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PCR Marker-Based Evaluation of the Eating Quality of *Japonica* Rice (*Oryza sativa* L.)

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Evaluation of eating quality in early breeding generations of rice is critical to developing varieties with better palatability. This paper reports DNA markers associated with eating quality of temperate *japonica* rice and an evaluation method aided by multiple regression analysis. A total of 30 markers comprising STSs, SNPs, and SSRs were tested for their association with palatability using 22 temperate *japonica* varieties with different palatability values. Eating quality-related traits of the 22 varieties were also measured. Of the 30 markers, 18 were found to be significantly associated with palatability and, consequently, a model regression equation with an R^2 value of 0.99 was formulated to estimate the palatability by the marker data set. Validation of the model equation using selected breeding lines indicated that the marker set and the equation are highly applicable to evaluation of the palatability of cooked rice in temperate *japonica* varieties.

KEYWORDS: Rice; eating quality; palatability; marker; regression analysis

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for half of the world's population. The eating quality of rice is increasingly important to meet the market demand. Therefore, one of the major goals in a breeding program is to develop rice varieties of better eating quality to satisfy the requirements of both the food industry and consumers. Even though *indica* rice varieties are popular worldwide, consumers in northeastern Asian countries such as Korea, Japan, northern China, and Taiwan prefer *japonica* rice, mainly due to its moderate elasticity and stickiness.

The eating quality of rice is a complex trait involving many physicochemical properties, and thus it has been challenging to accurately evaluate eating quality for selection in rice-breeding programs. Some key physicochemical properties affecting the eating quality are amylose content (AC) (*1*), pasting properties (PP) (*2*), gel consistency (GC), gelatinization temperature (GT) (*3*), and protein content (PC) (*4*). Good eating quality is also associated with stickiness, sweet flavor, glossiness of the cooked rice, and palatability. Palatability, the trait directly related to rice eating quality, is determined by aroma, appearance, taste, and texture (*4*). In addition to genetic determinants, such as genes 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 (*5*).

In breeding programs, accurate evaluation of eating quality in early generations is critical. A sensory test by trained panels is the most appropriate evaluation method. However, because this method both requires a large amount of rice per sample and allows the evaluation of only a few samples per day, the sensory test is more efficient when performed at a later stage when selected lines are homozygous (*6*). Moreover, the results of sensory evaluation are sometimes not consistent even for the same sample, presumably due to the physical and emotional condition of members of the panel or subtle differences in sample preparation. Recently, an instrument for evaluating the palatability value of rice has been developed and used for line selection in breeding programs (*7, 8*). However, it also requires a large amount of rice per sample, and thus the palatability test using this instrument is usually performed only for advanced breeding generations.

A number of genetic studies on eating quality traits have been conducted. These have revealed that some rice physicochemical

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Table 1. Previously Reported Markers for the Evaluation of Rice Eating Quality and Their Chromosomal Locations

PCR marker	marker type	chr[a]	primer sequence	
			forward (5′−3′)	reverse (5′−3′)
Ohtsubo et al. (*13, 17*)	Ohtsubo and Nakamura (*12*)			
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. (*2, 3*)				
Wx (SNP)	dCAPS/*Acc*I	6	CTTTGTCTATCTCAAGACAC	TTTCCAGCCCAACACCTTAC
SS1 (SSR)	SSR	6	GATCCGTTTTTGCTGTGCCC	CCTCCTCTCCGCCGATCCTG
SBE1 (SSR)	SSR	6	ATTTCTTTGGCCACAGGCGA	CCCAGATTCGGAACAAGAAC
SBE1 (STS)	STS	6	GAGTTGAGTTGCGTCAGATC	AATGAGGTTGCTTGCTGCTG
SBE3 (SNP)	dCAPS/*Spe*I	2	GTCTTGGACTCAGATGCTGGACTC	ATGTATAACTGGCAGTTCGAACGG
SSIIa	SNP	6	F7: CTGGATCACTTCAAGCTGTACGAC F22: CAAGGAGAGCTGGAGGGGGC	R1: GCCGGCCGTGCAGATCTTAAC R21: ACATGCCGCGCACCTGGAAA

[a] Chromosome location.

properties such as AC, GT, GC, and pasting viscosity are controlled by one to three major genes with one or more modifiers. The enzymes involved in starch biosynthesis, such as *starch branching enzyme* (SBE), *starch synthase* (SS), and *granule bound starch synthase* (GBSS) contribute greatly to the variation of starch physicochemical properties and thus eating quality (*2*). Major genes and/or quantitative trait loci (QTLs) associated with eating quality (*9*), PC (*10*), and palatability (*9, 11*) such as *Wx* (waxy gene) and *alk* (starch synthase II) (*3*) have been reported. In addition, interaction among these genes along with others may govern rice grain physicochemical properties, which in turn determine the eating quality of cooked rice. Collectively, the genetic complexity of eating quality, as well as the difficulty in accurate evaluation of eating quality at early breeding generations, has constrained the development of rice varieties with high eating quality.

To complement the physicochemical analyses and sensory tests available to evaluate eating quality, DNA marker-based approaches have been developed. These methods offer the additional advantages of screening at early breeding generations as well as simplicity and accuracy. Markers based on the Polymerase Chain Reaction (PCR) have been tested for quality evaluation of rice varieties (*12*). Recently, sequence-tagged site (STS) primers developed from random amplified polymorphic DNA (RAPD) analysis were able to differentiate rice varieties according to their palatability (*12, 13*). Several functional markers have also been developed to distinguish the physicochemical properties of rice, especially the effect of the waxy locus on PP (*14*), that of SBE on starch viscosity (*15*), and those of AC (*2*) and *starch synthase IIa* (SSIIa) on GT (*3*). Additional gene-tagged markers have also been developed from starch-synthesizing genes (*2, 14, 15*). Despite the recent progress in developing markers and identification of QTLs associated with eating quality, a marker-assisted breeding (MAB) system for better eating quality has not been established.

In this study, our aim was to develop DNA markers associated with eating quality and to formulate a marker-based evaluation and prediction method of eating quality of cooked rice in *japonica* varieties.

