools), assisted with CodonCode Aligner 1.3.4 (CodonCode Corporation, Dedham, MA, USA) and BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). To detect one base substitution in the specific fragment, a dCAPS primer was designed facilitated by dCAPS Finder 2.0 (http://helix.wustl.edu/dcaps). Previously reported markers (He et al. 2006, Zeng et al. 2007, Kobayashi et al. 2008) and some selected markers included in the marker sets comprising 13 and 14 markers in the regression equation for the evaluation of japonica rice eating quality (Lestari et al. 2009) were also utilized for analysis in this study. The list of markers previously reported and their sequences is presented in Table 1.

PCR amplification and sequencing: Standard PCR for further sequencing analysis using ExTakara *Taq* polymerase (Takara, Tokyo, Japan) was carried out in a PTC-200 Peltier Thermal Cycler (Bio-Rad, Hercules, CA, USA) in a total reaction volume of 50 μl. The amplifications were performed under the condition of 35 cycles of 1 min at 95°C, 30 s at 55°C and 1 min at 72°C. PCR amplification of DNA using the selected primers was performed in a total volume of 20 μl with the following PCR reagents: 2 μl of DNA at 20 ng/μl, 2 μl of 10X buffer containing 25 mM $MgCl_2$, 1 μl of 2.5 mM dNTPs, 1 U of *Taq* Polymerase (Intron Biotechnology, Seoul, Korea), and 1 μl each of forward and reverse primers (10 μM). The PCR amplifications were performed for a total of 35 cycles of 1 min at 95°C, 30 s at 55°C and 1 min at 72°C. Amplified PCR products were analysed by electrophoresis on 3% agarose gels stained with ethidium bromide and/or by non-denaturing electrophoresis on 8% polyacrylamide gels stained with ethidium bromide (Model MGV-202-33, CBS Scientific Co., Del Mar, CA, USA).

Evaluation of eating quality traits: For physicochemical analysis, the rice grains were hulled and milled to the yield of 91%. PC was calculated by total nitrogen multiplied by 5.95 after determining the nitrogen content of rice material using the micro-Kjeldahl method (AOAC 1995). The AC of milled rice was determined by the relative absorbency of starch–iodine colour in digested solution of 100-mesh rice flour by the method of Perez and Juliano (1978). RVA pasting properties were determined on a Rapid Visco Analyser (RVA) in accordance with the operation manual (Newport Scientific, Warriewood, Australia). Rice starch paste profile characteristics were described by six parameters, peak viscosity (PV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity (BDV = PV−HPV), setback viscosity (SBV = CPV−PV) and consistency viscosity (CTV = CPV−HPV), according to Bao and Xia (1999).

Sensory evaluation of rice varieties: Sensory evaluation of cooked rice samples was conducted by 11 professionally trained panel members (age from 38 to 55 years) with 5 replications at the Indonesian Center of Rice Research, Sukamandi. To produce reliable and valid data, the panellists should be treated as a scientific instrument; therefore, they must be free from mental features and physical conditions which might affect their judgement. All sensory panels must have an ability to conduct the sensory test and to repeat consistently. The overall palatability/eating quality was assessed according to appearance (glossiness), flavour, texture, taste and colour and scored from 1 to 5 (1: unacceptable and 5: excellent). Palatability score by sensory test of each variety was the average value scored by 11 panels. Cooked rice samples were prepared according to the protocol of NICS, Rural Development Administration, Korea. Dry-milled head rice (300 g) was rinsed four times and soaked for 30 min with distilled water and strained from the water for 10 min. Rice was cooked using an electric rice cooker with a ratio of rice water of 1 : 1.25 w/w. After completion of the automatic cooking cycle, the rice was allowed to remain in the cooker for 30 min. Samples were transferred to plates and kept at room temperature for about ten minutes until cooked rice samples cooled down to 35–37°C. The order of sample was presented randomly but not randomized across panellists due to the importance of serving temperature. Panel performance was monitored for repeatability and discriminative judgement. In this sensory evaluation, we ignored the environmental effect which may contribute to the eating quality. This sensory evaluation was performed at the same procedure for both the formulation of marker set and its validation.

Statistical analysis: The obtained data were subjected to analyses of variance using SAS 8.2 (Statistical Analysis Systems Institute 2001). The difference between trait means was evaluated using the least significant difference. Correlation and regression analyses were conducted to determine the relationship between traits. Multiple regression analysis was performed to determine the relationship between palatability scores by molecular markers and sensory test. As described in the previous

Table 1: List of markers previously reported and developed in this study for evaluation of indica rice eating quality

