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The biosynthesis, structure and gelatinization properties of starches from wild and cultivated African rice species (*Oryza barthii* and *Oryza glaberrima*)

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ABSTRACT

The molecular structure and gelatinization properties of starches from domesticated African rice (*Oryza glaberrima*) and its wild progenitor (*Oryza barthii*) are determined and comparison made with Asian domesticated rice (*Oryza sativa*), the commonest commercial rice. This suggests possible enzymatic processes contributing to the unique traits of the African varieties. These have similar starch structures, including smaller amylose molecules, but larger amounts of amylose chains across the whole amylose chain-length distribution, and higher amylose contents, than *O. sativa*. They also show a higher proportion of two- and three-lamellae spanning amylopectin branch chains (degree of polymerization 34–100) than *O. sativa*, which contributes to their higher gelatinization temperatures. Fitting amylopectin chain-length distribution with a biosynthesis-based mathematical model suggests that the reason for this difference might be because *O. glaberrima* and *O. barthii* have more active SSIIIa and/or less active SBEIIb enzymes.

1. Introduction

Rice is one of the most important cereal crops in the world, especially in the developing world. It is a major staple food for nearly half of the world population. There are two cultivated species of rice: *Oryza sativa* and *Oryza glaberrima* (Wambugu, Furtado, Waters, Nyamongo, & Henry, 2013). *O. sativa* was domesticated from the wild progenitor *Oryza rufipogon* around 10,000 years ago, and is now widely distributed throughout the world (Huang et al., 2012). *O. glaberrima*, on the other hand, was independently domesticated from the wild species *Oryza barthii* approximately 3000 years ago, and is traditionally found only in West Africa (Sweeney & Mccouch, 2007). Currently *O. glaberrima* is increasingly being replaced by *O. sativa* for higher yield and productivity. However, *O. glaberrima* has many desired traits that *O. sativa* lacks, such as greater tolerance to diseases (for example, African rice gall midge and rice yellow mottle virus) and stresses (including drought, soil acidity, iron and aluminum toxicity, and weed competitiveness) (Sarla & Swamy, 2005). Previous studies on starch structure and properties have been mainly focused on *O. sativa*, with only limited information being available on *O. glaberrima* and *O. barthii* species.

As the major component of rice grains, starch dominates the textural and nutritional qualities of rice. Starch is normally almost entirely composed of two types of polymers: mostly linear amylose and highly branched amylopectin. Both are made up of glucose units linearly extended by α-(1→4) bonds and connected by α-(1→6) bonds at branching points. Amylose has a small number of long-chain branches and relatively low molecular weights, whereas amylopectin accounts for ~75% of regular starch dry mass, and has an enormous number of short-chain branches and much larger molecular weights. The amylose content and the structure of

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CLD, chain length distribution; DBE, starch debranching enzymes; DMSO, dimethyl sulfoxide; DP, degree of polymerization; DSC, differential scanning calorimeter; FACE, fluorophore-assisted carbohydrate electrophoresis; GBSS, granule bound starch synthases; RID, refractive index detector; SBE, starch branching enzymes; SEC, size-exclusion chromatography; SS, starch synthases.

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amylose and amylopectin determine the factors controlling many physiochemical properties of starch, including eating, cooking and nutritional qualities of rice.

Starch is synthesized in the rice endosperm through a complex pathway that is catalyzed by several enzymes, including granule bound starch synthases (GBSS), soluble starch synthases (SS), starch branching enzymes (SBE) and starch debranching enzymes (DBE) (Ball & Morell, 2003). Each class of enzyme has multiple isoforms, and each isoform may play a distinct role in starch synthesis. Amylose is believed to be mainly elongated by GBSSI, with little branching or debranching activity, whereas amylopectin biosynthesis involves multiple enzymes, including several SS, SBE and DBE isoforms (Smith, Denyer, & Martin, 1997). Alterations in starch structure, and hence the nutritional and processing properties of rice, could be achieved by modifying the genes encoding starch biosynthetic enzymes.

Gelatinization properties are frequently used in rice breeding programs as a rapid method to gauge rice quality (Cuevas, Daygon, Corpuz, et al., 2010). Starch gelatinization is an order–disorder transition procedure that involves the disruption of the ordered semicrystalline structure of starch granules by heating in excess water, resulting in an amorphous matrix (Cooke & Gidley, 1992; Jane et al., 1999). Gelatinization properties, commonly analyzed by differential scanning calorimetry (DSC), could predict the cooking and eating qualities of rice (Juliano & Perez, 1983). Easily cooked rice is desirable for energy saving; however, rice grains that are relatively difficult to cook tend to have slower digestion rates, which can be beneficial to human health.

This study aims to determine the structural and gelatinization properties of starch from the African rice species *O. glaberrima* and *O. barthii*, and compare with those of the widely consumed *O. sativa*. This will help to find any advantages and potential applications of these less distributed African rice species. The molecular structures, including the molecular size distributions of whole starch molecules and chain-length distributions (CLDs) of debranched starch, of all three species were analyzed using size-exclusion chromatography (SEC) and fluorophore-assisted carbohydrate electrophoresis (FACE), respectively. Amylopectin CLDs were fitted with a biosynthesis-based mathematical model to identify possible enzymatic processes that are responsible for any unique structural and property characteristics of *O. glaberrima* and *O. barthii*. The resulting information could be beneficial in providing guidelines for gene engineering and rice breeding to produce rice with the desired traits from *O. glaberrima*, but similar or better eating and cooking qualities than *O. sativa*.

