



Full Length Article

Physicochemical Properties and Molecular Differences of Eating and Cooking Quality Formation in Low Amylose Content *japonica* Rice

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Abstract

To better understand the formation of eating and cooking quality (ECQ) in low amylose content (AC) *japonica* rice, the differences of starch physico-chemical properties, enzyme activity and expression pattern of starch biosynthesis genes in *japonica* normal, soft and waxy rice varieties was compared. Starch physico-chemical analysis showed that normal rice had the highest AC, retrogradation enthalpy and percentage (%R), pasting time and temperature, trough viscosity, final and setback viscosity, while waxy rice had the highest gel consistency (GC), gelatinization enthalpy and breakdown viscosity (BDV). Almost every ECQ parameter in soft rice was between normal and waxy rice and closer to normal rice, except %R, BDV and the fine structure of amylopectin. Granule-bound starch synthase I (GBSSI) activity was absent in waxy rice and significantly lower in soft rice than in normal rice, though the expression pattern of *Wx* mature mRNA in soft rice exhibited similar to normal rice. Soluble starch synthase activities performed opposite trends with GBSSI among three types of rice, but starch branching enzyme and debranching enzyme activities showed almost no differences among them. Soft and waxy rice showed higher expression levels of *SSI* and *SBEIIb*, which might cause the higher A chains of amylopectin in soft and waxy rice. The high amounts of A chains might contribute to the high BDV in soft and waxy rice. The results suggested that good ECQ of soft rice was mainly due to the polymerization of low AC, appropriate GC and pasting properties and low %R and gelatinization temperature. © 2019 Friends Science Publishers

Keywords: Eating and cooking quality; Enzyme activity; Starch biosynthesis genes; Rice

Introduction

Rice (*Oryza sativa* L.) is a crucial cereal crop, more than half of the people in the world take rice as a staple food. With the improvement of economy and living quality, rice grain quality especially eating and cooking quality (ECQ) are much concerned by both breeders and consumers nowadays. ECQ are mainly influenced by rice starch properties, because starch accounts for about 90% of the dry weight of the grain (Zhou *et al.*, 2015). Starch is basically composed of two forms of glucose polymers: amylopectin and amylose. The ratio of two components and their structures mainly determine the ECQ of rice (Teng *et al.*, 2015).

Amylose, catalyzed by the granule-bound starch synthase I (GBSSI) encoded by *Wx* gene, is a key factor in determining ECQ of rice (Tian *et al.*, 2009). Varieties of low amylose content (AC) are usually moist, sticky and soft, whereas those with high AC are dry, fluffy and hard (Bao, 2012). Besides AC, the *Wx* gene is also the major gene for gel consistency (GC) and other physicochemical properties of rice (Tian *et al.*, 2009; Bao, 2012). Therefore, the activity

of GBSSI and expression of *Wx* might play central roles of the wide diversity of rice AC, GC and other ECQ parameters. Amylopectin content and short chains of amylopectin are positively and significantly correlated with the stickiness of cooked rice, while the long-B chains in amylopectin rendered the hard texture of cooked rice (Ramesh *et al.*, 1999; Li *et al.*, 2016). Amylopectin is synthesized by multiple isoforms of three classes of enzymes including soluble starch synthase (SSS), starch branching enzyme (SBE), and starch debranching enzyme (DBE). The SSS enzyme is responsible for generating the different subclasses chains in amylopectin. There are total of eight SSS isoforms in rice, consisting of one SSI isoform, three SSII isoforms (SSIIa, SSIIb and SSIIc), two SSIII isoforms (SSIIIa and SSIIIb) and two SSIV isoforms (SSIVa and SSIVb); SBE is responsible to form new branches, rice SBE possess three isoforms: SBEI, SBEIIa and SBEIIb; DBE hydrolyzes branches to adjust the structure of the polymer, rice DBE has three isoforms of isoamylase (ISA) (ISA1, ISA2 and ISA3), whilst only one pullulanase (PUL) (Jeon *et al.*, 2010). Each enzyme plays a

unique role in the biosynthesis of rice amylopectin (Nakamura, 2002). Starch biosynthesis in rice grain is inseparable from the cooperation of these enzymes. Thus, it can be inferred that the changes in rice ECQ may due to the differences of starch biosynthetic genes expressions and enzymes activities varying levels.

In recent years, low AC varieties become more and more favorable by worldwide consumers, mainly due to moderate elasticity and stickiness (Yang *et al.*, 2013). *japonica* soft rice with Wx^{mq} (G/A mutation in the 4th exon of Wx^b) is a special type of low AC rice varieties (AC 8–12%) with good palatability in both hot and cool condition. And there is lacking clear information on the formation of this unique character. In this study, three types of *japonica* rice were used to analyze the differences of ECQ of mature grains, expression patterns and enzymes activities of key genes involved in starch biosynthesis during the grain filling. This study is expected to find the specific physicochemical parameters of starches in *japonica* soft rice distinctive to those in normal and waxy rice. The gene expression patterns and enzymes activities of starch biosynthesis could strengthen the explanation of variation of ECQ parameters among three types of rice.