MATERIALS AND METHODS

Plant Materials and DNA Extraction. A total of 22 *japonica* rice varieties, mostly bred in Korea, were evaluated for palatability. These consisted of 2 varieties from Japan (Koshihikari and Hitomebore), 1 variety from China (Hexi41), and 19 varieties from Korea (Gopum, Ilpum, Samgwang, Chucheong, Dongjin, Sinkeumo, Hwaseong, Hwacheong, Dobong, Samnam, Palkong, Baekjinju1, Seonong4, Onnuri, Manmi, Giho, Geuman, Nakdong, and Samdeok). These varieties were chosen because they represent diverse palatability scores among *japonica* rice (National Institute of Crop Science (NICS), personal communication). Varieties were grown using conventional cultural practices at the experimental farm of Seoul National University, Suwon, in 2006. In addition, 32 *japonica* breeding lines along with three check varieties grown in a regional yield trial plot in 2007 at NICS were also used for validation of the marker set developed in this study. For physicochemical analysis, rice grains were dried to 15% moisture content. All rice varieties and lines were grown in a light- and temperature-controlled greenhouse until the tillering stage, at which point tissue was collected for DNA extraction. Genomic DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) method based on the protocol of Murray and Thompson (*16*).

Previously Reported Markers. Twenty previously reported STS primer pairs developed from RAPD (*12, 13, 17*) were tested in this study. To identify the amplicon sequences, the amplified bands were excised, purified, TA cloned, and sequenced. In a homology search, each cloned sequence was compared against all sequences in the nonredundant databases using the BLASTX and BLASTN programs (http://www.ncbi.nlm.nih.gov/BLAST and www.gramene.org). How-

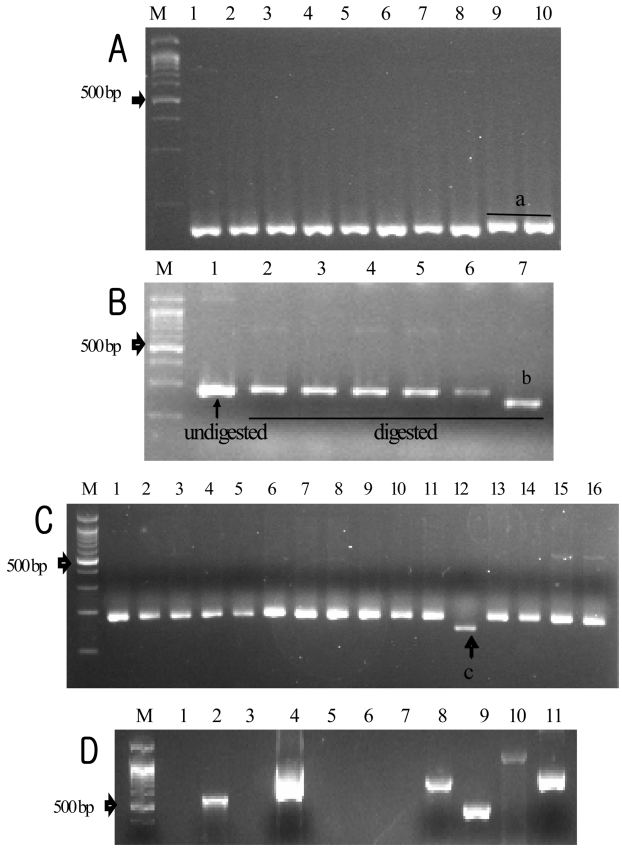

Figure 1. Examples of PCR amplicons of several types of markers. (**A**) Polymorphic indel in Tre (M, 100 bp DNA marker; 1, Koshihikari; 2, Samgwang; 3, Ilpum; 4, Sinkeumo; 5, Dobong; 6, Samnam; 7, Palkong; 8, Hitomebore; 9, Hexi41; 10, Samdeok); a, insertion of CTTT. (**B**) Polymorphic SNP in S3 (1, Koshihikari; 2, Samgwang; 3, Ilpum; 4, Sinkeumo; 5, Dobong; 6, Samnam; 7, Hexi41); b, point mutation T to G allele. (**C**) Variation in $(CTT)_n$ repeats in CBG (1, Koshihikari; 2, Gopum; 3, Samgwang; 4, Ilpum; 5, Chucheong; 6, Dongjin; 7, Sinkeumo; 8, Hwaseong; 9, Hwacheong; 10, Dobong; 11, Samnam; 12, Palkong; 13, Hitomebore; 14, Baekjinju1; 15, Seonong 4; 16, Manmi); c, $(CTT)_8$ alleles, others are $(CTT)_{19}$ alleles. (**D**) DNA banding patterns produced from 11 STS markers developed by Ohtsubo et al. (*13, 17*) and Ohtsubo and Nakamura (*12*) observed on Koshihikari (marker; 1, B7; 2, A7; 3, G81; 4, B43; 5, E30; 6, F6; 7, G4; 8, G22; 9, G28; 10, J6; 11, M11).

ever, only one of the six markers, SSIIa from Bao et al. (*3*), was polymorphic among the 22 rice varieties and was actually used in this study. The sequences and banding patterns of markers previously reported are presented in **Table 1** and **Figure 1**, respectively.

Development of New Markers. On the basis of selected regions either close to or within the genes linked to interesting QTLs (*11, 18, 19*) for rice eating quality traits (**Table 2**), 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) (*20*) was used. The purified PCR products from various *japonica* varieties were TA-cloned into pGEM-T Easy vectors and transformed into *Eschericia coli DH5*α-competent cells prepared according to the protocol of Sambrook and Russell (*21*). 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, insertions and deletions (Indels), and/or microsatellite repeats in the rice varieties, sequence results were aligned using the CLUSTAL W program (*22*) 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). To detect a 1 bp substitution in a specific fragment, a dCAPS (derived cleaved amplified polymorphic sequences) primer was designed, facilitated by dCAPS Finder 2.0 (*23*) (http://helix.wustl.edu/dcaps). Finally, nine molecular markers were successfully developed on the basis of Indels, SNPs, and microsatellite repeats identified in this study (**Table 3**).

