Molecular Aspect of Good Eating Quality Formation in Japonica Rice

Ming-Mao Sun1, Sailila E. Abdula1, Hye-Jung Lee1, Young-Chan Cho2, Long-Zhi Han3, Hee-Jong Koh4, Yong-Gu Cho1*

1 Department of Crop Science, Chungbuk National University, Cheongju, Korea, 2 National Institute of Crop Science, Rural Development Administration, Suwon, Korea, 3 Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China, 4 Department of Plant Science, Seoul National University, Seoul, Korea

Abstract

The composition of amyllopectin is the determinant of rice eating quality under certain threshold of protein content and the ratio of amylose and amyllopectin. In molecular biology level, the fine structure of amyllopectin is determined by relative activities of starch branching enzyme (SBE), granule-bound starch synthase (GBSS), and soluble starch synthase (SSS) in rice grain under the same ADP-Glucose level. But the underlying mechanism of eating quality in molecular biology level remains unclear. This paper reports the differences on major parameters such as SNP and insertion-deletion sites, RNA expressions, and enzyme activities associated with eating quality of japonica varieties. Eight japonica rice varieties with significant differences in various eating quality parameters such as palatability and protein content were used in this experiment. Association analysis between nucleotide polymorphism and eating quality showed that S12 and S13 loci in SBE1, S55 in SSS1, S58 in SSS2A were significantly associated with apparent amylose content, alkali digestion value, setback viscosity, consistency viscosity, pasting temperature, which explained most of the variation in apparent amylose content, setback viscosity, and consistency viscosity; and explained almost all variations in alkali digestion value and pasting temperature. Thirty-five SNPs and insertion-deletions from SBE1, SBE3, GBSS1, SSS1, and SSS2A differentiated high or intermediate palatability rice varieties from low palatability rice varieties. Correlation analysis between enzyme activities and eating quality properties revealed that SBE2S and SSS15/W15 were positively correlated with palatability, whereas GBSS10 and GBSS15 were negatively correlated. Gene expressions showed that SBE1 and SBE3 expressions in high palatability varieties tended to be higher than middle and low palatability varieties. Collectively, SBE1, SBE3, SSS1, and SSS2A, especially SBE1 and SBE3 could improve eating quality, but GBSS1 decreased eating quality. The results indicated the possibility of developing high palatability cultivars through modification of key genes related to japonica rice eating quality formation in starch biosynthesis.

Introduction

Rice (Oryza sativa L.) is the staple food for more than half of the world’s population. Each year many new rice varieties are being developed and released with high yield, and high resistance to biotic and abiotic stresses, but their eating quality remains to be improved. Eating quality has now become the primary consideration of final rice-eating consumers and breeding programs. Cultivars with better eating quality are also required for food industry and rice seed producers. Although indica rice varieties are widely planted worldwide, consumers in Northeast Asia preferred japonica rice. Also, japonica rice is becoming more and more popular with worldwide consumers due to its moderate elasticity and stickiness [1–3].

Polished rice is mainly made up of starch, protein, lipid, and moisture. The protein content of polished rice in 22 japonica rice varieties ranged from 5.9 to 7.9% [3], and 6.0–13.6% in brown rice among 1,518 Chinese japonica varieties [4]. Starch comprises 76.7–78.4% in polished rice with 14% moisture content [5]. Eating quality of rice is thus mainly influenced by starch property. Moreover, eating quality of rice is a very complex trait, and the palatability from Toyo taste meter is significantly and positively correlated with palatability from sensory test [3]. Therefore, palatability is directly related to rice eating quality. In physico-chemical level, apparent amylose content [6], gel consistency [7], gelatinization temperature [8] or alkali digestion value, pasting properties [9], chain length distribution of amyllopectin [10], and protein content [11] are thought to be important parameters affecting eating quality. Rice eating quality is partially affected by environmental factors such as growing temperature and soil fertility, but mainly determined by genetic control [12].

In molecular biological aspects, sucrose derived from glucose and fructose is synthesized in cytoplasm, and then transported into cytosol in which sucrose is degraded by invertase. Using degraded products of sucrose, fructose and UDP-glucose, starch is synthesized by multiple subunits or isoforms of five classes of enzymes: ADP-glucose pyrophosphorylase (AGP), soluble starch synthase (SSS), granule-bound starch synthase (GBSS), starch branching enzyme (SBE), and starch debranching enzyme (DBE) [13–15] (Figure 1). Moreover, GBSS is not only responsible for

Citation: Sun M-M, Abdula SE, Lee H-J, Cho Y-C, Han L-Z, et al. (2011) Molecular Aspect of Good Eating Quality Formation in Japonica Rice. PLoS ONE 6(4): e18385.

do:10.1371/journal.pone.0018385

Editor: Dorian Q. Fuller, University College London, United Kingdom

Received November 28, 2010; Accepted February 28, 2011; Published April 6, 2011

Copyright: © 2011 Sun et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by a grant from the BioGreen 21 Program, Rural Development Administration and by a grant (code # 306005-04-2-HD110) from MIFAFF, Korea. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: ygcho@chungbuk.ac.kr

Downloaded from http://journals.plos.org/plosone/article/info:doi/10.1371/journal.pone.0018385 by guest on July 9, 2022
amylase synthesis, but also involved in amylopectin synthesis, especially in forming the extra-long chains of amylopectin [16]. Among the five enzymes, SBE, GBSS, SSS, and DBE are contributing to the fine structure of amylopectin [17]. SBE in rice grain contains SBE1, SBE3 (QEIa, or BEIIb), and SBE4 (QEIb, or BEIla) isoforms [18–20]. GBSS has two isoforms, GBSS1 in rice grain and GBSS2 in rice leaf [21]. SSS contains SSS1, SSS2A (SSSII-3), SSS2B (SSSII-2), SSS2C (SSSII-1), SSS3A (SSSIII-2), SSS3B (SSSIII-1), and SSS4A (SSSIV-1), SSS4B (SSSIV-2) [22]. DBE contains isoamylase (ISA) and pullulanase (PUL) in rice. ISA has at least three isoforms, ISA1, ISA2, and ISA3, and only one PUL [23].

The enzymes and genes involved in fine structure of amylopectin play distinct roles, but jointly comprise of the genetic dissection of rice eating quality. Using 70 rice varieties, association analysis between 18 genes related to starch synthesis and amylose content, gel consistency, gelatinization temperature showed that eating and cooking quality by affecting amylose content, gel consistency and gelatinization temperature [24]. The correlations among amylose content, gel consistency, and gelatinization temperature were found in 30 rice varieties and GC/TT polymorphism could differentiate rice varieties with high or intermediate GT from those with low GT at a rate of about 90% correct prediction for 509 varieties [26].

Activities of AGP at all rice grain filling stages and activities of SBE at the early and middle grain filling stages were not correlated with rice eating quality components including gel consistency, alkali spreading value, and amylase content. SSS activity at the early grain filling stage was negatively correlated with gel consistency and alkali spreading value, and positively correlated with amylose content. Activities of SSS at middle and late grain filling stages and SBE at the late grain filling stage were positively correlated with gel consistency and alkali spreading value, and negatively correlated with amylose content [27]. The SSS activity at whole grain filling stages was not correlated with palatability, but the activities of AGP and SBE at 12, 24, and 30 days after heading were correlated with palatability. Moreover, the activities of AGP, SSS, and SBE were correlated with Rapid Visco Analyzer (RVA) pasting properties [28]. Changes in nucleotide sequences and mRNA expressions of genes involved in SBE, GBSS, and SSS, and activities of SBE, GBSS, and SSS in rice grain, and the relationship between enzyme activities of SBE and SSS and rice eating quality are studied little. There is no report on the correlations between nucleotide sequence variation and rice palatability, between mRNA expression and rice palatability, between GBSS activity and rice palatability.