Materials and Methods

Plant Material

Five varieties with closer growth period duration, two *japonica* soft rice varieties NJ9108 and NJ5055, two *japonica* normal rice varieties HD5 and WYJ23 and a *japonica* waxy rice cultivar ZN19, were used in the study. For analysis of gene expression and enzyme activities of starch biosynthesis at the heading stage, grains were marked on the flowering day and harvested at 5, 7, 10, 15, 20, 25, 32 days after flowering (DAF). The sampled grains were immediately frozen in liquid nitrogen and stored at -80°C until use. The field experiment was conducted in the experimental field of Jiangsu Academy of Agricultural Sciences with three replications, during rice growing season of 2016.

Measurement of Grain ECQ

Mature rice grains were de-hulled, milled and then ground into powder with a Foss CT410 Sample Mill (Foss Corporation, Hillerød, Denmark) and sieved through a 100-mesh sieve. These powders were used to measure the ECQ parameters of rice grain, and three replications were carried out in all experiments.

AC was determined using the method of Tan *et al.* (1999). GC was assayed as described by Cagampang *et al.* (1973). Rice pasting properties were measured using a rapid visco analyzer (RVA) (Perten Instruments, Stockholm, Sweden). The RVA profile was implemented following the

AACC 61-02 standard method. The viscosity was expressed in rapid visco units (RVU).

Thermal Properties

The thermal characteristics (gelatinization and retrogradation) were determined using differential scanning calorimetry DSC Q20 Series (TA Instrument, New Castle, USA) according to a previously utilized method of Zhang *et al.* (2017). Thermal transitions were defined as T_o (onset temperature), T_p (peak of gelatinization temperature), T_c (conclusion temperature), ΔH_{gel} (gelatinization enthalpy) and ΔH_{ret} (retrogradation enthalpies). The retrogradation percentage (%R) was calculated as $\%R = 100 \times \Delta H_{ret} / \Delta H_{gel}$.

Assessment of Amylopectin Chain Length Distribution

The amylopectin chain length distribution was studied according to the method of Han *et al.* (2012) using an ABI PRISM 3130XL (Thermo Fisher Scientific, Waltham, USA) DNA sequencing device.

Assay of Enzymes

The crude enzyme preparation was extracted according to the method of Teng *et al.* (2015). The activities of GBSS and SSS were measured according to the procedure of Nakamura *et al.* (1989). SBE activity was assayed using the methods of Zhao *et al.* (2007). The enzyme activity of DBE was assayed as described by Liu *et al.* (2010). Triplicate measurements were performed for each sample.

Gene Expression Analysis

Quantitative real-time RT-PCR (qRT-PCR) was performed on the StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, USA) with the SuperReal PreMix Plus (Tiangen, Beijing, China). The primers used for qRT-PCR were listed in Table S1. Expression data of the target genes was normalized to endogenous reference gene *OsUBQ*.

Statistical Analysis

The data were subjected to ANOVA, using Tukey's test ($p < 0.05$) with SPSS 23.0 statistical software program.

Results

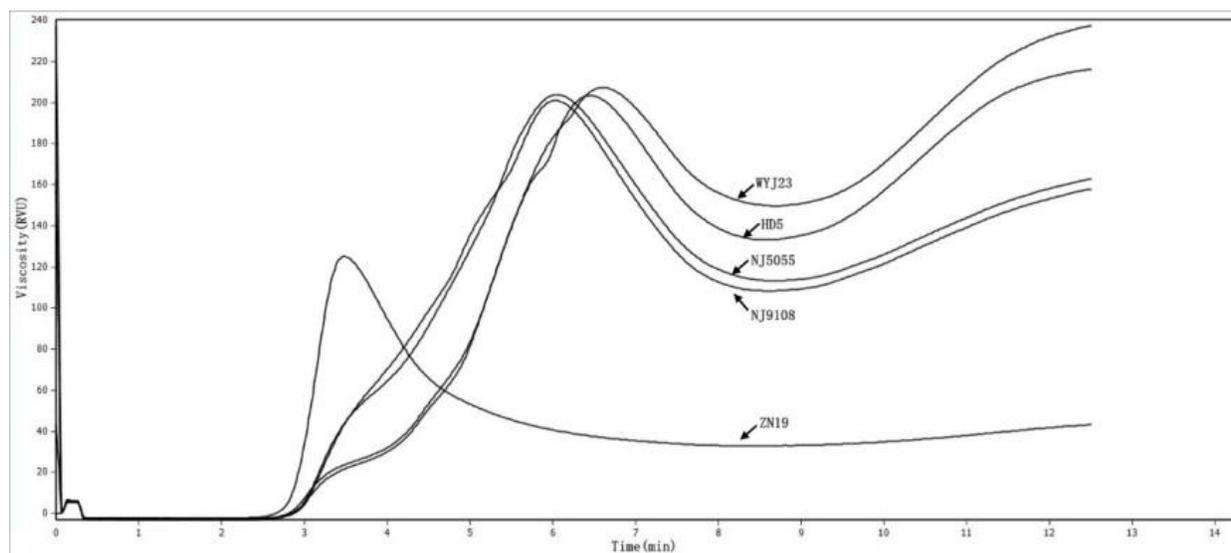
AC, GC and RVA Profile Parameters

There were significant differences in starch physicochemical properties of AC, GC and RVA profile among three types of *japonica* rice (Table 1). For AC, the variation ranged from 0.94% in waxy rice ZN19 to 17.56% and 18.87% in normal rice HD5 and WYJ23. The AC in two soft rice NJ9108 and NJ5055 was 9.43% and 9.60%, respectively. For GC, the value 109.3 mm in ZN19 was

