Analysis Strategy for Assisted Selection of Rice Eating Quality by Using Starch Properties

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Abstract

Background

Eating quality is the main factor affecting the commodity value of commercial rice. However, its determination relies on sensory evaluations, which require large samples, are easily influenced by subjective factors and are time consuming. In order to make the determination of eating quality more efficient, a set of recombinant inbred lines (RILs) obtained from the hybridization of *indica* and *japonica* rice was used as the test material to analyze the relationship between starch characteristics and the eating quality (EQ) score, from sensory evaluation. An implementation protocol was proposed for starch assisted-selection of eating quality.

Results

Among the RILs, the values of the measured parameters for amylose content (AC), trough viscosity (TV), final viscosity (FV), breakdown (BD) and setback (SB) were significantly correlated with the EQ values obtained by sensory evaluation. The RILs with higher EQ scores, generally had low AC, TV, FV and SB, but high BD. By normalizing and summing the AC, TV, FV, SB and BD indicators, a comprehensive indicator—viz., the starch properties quality (SPQ)—was established, to assess starch quality. The SPQ value was better correlated with EQ than any of the individually measured parameters, suggesting that the model is valid. This method requires much smaller rice samples than sensory evaluations, i.e., three grams of milled rice flour.

Conclusions

Therefore, the SPQ indicator is a rapid, objective and convenient technique for the rapid assessment of rice eating quality in a large number of crossbreeding progeny, especially early-generation progeny, from which only small samples of rice grains are available.

Background

Rice is the staple food of more than two-thirds of the global population [1]. As countries industrialize and living standards increase, there is more demand for rice with high eating quality [2]. Traditional rice eating-quality measurements mainly rely on sensory evaluations by trained human panels. This is time consuming, labor intensive and prone to subjective errors, because of differences in panelists’ geographic origin, age and gender. Sensory evaluations also require large rice samples, and they are difficult to carry out during plant crossbreeding, when only very small samples can be harvested [3]. Given these limitations, extensive studies have been conducted to find simple physicochemical indicators to replace human sensory evaluations [4, 5].
The amylose content of rice has the greatest influence on its eating quality [6, 7]. Rice with high amylose content generally has low viscosity, high hardness, fluffy dryness and poor palatability. Reducing the amylose content is a major goal of rice-breeding aiming to improve the palatability of rice [8, 9]. However, for those varieties that have similar amylose contents, the eating quality can still vary widely, indicating that other factors also contribute [3]. Rice analysis using Rapid Visco Analyzer (RVA) profiles can characterize physicochemical property changes in rice during starch pasting and shows a significant correlation with eating quality [10, 11]. The RVA profile includes determinations of peak viscosity (PV), TV, FV, BD, consistency (CO) and SB. The RVA profile is closely related to the amylose content and eating quality of rice [12, 13], but previous research mostly focused on the comparison of indica or japonica subspecies varieties, and feasible protocols for using the RVA profile to assist selection for eating quality have not been established [14, 15]. Based on the inbred lines of indica–japonica crosses with complex genetic backgrounds, this study aimed to derive a quantitative relationship between amylose content, RVA profile characteristics and eating quality determined by sensory evaluation, to establish an effective method to assess eating quality by assigning rice starch objective characteristics with numerical values. This would provide technical support for the rapid identification of the eating quality of a large number of crossbreeding progeny, especially at the early generation stages.

**Results**

**Performance of rice starch properties and eating quality value in recombinant inbred lines**

The rice starch properties and EQ distributions of recombinant inbred lines (RILs) and their parents are shown in Fig. 1. The AC distribution range of RILs was 12.90–23.30%, with a mean of 17.60%; and the EQ distributed from 37.9 to 87.4, with a mean of 66.95. The corresponding distribution range and the mean for RVA profile characteristics was shown as below: PV, 1712–3727 RVU, TV, 2745 RVU, FV, 1112–3235 RVU, 1723 RVU, BD, 191–2036, 952, CO, 580–2073, 1291, and SB 1242–1624, 292.5. Generally, the rice starch properties and EQ of RILs covered a wide range, and there was a super-parent distribution compared with the parent distribution.

**Relationship between rice amylose content and RVA profile characteristics**

The correlation between AC and TV, BD and SB, reached a very significant level of p < 0.01 (Fig. 2) and the correlation with FV reached a significant level of p < 0.05. The TV, FV, CO and SB of the RVA profile characteristics were positively correlated with AC content, whereas PV and BD were negatively correlated with AC content.

