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Genetic diversity of amylose content and RVA pasting parameters in 20 rice accessions grown in Hainan, China



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ABSTRACT

Starch physicochemical properties determine the eating and cooking quality of rice. The genetic diversity in the apparent amylose content (AAC) and pasting viscosity parameters of 20 geographically diverse rice accessions were investigated. It was found that AAC and pasting viscosities differed widely among different accessions, but each accession performed relatively stably across two environments. Analysis of variance (ANOVA) indicated that all traits were predominantly controlled by genotypic variance, but the genotype \times environment interaction effects were also significant except for AAC and PT. Significant correlations were found for each parameter between 2 years (P < 0.001). Association mapping identified a total of 22 main-effect quantitative trait loci (QTLs) responsible for all traits except for CPV. This study showed that starch physicochemical properties of rice were highly stable and mainly controlled by genetic factors, and gave insight into the molecular improvement of eating quality using marker assisted breeding with the identified QTLs/genes.

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1. Introduction

Rice (*Oryza Sativa* L.) is one of the fundamental cereal crops, which is planted by farmers for over 7000 years in the developing world, predominantly in Asian monsoon regions and Southeast Asian rainforest areas (http://faostat3.fao.org). Worldwide, more than half of the population consumes rice as the staple food. Thus, accelerating the improvement of rice quantity and quality is not only closely bound up with the amelioration of people's living conditions, but also one of central response measures for ensuring the global food security under the situation of prodigious population growth and abominable climate change.

The rice quality is mainly evaluated from four aspects, the milling quality, appearance quality, nutrition quality, cooking and eating quality (Bao, 2012). Among them, the eating and cooking quality is regarded as the most important trait affecting consumer acceptability of rice. The physicochemical measurements of the eating and cooking quality have been well established, for

example, apparent amylose content (AAC), gelatinisation temperature, gel consistency, and rapid visco-analyser (RVA) pasting viscosity (Bao, 2012; Bao, Kong, Xie, & Xu, 2004). Different cooking and eating quality among different rice may be not only due to different cultivars, but also environmental effects. At the genotypic level, many studies have shown that the physicochemical, cooking and textural properties of rice cultivars originated in different countries vary dramatically (Champagne, Bett-Garber, McClung, & Bergman, 2004; Singh, Kaur, Sodhi, & Sekhon, 2005), which may be owing to the difference in amylose content, amylopectin structure and protein content among different rice genotypes (Suwansri & Meullenet, 2004). At the environmental level, variations in soil moisture and nutrient availability, ambient temperature, and atmospheric composition affect starch functionality (Beckles & Thitisaksakul, 2014). Elevated temperature leads to the most significant changes in cereals, and amylose content is the most sensitive parameter under various environmental conditions (Beckles & Thitisaksakul, 2014). These studies greatly improve our understanding the wide diversity in eating and cooking qualities among different rice cultivars and the possible reasons why they are different.

However, how the genotype \times environment interaction affects the formation of the diverse starch physicochemical properties are less understood. Our previous study showed that amylose content, cool paste viscosity (CPV), breakdown viscosity (BD),



Abbreviations: AAC, apparent amylose content; ANOVA, analysis of variance; BD, breakdown; CPV, cool paste viscosity; CS, consistency; HPV, hot paste viscosity; PT, pasting temperature; PV, peak viscosity; QTL, quantitative trait locus; RVA, rapid visco-analyser; SB, setback.

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and setback viscosity (SB) of pasting viscosity parameters were mainly affected by genotypic variance, while peak viscosity (PV) and hot paste viscosity (HPV) were mainly affected by environmental variance. The year \times season, year \times variety, season \times variety and year \times season \times variety effects were significant for most traits, indicating significant genotype \times environment interactions (Bao et al., 2004). Genetic studies such as linkage mapping showed that AAC and all the pasting viscosity parameters are mapped at the Wx locus except for PV (Bao et al., 2000; Fan et al., 2005; He et al., 1999; Teng et al., 2012; Traore, McClung, Chen, & Fjellstrom, 2011). PV was mapped at different loci in two environments suggesting that it is under other genetic control and is mainly affected by environment (Bao et al., 2000). Recently, association mapping approach has been extensively used to research the genetic basis of complex traits of cereal crops, such as rice (Famoso et al., 2011; Huang et al., 2012; Zhao et al., 2011). Allelic diversity in starch synthesis related genes may explain genetic basis of the diversity in starch physicochemical properties among germplasm (Tian et al., 2009). Reports on use of association mapping to dissect the genotype by environment effects on amylose content and pasting viscosity in rice are rare.