Table 1: Eating and cooking qualities of *japonica* normal, soft and waxy rice flours^a

| Cultivar | AC (%) | GC (mm) | PV (RVU) | TV (RVU) | BDV (RVU) | FV (RVU) | SBV (RVU) | PT (min) | P _{temp} (°C) |
|----------|----------------|-----------------|------------------|------------------|-----------------|------------------|-----------------|----------------|------------------------|
| ZN19 | 0.94 ± 0.07 d | 109.33 ± 3.25 a | 128.83 ± 11.34 b | 31.54 ± 1.49 c | 96.88 ± 10.15 a | 41.96 ± 1.76 c | -78.54 ± 9.69 d | 3.44 ± 0.10 c | 69.85 ± 0.87 b |
| NJ9108 | 9.43 ± 0.03 c | 69.75 ± 1.06 b | 197.83 ± 4.47 a | 101.21 ± 10.08 b | 96.63 ± 5.60 a | 150.71 ± 10.08 b | -47.13 ± 5.60 c | 5.90 ± 0.14 b | 72.40 ± 0.50 a |
| NJ5055 | 9.60 ± 0.07 c | 69.60 ± 2.07 b | 208.46 ± 6.54 a | 118.21 ± 7.13 ab | 90.25 ± 0.59 a | 167.46 ± 6.66 b | -41.00 ± 0.12 c | 6.13 ± 0.09 ab | 72.70 ± 0.52 a |
| HD5 | 17.56 ± 0.67 b | 69.00 ± 3.12 b | 203.21 ± 5.66 a | 134.46 ± 18.40 a | 68.75 ± 12.80 b | 218.46 ± 11.84 a | 15.25 ± 6.50 b | 6.43 ± 0.18 a | 72.75 ± 0.52 a |
| WYJ23 | 18.87 ± 0.67 a | 67.00 ± 4.77 b | 201.13 ± 8.78 a | 149.54 ± 0.18 a | 59.83 ± 3.06 b | 229.79 ± 8.64 a | 26.92 ± 0.82 a | 6.50 ± 0.82 a | 72.85 ± 1.06 a |

^a Values in the same column with different letters are significantly different at $p \leq 0.05$

**Fig. 1:** Rapid viscosity profiles of *japonica* normal, soft and waxy rice flours

significantly distinguished from other four rice varieties, while the values were similar in two normal and two soft rice varieties.

The RVA pasting properties of rice flour were outlined in Fig. 1 and their detailed values were provided in Table 1. The multiple comparison of RVA properties revealed that there were no significant differences in Peak Viscosity (PV) and Pasting temperature (P_{temp}) between normal and soft rice varieties, while ZN19 showed much lower PV and P_{temp} values than other four varieties (Fig. 1). For Trough Viscosity (TV), Final Viscosity (FV), Setback Viscosity (SBV) and Peak Time (PT), the high values were always present in normal rice varieties, and the low values were always present in ZN19 (Table 1). The corresponding values in soft rice were always between normal and waxy rice and much closer to normal rice. In contrast, BreakDown Viscosity (BDV) presented low values in normal rice varieties and did not show significant difference between soft and waxy rice varieties.

Thermal Properties

The gelatinization and retrogradation properties could reflect the degree of difficulty during rice cooking and the energy requirement. The thermal parameters of the five rice varieties were listed in Table 2. For gelatinization properties, ZN19 showed the highest T_c and ΔH_{gel} values but the lowest

T_o and T_p values compared with other rice varieties. Two normal rice varieties showed the highest T_o and T_p values but the lowest ΔH_{gel} . The values of T_o , T_p and ΔH_{gel} in two soft rice varieties were between the values in normal and waxy rice, but the T_c values were lowest in two soft rice varieties. This indicated that the gelatinization progress ended earlier in soft rice varieties than other four rice varieties. After gelatinization properties were determined, the samples were cooled down for at least 7 days in refrigerator and then used for retrogradation determination. In retrogradation properties, the T_o , T_p and T_c values varied slightly, but ΔH_{ret} showed significant higher values in two normal rice varieties than in other three varieties. %R showed the lowest value in waxy rice, the highest value in normal rice and intermediate values in soft rice.

Amylopectin Chain Length Distribution

The chain length distribution of amylopectin was identified as another key element affecting the ECQ of rice. The DSA-FACE method was applied to analyze the chain length distribution of the amylopectin synthesized in five rice varieties. Four types of degree of polymerization (DP) fractions were divided according to amylopectin chain length. The values of all the four DP fractions in two soft rice varieties showed similar with that in ZN19, which were significantly distinguished from two normal rice varieties

