

Waxy gene haplotypes: Associations with apparent amylose content and the effect by the environment in an international rice germplasm collection[☆]

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Received 16 May 2007; accepted 13 June 2007

Abstract

Apparent amylose content (AAC), the key determinant of rice end-use quality attributes, is primarily controlled by the *Waxy* gene which codes for granule bound starch synthase (GBSS). We examined the combination of sequence variation in the *Waxy* gene and environmental effects, and their associations with AAC using 171 rice accessions originating from 43 countries. The combination of two single-nucleotide-polymorphism (SNP) markers in the *Waxy* gene allows for the identification of three marker haplotypes in this gene. The first SNP is at the leader intron splice site (In1 SNP), and the second polymorphism is in exon 6. The haplotypes explained 86.7% of the variation in AAC and discriminated the three market classes of low, intermediate and high AAC rice from each other. The environment affected the AAC of all haplotypes. Higher air temperature during grain development associated with a decrease in AAC of low and intermediate AAC-types, but with an increase in AAC of high AAC-type. The association of AAC with several *Waxy* RM190 microsatellite-(CT)_n alleles in combination with the In1 SNP was also examined. In conclusion, the *Waxy* haplotypes studied appear to be useful markers for selecting the AAC of breeding lines developed from the world's rice germplasm.

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Keywords: Amylose; *Waxy* gene; SNP; Single-nucleotide-polymorphism; SSR; Microsatellite marker; *Oryza sativa* L.

1. Introduction

Consumer preference for rice around the world, when eaten as an intact grain, is largely dependent on a desire for its cooked texture to be either firm-and-not-sticky or soft-and-sticky. For the food processing industry, different

functional properties such as freeze–thaw stability or flour thickening power are desired depending on the application. Apparent amylose content (AAC) is the key determinant of these different rice (*Oryza sativa*) cooking, sensory and processing properties (Bergman et al., 2004). Consequently, rice breeders develop cultivars with improved agronomic traits, while maintaining a targeted AAC that fits the desired end-use quality characteristics.

Milled rice samples are commonly categorized into an AAC-class or quality type similar to the following: glutinous/waxy- (AAC range: 0–5%), low AAC- (range: 6–18%), intermediate AAC- (range: 19–23%), or high AAC- (>23%) types. In the USA, the high AAC-class is further divided into two sub-classes: those quality types suitable for commercial thermal processing and those that are not (Bergman et al., 2004).

The *Waxy* gene, located on rice chromosome 6, encodes the enzyme—granule bound starch synthase (GBSS),

Abbreviations: AAC, apparent amylose content; ddF, dideoxyfingerprinting method; Ex6 SNP, exon 6 SNP; Ex6A, allele A of Ex6 SNP; Ex6C, allele C of Ex6 SNP; GBSS, granule bound starch synthase; In1 SNP, intron 1 SNP; In1G, allele G of intron 1 SNP; In1T, allele T of intron 1 SNP; In5 InDel, intron 5 insertion and deletion sequence variation; In5C, allele C of In5 InDel; SNP, single-nucleotide-polymorphism; SSCP, single-strand conformation polymorphism

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which plays a key role in apparent amylose synthesis (Smith et al., 1997). Two *Waxy* gene alleles, Wx^a and Wx^b , have traditionally been associated with the contents of GBSS and AAC in rice endosperm, with the Wx^a allele synthesizing higher contents of GBSS, and thus AAC, than the Wx^b allele (Sano, 1984; Sano et al., 1985). Wang et al. (1995) demonstrated that the AAC of rice is strongly associated with the ability of the enzymes to excise the intron 1 from the *Waxy* pre-mRNA to different degrees between the two *Waxy* alleles, suggesting the genetic control of this starch fraction is at least in-part at the post-transcriptional level. Subsequent studies identified that the single nucleotide change of G→T at the +1 position of the intron 1 consensus cleavage site is responsible for the characteristics of the Wx^b allele: the presence of pre-mRNA (containing intron 1), low level of mature *Waxy* transcript, GBSS and AAC (Bligh et al., 1998; Cai et al., 1998; Hirano et al., 1998; Isshiki et al., 1998; Larkin and Park, 1999). Rice *waxy* mutants, which have no detectable AAC and GBSS, were reported to contain a premature translation termination codon (Wanchana et al., 2003). In a study of 89 non-glutinous USA cultivars, an intron 1 single-nucleotide polymorphism (In1 SNP) discriminated low AAC-type cultivars from intermediate and high AAC-type cultivars (Ayres et al., 1997). However, the In1 SNP was unable to distinguish between intermediate and high AAC-type rices.