Based on the mean AC content of 17.6%, the RILs were classified into two groups with high (n = 70) and low (n = 74) AC content. Differences in TV, FV, BD and SB performance were compared in different AC content groups (Fig. 3). The performance of TV, FV and SB in the high AC content group was significantly
higher than that of the low AC content group, which was opposite to the BD performance; the low AC content group had a higher BD value.

**Relationship between starch properties and eating quality in RILs**

Figure 4 shows the correlation between starch properties and EQ values in the RILs. The AC content was negatively correlated with EQ value. Of the RVA profile characteristics, PV and BD were positively correlated with the EQ value, and the correlation between BD and EQ value reached a very significant level of \( p < 0.05 \). TV, FV, CO and SB were negatively correlated with EQ value, with a significance level of \( p < 0.01 \) for TV, FV and SB.

With EQ values of 60 and 70 as the limiting criteria, RILs were classified into high (EQ > 70, \( n = 48 \)), medium (EQ: 60–70, \( n = 53 \)) and low (EQ < 60, \( n = 43 \)) EQ groups. The AC, TV, FV, BD and SB values that were significantly related to the EQ value were compared between the three EQ groups (Fig. 5). The high EQ group generally had a low AC, TV, FV and SB, but a high BD. The TV value was the best of the starch characteristics for distinguishing between the medium and low EQ groups.

**Analysis of the assisted selection of eating quality of RILs by using starch properties**

It appeared that the indicators AC, TV, FV, BD and SB were closely related to the EQ value obtained by sensory evaluation. Based on the assessment criteria of EQ, the 25th, 50th and 75th of the distribution of AC, TV, FV, BD and SB values were used as classification standards, and the five indicators were assigned with numeric values i.e., 20, 15, 10 and 5, respectively. The five indicator scores were added to derive a new indicator—viz., the starch properties quality (SPQ) (Fig. 6A)—which represented the comprehensive characteristics of rice starch that contributed to eating quality. The SPQ and EQ values were positively correlated at the significance level of \( p < 0.001 \)(Fig. 6B).

**Discussion**

The TV, FV, BD and SB values obtained from the RVA profiles were significantly correlated with AC content. This is consistent with previous research findings [16, 17], but the correlation coefficient in this study was slightly lower than that of *indica* or *japonica* subspecies [18]. This is probably attributable to the complex genetic background of *indica–japonica* hybrid offspring, with more uncertainty regarding influencing factors [19].

Of the RVA profile characteristics, the TV, FV, BD and SB values were significantly correlated with the EQ value. Using EQ values of 70 and 60 as the limiting criteria to classify RILs into high, medium and low EQ groups revealed that the high EQ group had a profile of low TV, low FV and low SB, but high BD. Compared with different EQ groups separated by AC content, TV and FV were more significantly different
between the high and low EQ groups. The TV value can also be used to distinguish between the medium and low EQ groups.

The indicators that were closely related to the EQ value, i.e., AC, TV, FV, BD and SB, were assigned numeric values to derive the SPQ, an integrated indicator that can comprehensively describe the characteristics of starch in terms of EQ. The SPQ was superior to any individual indicator at correlating with the EQ value, at the significance level of \( p < 0.001 \). Therefore, we conclude that the SPQ value of a starch sample can be used as an accurate assisted-selection indicator of EQ. It requires much smaller rice samples and assessment is much faster. However, before wider application of the SPQ assay in assisted selection, it needs to be verified by testing a much wider range of crossbreeding progeny, with diverse genetic backgrounds, to ensure that the correlation between SPQ and EQ can be generalized. Our future work will aim to achieve this verification.

**Conclusions**

In this study, the SPQ indicator was proposed for starch assisted-selection of eating quality holding the advantage of rapid, simple and smaller rice samples. Therefore, this method should be a rapid, objective and convenient technique for the rapid assessment of rice eating quality in a large number of crossbreeding progeny, especially early-generation progeny, from which only small samples of rice grains are available.

**Methods**

**Experimental materials and field trial**

The test material was a recombinant inbred line (\( n = 144 \)) constructed by crossing *japonica* rice, i.e., Akihikari (AKI) with *indica* rice, i.e., Qishanzan (QSZ). The trial was carried out in 2019 in a research rice field of the Research Institute of Saline-Alkali Land Utilization in Liaoning Province (41.07° N; 122.03° E). A random block design with triplicates was performed, with each sub plot area measuring 12 m\(^2\), planted on 04/21/2019 and transplanted on 05/26/2019. Fertilizer was applied during the whole growth period over the 667 m\(^2\) main plot (Nitrogen, 15 kg; Phosphorus, 7 kg, Potassium 3 kg). Other crop management practices followed local production techniques.