Figure 1. Main pathway of starch biosynthesis including sucrose synthesis, sucrose degradation and starch synthesis in rice. FBP1, fructose-1,6-bisphosphatase1; PGM, phosphoglucomutase; AGPase, ADP-glucose pyrophosphorylase; PPI, pyrophosphate. doi:10.1371/journal.pone.0018385.g001
In this study, our aim was to find the relationships between nucleotide sequence variations of SBE1, SBE3, GBSSI, SSS1, and SSS2A and rice eating quality, between activities of SBE, GBSS1, and SSS at different grain filling stages and rice eating quality, between gene expressions of SBE1 and SBE3 at different grain filling stages and rice eating quality, to explore the molecular biology aspect of good eating quality formation in japonica rice which provide the basic ideas for breeding good eating quality japonica rice.

Results
Eating quality traits
Palatability from Toyo taste meter served as an important and direct index in estimating eating quality of japonica rice instead of sensory test [3,29]. The physicochemical properties of eight japonica rice varieties were summarized in Table 1 and Table S3.

All traits showed significant differences among varieties and exhibited a wide range of variation, especially palatability (P) with 49.7–70.4, apparent amylose content (AAC) of 14.35–18.67, and protein content (PC) of 5.78–8.54 (Figure 2). The palatability was higher in Gopum and Koshihikari followed by Ilpum and Palgong. Lowest palatability was in Samnam, Singeumo, and Dobong. Among middle palatability group, Palgong was relatively lower than Ilpum and Samgwang (Figure 2).

Correlation analysis revealed that palatability was negatively correlated with protein content (Table 2). However, no significant correlation was observed to the other traits including apparent amylose content, alkali digestion value (ADV), and RVA pasting properties indicating that the palatability is a complex trait in which many factors are involved, but mainly affected by protein content. Apparent amylose content and alkali digestion value were significantly correlated with RVA pasting properties such as breakdown viscosity, setback viscosity, consistency viscosity, and pasting temperature.

Table 1. Means and ranges of eating quality parameters in eight japonica rice varieties.

| Parameter * | Mean ± SD | Pr>F | Range | CV (%) b | Skewness | Kurtosis |
|-------------|-----------|------|-------|----------|----------|----------|
| P           | 60.4±7.28 | <0.0001 | 49.7–70.4 | 12.04 | -0.23 | -1.19 |
| AAC (%)     | 17.47±1.31 | <0.0001 | 14.35–18.67 | 7.49 | -1.53 | 1.90 |
| PC (%)      | 7.09±1.00 | <0.0001 | 5.78–8.54 | 14.12 | 0.22 | -1.44 |
| ADV (%)     | 5.8±1.65  | <0.0001 | 1.5–6.5 | 28.62 | -2.25 | 3.09 |
| PV *        | 282.70±24.69 | <0.0001 | 241.45–317.56 | 8.74 | -0.05 | -0.77 |
| HPV *       | 174.60±15.24 | 0.0003 | 158.79–203.68 | 8.73 | 0.73 | -0.56 |
| CPV *       | 280.17±18.74 | 0.0001 | 247.37–307.57 | 6.69 | -0.32 | -0.49 |
| BDV *       | 108.10±23.65 | <0.0001 | 75.60–148.65 | 21.87 | 0.05 | -0.68 |
| SBV *       | -2.53±32.04 | <0.0001 | -67.60 to 39.71 | 1268.70 | -0.64 | 0.14 |
| CTV *       | 105.58±10.63 | <0.0001 | 81.05–115.31 | 10.07 | -1.48 | 1.24 |
| PT (°C)     | 68.84±2.12 | <0.0001 | 68.00–74.28 | 3.08 | 2.27 | 3.14 |

*S, palatability value; AAC, apparent amylose content; PC, protein content; ADV, alkali digestion value; PV, peak value; HPV, hot pate viscosity; CPV, cool paste viscosity; BDV, breakdown value; SBV, setback viscosity; CTV, consistency viscosity; PT, pasting temperature;

Nucleotide sequence differences
Five genes related to fine structure formation of amylopectin were selected to identify the differences in the association analysis of eating quality. To identify base pair differences in genomic regions, the polymerase chain reaction (PCR) products of these genes were sequenced as follows: 8,756 bp for SBE1, 10,287 bp for SBE3, 3,444 bp for GBSSI, 6,914 bp for SSS1, and 2,314 bp for SSS2A (Table S1). Sequenced fragments were detected in 60 SNP/insertion-deletion (InDel) sites for 3 genes among eight japonica varieties. The polymorphic sites were listed in Table S4. Of the 60 polymorphic sites, 17 were located in coding regions, and the diversity of coding sequences was much lower than non-coding sequences throughout the genes investigated.

For SBE1 gene, the S6, S8, S12, S13, S16, S18 and S19 sites “AGGGTG-” were the same in Gopum, Koshihikari, Ilpum, and Samgwang, but they were partially replaced by “GAAAAGAT” in Palgong, Samnam, Singeumo, and Dobong. The S1, S10, S15, and S17 sites “CAT--” were the same in Palgong, Samnam, Singeumo, and Dobong, but they were partially replaced by “TGGG/GA” in Gopum, Koshihikari, Ilpum, and Samgwang. For SBE3 gene, the S23, S25–S29, S36, S42, S43, S45, S46 sites “CATGTAAAGCC” were same in Palgong, Samnam, Singeumo, and Dobong, but they were partially replaced by “ACCTG-TG–A” in Gopum, Koshihikari, Ilpum, and Samgwang. Though we detected sequences of GBSSI, SSS1, and SSS2A in some SNP/insertion-deletion sites, we did not find strong evidence as to differences related to eating quality compared to SBE1 and SBE3 in all japonica rice varieties. It indicated that the “AGGGTTG-” linkage sites may be advantageous for high eating quality formation, but the “CATGTAAAGCC” linkage sites may be disadvantageous for high eating quality formation in japonica rice varieties.

Association analysis between eating quality properties and SNP/insertion-deletions showed that S12 and S13 loci in SBE1, S55 in SSS1, S58 in SSS2A were significantly associated with apparent amylose content, alkali digestion value, setback viscosity (SBV), consistency viscosity (CTV), and pasting temperature (PT), which explained most of the variation in apparent amylose content, setback viscosity, and consistency viscosity, and almost all variations in alkali digestion value and pasting temperature ($R^2=0.99$) (Table 3). S40 in SBE3, and S48 and S49 in GBSSI

doi:10.1371/journal.pone.0018385.t001

Table 1. Means and ranges of eating quality parameters in eight japonica rice varieties.
were significantly associated with peak viscosity (PV) and consistency viscosity at P<0.05, which explained about 60% of total variation. S3, S4, and S5 in SBE1 were significantly associated with breakdown viscosity (BDV), indicating that the breakdown viscosity was partially controlled by SBE1. However, palatability was not significantly associated with any single SNP or insertion-deletion locus detected.

Phylogenetic analysis was performed using 35 SNPs and insertion-deletions filtered from SBE1, SBE3, GBSS1, SSS1, and SSS2A (Figure 3). In UPGMA tree, high and intermediate palatability varieties were placed in Clade1, and low palatability varieties were placed in Clade2, indicating that palatability was controlled by a few polymorphic sites together at the genomic level resulted in enzymatic differences. These results showed that the five genes would be very important to eating quality formation.

**Enzyme activities**

The enzyme activities of SBE, GBSS1, SSS, and the ratio of SSS activity to grain weight (SSS/W) in developing rice grains were presented in Figure 4. Eight varieties showed different enzyme activities with diverse maximum peak times during grain filling stage.

For starch branching enzyme, the peak values of enzyme activity were formed during 10–15 days after flowering in six varieties except for Palgong and Samnam. The activities of SBE at 25 days after flowering were higher in Gopum, Koshihikari, Ipum, and Samgwang than those in Palgong, Samnam, and Samnam.