PCR Protocols. PCR amplification of markers was carried out in a PTC-200 Peltier Thermal Cycler (MJ Research, Inc.) in a total volume of 20 μL with the following genotyping PCR reagents: 2 μL of DNA at 20 ng/μL, 2 μL of 10× buffer containing 25 mM $MgCl_2$, 1 μL of 2.5 mM dNTPs, 1 unit of Taq Polymerase (Intron Biotechnology, Korea), and 1 μL each of forward and reverse primers (10 μM). The PCR reaction for further sequencing analysis consisted of 1 unit of ExTaq polymerase (TaKaRa) in a total reaction of 50 μL. All 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. For STS markers developed by Ohtsubo et al. (*13, 17*) and Ohtsubo and Nakamura (*12*), the initial denaturation was at 95 °C for 5 min, followed by 40 cycles of 96 °C for 1 min, 62 °C for 1 min, and 72 °C for 2 min. All amplifications using primers reported by Bao et al. (*2, 3*) were performed under the following conditions: 5 min at 94 °C followed by 35 cycles of 45 s at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, with a final extension of 7 min at 72 °C. Amplified PCR products were analyzed by electrophoresis on 3% agarose gels stained with ethidium bromide and/or by nondenaturing electrophoresis on 8% polyacrylamide gels stained with ethidium bromide (model MGV, CBS Scientific Co.).

Evaluation of Eating Quality Traits of Rice Varieties. For palatability and physicochemical analyses, the rice grains were hulled and milled to 91% yield. Palatability was measured using a rice taste measuring system (Toyo taste meter, model MA-90) in accordance with the operation manual (TRCM Co.) (Toyo Rice Polishing Machine Factory, Japan). PC was calculated using total nitrogen multiplied by 5.95 after determination of the nitrogen content of rice material by the micro-Kjeldahl method (*24*). The AC of milled rice was determined by the relative absorbency of starch−iodine color in a digested solution of 100-mesh rice flour according to the method of Perez and Juliano (*25*). RVA pasting properties were determined on a rapid visco analyzer (RVA) in accordance with the operation manual (NewPort Sci. Co., 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) in accordance with the procedure of Bao and Xia (*26*).

Sensory Evaluation by a Taste Panel. The milled rice was cooked according to the protocol of NICS, the Rural Development Administration (RDA), Korea. Dry-milled head rice (300 g) was rinsed four times and soaked for 30 min with distilled water, and the water was then strained for 10 min. Rice was cooked using an electric rice cooker with the ratio of rice/water = 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 10 min until cooled to 35−37 °C. Sensory evaluation of cooked rice samples by 11 well-trained panel members was performed with five replications. The overall palatability was assessed according to appearance (glossiness), fragrance, taste, stickiness, texture, and palatability score (overall score; overall eating quality) and scored from +3 to −3 compared to a cooked rice reference sample, Chucheong (score = 0). The palatability score of each variety by the sensory test was the average value scored by 11 panel members.

Statistical Analysis. The collected data were analyzed and subjected to analysis of variance using SAS software version 8.2 (*27*). The least significant difference (Duncan) method was used to evaluate differences between trait means. Regression and correlation analyses were also performed to determine the relationships between rice eating quality traits. Multiple regression analysis was also conducted to determine the relationship between palatability scored both by taste analyzer and by sensory test and also palatability evaluated by molecular markers. STS marker data were scored as 1 (present) and 0 (absent). Similarly, the two different alleles resulting from each SSR and SNP marker were also converted into binary values of 1 or 0. By using the palatability scores as dependent variables and the binary data from molecular

Table 2. Candidate Genes for QTLs Related to Rice Eating Quality and a Search of the Genomics DB[a]

candidate gene	clone	markers developed	chr[b]	QTL	source
granule−bound starch synthase1 (GBSS1)	AP002542	GBSS1	6	OSR19−RM587	Kwon et al. (*18*)
sucrose synthase 3 (S3)	AP004988	S3cI, S3cII	7	RM234−RM47	Kwon et al. (*18*)
trehalose phosphatase(Tre)	AP004341	TreB	7	RM234−RM47	Kwon et al. (*18*)
UDP−N−acetylglucosamine pyrophosphorylase (AcPh)	AP003875	AcPh	8	RM547−RM72	Kwon et al. (*18*)
glucosamine−fructose−6−phosphate aminotransferase (GPA)	AC138454	GPA	11	RM20b−RM332	Kwon et al. (*18*)
aspartate aminotransferase (AM)	AP003991	Ams	2	OSR8−OSR9	Suh et al. (*19*)
noncyanogenic β−glucosidase (CBG)	AC074354	CBG	10	RM2887	Wada et al. (*12*)
ADP−glucose pyrophosphorylase/*shrunken* gene (SH)	AP004317	SH51	1		Genomics DB

[a] http://www.ncbi.nlm.nih.gov/, http://rgp.dna.affrc.go.jp/E/IRGSP/index.html, http://rice.plantbiology.msu.edu/, and http://www.gramene.org/. [b] Chromosome location.

Table 3. Markers Developed in This Study for Evaluation of Rice Eating Quality

			sequence		
marker name	marker type	chr[a]	forward (5′−3′)	reverse (5′−3′)	amplicon size (bp)
S3cI	Indel	7	CCACTCTCATGTCCTTGAAC	GCCATGACATTTGGACAT	153/150
S3cII	dCAPS/*TaqI*	7	TTCCATGATGTGCCACTCTC	GGACAAATGTTTTCAGTGAATAAAT	277/251 + 26
TreB	Indel	7	CACTCCAGTTCCTGCTCAAA	CACTCCAGTTCCTGCTCAAA	167/171
AMs	SSR	2	CTTCCAAGGACCCCATCCT	CCCAACATCTCCGTCAGAAT	187/179
GPA	SSR	11	AATACGCGGCCTTCTCCTAT	TTGATCCGAATGGGTCAAAT	178/149
GBSS1	SSR	6	CAAATAGCCACCCACACCAC	CTTGCAGATGTTCTTCCTGATG	172/170
AcPh	dCAPS/*MseI*	8	AGTTGTGGTTTAAGCATAGG	ATTGTCCTTTTCTTTAAAGTTTATTA	14 + 10 + **125** + 12/14 + **135** + 12
CBG	SSR	10	AGCTTCCCTAATGGCTTCGT	ATTTGCCAACTTTTGGATGG	184/151
SH51	dCAPS/*SpeI*	1	ATTCTTGATGAAAATAATTAACTAG	GGTTAACCATCTTATAAAATTTGTC	475/454 + 21

[a] Chromosome location.

markers as independent variables or regressors, the best model equation to predict rice palatability was obtained. The most accurate prediction gave the lowest standard error and significantly highest coefficient of determination (R^2), which consisted of the highly significant regression coefficient for each marker in the model. Using the binary matrix, a cluster analysis was performed with the Unweighted Pair Group Method (UPGMA) in NTSYS (Exeter Software, Setauket, NY) (*28*).