Table 2: Thermal properties of *japonica* normal, soft and waxy rice flours^a

| Cultivar | Gelatinization | | | | retrogradation | | | %R | |
|----------|---------------------|---------------------|---------------------|---------------|---------------------|---------------------|---------------------|---------------|----------------|
| | T _o (°C) | T _p (°C) | T _c (°C) | ΔH (J/g) | T _o (°C) | T _p (°C) | T _c (°C) | | ΔH (J/g) |
| ZN19 | 61.12 ± 0.08 e | 68.18 ± 0.32 b | 79.33 ± 0.21 a | 7.22 ± 0.10 a | 45.08 ± 0.08 b | 55.04 ± 0.12 d | 63.90 ± 0.10 c | 0.60 ± 0.13 b | 8.32 ± 0.12 c |
| NJ9108 | 61.81 ± 0.10 d | 67.77 ± 0.17 c | 77.12 ± 0.12 d | 6.80 ± 0.13 b | 45.78 ± 0.08 a | 55.99 ± 0.15 a | 64.69 ± 0.08 b | 0.60 ± 0.15 b | 8.89 ± 0.14 b |
| NJ5055 | 62.52 ± 0.05 c | 68.53 ± 0.13 b | 77.94 ± 0.18 c | 6.59 ± 0.22 b | 45.25 ± 0.06 b | 55.25 ± 0.14 c | 62.72 ± 0.12 d | 0.61 ± 0.16 b | 9.29 ± 0.19 b |
| HD5 | 62.88 ± 0.10 b | 69.33 ± 0.17 a | 78.80 ± 0.31 b | 5.79 ± 0.06 c | 45.84 ± 0.11 a | 54.90 ± 0.15 e | 64.66 ± 0.07 b | 0.91 ± 0.16 a | 15.88 ± 0.11 a |
| WYJ23 | 63.59 ± 0.08 a | 69.33 ± 0.17 a | 78.66 ± 0.19 b | 5.75 ± 0.14 c | 45.95 ± 0.06 a | 55.60 ± 0.16 b | 65.69 ± 0.13 a | 0.93 ± 0.15 a | 16.02 ± 0.14 a |

^a Values in the same column with different letters are significantly different at $p \leq 0.05$. T_o, T_p, T_c and ΔH correspond to the onset temperature, peak temperature, conclusion temperature, and enthalpy of gelatinization and retrogradation, respectively. Retrogradation percentage (%R): %R = 100 × ΔH_{ret} / ΔH_{gel}

Table 3: Amylopectin chain length fractions of *japonica* normal, soft and waxy rice flours^a

| Cultivar | DP 6-12 (A Chains, %) | DP 13-24 (B1 chains, %) | DP 25-36 (B2 chains, %) | DP 37-56 (B3+ chains, %) |
|----------|-----------------------|-------------------------|-------------------------|--------------------------|
| ZN19 | 39.27 ± 0.27 a | 55.84 ± 0.12 b | 4.54 ± 0.03 c | 0.95 ± 0.01 d |
| NJ9108 | 38.93 ± 0.41 a | 55.87 ± 0.20 b | 4.71 ± 0.03 b | 1.11 ± 0.01 b |
| NJ5055 | 39.14 ± 0.27 a | 55.82 ± 0.12 b | 4.63 ± 0.03 bc | 1.04 ± 0.01 c |
| HD5 | 37.44 ± 0.17 b | 57.17 ± 0.40 a | 4.94 ± 0.13 a | 1.18 ± 0.01 a |
| WYJ23 | 37.43 ± 0.17 b | 56.81 ± 0.20 a | 5.14 ± 0.13 a | 1.18 ± 0.01 a |

^a Values in the same column with different letters are significantly different at $p \leq 0.05$.

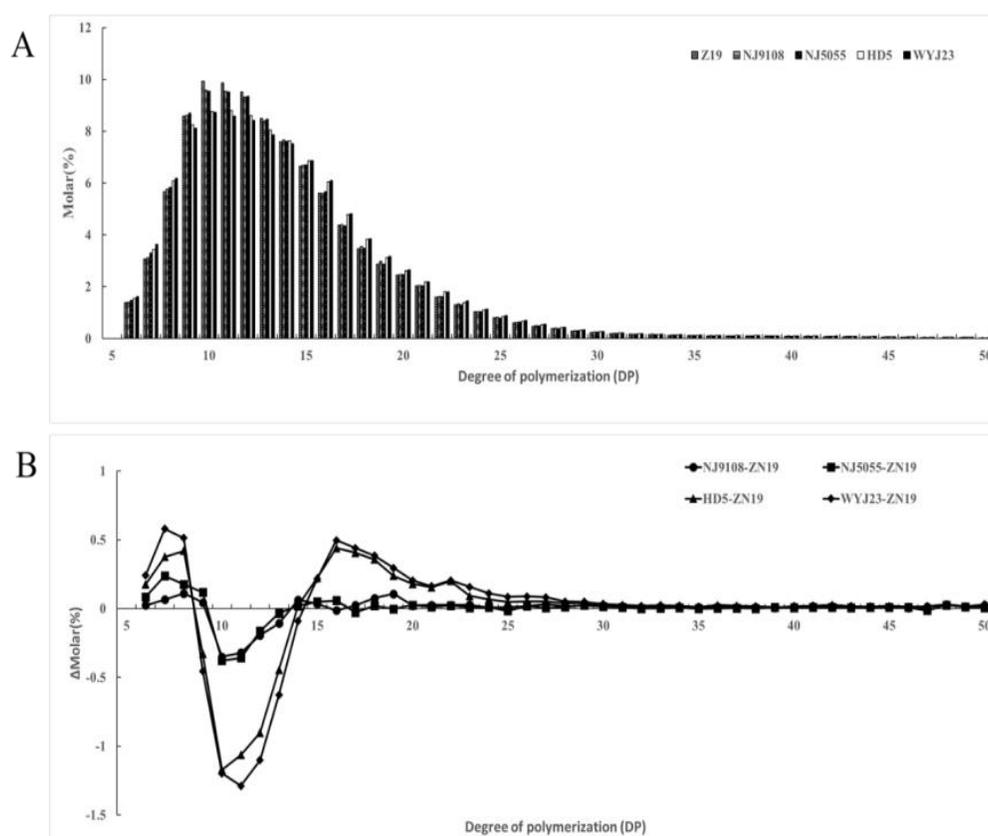


Fig. 2: Amylopectin chain length distribution of debranched starches from *japonica* normal, soft and waxy rice flours. (A) Estimated molar chain length distribution for DP 6–56 starch chains. (B) Difference in the amylopectin chain length distribution between ZN19 and other varieties