Table 2. Correlation matrix of rice eating quality parameters.

| Parameter | AAC | P | PC | ADV | PV | HPV | CPV | BDV | SBV | CTV |
|-----------|-----|---|----|-----|----|-----|-----|-----|-----|-----|
| P *       | 0.63|    |    |     |    |     |     |     |     |     |
| PC        | −0.64| −0.93**| |    |    |     |     |     |     |     |
| ADV       | 0.97**| 0.59| −0.58| |    |     |     |     |     |     |
| PV        | −0.58| 0.12| −0.09| −0.53| |     |     |     |     |     |
| HPV       | 0.19| 0.36| −0.21| 0.21| 0.36| |     |     |     |     |
| CPV       | 0.68| 0.53| −0.46| 0.70| −0.10| 0.82*| |     |     |     |
| BDV       | −0.72*| −0.10| 0.04| −0.68| 0.81*| −0.25| −0.62| |     |     |
| SBV       | 0.83**| 0.21| −0.20| 0.81*| −0.82*| 0.19| 0.65| −0.97**| |     |
| CTV       | 0.92**| 0.41| −0.51| 0.93***| −0.68| 0.02| 0.59| −0.72*| 0.86**|     |
| PT        | −0.96**| −0.58| 0.55| −1.00**| 0.51| −0.23| −0.70| 0.67| −0.79*| −0.91**|

*P, palatability value; AAC, apparent amylose content; PC, protein content; ADV, alkali digestion value; PV, peak value; HPV, hot pate viscosity; CPV, cool paste viscosity; BDV, breakdown value; SBV, setback viscosity; CTV, consistency viscosity; PT, pasting temperature.

**P, significant at 0.01; *, at 0.05 level.

10.1371/journal.pone.0018385.t002
### Table 3. Highly associated SNPs and insertion-deletions with eating quality properties identified under general linear model.

| Trait | Locus a | R² b | F value | P value | Trait | Locus | R² | F value | P value |
|-------|---------|------|---------|---------|-------|--------|-----|---------|---------|
| AAC   | S12     | 0.92 | 73.75   | 1.4 × 10⁻⁴| BDV   | S4     | 0.54| 7.18    | 3.7 × 10⁻²|
|       | S13     | 0.92 | 73.75   | 1.4 × 10⁻⁴|       | S5     | 0.53| 6.67    | 4.2 × 10⁻²|
|       | S55     | 0.92 | 73.75   | 1.4 × 10⁻⁴| SBV   | S12    | 0.63| 10.29   | 1.8 × 10⁻²|
|       | S58     | 0.92 | 73.75   | 1.4 × 10⁻⁴|       | S13    | 0.63| 10.29   | 1.8 × 10⁻²|
| ADV   | S12     | 0.99 | 1605.56 | 1.6 × 10⁻⁸|       | S4     | 0.63| 10.29   | 1.8 × 10⁻²|
|       | S13     | 0.99 | 1605.56 | 1.6 × 10⁻⁸|       | S5     | 0.63| 10.29   | 1.8 × 10⁻²|
|       | S55     | 0.99 | 1605.56 | 1.6 × 10⁻⁸| CTV   | S12    | 0.83| 28.93   | 1.7 × 10⁻³|
|       | S58     | 0.99 | 1605.56 | 1.6 × 10⁻⁸|       | S13    | 0.83| 28.93   | 1.7 × 10⁻³|
| PV    | S40     | 0.67 | 12.16   | 1.3 × 10⁻²|       | S55    | 0.83| 28.93   | 1.7 × 10⁻³|
|       | S48     | 0.67 | 12.16   | 1.3 × 10⁻²|       | S58    | 0.83| 28.93   | 1.7 × 10⁻³|
| PV    | S49     | 0.67 | 12.16   | 1.3 × 10⁻²|       | S40    | 0.55| 7.39    | 3.5 × 10⁻²|
|       | S53     | 0.57 | 7.94    | 3.0 × 10⁻²|       | S48    | 0.55| 7.39    | 3.5 × 10⁻²|
| HPV   | S22     | 0.57 | 7.89    | 3.0 × 10⁻²|       | S49    | 0.55| 7.39    | 3.5 × 10⁻²|
|       | S36     | 0.59 | 8.64    | 2.6 × 10⁻²| PT     | S12    | 0.99| 10928.62| 5.2 × 10⁻¹¹|
|       | S59     | 0.59 | 8.64    | 2.6 × 10⁻²|       | S13    | 0.99| 10928.62| 5.2 × 10⁻¹¹|
|       | S17     | 0.75 | 7.53    | 3.1 × 10⁻²|       | S55    | 0.99| 10928.62| 5.2 × 10⁻¹¹|
| CPV   | S51     | 0.58 | 8.12    | 2.9 × 10⁻²|       | S58    | 0.99| 10928.62| 5.2 × 10⁻¹¹|
| BDV   | S3      | 0.54 | 7.18    | 3.7 × 10⁻²|       |        |     |         |         |

1 AAC, apparent amylose content; ADV, alkali digestion value; PV, peak value; HPV, hot pate viscosity; CPV, cool paste viscosity; BDV, breakdown value; SBV, setback viscosity; CTV, consistency viscosity; PT, pasting temperature;
2 SNP and insertion-deletion (see Table S4);
3 Explained variation.

doi:10.1371/journal.pone.0018385.t003

Figure 3. UPGMA tree based on 35 SNPs and insertion-deletions from five genes in eight japonica varieties. The 35 SNPs and insertion-deletions are as follows: S2, S3, S4, S5, S6, S7, S8, S9, S11, S12, S13, S14, S16, S18, S19, S20, S21, S22, S24, S30, S31, S32, S33, S34, S35, S37, S38, S39, S40, S41, S44, S48, S55, S58, S60 (See Table S4).
doi:10.1371/journal.pone.0018385.g003
Singeumo, and Dobong. For GBSS1, the peak values of enzyme activity were formed during 15–20 days after flowering. For soluble starch synthase, the peak values of enzyme activity were showed at 10 days after flowering. The differences were existed among eight varieties, but there were no evident differences for SSS activities between high and inferior palatability groups. In GBSS1, Singeumo and Dobong showed much higher activities than the other varieties during grain filling, but unexpectedly high during 10–15 days after flowering. Also, Palgong was high during 20–25 days after flowering. This implies that the high enzyme activity of GBSS1 was reflected to low palatability based on the negative correlation between GBSS1 activity and palatability (Table 4).

Correlation analyses revealed that SBE25, GBSS10, GBSS15, and SSS15/W15 were significantly correlated with palatability (Table 4). Among them, SBE25 was positively correlated with palatability, suggesting that the SBE activity at late grain filling stage played an important role in good eating quality formation. However, the activities of GBSS10 and GBSS15 were negatively correlated with palatability and resulted in low eating quality. The correlation between SSS activity and palatability was not significant (data not shown), therefore SSS activity/grain weight was employed to detect the correlation (Table 4). The SSS15/W15 was positively correlated with palatability, implying that SSS

**Figure 4. Enzyme activities related to amylopectin biosynthesis during rice grain filling in eight japonica varieties.** Letters A–G indicate significant differences at 0.05 level.
doi:10.1371/journal.pone.0018385.g004

### Table 4. Correlation analysis between eating quality traits and enzyme activities during rice grain filling.

| Trait       | Enzyme          | Correlation coefficient |
|-------------|-----------------|------------------------|
| AAC         | SBE10*          | 0.74*                  |
|             | SBE20           | 0.74*                  |
|             | SBE25           | 0.79*                  |
|             | GBSS10          | −0.73*                 |
|             | GBSS15          | −0.79*                 |
|             | SSS15/W15       | 0.90**                 |
| BDV         | SBE10           | −0.71*                 |
| SBV         | SBE10           | 0.76*                  |
| CTV         | SBE10           | 0.73*                  |

AAC, apparent amylose content; P, palatability value; BDV, breakdown value; SBV, setback viscosity; CTV, consistency viscosity.

*SBE10, starch branching enzyme activity at 10 days after flowering; W15, grain weight at 15 days after flowering.