RESULTS

Evaluation and Development of Markers. DNA sequences of PCR amplicons produced by 20 previously reported STS primer pairs (*12, 13, 17*) were compared against all sequences in the nonredundant databases using the BLASTX and BLASTN programs. The amplicon sizes ranged from about 450 to 1800 bp in length. However, no candidate gene of any known function possibly related to quality traits was found among the sequences derived from STS primers. Therefore, a total of 21 markers, including SSIIa (*3*), were used directly in this study (**Table 1**) as 5 of the markers listed in **Table 1** from Bao et al. (*2*) were not polymorphic among the 22 varieties.

On the basis of QTL analyses for rice eating quality (*11, 18, 19*), we were able to select seven candidate genes underlying the QTL regions: *sucrose synthase 3* (S3, clone AP004988), *trehalose phosphatase* (Tre, clone AP004341), *granule bound starch synthase 1* (GBSS1/*Waxy* gene, clone AP002542), *UDP-N-acetylglucosamine pyrophosphorylase* (AcPh, clone AP003875), *glucosamine-fructose-6-phosphate aminotransferase* (GPA, clone AC138454), *aspartate aminotransferase* (AM, clone AP003991), and *noncyanogenic β-glucosidase* (CBG, clone AC074354) (**Table 2**). In addition, by searching the Genomics Date Base (DB), *ADP-glucose pyrophosphorylase* (*shrunken* gene, SH51, AP004317), a gene involved in starch biosynthesis and located on chromosome 1, was also chosen for marker development. By comparing the sequence of each gene (**Table 2**) among *japonica* varieties, we developed a total of nine DNA markers (**Table 3**). Examples of PCR amplicons of different marker types among *japonica* rice varieties are shown in **Figure 1**. Some unique alleles existed in only some varieties. The insertion of CTTT alleles in the Tre locus (**Figure 1A**) and the G allele in the S3 locus (**Figure 1B**) were rarely found in *japonica* varieties. In the CBG locus were distinguished two $(CTT)_n$ repeats of $(CTT)_8$ and $(CTT)_{19}$, but only a rice of low palatability (Palkong) possessed the $(CTT)_8$ allele (**Figure 1C**). Moreover, the DNA banding pattern from the STS primers for palatability on Koshihikari was similar to those of previous studies (*12, 13, 17*) (**Figure 1D**; **Table 4**).

Genotyping of Rice Varieties and Cluster Analysis. We genotyped 22 *japonica* rice varieties with diverse palatability values using a total of 30 markers comprising 21 markers developed previously and 9 markers developed in this study (**Table 4**). Cluster analysis was performed on similarity coefficient matrices calculated from molecular markers to generate a dendrogram (**Figure 2**). The varieties Chucheong and Hwacheong showed maximum genetic similarity (0.96), whereas Koshihikari and Seonong4 showed the least genetic similarity (0.41). When a cutoff value of 0.64 was used for genetic similarity among all varieties, three clusters were formed, I, II, and III (**Figure 2**), which contained 12, 7, and 3 varieties, respectively. The two Japanese cultivars, Koshihikari and Hitomebore, formed an independent subcluster, supporting the fact that Hitomebore was bred using Koshihikari as a parent. Similarly, other varieties sharing parentage grouped in the same subclusters.

Eating Quality Traits. The palatability value according to the Toyo taste meter (P), the palatability score according to the sensory test (ST), and the physicochemical properties of 22 *japonica* rice varieties are summarized in **Tables 5** and **6**. All traits except SBV showed significant differences among varieties. P and ST exhibited a wide range of variation as expected. Four varieties (Koshihikari, Ilpum, Samgwang, and Geuman) showed good palatability in both P and ST. Eight varieties exhibited better ST than Chucheong, which was used as a check variety in the sensory test. AC was similar among varieties except for two, Baekjinju1 and Seonong4, which were developed as extremely low AC varieties.

Correlation analysis among quality traits revealed that P and ST were significantly correlated ($r = 0.85^{**}$) as expected (**Table**

Table 4. Genotyping of 22 Rice Varieties Using 32 Markers

(**A**) Using Previously Reported Markers (*12, 13, 17*)