(Fig. 2). This indicated that soft rice owned waxy-like fine structure of amylopectin. Normal rice had lower DP 6–12 (A chains), higher DP 13–24 (B1 chains), DP 25–36 (B2 chains) and DP 37–50 (B3+ chains) than soft and waxy rice. This suggested that normal rice had more capacity of chain

elongation than ZN19 and soft rice (Table 3).

Enzyme Activities

The enzyme activities of GBSSI, SSS, SBE and DBE in

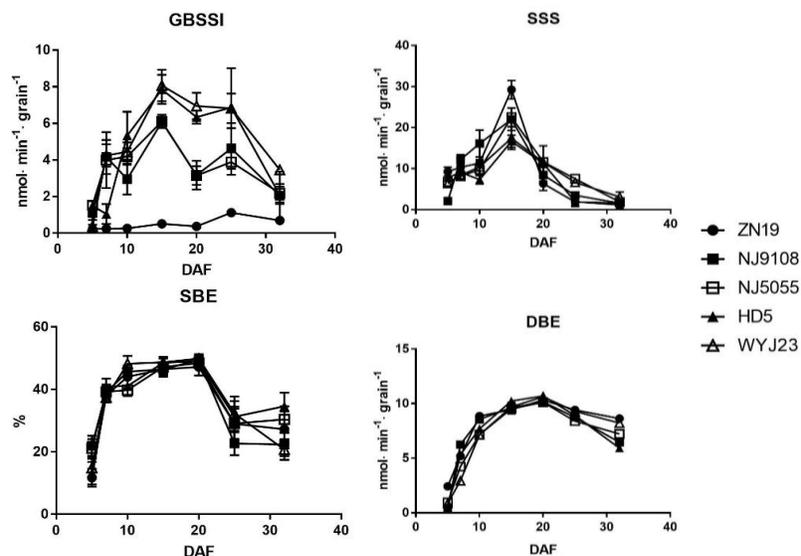


Fig. 3: Enzyme activities related to starch biosynthesis in grains of *japonica* normal, soft and waxy rice varieties during grain filling stage. Error bars indicate the SD for three independent measurements. DAF, days after flowering; GBSSI, granule bound starch synthase I; SSS, soluble starch synthase; SBE, starch branching enzyme; DBE starch debranching enzyme

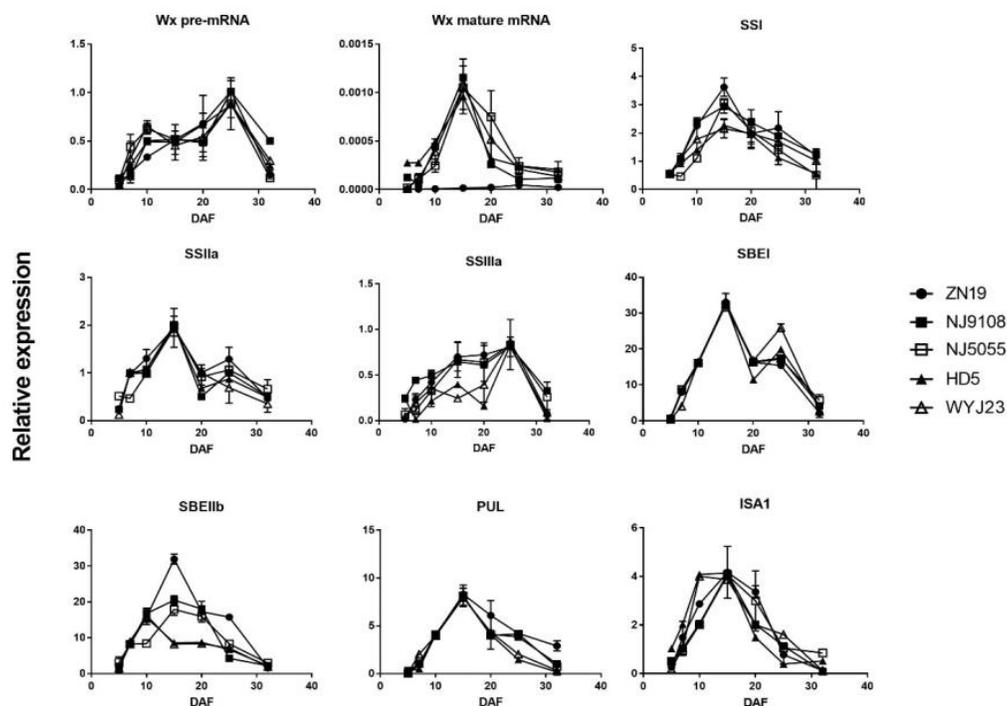


Fig. 4: Relative transcript levels of genes involved in starch synthesis in grains of *japonica* normal, soft and waxy rice varieties during grain filling stage. Error bars indicate the SD for three independent measurements. DAF, days after flowering

developing rice grains were presented in Fig. 3. Three types of rice showed different enzyme activities with diverse peak times during grain filling stage. There was an obviously opposite situation for GBSSI and SSS activities in all the investigated rice varieties. The peak values of GBSS activity were formed in 15 DAF in four rice varieties except for ZN19.