**: correlation is significant at 0.01 and 0.05 level, respectively.
doi:10.1371/journal.pone.0018385.t004
activity/grain weight at middle stage was reflected to form the high palatability of rice grain. SBE activities were positively correlated with apparent amylose content at 10 and 20 days after flowering. Partial apparent amylose content was from long strains of increased amylopectin, and resulted in high apparent amylose content within certain threshold. Moreover, SBE activity at 10 days after flowering was positively correlated with consistency viscosity and setback viscosity, but negatively correlated with breakdown viscosity. This result was consistent with report of Shen et al. [28], implying that SBE played an important role in rice amylpectin composition. These results indicated that SBE and SSS/W could be controlled by modifying gene expression to improve rice eating quality, but GBSS1 reduced eating quality during rice grain filling stage.

**mRNA expressions**

Since starch branching enzyme was important determinant of eating quality by association analysis, real-time RT-PCR of SBE1 and SBE3 was carried out to elucidate the effect of gene expression to eating quality (see the primer information in Table S2). Using eight japonica varieties, the mRNA expressions of SBE1 and SBE3 tended to increase gradually to peak values, thereafter descended during late grain filling period (Figure 5). The peak values of SBE1 expression formed during 10–15 days after flowering, and the peak values of SBE3 formed during 5–10 days after flowering, which were consistent with report of Ohdan et al. [22]. According to palatability, the eight japonica varieties were divided into three groups: high (Gopum, Koshihikari), middle (Ilpum, Samgwang, Palgong), and low (Sannam, Singeumo, Dobong) palatability. T-tests showed that there were significant differences among high, middle, and low palatability groups. Alkali digestion value and cool paste viscosity were significantly different between middle and low.

The differences of SBE1 and SBE3 expressions at 5, 10, 15, 20, and 25 days after flowering among high, middle, and low palatability groups were detected using t-tests (LSD), respectively (Table 5). SBE1 mRNA expression at 5 days after flowering (SBE1-5) and SBE3-5 expressions were significantly different between high and low, and between middle and low groups. SBE1-15 and SBE3-15 expressions were significantly different between high and middle groups, and between high and low groups. SBE3-20 expression was significantly different between high and low groups. SBE3-25 expression was significantly different between high and low groups, and between middle and low groups at P<0.05.

Correlation analysis between eating quality properties and mRNA expressions of SBE1 and SBE3 involving SBE1-15 and SBE3-15 played an important role in palatability difference between high and middle groups. Interestingly, mRNA expressions of SBE1-5, SBE1-15, SBE3-5, SBE3-15, SBE3-20, and SBE3-25 contributed a lot to the palatability variation between high and low groups. Among them, SBE1-5, SBE3-5, and SBE3-25 were important in palatability differentiation between middle and low groups. These results suggested that mRNA expressions of SBE1 and SBE3 were important to form good eating quality in japonica varieties, especially SBE1 and SBE3 expressions at both 5 and 15 days after flowering. SBE1-5, SBE3-5, and SBE3-25 also played an important role in alkali digestion value and cool paste viscosity variations between middle and low palatability groups.

**Discussion**

Rice (Oryza sativa L.) is the most important staple food of East Asia and the most densely populated landscapes of China, Korea, Japan, Southeast Asia, and South Asia [30], which contains two subspecies, japonica and indica. Molecular and archaeobotanical evidences have shown that japonica and indica rice originated from distinct gene pools in the wild ancestor Oryza rufipogon [31]. Consumers in northeastern Asian countries and regions such as Korea, northern China, Taiwan, and Japan prefer the gelatinous to sticky spectrum of japonica rice [2,3,32]. The preference for eating glutinous endosperm starch or sticky foods had been established fairly early in the evolution of cereal farming. This particular preference for eating glutinous foods maybe the effective basis for the later selection of sticky endosperm mutants from normal non-glutinous cereal crops such as rice, foxtail millet, common millet, barley, and maize [32]. This indicated the existence of rice eating quality preference which exerted selective pressure in favor of sticky endosperm form of rice in Northeast Asia [33].

The palatability from Toyo taste meter is positively and significantly correlated with palatability from sensory test, and thus the palatability from Toyo taste meter is considered an important and direct index in the evaluation of rice eating quality [3,29]. There was no correlation between palatability and any RVA pasting properties, between palatability and amylose content, between palatability and alkali digestion value, indicating that apparent amylose content, alkali digestion value, and RVA pasting properties could not be a good indicator for eating quality.

![Figure 5. SBE1 and SBE3 expressions based on real-time RT-PCR during grain filling in eight japonica varieties. Letters A–F indicates difference is significant at 0.05 level.](https://doi.org/10.1371/journal.pone.0018385.g005)
evaluation of japonica rice. The protein content which showed negative correlation was the only trait to affect the palatability as previously reported [3,34]. Setback viscosity in 23 rice varieties ranged from ~0.0 to 132, and the coefficient of variation was 275.47 [35]. Lestari et al. [3] reported that setback viscosity in 22 japonica rice varieties ranged from ~94.75 to 25.00, and the coefficient of variation was 318.71. In this study, setback viscosity ranged from ~67.60 to 39.71, and the coefficient of variation was 1268.70 which was produced by small number of varieties. The alkali digestion value of Dobong was much smaller than the other varieties. On the contrary, the pasting temperature of Dobong was much higher than the other varieties (Table S3).

Nucleotide sequence composition is the basis of gene expression and gene function, and the SNP and insertion-deletion sites of genes related to amylopectin structure among different rice varieties have been reported. The G/T polymorphism at 5’ splice site of GBSS1 1st intron affects the rice apparent amylose content. Cultivars with 18% or less amylose content have the sequence, AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36]. All eight japonica rice varieties have the sequence AGTTATA at the 5’ splice site, while cultivars with a higher proportion of amylose have AGGTTATA [36].

Table 5. mRNA expressions of SBE1 and SBE3 related to eating quality properties among high, middle, and low palatability groups.

| Parameter | High P group (2) * | Middle P group (3) | Low P group (3) | H-M | H-L | M-L |
|-----------|-------------------|--------------------|----------------|-----|-----|-----|
| P | 69.2 | 62.5 | 52.5 | ** | ** | ** |
| ADV | 6.3 | 6.5 | 4.7 | ns | ns | * |
| CPV | 288.73 | 288.03 | 266.61 | ns | ns | * |
| SBE1-5 | 1.02 | 0.94 | 0.58 | ns | ** | ** |
| SBE1-15 | 1.62 | 1.44 | 1.36 | ns | ** | ** |
| SBE3-5 | 3.54 | 2.94 | 1.73 | ns | ** | ** |
| SBE3-15 | 2.00 | 1.32 | 1.30 | ns | ** | ** |
| SBE3-20 | 1.51 | 1.21 | 0.97 | ns | ** | ns |
| SBE3-25 | 1.15 | 1.11 | 0.92 | ns | * | * |

1: P, palatability value; ADV, alkali digestion value; CPV, cool paste viscosity; SBE1-5, SBE1 mRNA expression at 5 days after flowering;
2: *, number in the parenthesis means the number of varieties;
3: **: difference is significant at 0.01 and 0.05 level, respectively; ns, not significant.

60 SNP and insertion-deletion sites were detected for SBE1, SBE3, GBSS1, SSSI, and SSS2A nucleotide sequences in eight japonica varieties. The SNP and insertion-deletion sites were not even in allele distribution among eight japonica varieties. It indicated that single SNP or insertion-deletion might not be a good measure to evaluate rice eating quality, and thus several SNP and insertion-deletion sites should be combined. Apparent amylose content, alkali digestion value, setback viscosity, and pasting temperature were associated with SBE1, SSSI, and SSS2A. Peak viscosity was associated with SBE1, SBE3, GBSS1, and SSSI. Hot paste viscosity was associated with SBE1, SBE3, and SSS2A. Cool paste viscosity was associated with GBSS1. Breakdown viscosity was associated with SBE1. Consistency viscosity was associated with SBE1, SBE3, GBSS1, SSSI, and SSS2A. 55 SNPs and insertion-deletions from SBE1, SBE3, GBSS1, SSSI, and SSS2A jointly differentiated the low palatability and high or middle palatability japonica groups. Tian et al. [24] reported the apparent amylose content was controlled by GBSS1, SSSI, and SSSI, whereas gelatinization temperature was controlled by SSS2A, GBSS1, ISA, SBE3, and SSS4B in 33 indica and 37 japonica rice varieties. Apparent amylose content, hot paste viscosity, breakdown viscosity, cool paste viscosity, consistency viscosity, setback viscosity, and pasting temperature were controlled by GBSS1 in 88 indica rice varieties, whereas apparent amylose content, cool paste viscosity, and consistency viscosity were controlled by AGPL1-4. Hot paste viscosity was controlled by SBE3, setback viscosity and consistency viscosity were controlled by GBSS1 in 41 japonica rice varieties [39].