2. Materials and methods

2.1. Materials

African rice grains from ten *O. glaberrima* accessions and five *O. barthii* accessions supplied by International Rice Research Institute (IRRI, Los Baños, Laguna, The Philippines) are listed in Table 1. Rice grains from four *O. sativa* breeding lines (YRR08-03-02, YRJ08-03-11, YRA08-04-10 and YRD08-01-22) were provided by Yanco Agricultural Institute, Department of Primary Industries, Yanco, NSW, Australia, and another *O. sativa* variety (Nipponbare) was provided by Dr. Matthew K. Morell, IRRI.

Protease from *Streptomyces griseus* (type XIV) was purchased from Sigma–Aldrich Pty. Ltd. (Castle Hill, NSW, Australia). Isoamylase (from *Pseudomonas* sp.) and a total starch (AA/AMG) assay kit were purchased from Megazyme International Ltd. (Bray, Co. Wicklow, Ireland). Pullulan standards with known peak molecular weights were purchased from Polymer Standards Service (PSS) GmbH (Mainz, Germany). Dimethyl sulfoxide (DMSO, GR grade for analysis) was purchased from Merck Co. Inc. (Kilsyth, VIC, Australia). All other chemicals were reagent grade and used as received.

Table 1
Information on *O. barthii* and *O. glaberrima* samples.

Species	Accession number	Country of origin	Provider
O. barthii	99565	Tanzania	IRRI
O. barthii	103895	Senegal	IRRI
O. barthii	104060	Nigeria	IRRI
O. barthii	104076	Nigeria	IRRI
O. barthii	86481	Zambia	IRRI
O. glaberrima	86825	Mali	IRRI
O. glaberrima	103450	Gambia	IRRI
O. glaberrima	104586	Cameroon	IRRI
O. glaberrima	103596	Cameroon	IRRI
O. glaberrima	96746	Nigeria	IRRI
O. glaberrima	103459	Senegal	IRRI
O. glaberrima	105023	Guinea	IRRI
O. glaberrima	104019	Tanzania	IRRI
O. glaberrima	104594	Burkina Faso	IRRI
O. glaberrima	104023	Guinea-Bissau	IRRI

2.2. Grinding of rice grains

Rice grains were dehulled and then ground into fine flour in a liquid nitrogen bath using a cryo-grinder (Freezer/Mill 6850 SPEX, Metuchen, NJ, USA) for 5 min at 10 s^{-1} (Tran, Shelat, Tang, Li, Gilbert, & Hasjim, 2011).

2.3. Size-exclusion chromatography

To prepare the starch sample for structural analysis, protein was removed by treating the flour with protease and sodium bisulfite solution, each followed by centrifugation using a method described elsewhere (Syahariza, Li, & Hasjim, 2010; Tran et al., 2011). The treated rice flour was then dissolved in a DMSO–0.5% (w/w) LiBr solution (denoted by DMSO/LiBr), and remaining non-starch polysaccharide components then removed by precipitating starch using a sufficient amount of ethanol (6 volumes of DMSO/LiBr) followed by centrifugation at 4000 g for 10 min. The precipitated starch was collected, and then dissolved in DMSO/LiBr at 80 °C overnight. The concentration of starch in DMSO/LiBr was analyzed using a Megazyme total starch assay kit, and then adjusted to 2 mg/mL for SEC analysis.

The SEC weight distribution of whole branched starch was characterized using an Agilent 1100 SEC system with a refractive index detector (RID; ShimadzuRID-10A, Shimadzu Corp., Kyoto, Japan) following a previously described method (Cave, Seabrook, Gidley, & Gilbert, 2009; Vilaplana & Gilbert, 2010b), and data were treated following the method given by Castro, Dumas, Chiou, Fitzgerald, and Gilbert (2005). Size separation of branched starch molecules was carried out using GRAM pre-column, GRAM 100 and GRAM 3000 columns (PSS) in a column oven at 80 °C, and starch molecules were eluted using DMSO/LiBr solution at 0.3 mL/min. A series of pullulan standards with molecular weights ranging from 342 to 2.35×10^6 were used for calibration, allowing elution volume to be converted to hydrodynamic volume (V_h, or the corresponding radius, R_h) using the Mark–Houwink equation (see Cave et al. (2009)). The Mark–Houwink parameters K and α of pullulan in DMSO/LiBr solution at 80 °C are 2.424×10^{-4} dL/g and 0.68, respectively. The SEC weight distribution, $w_{br}(\log V_h)$, of whole branched starch molecules was obtained from the RID signal and plotted as a function of R_h (Vilaplana & Gilbert, 2010a).

To characterize the CLDs of amylose and amylopectin, starch samples were extracted as described above for whole branched starch molecules and debranched using isoamylase following