The objectives of this study are (1) to investigate the genetic diversity in amylose content and pasting viscosity parameters among 20 diverse rice accessions used in OryzaSNP project in two environments (years); and (2) to identify the genetic loci or candidate genes for rice starch physicochemical properties across different environments. The results of study not only interpret how genotype, environment, and their interaction effects influence starch properties, but also provide some insights into genetic basis for rice eating quality which may be useful for molecular breeding for improvement of rice eating and cooking quality.

2. Materials and methods

2.1. Materials

Twenty rice accessions (*Oryza sativa* L.) including cultivars, germplasm lines, and landraces from 10 geographical areas was introduced from the OryzaSNP project (http://www.oryzasnp.org/) (McNally et al., 2009). All the accessions were planted in a randomised block design with two replications in two years at the same site in Lingshui, Hainan province, China. The seeds were sowed in early December 2010 and 2011, and harvested in April 2011 and 2012, respectively. Hereafter two years (environments) are denoted 2011 and 2012 for when the grain was harvested. Supplementary Table 1 summarises the major environmental conditions, i.e. the mean temperature, total radiation hours and total rainfall for each month during rice growth.

The grains were air-dried to a moisture content of about 12%, stored at room temperature for two months and later at 4 °C in the dark until all the grain materials were obtained. The samples were dehulled on a Satake Rice Machine (Satake Co., Tokyo, Japan) and milled to pass through a 100 mesh sieve on a Cyclone sample mill (UDY Corporation, Fort Collins, CO., USA).

2.2. Apparent amylose content (AAC)

Determination of AAC was carried out using the iodine staining method (Bao, Shen, Sun, & Corke, 2006). The absorbance of the solution was measured at 620 nm against the blank solution using a spectrophotometer (UNICAM UV300, Thermo scientific, UK). AAC was calculated using a standard curve made from four rice samples with known apparent amylose content.

2.3. Pasting viscosity

Rice pasting properties were determined using a Rapid Visco Analyser (RVA, Model 3-D, Newport Scientific, Warriewood, Australia). Flour (3 g, 12% m.b.) was mixed with 25 g of double-deionised water in the RVA sample can. The RVA was run using Thermocline for Windows software (Version 1.2). The Rice Method 1 program was used with heating and cooling cycle set as (1) holding at 50 °C for 1 min, (2) heated to 95 °C in 3.8 min, (3) holding at 95 °C for 2.5 min, (4) cooling to 50 °C in 3.8 min, (5) holding at 50 °C in 1.4 min. The RVA paddle speed was at 960 rpm for the first 10 s of the test, after which the speed was at 160 rpm. The peak (PV), hot paste (HPV), cool paste (CPV) viscosities and their derivative parameters breakdown (BD, =PV-HPV), setback (SB, =CPV-PV), and consistency (CS, =CPV-HPV) were recorded form the Thermocline for Windows software (Version 1.2). The pasting temperature (PT) was calculated according to the method of Bao (2008). The viscosity was measured in Rapid Visco Units (RVU).

2.4. Statistical analysis

All the physicochemical parameters were measured at least in duplicate. Data analyses were performed with the SAS program version 8 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was carried out to determine genotypic and environmental variation among the parameters using general linear model procedure (Procglm). Means of different genotypes and environments were determined using Proc means, followed by Tukey multiple comparison tests at P < 0.05. Correlation analysis among the parameters in (or between) the two environments were conducted by Proc correlations.

2.5. Genotype data and association mapping

The publically available genotype data were downloaded from the Gramene website (http://www.gramene.org/) on March 10, 2013. During downloading, the reference genome data were first removed, and other sites were filtered with following parameters: minimum count was 16 out of 20 sequences, and minimum frequency was set at 0.25. Finally, a total of 32,655 SNPs distributed in 12 chromosomes were downloaded (Xu et al., 2014).

The population structure (Q) among rice accessions was performed using the software package STRUCTURE version 2.2 and revealed that the optimum number of populations (K) was 3 (Xu et al., 2014). Principle component analysis (PCA) was calculated and input into association mapping which was performed with GAPIT (Lipka et al., 2012). To avoid the probability of spurious association between markers and traits, six models i.e. ANOVA, K (kinship), P (PCA), Q, P+K, Q+K were compared with GAPIT (Lipka et al., 2012), and the best model for each trait was chosen according to the Bayesian information criterion (BIC) value. The results with the best model (Supplementary Table 2) were presented at the significance level of P < 0.005.

3. Results

3.1. Genetic diversity in starch physicochemical properties of rice in 2011

The genetic structure analysis of 20 rice accessions indicated that eight belong to the japonica group and eight to the indica group; the other four accessions belong to the *aus* group (Table 1). Broad diversity of starch amylose and pasting viscosity properties were observed in 20 rice accessions, with an average of 22.2% of AAC, 237.9 RVU of PV, 156.3 RVU of HPV, 295.5 RVU of CPV, 81.6

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