PCR marker	Marker type	Chr[1]	Sequence	
			Forward (5′-3′)	Reverse (5′-3′)
Ohtsubo et al. (2002, 2003); Ohtsubo and Nakamura (2007)				
A6	STS	7	CCAGCTGTACGCCTGTACTAC	CCAGCTGTACGTCTTCCCCAGC
A7	STS	12	TGCCTCGCACCAGAAATAG	TGCCTCGCACCATGAG
B1	STS	11	GTTTCGCTCCTACAGTAATTAAGGG	GTTTCGCTCCCATGCAATCT
B43	STS	9	GGCCGGCATGACTCAC	ACTGGCCGGCATCAAGAC
F6	STS	4	ACCACTCCATATATATCATCCAAAG	ACCACTCCATATCACCACAAGG
G4	STS	1	GAGACCGATATGCGATTC	GTGGTGTTTAGATCCAGAGACTTA
G22	STS	9	CTCACTCAAATTTACAGTGCATTTTCTTG	AGGGCCATGATACAAGACTCTGT
G28	STS	1	GGCGGTCGTTCTGCGAT	GGAGAATCCCACAGTAAGTTTTTCTTTG
J6	STS	11	GTCGGAGTGGTCAGACCG	GTCGGAGTGGATGGAGTAGC
M2CG	STS	8	ACAACGCCTCCGATGA	ACAACGCCTCCGACAACAAGAT
M11	STS	6	GTCCACTGTGACCACAACAT	GTCCACTGTGGGGATTGTTC
P5	STS	10	ACAACGGTCCGTCCTTGCTT	ACAACGGTCCAACAGATACTTTTGA
S13	STS	1	GTCGTTCCTGTGGTTAGGACAGGGT	GTCGTTCCTGCTGGTGTCTCAGAT
T16	STS	12	GGTGAACGCTGTAGTTGGAATATA	GGTGAACGCTCAGATTTAAATATAAT
WK9	STS	9	CCCGCAGTTAGATGCACCATT	CCGCAGTTAGATCAAGTGGC
E30	STS	1	TACCTGGTTGATGTATACAGATCTGGTT	ATCCCTCGATCCCTCTAGCATTAT
B7	STS	2	CAGGTGTGGGTTACAAGGATGA	CAGGTGGTTCACGGCCTTT
G49A	STS	11	AATCCAGACATGAAATTTATATGCAGATA	AATCCAGACATGTTGTCCTCAATTTTTG
G81	STS	6	TACCTGAACCAGCAAGCATGCGCG	TACCTGAACCAGTATAATCTTTG
P3	STS	5	AACGGGCCAAAAACGGAGGT	AACGGGCCAACGCAG
Bao et al. (2006a,b)				
Wx (SNP)	CAPS/AccI	6	CTTTGTCTATCTCAAGACAC	TTTCCAGCCCAACACCTTAC
SS1 (SSR)	SSR	6	GATCCGTTTTTGCTGTGCCC	CCTCCTCTCCGCCGATCCTG
SBE1 (SSR)	SSR	6	ATTTCTTTGGCCACAGGCGA	CCCAGATTCGGAACAAGAAC
SBE1 (STS)	STS	6	GAGTTGAGTTGCGTCAGATC	AATGAGGTTGCTTGCTGCTG
SBE2 (SNP)	dCAPS/SpeI	2	GTCTTGGACTCAGATGCTGGACTC	ATGTATAACTGGCAGTTCGAACGG
SSIIa	SNP	6	F7:CTGGATCACTTCAAGCTGTACGAC	R1:GCCGGCCGTGCAGATCTTAAC
			F22:CAAGGAGAGCTGGAGGGGGC	R21:ACATGCCGCGCACCTGGAAA
Lestari et al. (2009)				
S3cI	Indel	7	CCACTCTCATGTCCTTGAAC	GCCATGACATTTGGACAT
S3cII	dCAPS/TaqI	7	TTCCATGATGTGCCACTCTC	GGACAAATGTTTTCAGTGAATAAAT
TreB	Indel	7	CACTCCAGTTCCTGCTCAAA	CACCTCCAAAACGAATATGG
AMs	SSR	2	CTTCCAAGGACCCCATCCT	CCCAACATCTCCGTCAGAAT
GPA	SSR	11	CCAAATACGCGGCCTTCT	AGTTTCTGGGCTCGGAGGA
GBSS1	SSR	6	CAAATAGCCACCCACACCAC	CTTGCAGATGTTCTTCCTGATG
AcPh	dCAPS/MseI	8	AGTTGTGGTTTAAGCATAGG	ATTGTCCTTTTCTTTAAAGTTTATTA
CBG	SSR	10	AGCTTCCCTAATGGCTTCGT	ATTTGCCAACTTTTGGATGG
SH51	dCAPS/SpeI	1	ATTCTTGATGAAAATAATTAACTAG	GGTTAACCATCTTATAAAATTTGTC
He et al. (2006)				
SS1 (STS)	STS	6	TCTAGATTGCTACACGTGAGAGG	TCTCCACGATAACTTCCACC
SBE3 (STS)	STS	2	TCGGTCAATTCGGTTAGTCTCCTC	ACATCCTCTAGCATACTGGCGACTC
SssIIa	STS	6	TCTAGATTGCTACACGTGAGAGG	GGAGCCACCTGTAAAGCGTG
Isa	STS	8	CCTGTCTTGCACGTGCGGTA	GCACGGTTCTGATGTACGAGAG
Pul3 (3′end)	STS	4	GGGTTCGCTTTCACAACACAG	GTCACGACATAAGAGAAGCTGC
Pul5 (5′end)	STS	4	AGTTCGCTAGTCATCTGCTCG	CCACATGTCCTTGTCTCCACTT
Zeng et al. (2007)				
P2	STS	10	ATTAGCCGGTAAATGGATGAGTTC	AAGCAATACTAATCCCTCCAAACC
P3A	STS	10	AATCCAACGCATCAAGGCTGGC	ACAATGCCAAACACCAGGAACTCG
P4	STS	10	TGAGCTTTACCTCCCCTCCTAACC	TCCACCTTTCTCTCTCATCCCAC
P7	STS	10	AGTTAAACAACCTCCCCACTGC	GGGTAGGATAGGGGATAAGGAGC
Kobayashi et al. (2008)				
KA43	SSR	2	CCTTTCTGAATGCGGAATTT	GAAATGATGGCATGGGAGAT
This study				
MAD[2]	STS	12	TAACAACCACGGCCGAGAA	GAGCGTTCTTTTCTTTCGGTA
HP[2]	STS	3	TGGAGGAGATGTACGTCGAG	GAAGTCGAGGTGGTCCATGA
PP2[2]	CAPS/MseI	12	TTTGAATAGGTCCACTGCTT	CCATGCATCTCATTAGTCAA
PFruc[3]	dCAPS/EcoRI	1	CTTCTTCTTCGGGTGTCTCG	TGTTAAGTCCAGGGCAGAGG
Aglu[3]	STS	1	CCTCTGGAATCTTGCTATTTAGG	ATCCGCTAGATCACTGACAAA
LDS[3]	STS	1	CGAGGAGACAGACAGCATCA	GATGCAGCAGCCATGAGTT
BE2[3]	CAPS/SpeI	2	GCCCCGAACATGATTCTA	GGCTTTACCGACCTTACTGT
BE6[3]	dCAPS/HphI	6	TACCCCAGTTAAGTGTCTGTAAAGG	GAAGAGAGCGCAAGAATCCATTGTT