The peak values of starch branching enzyme activity appeared during 10–15 days after flowering in Gopum, Koshikihari, Ilpum, Samgwang, Singeumo, and Dobong, whereas Palgong and Samnam appeared at 20 days after flowering, which were consistent with previous reports [28,40–42]. The peak values of GBSS1 activity in eight japonica varieties appeared during 15–20 days after flowering, which were in good agreement with the report of Liu et al. [43]. The peak values of SSS activity in all japonica varieties appeared at 10 days after flowering, and then descended gradually during rice grain filling stage, which were consistent with previous reports [41–43].

SBE25 and SSS15/W15 were positively correlated with palatability, whereas GBSS10 and GBSS15 were negatively correlated with palatability. The results indicated that SBE and SSS/W could improve rice eating quality, but GBSS1 decreased the eating quality. Shen et al. [28] reported that palatability was...
negatively correlated with SBE activity at 12 days after heading, and positively correlated with SBE activities at 24 and 30 days after heading, respectively, but no correlation between SSS activity and palatability was detected.

The peak values of SBE1 expression formed during 10–15 days after flowering, and the peak values of SBE3 formed during 5–10 days after flowering were consistent with the report of Ohdan et al. [22]. mRNA expression is located in an intermediate process between initial nucleotide diversity and final enzyme activity, which is important in rice eating quality formation.

We divided eight japonica varieties into three groups according to palatability. The differences in eating quality properties, SBE1 and SBE3 expressions between high and middle, between high and low, between middle and low palatability groups were detected. This method was used to compare the gene expressions between indica and japonica variety groups, and found the relationships between cooking and eating quality properties and gene expressions [39]. Therefore, this method is feasible in gene expression analysis. SBE1-15 and SBE3-15 expressions in high palatability group were higher than those in middle palatability group, and SBE1-5, SBE3-5, SBE1-15, SBE3-15, SBE3-20, and SBE3-25 expressions in high palatability group were higher than those in low palatability group. SBE1-5, SBE3-5, and SBE3-25 expressions in middle palatability group were higher than those in low palatability group, indicating that SBE1 and SBE3 expressions tended to be higher in high palatability varieties compared to middle and low palatability varieties. The SBE1 and SBE3 were important to good eating quality formation in japonica varieties.

Materials and Methods

Plant Materials and Field Trials

The field experiment was conducted in the rice field of Chungbuk National University in 2009. Eight japonica rice varieties, Koshihikari, Gopum, Ilpum, Samgwang, Palgong, Samnam, Singeumo, and Dobong, with different eating quality varieties, Koshihikari which was developed in Japan.

The varieties were sown in a seedling-growing tray and placed in the greenhouse for 3 weeks in May, 2009. Grown seedlings were transplanted in the experimental farm space at 30×15 cm with one seedling per hill and arranged in randomized complete block design replicated three times. The cultivation and management were performed according to the rice cultivation standards of the experimental farm in Chungbuk National University.

The young leaf tissues of eight varieties were collected at the tillering stage for genomic DNA extraction. Thirty uniformly flowered panicles were chosen and recorded the flowering date for each variety in each plot. Six tagged panicles from each variety in each plot were sampled at 5, 10, 15, 20, and 25 days after flowering. Collected samples were immediately frozen in liquid nitrogen for 5 min, and then stored at −80°C for enzyme activity and mRNA expression analyses. Matured rice seeds were harvested separately in each plot, and air-dried for 2 weeks before measuring the palatability and physicochemical properties.

DNA Extraction and Purification

Genomic DNA was extracted in extraction buffer (100 mM Tris-HCl pH 8.0, 500 mM NaCl, 50 mM EDTA, 1.25% SDS, Sodium bisulfate 0.38 g/100 ml) as described by Cho et al. [44] with some modifications. 0.2 g of ground powder of fresh rice leaf tissue was placed in 2 ml microcentrifuge tube, and 900 μl of 65°C preheated extraction buffer (pH 7.8–8.0) was added. After incubation in water bath at 65°C for 30 min, 700 μl of phenol: chloroform: isoamyl alcohol (25:24:1) was added into the tube and mixed for 10 min, and centrifugation was carried out at 13,000 rpm for 10 min. The upper phase was transferred into a new 2 ml tube and equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed for 10 min. The mixed samples were centrifuged at 13,000 rpm for 10 min and the upper phase was transferred into a new 1.5 ml tube containing 4 μl of 10 mg/ml RNase1. After incubation at 37°C for 30 min, 0.7 volume of precooled isopropanol was added and mixed well, and centrifuged at 13,000 rpm at 4°C for 15 min. The supernatant was discarded and 1 ml of 70% ethanol was applied to wash the DNA pellet and centrifuged at 13,000 rpm for 5 min. DNA pellet was air-dried and suspended in 50 μl of double-distilled water.

Primer Design, PCR Reaction, and Sequencing

The nucleotide sequences of SBE3 LOC_Os06g51084, SBE3 LOC_Os02g32660, GBSSI LOC_Os06g04200, SSSI LOC_Os06g06360, and SSS2 LOC_Os06g12450 were obtained by searching the Gramene database (http://www.gramene.org). Using genomic DNA sequences, primers were designed with Primer3 online software (http://bioinformatics.ummassmed.edu/bioapps/primer3_www.cgi). Primer analysis was carried out using Oligo 6 software. The primers used for sequencing were listed in Table S1. Polymerase chain reaction (PCR) was carried out in a 50 μl reaction mixture containing 80 ng of template DNA, 5 μl of 10×buffer, 4 μl of dNTP mixture (2.5 mM each), 1.25 units of Ex Taq polymerase (Takara), and 1.25 μl each of forward and reverse primers (10 μM). The PCR reaction was performed at 94°C for 5 min as a hot start, followed by 25 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec. Moreover, the annealing temperature and extension time were adjusted according to different primer pairs and expected PCR product sizes. The final extension was followed by one cycle of 10 min at 72°C. The reactions were carried out in PTC-100 programmable thermal controller (MJ Research Inc.). Amplified PCR products were analyzed by electrophoresis on 1% agarose gel stained with ethidium bromide, and purified by PCR DNA purification kit (GeneAll). After that, the purified PCR products were sequenced at COSMO Genetech Company, and the sequences reported in this paper have been deposited in the GenBank database (accession nos. HQ712126-HQ712165). DNA fragments sequenced of 8 rice varieties were analyzed with clustalw of DDBJ (http://clustalw.ddbj.nig.ac.jp/).

RNA Extraction and First-Strand Synthesis of cDNA

Total RNAs were isolated from rice grains using the RNaseasy mini kit (Qiagen) according to the manufacturer’s instruction with some modifications. 100 mg of frozen ground seed powder was placed into cold 1.5 ml tube, and then 600 μl of RLC buffer was added. After immediately rapid and strong vortex, centrifugation was carried out at 13,000 rpm for 2 min. The supernatant was rapidly transferred into 1 ml spin column, and then centrifuged at 12,000 rpm for 2 min. 280 μl of 100% ethanol was added into the flowthrough, mixed 4–5 times by pipetting, and then the sample was transferred into pink RNase spin column. After centrifugation at 10,000 rpm for 1 min, 700 μl of RW1 buffer was added into the pink spin column. Centrifugation was carried out at 10,000 rpm for 1 min, and then 300 μl of RPE buffer was used to wash RNA twice at 10,000 rpm for 1 min. After centrifugation for 2 min at 10,000 rpm, 50 μl of RNase-free water was added into the pink spin column in 1.5 ml tube, and centrifuged again at 10,000 rpm for 1 min. The RNA concentration was detected in Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc. USA), and stored at −80°C.
freezer. Total RNAs were cleaned using DNase 1 kit (Cat. No.
18066-015, Invitrogen), and the first-strand cDNA synthesis was
performed by reverse transcription of mRNA using Oligo(T)20
primer and SuperScript™ III Reverse Transcriptase (Cat. No.
18080-051, Invitrogen).