	primer																			
variety	A6	A7	B1	B43	E30	F6	G4	G22	G28	J6	M2CG	M11	P5	S13	T16	WK9	B7	G49A	G81	P3	digitized value
Koshihikari	+	+	+	+	−	−	−	+	+	+	−	+	+	−	−	−	−	−	−	+	11,110,001,110,110,000,001
Gopum	+	+	+	+	+	−	−	−	+	−	+	−	−	−	−	−	−	−	+	+	11,111,000,101,000,000,011
Samgwang	+	+	+	+	+	−	−	−	+	+	+	−	−	+	−	−	+	+	−	+	11,111,000,111,001,001,101
Ilpum	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	−	+	−	+	11,111,000,100,000,010,101
Chucheong	+	+	+	+	+	+	−	−	+	−	+	−	−	−	−	−	−	+	−	+	11,111,100,101,000,000,101
Dongjin	+	+	+	+	−	+	−	−	−	+	−	−	−	+	−	−	−	−	+	+	11,110,100,010,001,000,011
Sinkeumo	+	+	+	+	−	−	−	−	+	+	−	−	−	−	−	+	−	+	−	+	11,110,000,110,000,010,101
Hwaseong	+	+	+	+	−	+	−	−	−	+	−	−	−	−	−	−	−	+	−	−	11,110,100,010,000,000,100
Hwacheong	+	+	+	+	+	−	−	−	+	−	+	−	−	−	−	−	−	+	−	+	11,111,000,101,000,000,101
Dobong	+	+	+	+	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	+	11,110,000,101,100,000,001
Samnam	+	+	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−	+	−	+	11,111,000,001,000,000,101
Palkong	+	−	+	+	−	−	−	−	+	+	−	+	−	−	−	−	−	+	−	+	10,110,000,110,100,000,101
Hitomebore	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	+	11,110,001,111,111,110,001
Baekjinju1	+	+	+	−	+	+	−	−	+	+	+	+	−	−	−	+	−	+	+	−	11,101,100,111,100,010,110
Seonong4	+	+	+	−	+	+	−	+	−	+	+	−	−	+	+	+	−	+	+	+	11,101,101,011,001,110,111
Onnuri	+	+	+	−	+	+	−	−	−	+	+	+	−	+	−	+	−	−	−	−	11,101,100,011,101,010,000
Manmi	+	+	+	+	+	−	−	+	+	+	−	+	−	+	−	−	−	+	−	+	11,111,001,110,101,000,101
Giho	+	+	+	−	+	−	−	+	−	+	+	−	−	+	−	−	−	+	+	+	11,101,001,011,001,000,111
Geuman	+	+	+	+	+	−	−	+	+	+	+	+	−	−	−	−	−	−	+	+	11,111,001,111,100,000,011
Nakdong	+	+	+	+	−	−	−	+	+	+	+	−	−	+	−	−	−	−	−	−	11,110,001,111,001,000,000
Hexi41	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	−	−	−	−	+	11,111,111,111,000,100,001
Samdeok	+	+	+	−	+	−	−	+	+	+	+	−	−	+	−	+	−	−	−	+	11,101,001,111,001,010,001

(**B**) Using SSIIa (*3*) and Nine Newly Developed Markers

	binary data of each primer									
variety	SSIIa[a]	TreB[b]	S3cII[c]	S3cI[d]	AMs[e]	GPA[f]	GBSSI[g]	AcPh[h]	SH51[i]	CBG[j]
Koshihikari	1	0	1	1	0	0	0	1	0	1
Gopum	1	1	0	0	1	0	1	0	1	1
Samgwang	1	0	1	1	1	0	1	0	1	1
Ilpum	0	0	1	1	1	0	1	1	1	1
Chucheong	0	1	0	0	1	0	1	1	1	1
Dongjin	1	1	0	0	1	1	1	1	1	1
Sinkeumo	0	0	1	1	1	0	1	1	1	1
Hwaseong	1	1	0	0	1	1	1	1	1	1
Hwacheong	0	1	0	0	1	0	1	1	1	1
Dobong	1	0	1	1	1	0	1	1	1	1
Samnam	0	0	1	1	1	0	1	1	1	1
Palkong	1	0	1	1	1	0	1	1	1	0
Hitomebore	0	0	1	1	1	0	1	1	1	1
Baekjinju1	0	0	1	1	1	0	1	1	1	1
Seonong4	1	0	1	1	1	0	1	0	1	1
Onnuri	0	0	1	1	1	0	1	0	1	1
Manmi	0	0	1	1	1	0	1	1	1	1
Giho	0	0	1	1	1	0	1	1	1	1
Geuman	0	0	1	1	1	0	1	1	1	1
Nakdong	1	0	1	1	1	1	1	1	1	1
Hexi41	0	1	0	0	1	0	1	1	1	1
Samdeok	1	1	0	0	1	0	1	0	1	1

[a] TT:GGTTTC (0) and GC:GGGCTC (1) at nt 4329−4330 (exon). [b] Insertion of CTTT (1) and no insertion (0) at nt 79−82 of consensus region (intron). [c] Point mutation from T (1) to G allele (0) at nt 1454 of consensus region (intron). [d] No deletion (1) and deletion (CTC) (0) at nt 1255−1257 of consensus region (intron). [e] $(CT)_{31}$ (1) and $(CT)_{27}$ (0). [f] $(CT)_{26}$ (0) and $(CT)_{11}$ (1). [g] $(CT)_{18}$ (1) and $(CT)_{17}$ (0). [h] Point mutation from T (1) to G allele (0) at nt 397 of consensus region (intron). [i] Point mutation from A (0) to T allele (1) at nt 51 (intron). [j] $(CTT)_{19}$ (1) and $(CTT)_8$ (0).

7). However, P and ST were not significantly correlated with other traits, indicating that palatability is a complex trait in which a number of factors are involved. AC was significantly correlated with RVA pasting properties.

Regression Analysis between Marker Genotypes and Eating Quality Traits. Association analyses between marker genotypes based on 30 primer sets (**Table 4**) and palatability values according to the Toyo taste meter (P) (**Table 6**) and sensory test (ST) were performed (**Table 8**). General linear model regression analysis indicated that individual molecular markers could not differentiate the eating qualities. Thus, multiple regression analysis of the entire set of markers was performed. By using only significant markers as independent variables, model equations were generated that could predict the eating quality with highly significant resolution ($R^2 = 0.99$) (**Table 8**). This indicates that palatability values of 22 varieties estimated by the Toyo taste meter and/or by the sensory test can be estimated with high resolution by these regression equations based on the marker genotypes. The marker sets for the equations consisted of 13 markers for palatability by the Toyo taste meter and 14 markers for palatability by the sensory test, with 9 markers shared for both equations. Three newly