[1]Chr: chromosome location.
[2]Markers developed from the candidate genes residing QTL regions identified by Wada et al. (2008): MAD, OsMAD20 MADS box family; HP, *Homeobox domain containing protein*; PP2, *Phosphoserine phosphatase*.
[3]Markers developed from randomly chosen candidate genes based on their potential association with palatability by their functions: PFruc, *6-phospho-fructokinase 2*; Aglu, *Acyl UDP N acetylglucosamine O acyltransferase*; LDS, *Lipid A disaccharide synthase*; BE2, *1.4 alpha-glucan branching enzyme IIB*; BE6, *1.4 alpha-glucan branching enzyme*.

study (Lestari et al. 2009), marker data scores were converted to binary values. The best model equation was predicted using palatability scores as dependent variables and the binary data from molecular markers as regressors. The highest accuracy of prediction showed the lowest standard error and significantly highest coefficient of determination (R^2). Cluster analysis also was generated with the unweighted pair group method (UPGMA) in NTSYS (Exeter Software, Setauket, NY, USA) (Rohlf 1993).

Results

Development of new markers and marker evaluation

Based on QTL analysis for rice eating quality (Wada et al. 2008), three genes, OsMAD20 MADS box family gene (MAD, clone AL731752), a Homeobox domain containing protein (HP, clone AC119747) and a Phosphoserine phosphatase (PP, clone AP003727), were selected as candidates for the marker development. Additionally, by searching the genomics database (DB), five candidate genes, 6-phosphofructokinase 2 (PFruc, clone AP002743), Acyl UDP-N acetyltransferase (Aglu, clone AP003453), Lipid A disaccharide synthase (LDS, clone AP003237), 1.4 alpha-glucan branching enzyme IIB (BE2, clone AP004879) and 1.4 alpha-glucan branching enzyme (BE6, AP005763), were chosen due to their involvement in starch biosynthesis, suggesting they may play roles in determining rice starch physicochemical properties. After comparing these gene sequences in japonica with those of indica varieties (Table 1), a total of eight DNA markers (STS, CAPS/dCAPS) were developed (Table 1).

