Genetic Diversity of Rice Grain Quality

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1. Introduction

Rice (Oryza spp.) is one of the most important food crops in the world, being planted on almost 11% of the Earth’s cultivated land area over a wide number of ecosystems (Khush, 2005; Maclean et al., 2002). Two species, Oryza sativa and O. glaberrima, are cultivated while other species are wild. Human selection and environmental factors have contributed to the genetic diversity in rice, particularly in O. sativa cultivars (Maclean et al., 2002). A significant amount of this genetic diversity is housed in rice gene banks around the world, with a copy also conserved in the snowy depths of Svalbard, Norway. The diversity of rice offers a valuable resource to understand grain quality and how different agronomic backgrounds alter those traits. Rice is consumed mainly as milled, white grains or as brown grains (unpolished), and also as ingredients in food products. The cooking and sensory properties of a variety are key components that affect its acceptability to consumers (Cramer et al., 1993). Consumer preferences shift from low-quality to high-quality rice with increased income and market liberalisation (Cramer et al., 1993; Dawe & Slayton, 2004). Improvements in post-harvest technologies have also contributed to this shift in consumer preference by decreasing the price difference between low- and high-quality rice (Dawe & Slayton, 2004).

With the exponential and breathtaking progress in sequencing and genotyping technologies this decade is bearing witness to in rice, research programs delivering to breeding programs should perhaps focus on understanding the genetic control of different traits of eating quality, and offering genetic markers to breeding programs for inclusion on the new generation chips being developed (Boualaphanh et al., 2011). However, understanding the genetics of eating quality is difficult because consumers are not easily able to describe the sensory experience. Without a way to measure the trait, it is not possible to find an associated locus.

Thus, in order to assess consumer acceptability of breeding materials and rice cultivars, and in the absence of a clear knowledge of sensory properties, quality evaluation and breeding programs rely heavily on three indirect indicators that predict, to some degree, the cooking and sensory properties of rice: amylose content, gelatinisation temperature, and gel consistency. An understanding of the genetics behind these quality indicators can aid in screening early in breeding programs. However, as research has progressed into the genetics of those indicators, the data reveal that one major gene can be found for each trait, but the genes underlying the finer phenotypic classifications for each of those traits remain undiscovered. In this chapter, the genetic diversity of rice for these three indicators of quality and future avenues for research are reviewed.
2. Amylose content

Amylose content (AC) is regarded as the most important indicator in classifying rice varieties (Juliano, 1985) because it influences texture and retrogradation potential of cooked grains (Champagne et al., 2004; Ong & Blanshard, 1995a). It is measured in breeding programs as soon as heterozygosity is minimised and is the first tool used in the selection process for eating quality. Rice varieties are classified into high (>25%), intermediate (20 – 25%), low (10 – 19%), very low (3 – 9%), or waxy (0 – 2%) amylose classes (Kumar & Khush, 1987).

Since waxy rice varieties do not have amylose molecules, they are of particular interest in research because they provide a simple way to unravel the effects of components of the rice grain, other than amylose, that affect cooking and eating properties. However, waxy rice varieties have been reported to have very low amounts of amylose (i.e., Chung et al., 2011; Juliano, 1971; Landers et al., 1991; Sanchez et al., 1988; Varavinit et al., 2003). In an international cooperative test conducted recently, waxy rice samples were reported to have as high as 11% amylose using the iodine colorimetric method (Fitzgerald et al., 2009a). Reports have also indicated the presence of amylose chains within the hilum of immature waxy grains based on the colour reaction with iodine (Badenhuizen, 1956; Baker & Whelan, 1951; Juliano & Villareal, 1987). The difference between the expected amount and the observed amylose values indicate either (1) amylose is truly present in waxy rice varieties or (2) the values obtained by iodine colorimetry stemmed from technical issues relating to the method. Therefore, there is a need to further define the waxy phenotype. In this review, the definition of amylose is based on its genetics, its structure, and its effect on the functional properties of rice.

In order to clarify whether amylose is truly present in the waxy mutants, 53 traditional Lao waxy varieties and IR29 (a waxy mutant) were used along with 75 non-waxy varieties. These non-waxy varieties consisted of 8 very low AC, 7 low AC, 19 intermediate AC, and 42 high AC rice varieties. The set also included milled rice flour of Calmochi-101 (waxy), Calamylow-201 and BL-2 (opaque mutants of Calhikari-201), BR-2, BR-5, and BR-7 (waxy mutants of Calhikari-201) kindly provided by Dr McKenzie (California Cooperative Rice Research Foundation, Inc) (McKenzie et al., 2006a, 2006b) and RS111, a high-amylose content mutant (37%) of a Chinese variety (Shu et al., 2006a) provided by Dr Wu Dianxin (Zhezhiang University, Hangzhou, China). These varieties were tested in many different ways to determine whether or not amylose was present (Cuevas, 2009).