A sequence change was reported in exon 6 of the *Waxy* gene, comprised of an A→C substitution, that results in an amino acid substitution (Larkin and Park, 2003). The intermediate AAC-type rice accessions studied contained cytosine (Ex6C) at this Ex6 SNP while both adenine (Ex6A) and cytosine (Ex6C) were identified in the high and low AAC-types. The association of Ex6 SNP to AAC warrants further investigation.

A polymorphic DNA microsatellite marker having a dinucleotide (cytosine–thymine) repeat (CT_n), located at exon 1 in the non-translated region of the rice *Waxy* gene was identified by Bligh et al. (1995). The CT_n alleles of this microsatellite, subsequently renamed RM190 (Temnykh et al., 2000), in an historical collection of 89 non-glutinous USA rice cultivars explained 82.9% of the variation in AAC (Ayres et al., 1997). This association dropped to 68% when 170 non-glutinous rice accessions from 53 countries were examined (Bergman et al., 2000). Both of these studies were performed using samples of rice accessions that were not grown under the same conditions. Bergman et al. (2001) studied 198 non-glutinous USA cultivars and breeding lines of diverse parentage, grown in four locations, and determined that 88% of the variation in AAC was explained by the RM190 alleles. Thus, the association of this microsatellite marker with AAC across a genetically diverse germplasm collection, grown in multiple locations is yet to be determined.

In addition to the major effect of the *Waxy* gene, minor genes and the environment are also thought to influence rice AAC (Bollich and Webb, 1973; Juliano and Pascual,

1980; McKenzie and Rutger, 1983). The AAC is therefore a quantitative trait that requires rice breeders to grow out their breeding material during multiple years and multiple locations to ensure that advanced lines have the desired AAC level. The molecular markers discussed above are capable of more quickly identifying the AAC-class of breeding lines in some crosses. However, the utility of these markers individually or in combination for cultivar development using non-US germplasm has not been studied. The purpose of this research effort was to examine the association of *Waxy* sequence variants with the AAC of rice accessions from many rice-growing regions of the world. The effects of these sequence polymorphisms alone, and in combinations (as haplotypes), versus environmental effects were also studied.

2. Materials and methods

2.1. Materials

A collection of 171 rice accessions were grown in 2000 and 2001 under field conditions in Beaumont, TX, using cultural management practices common for the region. This collection included historical and present-day USA cultivars as well as Asian, European, South American, African, and Australian accessions. Rough rice samples were obtained from the National Small Grains Collection of the US Department of Agriculture, the International Rice Research Institute Genebank and the Rice Research Unit of the USDA ARS. The accessions were grown in single plots arranged in blocks. Accessions were assigned to one of three blocks depending on their previously documented days to anthesis. The blocks were planted at different times in order to synchronize anthesis and thus grain filling of the accessions. The plots consisted of six rows, 3.5 m long, spaced 15 cm apart. Average within-row spacing was approximately 10 cm. The plants were kept continuously flooded after good tiller initiation in about 10 cm standing water. Mature seeds were harvested and threshed by hand, the grains were dehulled, and then all broken, diseased and immature kernels were removed by hand. Approximately, 50 g of dehulled kernels were milled for 30 s using a McGill Mill #1 with an 858-g weight in positions 12 and 6 for long- and medium-grain types, respectively (Chen and Bergman, 2005). The milled rice was ground using a Cyclone Sample mill (UDY Corp., Boulder, CO) and sieved through a 100-mesh screen. All the rice accessions were genotyped when first received from the original sources using the whole kernel alkali DNA extraction method (Bergman et al., 2001) and RM190 microsatellite CT_n markers (Ayres et al., 1997). The grains of each accession collected from both years were verified with the same methods to ensure the correct materials were carried out through planting, harvesting and grain chemical analysis. Multiple plots of control cultivars, Lemont (7–15 plots/block) and Bengal (1–3 plots/block), were planted at random interspersed among the diverse rice

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