Quantitative Real-Time RT-PCR

The deduced amino acid sequences of SBE1 and SBE3 were
blasted at NCBI/Blast website, and the clustalw (DDBJ/
Clustalw site) of amino acid sequences from 15 species was
searched. Using cDNA sequences responsible for the conserved
domains of proteins, primers were designed with Primer3 online
software, and primer analysis was carried out using Oligo 6 software.
Quantitative real-time RT-PCR was performed on the DNA Engine
Opticon 2 instrument (MJ Research, Waltham, MA) in 20 μl reaction
volume containing SYBR Green, a fluorophore which binds to all
double-strand DNA (F-410Lm, qPCR kit from FINNZYMES, Finland).
The primers used for real-time RT-PCR were shown in Table S2.
PCR conditions were as follows: 9 min at 95°C followed by 42
cycles of 33 sec at 94°C, 32 sec at 55°C, 22 sec at 72°C, and final
extension of 10 min at 72°C. Finally, the relative quantification of
gene expression was analyzed using the 2-ΔΔCt method [45] by
normalization to internal control Actin-1.

SBE Activity Assay

Enzyme activity of starch branching enzyme including SBE1,
SBE3, and SBE4 together in rice grains was assayed as described
by Yamanouchi and Nakamura [18] with some modifications.
Fifteen frozen rice endosperms were weighed and homogenized in
4 ml of cold extraction buffer (50 mM HEPES-NAOH pH 7.4,
4 mM MgCl₂, 50 mM 2-mercaptoethanol, 12.5% (v/v) glycerol)
contained in a precooled mortar with a pestle on ice. The
homogenate was transferred into a precooled 40 ml tube, and
6 ml of extraction buffer was added for two times to clean the
mortar. 40 ml tubes were centrifuged at 15,000 rpm at 2°C for
20 min, and supernatants were filtered through a filter paper
(0.45 μm, Whatman, Whatman International LTD). The filtrates
were used for enzyme preparations.

Starch branching enzyme was assayed by monitoring stimula-
tion of α-glucan synthesis from glucose-1-P by rabbit muscle
phosphorylase a [46–47]. 100 μl of enzyme preparation was added
into 100 μl of cold reaction buffer (50 mM HEPES-NAOH
pH 7.0, 50 mM glucose-1-P, 2.5 mM AMP, 1.2 units of
phosphorylase a from rabbit muscle), and was incubated at 30°C
for 30 min. The reaction was terminated by adding 50 μl 1 M
HCl, and mixed with 500 μl of dimethylsulfoxide. 700 μl of fresh
iodine-potassium iodide solution (2 g KI
D
0.01 optical density as 1 unit which has similar function as
%E; V_R, reaction volume (enzyme sample volume + working
reagent volume + stop solution volume) (ml); V_s, enzyme sample
volume (ml); V_T, total enzyme sample volume (ml). So SBE
activity = ΔA × 100 × 1.45 × 10/(30 × 15 × 1 × 0.1 × 0.1) = 322.22 × ΔA
(Units grain⁻¹ min⁻¹).

GBSS1 and SSS Extraction

GBSS1 and SSS enzyme extractions were conducted as
described by Umemoto and Teraslma [48] with some modifica-
tions. Fifteen frozen rice endosperms were weighed and hand-
homogenized in 2 ml of ice-cold extraction buffer (100 mM Tris
pH 7.2, 2 mM EDTA, 2 mM DTT, and 10% (v/v) ethanolid in
a precooled mortar with a pestle on ice. The homogenate was
transferred into a precooled 40 ml tube, and 1 ml of extraction
buffer was used to clean the mortar, and then transferred to 40 ml
tube. The homogenate was then centrifuged at 15,000 rpm and
2°C for 20 min. The supernatant was collected and filtered through
a membrane filter (0.45 μm, Whatman, Whatman International LTD),
and the filtrate was used for the assay of
SSS. The pellet was washed three times by suspension and
centrifugation using extraction buffer. The washed pellet was
resuspended in 3 ml extraction buffer and used for GBSS1 assay.

GBSS1 Activity Assay

The activity of GBSS1 was assayed as described by Nakamura
et al. [47] with some modifications. 0.15 ml of GBSS1 enzyme
preparation was added into 0.15 ml of solution A (50 mM
HEPES-NAOH pH 7.4, 1.6 mM ADP, glucose, 0.7 mg amryo-
pectin, 13 mM DTT), and incubated at 30°C for 1 hour (mixed
every 10 min). The enzyme was inactivated by placing the mixture
in a boiling-water bath for 30 s, and incubated on ice for 10 min.
The mixture was then added by 0.1 ml of solution B (50 mM
HEPES-NAOH pH 7.4, 4 mM PEP, 200 mM KCï, 10 mM
MgCl₂, and 1.5 units of pyruvate kinase), and incubated at 30°C
for 30 min. The ADP produced by the starch synthase reaction
was converted to ATP and the resulting solution was heated in a
boiling-water bath for 30 s, and incubated on ice for 10 min,
and then subjected to centrifugation at 15,000 rpm and 2°C for
15 min. The supernatant (0.3 ml) was mixed with 0.3 ml of
solution C (50 mM HEPES-NAOH pH 7.4, 10 mM glucose,
20 mM MgCl₂, 2 mM NADP). The GBSS1 activity was measured
as the increase in absorbance of 340 nm after the addition of 1 μl
each of hexokinase (1.4 units) and G-6-P dehydrogenase (0.35
units) at 25°C for 30 min.

The controls were prepared as follows: 0.15 ml of GBSS1
enzyme preparation was inactivated in a boiling-water bath for
30 s, and incubated on ice for 10 min, and then solution A was
added. The other steps were same as reaction samples. The blank
solution was made using 0.15 ml of cold extraction buffer to
replace 0.15 ml GBSS1 preparation, and the other steps were
same as reaction samples. Three replicates were carried out in
this experiment.

The enzyme activity of granule-bound starch synthase 1 was
calculated as follows: GBSS1 activity = ΔA × 100 × V_R × V_T/
(T × N × d × V_s × V_s); ΔA, absorbance of samples at 30 min minus
absorbance of samples at 0 min; T, time (minute); N, grain number;
d, light path in cm; 100, 0.01 optical density as 1 unit which has
similar function as %E; V_R, reaction volume (enzyme sample volume + working
reagent volume + stop solution volume) (ml); V_s, enzyme sample volume (ml); V_T, total enzyme sample volume (ml). So GBSS1 activity = ΔA × 100 × 0.602 × 3/(60 × 15 × 15.3/4 × 0.15 × 3/4) = 15.86 × ΔA
(Units grain⁻¹ min⁻¹).
SSS Activity Assay

The enzyme activity of SSS was assayed by Nishi et al. [49] with some modifications. 0.15 ml of SSS preparation was added into 0.15 ml of buffer A (0.5 M citrate-Na pH 7.5, 50 mM Bicine-NaOH pH 7.5, 1.7 mM ADP-Glucose, 0.7 mg oyster glycogen (Type II, Sigma), 16.7 mM DTT), and incubated at 30°C for 20 min. The enzyme was inactivated by placing the mixture in a boiling-water bath for 30 s, and incubated on ice for 10 min. The mixture was supplemented with 0.1 ml of Buffer B (50 mM HEPES-NaOH pH 7.4, 10 mM phosphocreatine, 200 mM KCl, 10 mM MgCl2, and 10 μl of 5 mg ml−1 creatine phosphokinase (5 units, Type I, Sigma)), and then incubated at 30°C for 30 min. The ADP produced by the soluble starch synthase reaction was converted to ATP, and the resulting solution was heated in a boiling-water bath for 30 s, and incubated on ice for 10 min, and then subjected to centrifugation at 15,000 rpm and 2°C for 15 min. The supernatant (0.3 ml) was mixed with 0.3 ml of buffer C (125 mM HEPES-NaOH pH 7.4, 10 mM glucose, 20 mM MgCl2, and 150 μg NADP). The SSS activity was measured as the increase in absorbance of 340 nm after the addition of 1.1 μl of hexokinase (1.4 units/μl, Roche Diagnostics, Tokyo) and 1.5 μl of G-6-P dehydrogenase (0.35 units/μl, Type XV, Sigma) at 25°C for 30 min.