The GBSSI activity was significantly higher in HD5 and WYJ23 than in NJ9108 and NJ5055 during 15–25 DAF. ZN19 had almost no GBSSI activities during whole grain filling stage. The peak value of SSS activity was formed in 15 DAF, while the two normal rice varieties had the lowest

peak values. The peak values of SBE and DBE activities were formed in 15 DAF and 20 DAF, respectively. All the five rice varieties showed similar trends and reached the close summit values. The enzyme activities suggested that GBSSI and SSS but not SBE and DBE played critical roles in rice ECQ formation among these diverse rice varieties.

Gene Expressions

Expression pattern of key genes of starch biosynthesis played an important role during rice grain filling stage. The differences of mRNA transcript levels were summarized in Fig. 4. *Wx* pre-mRNA expressed similarly in three types of rice varieties with a consistent peak at 25 DAF. However, the *Wx* mature mRNA expressed barely in ZN19 in the whole grain development period, while it showed similar expression profiles in other four rice varieties. This indicated that the lack of AC and GBSS activity in waxy rice was attributed to the missing of *Wx* mature mRNA.

The *SSI* expression for three types of rice showed the same variation tendency and peak time that appeared at 15 DAF. As a whole, the *SSI* gene expression showed the highest value in ZN19, followed by two soft rice varieties and the lowest values in two normal rice varieties. The expression profiles of *SSIIa* were similar in three types of rice with the consistent peak values at 15 DAF. For *SSIIIa* expression, three types of rice showed different expression profiles but closer peak values at 25 DAF. Their gene expression had the same tendency with the *SSI* gene, exhibiting the highest level in ZN19, intermediate levels in two soft rice varieties and the lowest levels in two normal rice varieties.

The expression profiles of *SBEI*, *PUL* and *ISA1* showed a similar variation tendency during rice grain filling stage in three types of rice. The only obvious differences were that ZN19 presented lower expression of *SBEI* gene and higher expression of *PUL* gene than other four rice varieties at 25 DAF. However, the expression profiles of *SBEIIb* were different in three types of rice. Totally, two soft rice varieties and ZN19 had significantly higher expression levels than two normal rice varieties during 5–25 DAF. The peak value of *SBEIIb* expression in two soft rice varieties and ZN19 presented at 15 DAF, but the expression values were relatively low in two normal rice varieties.

Discussion

ECQ is an important indicator of palatability evaluation and now becoming the core value of rice grain quality. AC, GC, thermal properties and pasting properties are generally considered to be main parameters for evaluating ECQ (Bao, 2012). For example, rice varieties with good ECQ usually had low or medium AC and GT, high GC and BDV. In this study, starch physicochemical parameters were measured and compared detailed in *japonica* normal, soft and waxy rice varieties. Normal rice had high AC, TV, FV, SBV, PT

and %R and low GC and BDV. Waxy rice had high GC and BDV and low AC, PV, TV, FV, SBV, PT, P_{temp} and %R. Soft rice had high PV, BDV and P_{temp} , medium AC, TV, FV and PT and low GC and %R. In soft rice, the parameters such as GC, PV, TV, FV, PT and P_{temp} were much closer to normal rice, but the parameters such as BDV, SBV and %R were similar with waxy rice. The intermediate state of ECQ parameters in *japonica* soft rice may indicate that this rice type is the medium type between *japonica* normal and waxy rice, combined with high pasting properties and BDV and low SBV and %R which performed in rice varieties with good ECQ (Shu *et al.*, 1998; Wu *et al.*, 2001). This may give an explanation “why soft rice has good palatability and soft texture”.

Amylopectin chain length distribution that differed among rice varieties was recognized to influence rice ECQ largely. Researches have characterized the relationship between rice amylopectin chain length distribution and starch physicochemical properties (Han and Hamaker, 2001; Nakamura *et al.*, 2006, 2010; Patindol *et al.*, 2009). The chain length distributions of the debranched amylopectin were compared among three types of rice varieties in this study. Normal rice had lower DP 6–12 (A chains), higher DP 13–24 (B1 chains) and DP 25–36 (B2 chains) than other investigated rice varieties, while ZN19 and soft rice showed a similar curve with high amounts of A chains (DP 6–12) and low amounts of B1 and B2 chains (DP 13–36). GBSSI was reported for being responsible for the synthesis of B1 and B2 chains (DP 15–35) *via* elongation of A chains (DP 6–14) (Zhang *et al.*, 2017). Thus, the higher A chains remaining in ZN19 and soft rice might be due to the decreased GBSSI activity in part. On the other hand, Fujita (2014) revealed *SSI* is primarily responsible for the synthesis of the shortest glucan chains (DP 8–12). The higher gene expression of *SSI* and SSS activity in waxy rice and soft rice might cause higher proportion of amylopectin short chains with DP 6–12 than in normal rice. In addition, the values DP 6–12 significantly positively correlated with paste breakdown of RVA profile in this study and according to the description by Han and Hamaker (2001). Therefore, soft rice harboring waxy-like amylopectin chain length might partly be attributed to the similar and high BDV values with waxy rice.