In total, we used 54 markers, which included previously reported markers (Lestari et al. 2009), plus those identified in this study for evaluation of indica rice eating quality (Table 1). Thirty of these markers showed polymorphism among indica rice varieties. Twenty-four of these were reported previously (Ohtsubo et al. 2002, 2003, He et al. 2006, Ohtsubo and Nakamura 2007, Zeng et al. 2007), including eight markers used for evaluation of japonica rice eating quality (Lestari et al. 2009) and six markers newly developed in our study (PP2, PFruc, Aglu, LDS, BE2, HP). The list of candidate genes for QTLs related to rice rating quality, the search of the genomic database, and the new primer sequences are presented in Table 1. Some developed primers were polymorphic both in japonica and in indica varieties, but others showed polymorphism only in japonica varieties. A number of unique alleles were observed in some rice varieties. The insertion of CTTT alleles in the Tre locus was rarely found in indica varieties, similar to the case of japonica rice for the same allele. In contrast to japonica rice, which is rich in T alleles in the S3c locus, the T allele was rare in indica and the occurrence was rather limited to the varieties of low palatability. On the other hand, Kalimutu, which is considered to be a highly palatable rice, also showed specific alleles on S3c (G allele) and PP loci (A allele).

Genotyping and cluster analysis

Not all primers derived from the japonica rice genome showed polymorphism in indica varieties. Nine of 20 STS primers reported to be associated with japonica rice palatability in previous studies (Ohtsubo et al. 2002, 2003, Ohtsubo and Nakamura 2007) demonstrated variation among indica varieties. Some primers corresponding to the genes including Isa (isoamylase), Pul3 (pullulanase) and starch-synthesizing genes, SS1 and SBE2 (Bao et al. 2006a,b, He et al. 2006), as well as those associated with starch biosynthesis (Zeng et al. 2007), displayed polymorphism among indica varieties. Twenty-four indica rice varieties were genotyped using 30 markers, revealing specific identities for different indica varieties (Table 2). For example, Rojolele, the variety of highest palatability, could be differentiated from other Indonesian indica varieties in this way based on sensory evaluation.

Cluster analysis was performed on similarity coefficient matrices calculated from the 30 molecular markers to generate a dendrogram (Fig. 1). Based on genetic similarity among all varieties, we used a cut-off value of 0.66 to generate three clusters (I, II and III), grouping 2, 21 and 1 varieties, respectively. Some varieties with close ST values were in the same subcluster; however, the variety of lowest palatability with low PC (Jatiluhur) seemed to be far distanced from higher palatable varieties. Kalimutu with medium ST but low PC was independently clustered. Interestingly, using total markers, japonica and indica rice varieties could be differentiated into two clusters (data not shown). However, these markers were still not able to differentiate indica varieties according to palatability. Therefore, an analysis of interaction among markers to evaluate rice palatability was needed.

Eating quality traits of indica rice

The palatability scores based upon the sensory test (ST) and the physicochemical properties of 24 indica rice varieties are summarized in Table 3. Similar to japonica rice results, ST and other physicochemical properties gave a wide range, as expected. The palatability value of indica rice could not be determined quantitatively due to the glossiness of indica rice, which cannot be precisely estimated with the Toyo taste meter specifically designed for japonica. Seven varieties (Rojolele, Memberamo, Cimelati, Conde, Batang Gadis, Pepe and Tukad Balian) showed good palatability with a hedonic score higher than 4, while two varieties (Singkil and Jatiluhur) possessed low palatability (score less than 3). The amylose content (AC) of Tukad Balian turned out to be the highest, while four varieties (Jatiluhur, Kalimutu, Sintanur and Logawa) showed a low value for protein content (PC).

Correlation analysis among the eating quality traits revealed that ST and PC were significantly correlated, but there was no significant correlation between AC and pasting properties (Table 4). On the other hand, AC was not significantly correlated with ST and PC, although it was significantly correlated with most of the pasting properties, demonstrating that palatability is a complex trait both in indica and in japonica rice. Moreover, it should be noted that because rice quality is determined by complex traits of physical and chemical characteristics, thus, in this study, we assume that sensory test is able to estimate the palatability (overall eating quality) of each Indonesian variety/local cultivar to represent eating quality.

Equation model to predict rice eating quality and its validation

Association analyses between the marker genotypes of the aforementioned 30 primer sets and the palatability values obtained from the sensory test (ST) were performed (Table 5) to generate equations using only significant markers as independent variables, in the same way they were used to predict eating quality of japonica rice (Lestari et al. 2009). The results demonstrated a highly significant correlation between the palatability values of 24 indica rice varieties and the genotypes of 18 markers (R^2: 0.997), including the ones we have previously developed (GPA,

Table 2: Genotyping of 24 indica rice varieties using 24 previously reported markers and 6 newly developed markers