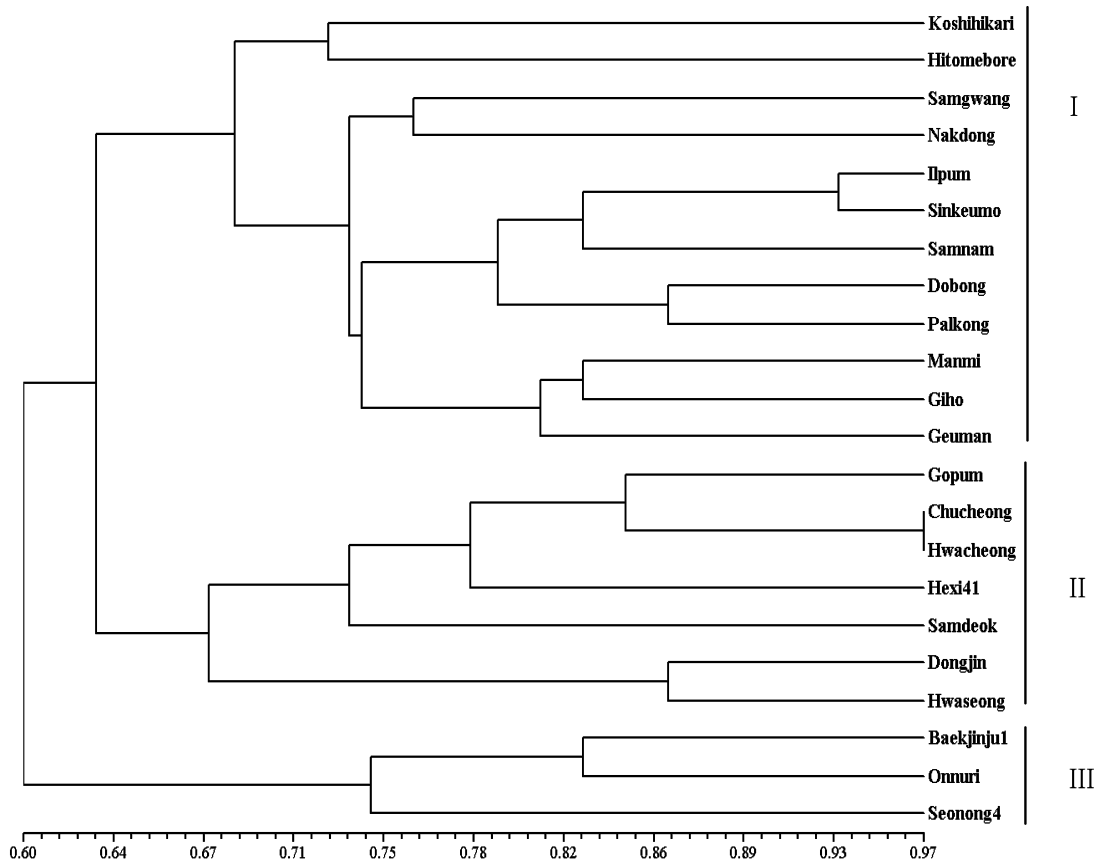

Figure 2. Dendrogram of 22 *japonica* varieties constructed on the basis of similarity coefficients in UPGMA analysis.

Table 5. Means and Ranges of Eating Quality Parameters Detected for the *Japonica* Rice Varieties under Study

parameter[a]	mean ± SD[b]	range	CV (%)	skewness	kurtosis
P	74.46 ± 8.11 **	49.83−84.00	10.90	−1.56	2.09
ST	−0.22 ± 0.48 *	−1.24 to 0.39	46.61	−0.87	−0.27
M	74.44 ± 8.09 **	49.99−83.98	10.8 6	−1.55	2.07
AC (%)	17.33 ± 3.10 **	8.75−19.93	17.87	−1.80	2.23
PC (%)	6.83 ± 0.61 **	5.91−7.89	8.89	0.30	−0.94
CPV^{RVU}	232.62 ± 42.08 **	93.53−284.39	18.09	−1.91	3.88
BDV^{RVU}	89.19 ± 21.16 **	39.83−123.08	23.73	−0.49	−0.23
PV^{RVU}	242.37 ± 28.52 **	181.89−298.95	11.77	0.05	0.27
SBV^{RVU}	−9.75 ± 31.08 ns	−94.75 to 25.00	36.56	−1.39	1.40
HPV^{RVU}	153.17 ± 30.99 **	65.20−208.80	20.23	−0.72	1.28
CTV^{RVU}	79.44 ± 18.03 **	28.33−98.92	22.70	−1.37	1.49

[a] P, palatability value; 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. [b] ns, nonsignificant at 5% level; ** and *, significant at 1 and 5% level, respectively.

developed markers (GPA, S3cI, and CBG) were included among these 9 markers. The other 3 markers (AMs, SSIIa, and AcPh) were included among either 13 markers for palatability by the Toyo taste meter or 14 markers for palatability by the sensory test.

Validation of the Regression Equation Using Breeding Lines. To evaluate the validity of the regression equation, 32 breeding lines along with 3 *japonica* check varieties were genotyped with the set of 13 markers mentioned above and their palatability values were evaluated by the Toyo taste meter (**Table 9**). Palatability estimates were calculated by the regression equation based on marker genotypes. There was a highly significant correlation ($R^2 = 0.715^{**}$) between palatability estimates by the regression equation and palatability values according to the Toyo taste meter (**Figure 3**), indicating that the model equation we developed reliably predicts the palatability of random breeding lines of *japonica* rice.

DISCUSSION

Evaluation of Rice Eating Quality Traits. The highly significant correlation between palatability values according to the Toyo taste meter and palatability values according to the sensory test is in good agreement with previous studies (*9*). Japanese researchers have also reported that palatability values from the Toyo taste meter and palatability scores from the sensory test exhibit a high positive correlation (*7, 8*). This indicates that the palatability value according to the Toyo taste meter can be used as a good measure of the eating quality of rice. For breeding better eating quality rice, the Toyo taste meter is the measurement method of choice because of its relative simplicity and reproducibility compared to the sensory test (*7*−*9*). This is why the Toyo taste meter has been widely used in breeding programs for eating quality in Korea, Japan, and northeastern Asia. No correlation between palatability and any pasting property was found in this study, suggesting that pasting properties could not be a good indicator for palatability value due to its complex nature. PC was the only trait to affect the palatability value as reported previously (*29*).