The controls were prepared as follows: 0.15 ml of soluble starch synthase preparation was inactivated in a boiling-water bath for 30 s, and incubated on ice for 10 min, and then buffer A was added. The other steps were same as reaction samples. The blank solution was made using 0.15 ml of cold extraction buffer to replace 0.15 ml of soluble starch synthase preparation, and the other steps were same as reaction samples. Three replications were carried out in this experiment.

The enzyme activity of soluble starch synthase was calculated as follows: SSS activity = ΔA ×100 × Vr × Vf / (T × N × d × Vs × Vf); ΔA, absorbance of samples at 30 min minus absorbance of samples at 0 min; T, time (minute); N, grain number; d, light path in cm; 100, 0.01 optical density as 1 unit which has similar function as ε or %E; Vr, reaction volume (enzyme sample volume + working reagent volume + stop solution volume) (ml); Vs, enzyme sample volume (ml); Vf, total enzyme sample volume (ml). So SSS activity = ΔA ×100 × 0.6626 × 3/20 × 15 × 0.15 × 3/4 × 0.15 × 3/4 = 47.61 × ΔA (Units grain−1 min−1). SSS10/W10, SSS15/W15, SSS20/W20, and SSS25/W25 were calculated by SSS activities at 10, 15, 20, and 25 days after flowering were divided by 15 grain weight at 10, 15, 20, and 25 days after flowering, respectively.

Eating Quality Traits Evaluation

For palatability and physicochemical analyses, rice grains were dehulled and milled to 91% yield. The analyses were performed as described by Lestari et al. [3] with some modifications. Palatability was detected by a rice taste measuring system (Toyo taste meter, Japan) and calculated with the determined value using total nitrogen multiplied by the conversion coefficient 5.95. The amylose content of milled rice was measured using the relative absorbance of starch-iodine color in a digested solution of 100-mesh rice flour as described by Perez and Juliano [51]. The amylose content measured from iodine-binding capacity was named as apparent amylose content. Thus, actual amylose content was derived from apparent content by subtracting the contribution of the long chains of amylopectin [52]. The alkali digestion value was estimated using the alkali digestion test and spreading scores [8].

RVA pasting properties were detected with a Rapid Visco Analyzer (RVA) according to the manufacturer’s instruction (NewPort Sci. Co., Australia). Rice starch paste profile was described by seven parameters. They were as follows: peak viscosity (PV), through viscosity or hot paste viscosity (HPV), final viscosity or cool paste viscosity (CPV), breakdown viscosity (BDV = PV-HPV), setback viscosity (SBV = CPV-PV), consistency viscosity (CTV = CPV-HPV), and pasting temperature as described by Bao and Xia [53] and Shen et al. [28]. All the viscosity parameters were expressed in rapid visco units.

Statistical Analysis

Analysis of variance was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Significant differences were determined using t-tests (LSD) for comparison of means. Correlation analyses among rice eating quality parameters and enzyme activities were conducted using Statistix version 8.0 (http://www.statistix.com/). The general linear model (GLM) was used in the analysis of associations between nucleotide polymorphisms and various rice eating quality parameters, which was performed with Tassel version 2.1 (http://www.maizegenetics.net/tassel). Moreover, phylogenetic analysis was also performed using Tassel 2.1 software.

Supporting Information

Table S1 Primers used in the sequencing of genes related to rice amylopectin biosynthesis.

Table S2 Primers for SBE1 and SBE3 expressions in developing rice grains.

Table S3 Eating quality parameters for eight japonica rice varieties.

Table S4 Polymorphic sites of genes related to rice amylopectin biosynthesis of eight japonica varieties.

Author Contributions

Conceived and designed the experiments: YGC. Performed the experiments: MMS SEA HJL YCC LZH HJK YGC. Analyzed the data: MMS YGC. Contributed reagents/materials/analysis tools: MMS SEA HJL YCC LZH HJK YGC. Analyzed the data: MMS YGC. Performed the experiments: MMS SEA HJL YCC LZH HJK YGC. Contributed reagents/materials/analysis tools: MMS SEA HJL YCC YGC. Wrote the paper: MMS YGC.