Variety	A7[1]	E30[1]	F6[1]	G4[1]	G28[1]	S13[1]	T16[1]	WK9[1]	P3[1]	Isa[2]	SS1[3]	SBE2[4]	P3A[5]	P4[6]	P7[7]	Pul3[8]
Rojolele	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1
Ciliwung	0	0	1	0	1	0	0	0	0	1	1	1	1	0	1	1
Cisokan	0	0	1	0	1	1	0	0	1	1	1	1	1	0	1	1
Cibodas	0	0	1	0	1	1	0	0	1	1	1	1	1	0	1	1
Jatiluhur	0	0	1	0	1	0	0	0	0	1	1	0	1	0	1	1
Kalimutu	1	1	1	1	1	1	1	0	1	0	1	0	1	0	1	1
Cirata	0	0	1	0	0	0	0	0	1	1	1	1	1	0	1	1
Memberamo	0	1	0	1	1	1	0	1	1	1	1	0	1	0	1	1
Ciherang	0	0	0	0	1	0	0	0	0	1	1	1	1	1	1	1
Sintanur	0	0	0	0	1	1	0	0	0	1	1	1	1	0	1	1
Cimelati	0	1	0	1	1	1	0	1	1	1	1	0	1	0	0	1
Maros	0	0	1	1	0	1	0	0	0	1	1	1	1	1	1	0
Singkil	0	0	1	0	0	1	0	0	1	1	1	1	1	0	1	1
Batanghari	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	0
Conde	0	0	1	0	1	1	0	0	0	1	1	1	1	0	1	1
Angke	0	0	1	0	1	1	0	1	0	1	1	1	1	0	1	1
Batang Gadis	0	1	1	0	0	1	0	0	1	1	1	1	1	0	1	1
Batang Piaman	0	0	1	1	1	1	0	0	1	1	1	1	1	0	1	0
Cigeulis	0	0	1	0	1	0	0	0	1	1	1	1	0	0	1	1
Fatmawati	0	0	1	1	1	0	0	0	1	1	1	1	1	0	1	1
Konawe	0	0	1	0	0	1	0	0	0	1	1	1	1	0	1	1
Logawa	0	1	1	0	1	1	0	0	0	1	1	1	1	0	1	1
Pepe	0	0	1	0	1	1	0	0	0	1	1	0	0	0	1	1
Tukad Balian	1	0	1	1	1	1	0	0	1	1	0	1	1	0	1	1

Variety	TreB[9]	AMs[10]	GPA[11]	AcPh[12]	S3cI[13]	S3cII[14]	GBSS1[15]	PP2[16]	PFruc[17]	Aglu[18]	LDS[19]	BE2[20]	CBG[21]	HP[22]
Rojolele	1	1	0	0	0	1	1	1	0	0	1	1	0	1
Ciliwung	1	1	1	1	0	1	0	1	0	0	1	1	0	1
Cisokan	1	1	1	0	0	1	0	1	1	0	1	1	0	1
Cibodas	1	1	1	0	0	1	0	1	1	0	1	1	0	1
Jatiluhur	1	0	0	0	0	1	1	1	1	1	1	0	0	1
Kalimutu	0	1	0	1	0	0	0	0	0	0	0	1	1	1
Cirata	1	0	1	0	0	1	0	1	1	0	1	1	0	1
Memberamo	1	0	1	0	0	1	0	1	0	0	1	1	0	1
Ciherang	1	1	1	0	0	1	0	1	0	0	1	1	0	1
Sintanur	1	0	1	0	0	1	0	1	1	0	1	1	0	1
Cimelati	1	0	1	0	0	1	0	1	0	0	1	0	0	1
Maros	1	0	1	0	0	1	0	1	1	0	1	1	0	1
Singkil	1	0	1	1	0	1	0	1	0	0	1	1	1	1
Batanghari	1	1	1	0	0	1	0	1	1	0	1	1	1	1
Conde	1	0	1	1	0	1	0	1	1	0	1	1	0	1
Angke	1	0	1	0	0	1	0	1	1	0	1	1	1	1
Batang Gadis	1	0	1	0	0	1	0	1	0	0	1	1	0	0
Batang Piaman	1	0	1	1	0	1	1	1	1	0	1	1	0	0
Cigeulis	1	1	1	0	0	1	1	1	0	0	1	1	0	1
Fatmawati	1	0	1	0	0	1	1	1	1	0	1	1	0	1
Konawe	1	0	1	1	1	1	1	1	0	0	1	1	1	1
Logawa	1	0	1	1	0	1	1	1	0	0	1	1	1	1
Pepe	1	1	1	0	0	1	1	1	0	0	1	1	0	1
Tukad Balian	1	1	1	1	0	1	1	1	1	0	1	0	0	1

[1]presence (1), absence (0); [2]fragment of 230 bp(0), 220 bp(1); [3]No insertion(1), insertion (0); [4]allele of C(1), allele of G(0); [5]fragment of 268 bp(1), 278 bp(0); [6]fragment of 139 bp(0), 153 bp(1); [7]fragment of 171 bp(1), 195 bp(0); [8]fragment of 297 bp(1), 281 bp(0); [9]Insertion of CTTT (1) and no insertion (0) at nt 79-82 of the consensus region (intron); [10](CT)31 and other (CT) repeat (1) and (CT)27 (0); [11](CT)26 (0) and (CT)11 (1); [12]Point mutation from T (1) to G allele (0) at nt 397 of consensus region (intron); [13]No deletion (1) and deletion (CTC) (0) at nt 1255–1257 of consensus region (intron); [14]Point mutation from T (1) to G allele (0) at nt 1454 of consensus region (intron). [15](CT)18 (1) and (CT)17 (0); [16]Point mutation from G(1) to A(0); [17]Point mutation from A(0) to G(1); [18]fragment of 231 bp(0), 250 bp(1); [19]fragment of 191 bp(0), 204(1); [20]Point mutation from C(0) to G(1); [21](CTT)19 (1) and other (CTT) repeat (0); [22]fragment of 296 bp(1), other size (0).

