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Rice grain protein composition influences instrumental measures of rice cooking and eating quality

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ABSTRACT

Rice cultivar starch composition differences do not completely explain variation in rice cooking and eating quality. Rice grain storage proteins possess divergent solubility properties suggesting they may contribute to cultivar differences in rice grain quality. Application of high-performance liquid chromatography (HPLC) analysis to protein extracts derived from medium and long grain advanced rice breeding lines revealed rice grain protein composition differences which were associated with instrumental measures of grain quality. Globulin content displayed little variation in both grain types. The mean glutelin content was higher in long grain rice lines than medium grains. Although the mean content of prolamins in medium and long grain rice were similar, the prolamin content of medium grains was more variable. Individual medium grain prolamin HPLC peaks, total prolamin content and the prolamin:glutelin + prolamin ratio were positively correlated with several rapid visco analyser (RVA) parameters. The long grain HPLC data displayed a similar pattern of correlations with RVA parameters but of reduced magnitude. Protein composition was associated with instrumental measures of grain quality in this set of germplasm and although the textural properties of rice are complex, these data suggest consideration of rice grain protein composition could contribute to breeding high quality rice.

1. Introduction

Rice (*Oryza sativa* L.) is the staple food of many countries, particularly in Asia, and is increasing in popularity in many other parts of the world. Consumers pay attention to rice grain quality, displaying a preference for rice with a particular visual appearance, texture, flavour and aroma (Fitzgerald et al., 2009). There is a strong cultural dimension to these preferences resulting in a wide array of different types of cultivated rice originating from around the globe.

The texture of cooked rice is considered by many consumers to be a quality trait of primary importance. Texture is a complex trait perceived by humans and several instrumental tests are used to predict these sensory attributes or textural properties of rice. These tests involve assays that measure the physico-chemical properties of rice such as alkali spreading value, gel consistency, rapid visco analyser (RVA) and texture analyser (TA).

Rice grains are composed of approximately 80–85% starch, 4–10% protein, 1% lipid and 10% moisture. Amylose content, amylopectin fine structure, and intra and/or inter-molecular interactions of starch with other components such as proteins, lipids and non-starch polysaccharides, are some of the factors involved in determining rice grain quality. Among these, amylose content is perhaps the most important factor that determines cooking behaviour. Low amylose cooked rice is generally soft with individual grains adhering closely while high amylose rice is harder and less sticky (Rani and Bhattacharya, 1989). Amylopectin chain length

Abbreviations: AAC, apparent amylose content; AACC, American Association of Cereal Chemists; ACN, acetonitrile; CV, coefficient of variation; GI, glycaemic index; GT, gelatinisation temperature; HPLC, high-performance liquid chromatography; LG, long grain; MG, medium grain; QTL, quantitative trait loci; RVA, rapid visco analyser; TA, texture analyser.

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distribution also has an impact on rice grain quality through its effect on starch gelatinisation temperature (GT) (Umemoto et al., 2004) which is associated with cooking time.

The primary genetic determinants of amylose content and amylopectin chain length distribution are known. The *Waxy/waxy* gene codes for granule-bound starch synthase I and is the most important controller of amylose content (Wang et al., 1995), while *Alk/alk* codes for starch synthase IIa and is responsible for the most important differences in amylopectin chain length distribution (Umemoto et al., 2004). Although several alleles of the *Waxy/waxy* and *Alk/alk* genes associated with different forms of starch have been identified (Waters et al., 2006), other starch biosynthesis genes in addition to *Waxy/waxy* and *Alk/alk* affect rice cooking and eating quality (Kharabian-Masouleh et al., 2013). However, starch structure does not explain all variation in rice grain quality parameters in all rice germplasm (Kharabian-Masouleh et al., 2013), and flour derived from milled grain of cultivars with similar amylose content may have different pasting and textural properties (Champagne et al., 1999). Furthermore, viscosity differences between rice flour and rice starch (Singh et al., 2000) suggest components other than starch affect these properties of rice.

Rice grain protein is the second most abundant component of milled rice grain and has been studied extensively in the context of its important role as a nutrient. The net protein utilisation of rice is the highest among the cereal grains, despite rice having the lowest protein content (Juliano, 1992). In the context of eating quality, several studies have reported total protein content is related to cooked rice texture (Xie et al., 2008) with high protein content associated with harder cooked rice (Lyon et al., 1999). Martin and Fitzgerald (2002) demonstrated total protein content had a significant influence on rice eating quality by digesting the rice flour proteins of six cultivars with protease and observing the RVA profiles differed between samples of similar amylose content. Experiments which have removed and replaced each of the protein fractions also suggest protein composition may influence rice eating quality (Baxter et al., 2004, 2010; 2014).