References

1. Hansen J, Fuller F, Gale F, Crook F, Wails E, et al. (2002) China’s japonica rice market: growth and competitiveness. Rice Situation and Outlook Yearbook, Economic Research Service/USDA. pp 32–37.
2. Fuller DQ, Qin L, Zheng Y, Zhao Z, Chen X, Hosoya LA, Sun G (2009) The domestication process and domestication rate in rice: spikelet bases from the Lower Yangtze. Science 323(5921): 1607–1610.
3. Lestari P, Ham TH, Lee HH, Woo MO, Jiang W, et al. (2009) PCR marker-based evaluation of the eating quality of japonica rice (Oryza sativa L.). J Agric Food Chem 57: 2754–2762.
4. Chen N, Luo YK, Xie LH, Zhu ZW, Duan BW, et al. (2006) Protein content and its correlation with other quantity parameters of rice in China. Acta Agronomica Sinica 32(8): 1193–1196.
5. Huang YS, Sun ZX, Hu PS, Tang SQ (1996) Present situation and prospects for the research on rice grain quality forming. Chinese J Rice Sci 12(3); 172–176.
6. Juliano BO (1985) Criteria and tests for rice grain qualities. In: Juliano BO, ed. Rice Chemistry and Technology, 2nd edition. American Association of Cereal Chemists, St. Paul, MN, pp 443–513.
7. Fuller DQ, Qin L, Zheng Y, Zhao Z, Chen X, Hosoya LA, Sun G (2009) The domestication process and domestication rate in rice: spikelet bases from the Lower Yangtze. Science 323(5921): 1607–1610.
8. Lestari P, Ham TH, Lee HH, Woo MO, Jiang W, et al. (2009) PCR marker-based evaluation of the eating quality of japonica rice (Oryza sativa L.). J Agric Food Chem 57: 2754–2762.
7. Cagampang GR, Perez CM, Juliano BO (1973) A gel consistency test for eating quality of rice. J Sci Food Agric 24: 1509-1594.
8. Little RR, Hilder GR, Dawson EH (1958) Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chem 35: 111-126.
9. Bao JS, Corke H, Sun M (2006) Microsatellites, single nucleotide polymorphisms and a sequence tagged site in relation to starch physiochemical properties in nonwaxy rice (Oryza sativa L.). Theor Appl Genet 113: 1185-1196.
10. Hong HC (2006) Studies on the degree of polymerization of amylopectin and eating quality characteristics in rice (Oryza sativa L.). PhD dissertation. Dongguk University, Korea. pp 1-142.
11. Ramesh M, Bhattacharya KR, Mitchell JR (2000) Developments in understanding the basis of cooked-rice texture. Crit Rev Food Sci Nutr 40: 49-160.
12. Iumi OE, Yui M, Kuniyuki S, Toshiro K (2007) Effects of rising temperature on grain quality, palatability and physicochemical properties of rice. Scientific Reports of the Faculty of Agriculture, Okayama University 96: 13-18.
13. Piao AR, Kelder T, van mesel MF, Hanspers K, Creukink BR, et al. (2008) WikiPathways: Pathway editing for the people. PLoS Biol 6(7): e1403-1407.
14. Satoh H (2000) The possible pathway of starch biosynthesis in endosperm of rice. Available: http://www.glycosforum.or.jp/science/word/saccharide/SA-COIE.html via the Internet.
15. Compart-Moss S, Denyer K (2009) The evolution of the starch biosynthetic pathway in cereals and other grasses. J Exp Bot 60(9): 2481-2492.
16. Hanashiro I, Itoh K, Kurazumi Y, Yamazaki M, Igarashi T, et al. (2008) Granule-bound starch synthase IIa is responsible for biosynthesis of extra-long unit chains of amylopectin in rice. Plant Cell Physiol 49(3): 718-725.
17. Nakamura Y (2002) Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: Rice endosperm as a model tissue. Plant Cell Physiol 43: 1219-1228.
18. Yamanouchi H, Nakamura Y (1992) Organ specificity of isoforms of starch branching enzyme (Q-enzyme). Plant Cell Physiol 33(7): 985-991.
19. Tanaka N, Fujita N, Nishi A, Satoh H, Hosaka Y, et al. (2004) The structure of starch can be manipulated by changing the expression levels of starch branching enzymeIIb in rice endosperm. Plant Biotechnol J 2: 507-516.
20. Dian WM, Jiang HW, Wu P (2005) Evolution and expression analysis of starch synthase III and IV in rice. J Exp Bot 56(412): 623-632.
21. Dian WM, Jiang HW, Chen QS, Liu FY, Wu P (2003) Cloning and characterization of the granule-bound starch synthaseIIgene in rice: gene expression is regulated by the nitrogen level, sugar and circadian rhythm. Planta 218: 261-268.
22. Ohdan T, Francisco PB, Saudara T, Hirose T, Teraz T, et al. (2005) Expression profiling of genes involved in starch synthesis in sink and source organs of rice. J Exp Bot 56(422): 3229-3244.
23. Fujita N, Toyosawa Y, Usimoto U, Higuchi T, Hanashiro I, et al. (2009) Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. Proc Natl Acad Sci U S A 106 (15): 21760-21765.
24. Nakamura Y, Francisco Jr. PB, Hosaka Y, Sato A, Saudara T, et al. (2005) Essential amino acids of starch synthase IIa differentiate amylopectin structure and starch quality between japonica and indica rice varieties. Plant Mol Biol 58: 215-227.
25. Bao JS, Corke H, Sun M (2006) Nucleotide diversity in starch synthaseIIa and validation of single nucleotide polymorphism in relation to starch gelatinization temperature and other physicochemical properties in rice (Oryza sativa L.). Theor Appl Genet 113: 1171-1183.
26. Zhao BH, Zhang WJ, Wang ZQ, Zhu QS, Yang JC (2005) Changes in activities of the key enzymes related to starch synthesis in rice grains during grain filling and their relationships with the filling rate and cooking quality. Agricultural Sciences in China 4(1): 26-33.
27. Shen P, Qian CR, Jin ZX, Luo QX, Jin XY (2006) Relationship between variation in activities of key enzymes related to starch synthesis during grain filling period and quality of eating and cooking in rice. Rice Science 13(1): 43-50.
28. Tanaka R, Ino K, Kanagawa M (1992) Cultivation method and eating quality of paddy rice 1. Evaluation with mechanical tester of boiled rice so called “MIBDO meter”. Tohoku Journal of Agricultural Science 35: 34-46.
29. Hosoya LA, Sato YI, Fuller DQ (2010) Editorial: the archaeobotany of early agriculture in Asia. Archaeol Anthropol Sci 2: 57-59.
30. Harushima Y, Nakagawa M, Yano M, Sasaki T, Kurata N (2002) Diverse variation of reproductive barriers in three intraspecific rice crosses. Genetics 160: 515-522.
31. Sakamoto S (1996) Glutinous-endosperm starch food culture specific to Eastern and Southeastern Asia. In: Ellen R, Fukui K, eds. Redefining nature. Ecology, culture and domestication. Berg, Oxford. pp 215-231.
32. Fuller DQ, Sato YI, Castillo C, Qin L, Weisskopf AR, et al. (2010) Consilience of genetics and archaeobotany in the entangled history of rice. Archaeol Anthropol Sci 2: 115-131.
33. Yu TQ, Jiang WZ, Han TH, Chu SH, Lestari PJ, et al. (2008) Comparison of grain quality traits between japonica rice cultivars from Korea and Yunnan Province of China. Journal of Crop Science and Biotchnology 11: 135-140.
34. Shinn QY, Wu DX, Xia YW, Gao MW, Anna MC (1998) Relationship between RVA profile character and eating quality in Oryza sativa L. Scientia Agricultura Sinica 31(3): 25-29.
35. Ayres NM, McCling AM, Larkin PD, Bligh HEJ, Jones CA, et al. (1997) Microsatellites and a single-nucleotide polymorphism differentiate apparent amylose content classes in an expanded pedigree of US rice germplasm. Theor Appl Genet 94: 773-781.
36. Han YP, Xu ML, Liu XY, Yan CJ, Korban SS, et al. (2004) Genes coding for starch branching enzymes are major contributors to starch viscosity characteristics in waxy rice (Oryza sativa L.). Plant Sci 166: 357-364.
37. Bae JS, Jin L, Xiao P, Shen SQ, Sun M, et al. (2008) Starch physicochemical properties and their associations with microsatellite alleles of starch-synthesizing genes in a rice RIL population. J Agric Food Chem 56: 1389-1394.
38. Zhang Z (2009) The study on the relation of the SNP polymorphism and expression of starch-synthesis genes and rice grain quality. MS dissertation. Huazhong Agricultural University, China. pp 1-89.
39. Shen B, Chen Q, Li TG, Luo YK (1997) Effect of temperature on rice chalkiness formation and changes of materials in endosperm. Chinese J Rice Sci 11(3): 183-186.
40. Jiang HW, Dian WM, Wu P (2005) Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme. Phytochemistry 63: 53-59.
41. Nakamura Y, Yuki K (1992) Changes in enzyme activities associated with carbohydrate metabolism during the development of rice endosperm. Plant Sci 62: 15-20.
42. Liu B, Guo ZQ, Liang JS (2008) Effect of enzyme activities related to starch synthesis in rice endosperm on fine structure of amylopectin. Scientia Sinica Vitae 38(3): 766-773.
43. Cho YG, Kang HJ, Lee JS, Lee YT, Lim SJ, et al. (2007) Identification of quantitative trait loci in rice for yield, yield components, and agronomic traits across years and locations. Crop Sci 47: 2403-2417.
44. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408.
45. Hawker JS, Ozbun JL, Ozaki H, Greenberg E, Pruss J (1974) Interaction of spinach leaf adenosine diphosphate glucose 1,4-1,4-glucan and 1,4-glucosyl transferase and 1,4,1,4-glucan-6-glucosyl transferase in synthesis of branched 1,4-glucan. Arch Biochem Biophys 128: 530-531.
46. Nakamura Y, Yuki K, Park SY, Ohyu T (1989) Carbohydrate metabolism in the developing endosperm of rice grains. Plant Cell Physiol 30(3): 833-839.
47. Unemoto T, Terashima K (2002) Activity of granule-bound starch synthase is an important determinant of amylose content in rice endosperm. Funct Plant Biol 29: 1121-1124.
48. Nishi A, Nakamura Y, Tanaka N, Satoh H (2001) Biochemical and genetic analysis of the effects of amylase-extender mutation in rice endosperm. Plant Physiol 127: 459-472.
49. Cuniff P (1995) Official Methods of analysis of AOAC international, 16th edition. Arlington, VA: AOAC international. 30 p.
50. Perez, CM, Juliano BO (1978) Modification of the simplified amylose test for milled rice. Starch 30: 494-496.
51. Takeda Y, Hizakuri S, Juliano BO (1987) Structure of rice amylopectins with low and high affinity for iodine. Carbohydr Res 168: 79-88.
52. Bao JS, Xia YW (1999) Genetic control of the paste viscosity characteristics in indica rice (Oryza sativa L.). Theor Appl Genet 98: 1129-1124.