**Determination of rice amylose content and RVA profile characteristics**

Rice harvested from the field trial was stored at room temperature for 3 months, after which it was polished and milled into rice flour. AC was then determined by using size-exclusion chromatography (SEC) on Ultrahydrogel (Waters, Milford, MA) as described previously [20]. RVA profile characteristics were determined by 3-D viscosity rapid measurement on a Rapid Visco Analyzer (Newport Scientific Instruments, Warriewood, NSW, Australia), equipped with TCW (Thermal Cycle for Windows) software for
For sample preparation, polished rice flour (3.00 g, water content 14%), was mixed with distilled water (25.00 ml). During the measurement, the temperature inside the measurement jar was maintained at 50°C for 1 min, then increased to 95°C at 12°C min\(^{-1}\), maintained for 2.5 min, then decreased to 50°C at 12°C min\(^{-1}\) and maintained for 1.4 min. The rotation speed of the agitator was 960 r min\(^{-1}\) during the initial 10 s and then maintained at 160 r min\(^{-1}\). Viscosity values are expressed as rapid visco units (RVUs). The RVA profile characteristic values mainly include several initial values of PV, TV, FV and their derivatives, including BD (PV-TV), CO (FV-TV) and SB (FV-PV). The meaning of the RVA profile characteristics was defined (Fig. 1) based on a previous report [22].

**Determination of eating quality**

The EQ value was determined by sensory evaluation, as described previously [23], but with minor modifications. The sensory panel consisted of 12 individuals of different ages, occupations and genders, who had been trained to evaluate EQ. The scoring standard was based on five indicators, viz., odor, appearance, palatability, taste and texture of cold rice. Each indicator had a maximum score of 20 points and was classified into 4 levels: excellent, good, poor and bad, with a corresponding score 20, 15, 10 and 5 points, respectively. The scores obtained from each indicator, were added together to obtain the overall EQ score.

**Abbreviations**

- **RILs** recombinant inbred lines
- **EQ** eating quality
- **AC** amylose content
- **TV** trough viscosity
- **FV** final viscosity
- **BD** breakdown
- **SB** setback
- **SPQ** starch properties quality
- **PV** peak viscosity
- **CO** consistency
AKI
Akihikari
QSZ
Qishanzan

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors have read and approved the final version of the manuscript. XL and TM performed most of the experiments, YW and XS performed the field planting, HR, XHL and CM performed part of the quality analysis, MD provided the rice materials used in this study, LDF designed this experiment.

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Figures
Figure 1

Starch properties and EQ distributions of RILs. A-H denotes the distribution range of RILs’ AC, PV, TV, FV, BD, CO, SB and EQ, respectively. Data is presented as the mean ± standard deviation (n = 144).
Figure 2

Correlation analysis of AC content and RVA profile characteristics in RILs. A-F denotes the correlation between AC content and PV, TV, FV, BD, CO, SB, respectively, in RILs. The signs * and ** indicate significance at levels of 0.05 and 0.01 respectively.

Figure 3

Comparison of TV, FV, BD and SB values of RILs with different AC content. A-D denotes the comparison of TV, FV, BD and SB values of high and low AC content groups in RILs, respectively. The signs * and ** indicate the significance at the 0.05 and 0.01 levels, respectively.
Figure 4

Correlation analysis of starch properties and EQ value in RILs. A-G denotes the correlation between AC, PV, TV, FV, BD, CO and SB content in RILs and EQ, respectively. The signs * and ** indicate significance at the 0.05 and 0.01 levels, respectively.

Figure 5

Comparison of AC, TV, FV, BD and SB values between different EQ groups in RILs A-E denotes the comparison of AC, TV, FV, BD and SB in different EQ groups in RILs. The signs * and ** indicate significance at the 0.05 and 0.01 levels, respectively.
Figure 6

Correlations between individual indicators of SPQ and EQ. A denotes the standard of assigning numeric values for AC, TV, FV, BD and SB in SPQ. B presents the correlation between SPQ and EQ. *** denotes the significance level at p < 0.001.

Figure 7

Determination of PV, TV, FV, BD, CO and SB of the RVA profile characteristics.