Feasibility of PCR Marker-Based Evaluation of Eating Quality of Cooked Rice. As nucleotide differences among genotypes are a major source of heritable variation, molecular markers derived from them should provide an effective measure of genotypic variation and hence phenotypic differences among varieties. When 22 varieties were classified on the basis of the cluster analysis of 30 markers, they formed three major clusters at a genetic similarity level of 0.64−0.67. However, the clusters

Table 6. Characteristics Associated with Eating Quality of Rice Varieties in This Study

cultivar	P	ST	M	AC (%)	PC (%)	PV (RVU)[a]	HPV (RVU)	BDV (RVU)	CPV (RVU)	SBV (RVU)	CTV (RVU)
Koshihikari	84.00	0.25	83.98	18.38	5.91	285.39	208.80	76.58	284.39	−1.00	75.59
Gopum	78.00	0.18	76.86	19.93	6.40	255.11	172.95	82.17	251.72	−3.39	78.77
Samgwang	82.60	0.28	82.35	18.67	6.30	248.86	168.47	80.39	258.39	9.53	89.92
Ilpum	83.10	0.39	83.50	18.87	6.28	235.92	182.97	52.95	252.86	16.94	69.89
Chucheong	75.40	0.00	76.86	19.44	6.31	223.83	146.08	77.75	236.67	12.83	90.59
Dongjin	78.40	−0.05	77.72	19.36	6.11	239.61	131.86	107.75	223.39	−16.22	91.53
Sinkeumo	61.00	−0.40	60.82	16.62	7.32	231.61	157.69	73.92	256.61	25.00	98.92
Hwaseong	77.10	−0.20	77.72	18.73	6.19	245.78	141.31	104.47	238.72	−7.05	97.41
Hwacheong	77.77	−0.32	76.86	18.85	7.00	222.28	150.94	71.33	237.86	15.58	86.92
Dobong	49.83	−1.24	49.99	15.11	7.89	298.95	192.47	106.47	257.14	−41.81	64.67
Samnam	65.03	−0.95	65.03	16.51	7.10	246.00	181.64	64.36	263.19	17.20	81.55
Palkong	68.60	−0.83	68.59	17.33	6.93	275.2	181.08	94.11	276.47	1.28	95.39
Hitomebore	75.50	0.15	75.48	18.56	6.90	292.33	186.22	106.11	264.42	−27.91	78.20
Baekjinju1	76.63	0.10	76.62	8.75	7.82	231.28	110.11	121.17	151.72	−79.56	41.61
Seonong4	79.03	0.12	78.56	9.51	6.60	188.28	65.20	123.08	93.53	−94.75	28.33
Onnuri	77.00	−0.05	76.62	18.82	7.51	229.36	144.75	84.61	231.14	1.78	86.39
Manmi	76.60	−0.10	76.40	13.33	7.88	242	136.50	105.50	204.83	−37.17	68.33
Giho	75.50	−0.40	75.94	18.64	6.81	234.28	144.53	89.75	237.25	2.97	92.72
Geuman	80.00	0.20	80.14	19.83	7.22	255.75	149.75	106.00	246.69	−9.06	96.94
Nakdong	76.90	−0.25	76.94	19.83	6.70	228.55	134.94	93.61	226.83	−1.72	91.89
Hexi41	63.77	−1.20	63.76	17.60	6.02	181.89	142.05	39.83	205.56	23.67	63.51
Samdeok	76.33	−0.54	76.94	18.56	7.01	239.83	139.53	100.30	218.17	−21.67	78.64

[a] The value is presented as a Rapid Visco Unit (RVU).

Table 7. Correlation Matrix of Eating Quality Parameters[a]

parameter	AC	P	ST	PC	CPV	BDV	PV	HPV	SBV	CTV
P	0.18 ns									
ST	0.06 ns	0.85 **								
PC	−0.48 *	−0.43 *	−0.24 ns							
CPV	0.74 **	−0.14 ns	−0.12 ns	−0.17 ns						
BDV	−0.46 *	0.12 ns	0.23 ns	0.40 ns	−0.44 *					
PV	0.22 ns	−0.14 ns	0.02 ns	0.18 ns	0.67 **	0.25 ns				
HPV	0.52 *	−0.21 ns	−0.14 ns	−0.11 ns	0.92 **	−0.45 *	0.75 **			
SBV	0.79 **	−0.07 ns	−0.18 ns	−0.39 ns	0.74 **	−0.83 **	−0.01 ns	0.56 **		
CTV	0.82 **	0.02 ns	−0.04 ns	−0.20 ns	0.75 **	0.25 ns	0.28 ns	0.43 *	0.75 **	
M	0.19 ns	1.00 **	0.85 **	−0.43 *	−0.14 ns	0.11 ns	−0.14 ns	−0.21 ns	−0.06 ns	0.03 ns

[a] ns, nonsignificant at 5% level; * and **, significant at 5 and 1% level, respectively.

Table 8. Model Equations for Evaluating Rice Eating Quality Containing the Significant Coefficient of Each Marker *t* Value Aided by Multiple Regression Analysis[a]

	palatability by Toyo taste meter (P)			palatability by sensory test (ST)		
PCR primer	parameter estimate	*t* value	R^2	parameter estimate	*t* value	R^2
G4	−16.97 ± 1.19	−14.22 **	0.087	−1.20 ± 0.06	−21.77 **	0.212
M11	−1.94 ± 0.60	−3.25 **	0.096	−0.14 ± 0.03	−5.03 **	0.010
E30	26.55 ± 0.83	32.12 **	0.104	0.86 ± 0.04	19.62 **	0.059
M2CG	−2.40 ± 0.56	−4.33 **	0.060	−0.38 ± 0.03	−15.21 **	0.041
GPA	−21.14 ± 1.11	−19.12 **	0.129	−0.82 ± 0.05	−17.28 **	0.021
S3cl	−1.62 ± 0.62	−2.60 *	0.017	−0.38 ± 0.03	−13.41 **	0.005
P5	19.01 ± 1.32	14.44 **	0.307	1.09 ± 0.04	26.81 **	0.288
B1	6.42 ± 0.77	8.30 **	0.047	0.41 ± 0.03	12.90 **	0.015
CBG	13.45 ± 1.11	12.12 **	0.087	0.68 ± 0.07	10.27 **	0.228
J6	3.87 ± 0.74	5.21 **	0.083			
WK9	2.62 ± 0.59	4.42 **	0.003			
A7	−12.33 ± 1.27	−9.72 **	0.031			
AMs	−8.72 ± 1.56	−5.58 **	0.021			
G81				0.27 ± 0.03	10.41 **	0.021
F6				0.32 ± 0.03	10.36 **	0.033
SSIIa				−0.27 ± 0.03	−8.06 **	0.001
G28				0.33 ± 0.04	8.81 **	0.005
AcPh				−0.48 ± 0.04	−11.44 **	0.060
intercept	76.66 + 2.71	28.29 **		−0.54 ± 0.11	−4.74 **	
total			0.990			0.990
eq	$Y = 76.66 - 16.97(G4) - 1.94(M11) + 26.55(E30) - 2.40(M2CG) - 21.14(GPA) - 1.62(S3cl) + 19.01(P5) + 6.42(B1) + 13.45(CBG) + 3.87(J6) + 2.62(WK9) - 12.33(A7) - 8.72(Ams)$			$Y = -0.54 - 1.20(G4) - 0.14(M11) + 0.86(E30) - 0.38(M2CG) - 0.82(GPA) - 0.38(S3cl) + 1.09(P5) + 0.41(B1) + 0.68(CBG) + 0.27(G81) + 0.32(F6) - 0.27(SSIIa) + 0.33(G28) - 0.48(AcPh)$		