AcPh, CBG, S3cI, GBSS1) (Lestari et al. 2009) and one newly developed in the present study (HP).

Palatability estimate according to the sensory evaluation was calculated by the marker-based regression equation (Table 6). A highly significant correlation ($r = 0.805$**) was observed between palatability diagnosed by the regression equation and sensory evaluation, demonstrating that this regression equation could be a good candidate diagnostic kit to predict the palatability of *indica* rice varieties and local accessions in Indonesia.

Discussion

Rice is one of the most important staple foods in East Asia. The two subspecies of rice, indica and japonica, are thought that they have originated from different gene pools of the wild relative ancestor, *Oryza rufipogon* (Hosoya et al. 2010). Indica rice is more widely consumed than japonica in the world, especially in Southeast Asian countries including Indonesia. To keep up with the increased consumer demand for higher eating quality of rice,

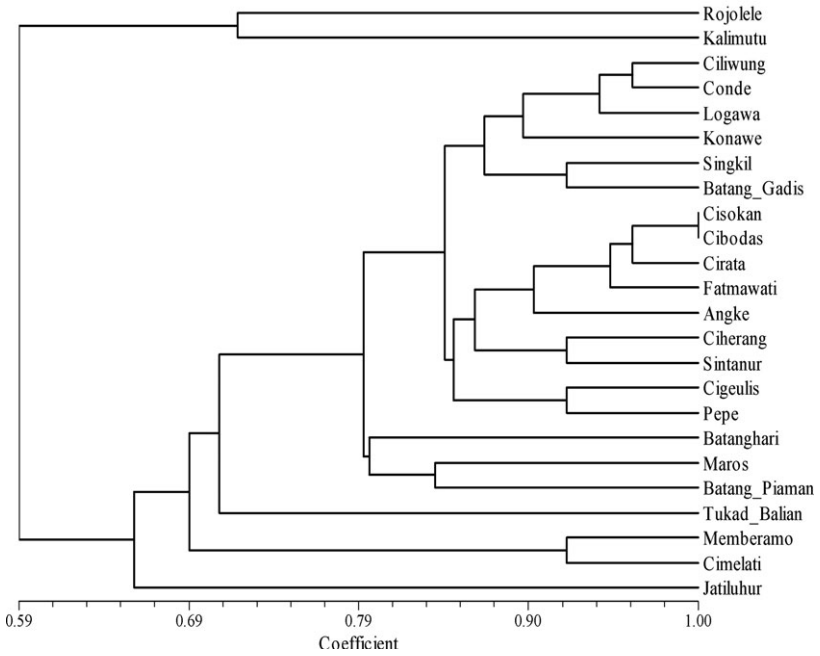

Fig. 1: Dendrogram of 24 indica varieties constructed on the basis of similarity coefficients in UPGMA analysis

Table 3: Means and ranges of eating quality parameters detected for the indica rice varieties examined in this study

Parameter	Mean ± SD	Range	CV (%)	Skewness	Kurtosis
ST	3.69 ± 0.49**	2.2–4.25	13.25	−1.47	2.3
AC (%)	21.38 ± 2.33**	16.63–26.51	10.91	0.05	−0.07
PC (%)	8.84 ± 1.40**	5.5.3–10.33	15.83	−1.08	0.14
PV^{RVU}	219.64 ± 41.09**	124.16–305.03	18.71	−0.58	0.68
HPV^{RVU}	127.58 ± 21.10**	91–169.1	16.54	−0.04	−0.6
BDV^{RVU}	92.06 ± 15.83**	32.91–150.08	28.06	−0.27	0.72
CPV^{RVU}	255.24 ± 38.53**	142.08–323.31	15.10	−0.74	2.09
SBV^{RVU}	35.6 ± 10.02**	−26.25 to 100.75	40.71	−0.33	0.15
CTV^{RVU}	127.66 ± 25.45**	31.06–172.45	19.94	−2.03	8.05

ST, palatability score from sensory test; AC, amylose content; PC, protein content; CPV, cold paste viscosity; BDV, breakdown viscosity; PV, peak viscosity; HPV, hot paste viscosity; SBV, setback viscosity; CTV, consistency viscosity; M, palatability value estimated from the equation based on marker data; RVU, Rapid Visco Unit.
**Significant at level of 1%.