It is well documented that the enzymes GBSSI, SSS, SBE and DBE are involved in starch synthesis in rice grain, and usually they interacted with each other (Zeeman *et al.*, 2010; Fujita, 2014). In this study, GBSSI showed the most variation in all the investigated enzymes among the three types of rice. Waxy rice ZN19 had a near zero AC value showed almost no GBSSI activity and normal rice had a relative high AC value showed the highest activity. The GBSSI activity in soft rice that had low AC value was intermediate. These indicated that GBSSI activity directly influenced AC synthesis. However, the gene expression of *Wx* pre-mRNA and mature mRNA presented no differences between normal and soft rice, suggesting that the G/A

mutation in the 4th exon of *Wx* in soft rice reduced GBSSI activity in comparison with normal rice (Sato *et al.*, 2002). ZN19 exhibited the normal expression of *Wx* pre-mRNA, but the lack expression of *Wx* mature mRNA, indicating that its missing of AC and GBSSI activity was due to the absence of *Wx* mature mRNA. This result was consistent with the previous report that *Wx* mature mRNA could not be detected in waxy rice (Ge *et al.*, 2000). The *Wx* gene is the sole major gene controlling both AC and GC. Furthermore, based on our work and previous studies, RVA parameters are highly associated with AC (Zhou *et al.*, 2015; Zhang *et al.*, 2017). Thus, it is the normally expressed *Wx* gene and remained GBSSI activity that kept AC at a relative low level in soft rice brought about the more similar values of GC, TV, FV, PT and P_{temp} with normal rice.

The activities of SSS involved in amylopectin synthesis showed an opposite situation to GBSSI among three types of rice: the highest in ZN19, the lowest in normal rice and intermediate in soft rice. However, among the three main SSS genes, the expression level of *SSI* was the highest and its expression tendency was consistent with the SSS activities among the three types of rice investigated, suggesting that *SSI* played a critical role in SSS formation. Cao *et al.* (1999) considered *SSI* was the major SSS component in cereal endosperm. The *SSIIIa* gene may play minor roles in regulating SSS activity, since its expression pattern varied similarly with *SSI* among the three types of rice. Besides SSS, SBE was another important part involved in amylopectin synthesis. Ohdan *et al.* (2005) approved that *SBEI* and *SBEIIb* were the most vigorously expressed amylopectin synthesis related genes, and expressed in great amounts during grain development. In this study, SBE activity showed almost no differences among the investigated rice types. However, *SBEIIb* expressed obvious and similar variation pattern with *SSI*: the highest in ZN19, intermediate in soft rice and the lowest in normal rice. Previous studies proved that *SSI* and *SBEIIb* played an important role in the formation of amylopectin A chains (DP 6–12) (Jeon *et al.*, 2010) and *SBEIIb* expression had consistency with *SSI* since the amounts of *SSI* active protein was significantly lower in the *SBEIIb* single mutant than in wild type (Nishi and Satoh, 2001). Therefore, besides *SSI*, the higher expression of *SBEIIb* might result in the higher proportion of amylopectin short chains with DP 6–12 in waxy rice and soft rice than in normal rice. The expression of *SBEI* almost unchanged among different rice types. This was the most possible that the consistent *SBEI* activity among three types of rice covered the variation of *SBEIIb* activity during the formation of SBE enzyme complex.

The key DBE genes *PUL* and *ISA1* were also vigorously expressed in grain development (Ohdan *et al.*, 2005), but there was also no significant difference in DBE activities and differences of the expression levels of *PUL* and *ISA1* were hardly identified among three types of rice in this study. This might be due to the slight variation of DBE genomics among different rice varieties.

Conclusion

Altogether, *Wx* mature mRNA expression level and GBSSI activity might be the main reason influencing the significant variation of ECQ (*e.g.*, AC, GC, RVA and %R) among the investigated three types of rice. The variation in gene expression of *SSI* and *SBEIIb* might cause the alternation in SSS activity and then amylopectin fine structure. The good ECQ of *japonica* soft rice was mainly due to the polymerization of low AC, appropriate GC and pasting properties and low %R and gelatinization temperature.