In common with all cereal grain, rice grain storage proteins are composed of globulins, glutelins and prolamins which are traditionally defined by their differences in solubility. Globulins are salt-soluble, the glutelins dissolve in dilute acid or alkali solutions while the prolamins, including those of rice which are poorly digested by monogastric animals including humans (Kubota et al., 2010), are solubilised by aqueous alcohol. Rice grain protein content differs between cultivars and within populations derived from two parental cultivars (Zhang et al., 2008), however, the relationship between rice grain protein composition and rice eating quality is not well understood. Here we report upon the relationship between rice grain protein composition and instrumental measures of rice eating quality in a range of advanced rice breeding lines.

2. Materials and methods

2.1. Rice samples

Flour from the milled grain of 80 medium grain and 80 long grain advanced rice breeding lines within a narrow range of 17%–20% apparent amylose content (AAC) from the 2014 rice breeding and quality trials at Yanco, New South Wales, Australia (34° 36′S; 146° 23′ E) was used in this study.

2.2. Accumulated thermal time

Temperature data (daily maxima and minima) were obtained at the Yanco Agricultural Institute (YAI) automated weather station (station number 074037; latitude 34.62°S, longitude 146.43°E) from the Australian Bureau of Meteorology website: (http://www.bom.gov.au/climate/data/). This weather station is located 7 km east of the field trial site at approximately the same elevation above sea level. The accumulated thermal time during the 30 days of grain filling after flowering for each season in years 2011–2014 appear in Supplementary Fig. 1. Accumulated thermal time during the grain filling period was estimated by calculating a 30 day forward rolling average of accumulated growing degree days. Growing degree days with a threshold of 10 °C were calculated from average daily temperatures. Accumulated growing degree days for 30 days after a given date were summed and used as an estimate of the accumulated thermal time for the given date.

2.3. Rice grain grinding, RVA and TA analysis

Milled rice grains (10 g) were ground to pass through a 0.5 mm screen (Cyclotec 1093 sample mill, Tecator, Hoganas, Sweden). Amylose content was measured using a modification of American Association of Cereal Chemists (AACC) Method 61-03 and UV5 spectrophotometer (Mettler-Toledo). Gelatinisation temperature was measured using differential scanning calorimetry (DSC) (Mettler-Toledo) (Waters et al., 2006) while the viscosity was measured by RVA (TecMaster and 4500 model) supported by Thermocline software following AACC Method 61-02. Peak viscosity, trough viscosity, final viscosity, breakdown viscosity, setback, peak time and pasting temperature were measured by a RVA to evaluate rheological properties of starch structure (Perten RVA 4500, Segeltorp, Sweden) according to the manufacturer's instructions. After RVA analysis, texture parameters were measured using a Perten TVT6700 texture analyser fitted with a 5 kg load cell and 20 mm probe, and supported by TexCalc software.

2.4. Rice grain protein extraction

Duplicate 250 mg sub-samples of rice flour were transferred to 2 mL microfuge tubes. Prolamins and albumins were extracted with 60% n-propanol while 5 M acetic acid was used to isolate glutelins and globulins. Each extraction was done independently following the modified protocol of Balindong et al. (2016).

2.5. Characterisation of protein composition

Two high-performance liquid chromatography (HPLC) methods utilising a C8-5 column were used to separate the proteins. The HPLC gradient for prolamins and albumins was as follows: 25% aqueous acetonitrile (ACN) at 0 min, 40% at 5 min, 45% at 15 min, 60% at 25 min, concluding at 95% at 26 and 27 min and returning to 25% at 28–33 min (Balindong et al., 2016). For glutelins and globulins, the HPLC gradient commenced at 25% ACN, increased to 35% at 5 min, 40% at 10 and 15 min, 50% at 25 min and reached a maximum of 95% at 26 and 27 min before returning to 25% between 28 and 33 min (Balindong et al., 2016).

The HPLC analysis was carried out using an Agilent 1260 HPLC System equipped with a vacuum degasser, quaternary pump, auto-injector, and diode array detector. Column temperature was set at 50 °C and absorbance was measured at 280 nm (Balindong et al., 2016).

2.6. Data analysis

Each protein peak was analysed using the ChemStation software B.04.03. Storage proteins (prolamins, glutelins and globulins) were selected for quantification and association with RVA and TA parameters. Data were compiled in Microsoft Excel 2013 and imported into GenStat 64-bit Release 17.1 for statistical analysis.