Table 4: Correlation matrix of eating quality parameters determined in indica rice varieties in this study

Parameter	ST	AC	PC	PV	HPV	BDV	CPV	SBV
ST								
AC	-0.324^{ns}							
PC	0.412*	-0.262^{ns}						
PV	0.249^{ns}	-0.343^{ns}	0.318^{ns}					
HPV	0.077^{ns}	-0.116^{ns}	0.222^{ns}	0.845**				
BDV	0.333^{ns}	−0.450*	0.324^{ns}	0.900**	0.528**			
CPV	-0.193^{ns}	0.381^{ns}	0.189^{ns}	0.496*	0.769**	0.161^{ns}		
SBV	−0.400*	0.718**	-0.0144^{ns}	−0.549**	-0.127^{ns}	−0.769**	0.453*	
CTV	-0.378^{ns}	0.657**	0.100^{ns}	0.049^{ns}	0.328^{ns}	-0.19^{ns}	0.856**	0.773**

ns, not significant at the 5% level; * and **, significant at 5 and 1% level, respectively.

the availability of a standardized rice eating quality reference is important. To date, several attempts to evaluate japonica rice eating quality have been made with a number of established molecular marker sets (Ohtsubo et al. 2002, 2003, Lestari et al. 2009), but no such data on indica rice are available yet. This study is the first report on the development of marker sets to estimate indica rice eating quality according to sensory test.

Evaluation of rice palatability has long been conducted on japonica rice, but little has been performed on indica rice, especially by means of the taste meter that has been designed specifically based upon japonica rice starch properties. Both the Toyo taste meter and sensory evaluation can constitute a good measure of the eating quality of rice. The existence of a strong positive correlation between the palatability measured with the Toyo taste

Table 5: Model equation for evaluating *indica* rice eating quality containing the significant coefficient of each marker t-value aided by multiple regression analysis

	Palatability by sensory test (ST)		
PCR primer	Parameter estimated	*t*-value	R^2
A7	−3.26 ± 0.21	−15.85**	0.074
E30	−0.33 ± 0.05	−6.78**	0.045
F6	0.65 ± 0.05	13.76**	0.029
S13	0.13 ± 0.04	3.20*	0.010
T16	6.11 ± 0.25	24.27**	0.029
WK9	1.00 ± 0.06	16.69**	0.048
P3	−0.23 ± 0.03	−6.58**	0.01
GPA	2.36 ± 0.11	22.23**	0.32
AcPh	0.27 ± 0.04	5.91**	0.048
Pul3	1.62 ± 0.08	18.92**	0.001
CBG	−0.85 ± 0.05	−18.27**	0.060
S3cI	−0.53 ± 0.07	−7.45**	0.010
GBSS1	0.42 ± 0.04	10.01**	0.0001
SS1	−3.20 ± 0.15	−21.80**	0.167
SBE2	−0.52 ± 0.05	−9.69**	0.012
P3A	0.45 ± 0.07	6.48**	0.008
P4	0.97 ± 0.08	11.65**	0.032
HP	−0.81 ± 0.06	−13.90**	0.094
Intercept	3.07 ± 0.12	26.49**	
Total			0.997
Equation	Y = 3.07−3.26(A7) −0.33(E30) + 0.65(F6) + 0.13 (S13) + 6.11(T16) + 1.00(WK9)−0.23 (P3) + 2.36(GPA) + 0.27(AcPh) + 1.62(Pul3)− 0.85(CBG)−0.53(S3cI) + 0.42(GBSS1)−3.20 (SS1)−0.52(SBE2) + 0.45(P3A) + 0.97(P4)− 0.81(HP)		

* and **, significant at 5 and 1% level, respectively.

Table 6: Palatability values of 23 local *indica* rice from Indonesia measured by the sensory test and estimated by the regression equation

	Palatability value by	
Accession name	Sensory test (A)	Regression equation (B)
Abang Busur	2.27	2.12
Cantik Manis	2.57	3.46
Ceru	2.35	1.89
Dayang Rindu	2.45	3.12
Gedagai Hitam	2.74	1.89
Merong	2.58	3.44
Padi Merah	3.32	4.25
Palao Bunan	2.53	3.45
Palawak Manur	2.26	1.98
Peria	2.60	3.12
Reket Abang	2.37	1.81
Reket Bideng	3.23	4.09
Reket Bideng Bayan	3.06	3.12
Reket Bontok	3.19	3.67
Reket Bune	2.60	3.64
Reket Sentul	2.61	2.70
Sango	2.29	2.42
Santang	3.42	4.06
Seluang	2.49	2.89
Serendah	2.04	1.89
Siam Mayang	2.20	2.41
Siam Saba	3.16	3.77
Sukung	2.22	1.92
Correlation between A and B	r = 0.805**	

**, significant at 1% level.

meter and sensory tests has been confirmed (Azuma et al. 1994, Tanaka et al. 2006, Takeuchi et al. 2007, Lestari et al. 2009), allowing sensory evaluation to be widely used on japonica rice as a direct index of the evaluation of rice eating quality. Protein content (PC) showed a significant effect with a positive correlation on indica rice palatability in this study. However, our palatability score showed no correlation with RVA pasting properties, except for setback viscosity (SBV) as in the previous reports (Yu et al. 2008, Lestari et al. 2009). In addition, no significant effect of amylose content (AC) has been shown on either indica palatability or japonica palatability (Allahgholipour et al. 2006, Lestari et al. 2009, Sun et al. 2011); therefore, AC and pasting properties may not be a solid indicator to measure rice palatability.