[a] ** and *, significant at 1% and 5% level, respectively.

did not appear to be related to eating quality, but to the parental origin of the strains, although the 30 markers were derived from quality-associated traits or QTLs for eating quality. This may be explained by the fact that the cluster diagram was constructed

Table 9. Palatability Values of 32 Breeding Lines and 3 Check Varieties Measured by the Toyo Taste Meter and Estimated by the Regression Equation

	palatability	
breeding line/variety	Toyo taste meter (P)	regression eq (M)
Suweon503	72.40	72.93
Suweon507	80.51	79.34
Suweon508	76.40	76.94
Suweon509	73.21	75.55
Suweon510	72.02	75.55
Suweon511	78.51	79.56
Suweon513	66.32	68.69
Suweon514	68.79	73.76
Suweon515	66.02	75.38
Suweon516	79.81	81.79
Suweon518	64.80	67.35
Nampyeong[a]	76.18	81.87
Iksan486	86.71	94.32
Iksan488	76.89	75.47
Iksan490	78.51	84.89
Iksan493	76.60	74.61
Iksan494	73.62	73.07
Iksan495	79.02	80.92
Iksan496	71.67	75.32
Iksan497	72.11	74.61
Iksan502	71.32	78.13
Iksan504	65.58	68.91
Keumo[a]	63.53	72.06
Juan[a]	77.41	73.07
Milyang211	70.90	75.83
Milyang215	59.81	60.10
Milyang218	70.02	73.15
Milyang219	74.58	81.65
Milyang220	64.31	58.20
Milyang222	60.52	72.06
Milyang223	62.80	68.34
Milyang224	61.00	61.93
Milyang230	78.60	79.16
Milyang231	66.41	69.31
Milyang232	67.03	64.52

[a] Three check varieties.

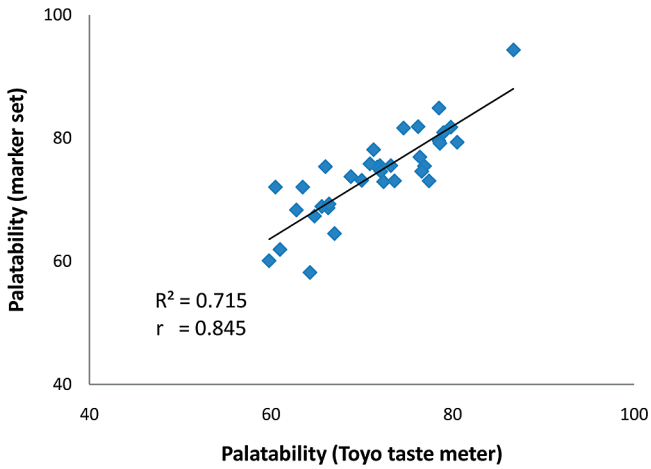

Figure 3. Correlation between palatability values of 32 breeding lines and 3 check varieties measured by the Toyo taste meter and estimated by the regression equation.

on the basis of genetic similarities simply calculated on the basis of the assumption that each of the markers had the same effect on the genotype determination. This demonstrates that simple genotyping and similarity analysis might not be a good measure to evaluate the eating quality of rice, and thus there should be differential weighting of markers. Therefore, multiple regression analysis was performed. Multiple regression equations developed in this study (**Table 8**) proved to be effective in predicting the palatability of rice varieties and breeding lines (**Figure 3**). Ohtsubo et al. (*13, 17*) and Ohtsubo and Nakamura (*12*) were able to estimate rice palatability (sensory test of Japanese people) by using a combination of STS markers and estimation equations with significant multiple regression coefficients. Similarly, other marker sets derived from starch synthesizing genes (GBSS, SS, SBE, and SDBE) (*2, 3*) and storage protein genes (glutelin and prolamin) were also tested to evaluate AC, PC, and the adhesiveness of cooked rice (*30*).

Of the significant markers in the equations (**Table 8**), nine were important for palatability as measured by both the Toyo taste meter (P) and the sensory test (ST), and the R^2 values of the nine markers explained most of the variation in both equations. This indicates that palatability by the Toyo taste meter and palatability by the sensory test are in good accordance as revealed by the significant correlation ($r = 0.85$ **) (**Table 7**). Of the nine significant markers, the STS marker, P5, showed the highest partial R^2 in both equations, implying that it might represent a major QTL for palatability.

Because we used most of the reported markers developed for taste evaluation in *japonica* rice, the regression equations (**Table 8**) are of sufficiently high resolution to estimate the palatability of *japonica* rice in Korea. However, because palatability varies among countries and varietal groups even within *japonica* rice, the equations may need to be modified to be applicable in other countries. Particularly in countries where *indica* rice is preferred, the markers should be re-evaluated. We expect that the set of 13 markers used in this study will be useful for the selection of early breeding or even individual F_2 plants to accelerate breeding for improvement of rice eating quality in Korea or other *japonica* rice-consuming countries.

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