Acknowledgements

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References

- Bao, J.S., 2012. Toward understanding the genetic and molecular bases of the eating and cooking qualities of rice. *Cereal Foods World*, 57: 148–156
- Cagampang, G.B., C.M. Perez and B.O. Juliano, 1973. A gel consistency test for eating quality of rice. *J. Sci. Food Agric.*, 24: 1589–1594
- Cao, H., J. Imparlradosevich, H. Guan, P.L. Keeling, M.G. James and A.M. Myers, 1999. Identification of the soluble starch synthase activities of maize endosperm. *Plant Physiol.*, 120: 205–215
- Fujita, N., 2014. Starch biosynthesis in rice endosperm. *Agric-Biosci. Monogr.*, 4: 1–18
- Ge, H., Z. Wang and M. Hong, 2000. Studies on the splicing ability of *Wx* gene intron 1 in glutinous rice. *Acta Phytophysiol. Sin.*, 26: 237–240
- Han, X., Y. Wang, X. Liu, L. Jiang, Y. Ren, F. Liu, C. Peng, J. Li, X. Jin, F. Wu, J. Wang, X. Guo, X. Zhang, Z. Cheng and J. Wan, 2012. The failure to express a protein disulphide isomerase-like protein results in a floury endosperm and an endoplasmic reticulum stress response in rice. *J. Exp. Bot.*, 63: 121–130
- Han, X.Z. and B.R. Hamaker, 2001. Amylopectin fine structure and rice starch paste breakdown. *J. Cereal Sci.*, 34: 279–284
- Jeon, J.S., N. Ryoo, T.R. Hahn, H. Walia and Y. Nakamura, 2010. Starch biosynthesis in cereal endosperm. *Plant Physiol. Biochem.*, 48: 383–392
- Li, H., S. Prakash, T.M. Nicholson, M.A. Fitzgerald and R.G. Gilbert, 2016. The importance of amylose and amylopectin fine structure for textural properties of cooked rice grains. *Food Chem.*, 196: 702–711
- Liu, X., S.B. Tan, J.C. Tian and X.Z. Wang, 2010. Activity and types of starch debranching enzyme in wheat cultivars containing different starch contents during the grain filling period. *Sci. Agric. Sin.*, 43: 850–854
- 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: 718–725
- Nakamura, Y., A. Sakurai, Y. Inaba, K. Kimura, N. Iwasawa and T. Nagamine, 2010. The fine structure of amylopectin in endosperm from asian cultivated rice can be largely classified into two classes. *Starch/Stärke*, 54: 117–131
- Nakamura, Y., A. Sato and B.O. Juliano, 2006. Short chain length distribution in debranched rice starches differing in gelatinization temperature or cooked rice hardness. *Starch/Stärke*, 58: 155–160
- Nakamura, Y., K. Yuki, S.Y. Park and T. Ohya, 1989. Carbohydrate metabolism in the developing endosperm of rice grains. *Plant Cell Physiol.*, 30: 833–839
- Nishi, A. and H. Satoh, 2001. Biochemical and genetic analysis of the effects of amylose-extender mutation in rice endosperm. *Plant Physiol.*, 127: 459–472

- Ohdan, T., P. Francisco, T. Sawada, T. Hirose, T. Terao, H. Satoh and Y. Nakamura, 2005. Expression profiling of genes involved in starch synthesis in sink and source organs of rice. *J. Exp. Bot.*, 56: 3229–3244
- Patindol, J., X.F. Gu and Y.J. Wang, 2009. Chemometric analysis of the gelatinization and pasting properties of long-grain rice starches in relation to fine structure. *Starch/Stärke*, 61: 3–11
- Ramesh, M., S.Z. Ali and K.R. Bhattacharya, 1999. Structure of rice starch and its relation to cooked-rice texture. *Carbohydr. Polym.*, 38: 337–347
- Sato, H., Y. Suzuki, M. Sakai and T. Imbe, 2002. Molecular characterization of *Wx-mq*, a novel mutant gene for low-amylose content in endosperm of rice (*Oryza Sativa L.*). *Breed. Sci.*, 52: 131–135
- Shu, Q., D. Wu, Y. Xia and M. Gao, 1998. Relationship between RVA profile character and eating quality in *Oryza sativa L.* *Sci. Agric. Sin.*, 31: 25–29
- Tan, Y.F., J.X. Li, S.B. Yu, Y.Z. Xing, C.G. Xu and Q. Zhang, 1999. The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid, Shanyou 63. *Theor. Appl. Genet.*, 99: 642–648
- Teng, B., C. Zhang, Y. Zhang, J. Wu, Z. Li, Z. Luo and J. Yang, 2015. Comparison of amylopectin structure and activities of key starch synthesis enzymes in the grains of rice single-segment substitution lines with different *Wx* alleles. *Plant Growth Regul.*, 77: 117–124
- Tian, Z., Q. Qian, Q. Liu, M. Yan, X. Liu, C. Yan, G. Liu, Z. Gao, S. Tang, D. Zeng, Y. Wang, J. Yu, M. Gu and J. Li, 2009. Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. *Proc. Natl. Acad. Sci. USA*, 106: 21760–21765
- Wu, D.X., Q.Y. Shu and Y.W. Xia, 2001. Assisted-selection for early indica rice with good eating quality by RVA profile. *Acta Agron. Sin.*, 27: 165–172
- Yang, J., J. Wang, F.J. Fan, J.Y. Zhu, T. Chen, C.L. Wang, T.Q. Zheng, J. Zhang, W.G. Zhong and J.L. Xu, 2013. Development of AS-PCR marker based on a key mutation confirmed by resequencing of *Wx-mp*, in milky princess and its application in Japonica, soft rice (*Oryza Sativa L.*) breeding. *Plant Breed.*, 132: 595–603
- Zeeman, S.C., J. Kossmann and A.M. Smith, 2010. Starch: its metabolism, evolution, and biotechnological modification in plants. *Annu. Rev. Plant Biol.*, 61: 209–234
- Zhang, C., S. Chen, X. Ren, L. Yan, D. Liu, X. Cai, Q. Li, J. Gao and Q. Liu, 2017. Molecular structure and physicochemical properties of starches from rice with different amylose contents resulting from modification of *OsGBSSI* activity. *J. Agric. Food Chem.*, 65: 2222–2232
- Zhao, F.M., X. Qi, J. Xiao and X.Z. Wang, 2007. Improved method for determining starch branching enzyme activity. *Plant Physiol. Commun.*, 43: 1167–1169
- Zhou, L.J., W.T. Sheng, J. Wu, C.Q. Zhang, Q.Q. Liu and Q.Y. Deng, 2015. Differential expressions among five waxy alleles and their effects on the eating and cooking qualities in specialty rice cultivars. *J. Integr. Agric.*, 14: 1153–1162

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