Sequence variations including SNPs, indels and SSRs randomly located on the rice genome, in addition to the nucleotide sequences representing QTLs/genes of interest, could be a major source of molecular markers. With its high fidelity and efficacy, molecular markers developed from nucleotide variation in the genome have been instrumental for genotyping and differentiating varieties. SNPs and/or indels in starch-branching enzyme (SBE), sucrose starch synthase (SSS), granule bound starch synthase (GBSS), Waxy gene and other starch-synthesizing genes or loci are associated with rice eating quality traits (Bao et al. 2006a,b, He et al. 2006). As each single SNP or indel site generally showed little significant association with rice palatability, a combination of markers rather than a single marker could be better for evaluating rice eating quality.

The 24 indica rice varieties fell into three main clusters at a genetic similarity level of 0.67–0.69 using 30 markers. Even though these markers were all derived from QTLs for eating quality and associated traits (Lestari et al. 2009), the clusters produced were not projected to be related to eating quality traits. These cluster results suggest that unweighing markers with the same effect on the genotypic determination could result in their biased contribution in the genetic similarity calculation. Although that is a common way of genotyping and performing genetic similarity analysis, it may not be a good approach as reported on japonica rice evaluation (Lestari et al. 2009) and differential integrity of markers is required to evaluate indica rice eating quality. A successful multiple regression analysis on evaluation of japonica rice eating quality was reported (Ohtsubo et al. 2003, Lestari et al. 2009), and multiple regression equations with the highest R^2 and the lowest standard error were performed and proven to be effective in predicting the indica rice palatability using sensory evaluation. With a similar approach in this study, combined with STS markers developed previously (Ohtsubo et al. 2002, 2003, Ohtsubo and Nakamura 2007), we were able to estimate rice palatability on the basis of a sensory test. Markers derived from starch-synthesizing genes, particularly starch-branching enzyme, starch-debranching enzyme, granule bound starch synthase (Bao et al. 2006a,b, He et al. 2006) and other eating quality traits (Nakamura et al. 2004) together with our developed markers are good sources for development of PCR-based evaluation of rice eating quality.

The regression equation contained seven markers (E30, F6, WK9, GPA, AcPh, CBG, S3cI) (Table 5) that seemed to significantly contribute to palatability on both japonica and indica varieties. Regression values (R^2) of the seven markers explained most of the variation in the equations of both indica and japonica rice. With the exception of WK9, six of these markers were shared in common for predicting palatability using a sensory test on both japonica and indica. While P5 showed the highest partial

R^2 in both equation models according to the Toyo taste meter and sensory test on japonica, the GPA marker developed previously (Lestari et al. 2009) gave the highest, possibly representing a major QTL for palatability in indica rice.

In the equation model comprising 18 markers, most of the reported markers developed for evaluation in japonica rice with some modified SNP markers for simpler application (Lestari and Koh 2013) were included. Some previously developed STS markers used in this study were based on the japonica rice genome. The regression equations had high enough resolution to predict the palatability of japonica rice (Lestari et al. 2009). Considering the various palatabilities of varietal groups among countries, broad application of the markers with some modifications is required, such as with indica, as demonstrated in this study. Some additional markers developed based on both japonica and indica rice genomes facilitate a new applicable regression equation to evaluate indica rice palatability. Surprisingly, validation of the equation using local rice accession from Indonesia indicates its sufficient resolution to estimate the palatability of *indica* rice originated from Indonesia. This is also due to the fact that 23 local rice accessions were cultivated using the same cultural practices at the same field as 24 training varieties, and therefore, mostly genetic factors involved in palatability were evaluated while environmental factors could be excluded. This set marker with significant correlation ($r = 0.805$**) is in good agreement with previously formulated markers for eating quality of *japonica* rice mostly originating from Korea and Japan (Ohtsubo et al. 2002, 2003, Lestari et al. 2009). This formulated marker set is like an initial step; thus, more validations should be carried out in different laboratories across Indonesia regions using more diverse indica rice varieties and breeding lines to encounter the consistent estimate. Similar to the marker set that works well on japonica rice, this marker set in the regression equation developed in our study will be useful for selection during early breeding generation to enhance breeding for improved rice eating quality and prediction of eating quality of *indica* rice varieties in Indonesia. Moreover, these markers in our study could be valuable resources for formulation of other marker sets to estimate *indica* rice eating quality by sensory test depending on the taste preference of rice-consuming countries' people.

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