2.1 Genetic definition of amylose

The genetic basis of AC has been studied extensively. The major gene responsible for amylose synthesis in rice is the Waxy gene on chromosome 6 (Nagao & Takahashi, 1963; Sano, 1984). Amylose classes associate with polymorphisms in the Waxy gene (Wx) (Table 1). The functional alleles Wxa and Wxb, caused by a single base change at the 5′ splice site of intron 1, distinguish low amylose contents from high and intermediate classes (Hirano et al., 1998; Wang et al., 1995). The G→T mutation in the splice site of intron 1 causes inefficient transcription because of alternate splicing (Bligh et al., 1998; Cai et al., 1998; Isshiki et al., 1998). However, these two alleles are not enough to explain the continuous variation in AC across the different classes nor do they define the waxy class. A single nucleotide polymorphism
(SNP) in exon 6 (A→C), Wx\textsuperscript{in}, was associated with intermediate AC (Chen et al., 2008; Larkin & Park, 2003; Mikami et al., 2008). Seeds that are opaque despite the production of amylose, Wx\textsuperscript{op} (or Wx\textsuperscript{hp}), carry the same SNP in the intron 1 splice site as Wx\textsuperscript{a} and an A→G SNP on exon 4 (Liu et al., 2009; Mikami et al., 1999). Further, premature termination of translation caused by a 23-bp duplication in exon 2 of Wx causes a shift in the reading frame; this leads to a premature stop codon and the waxy phenotype (Mikami et al., 2008; Wanchana et al., 2003).

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Intron 1</th>
<th>Exon 2 (23-bp dupl’n)</th>
<th>Exon 4</th>
<th>Exon 6</th>
<th>Phenotype</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>T\textsuperscript{a}</td>
<td>+</td>
<td></td>
<td></td>
<td>Waxy</td>
</tr>
<tr>
<td>2</td>
<td>T</td>
<td>-</td>
<td>G</td>
<td></td>
<td>Low AC</td>
</tr>
<tr>
<td>3</td>
<td>G</td>
<td>-</td>
<td>A</td>
<td>C</td>
<td>Very low AC</td>
</tr>
<tr>
<td>4</td>
<td>G</td>
<td>-</td>
<td>A</td>
<td>A</td>
<td>Intermediate AC</td>
</tr>
<tr>
<td>5</td>
<td>G</td>
<td>-</td>
<td></td>
<td></td>
<td>High AC</td>
</tr>
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</table>

\* Waxy rice predominantly carry the Wx\textsuperscript{b} allele; however, there are waxy rice varieties that do carry the Wx\textsuperscript{a} allele (Yamanaka et al., 2004)

Table 1. Known haplotypes of rice based on mutations in the \textit{Waxy} gene

In rice, Granule-Bound Starch Synthase I (GBSSI) enzyme, the product of the Wx gene, is expressed in the endosperm and encodes a 66-kDa protein (Hirose & Terao, 2004). The SNPs defining each haplotype are at important sites for gene expression, therefore each SNP lowers the amount of GBSSI protein produced (Figure 1).

Fig. 1. Comparison of GBSSI bands in rice varieties with decreasing amounts of amylose: (1) RS 111, (2) IRGC111746 B, (3) IR8, (4) IR64, (5) Calamylow-201, (6) Calhikari-201, (7) BL-2, and (8) Calmochi-101. The ladder is composed of phosphorylase B (94 kDa), albumin (67 kDa), and ovalbumin (43 kDa).

In contrast to non-waxy varieties, Calmochi-101, a waxy rice variety, does not express GBSSI (Figure 1). Bands of GBSSI were also not observed in IR65, a waxy variety used as a standard, nor in the set of 54 waxy varieties (Cuevas, 2009). Hence, it can be concluded that the waxy mutants of rice do not express GBSSI protein.

2.2 The molecules of amylose

GBSSI synthesises amylose molecules in storage organs (Ball & Morell, 2003; Sano & Katsumata, 1984; Smith et al., 1997). Amylose is a linear (sparsely branched) polymer of glucose units linked by α-1,4 linkages (Ball et al., 1996), that reaches up to chain lengths
ranging from 200 to 10,000 degrees of polymerisation (Hizukuri et al., 1989; Takeda et al., 1986; Takeda et al., 1992a, 1992b; Ward et al., 2006) and with molecular weights in the $10^5$ range (Ebermann & Praznik, 1975; Roger & Colonna, 1996; Shelton & Lee, 2000). These molecules are distinct from amylopectin molecules, the hyperbranched glucose homopolymer of starch.

Amylose chains form helical coils when suspended in water. Iodine then enters these coils, subsequently forming stable amylose-iodine complexes that are characterised by a blue colour reaction (Rundle et al., 1944). Thus, amylose content in rice varieties can be quantified based on the intensity of the color reaction with iodine. In a previous study (Cuevas, 2009), amylose content of the 54 waxy rice varieties was measured using the a modified method based on the ISO 6647 routine method (International Organization for Standardization, 2007), and the method returned positive values (Table 2).

<table>
<thead>
<tr>
<th>Amylose Content (%)</th>
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<tr>
<td>Maximum 8.15</td>
</tr>
<tr>
<td>Minimum 3.88</td>
</tr>
<tr>
<td>Mean 6.21</td>
</tr>
<tr>
<td>Standard Deviation 1.12</td>
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</table>

Table 2. Range of amylose content values of the waxy rice varieties following modified ISO 6647, using non-waxy standards with known amylose contents (Cuevas, 2009).

The range of amylose contents for the waxy varieties measured in Table 2 fell within the very low amylose class (Kumar & Khush, 1987). Based on these results, it is easy to conclude that waxy rice grains do have some amount of amylose molecules despite the absence of GBSSI expression within the grains of these varieties. It is also easy to classify very low amylose varieties as waxy, or group these two types into one class (Varavinit et al., 2003). However, the results in Table 2 were obtained using calculations based on standard curves that might not be chemically appropriate: 0% amylose was extrapolated from absorbance readings of non-waxy materials, but a waxy variety for the zero reading is more appropriate and delivers matrix control. A pure amylopectin matrix represents 0% amylose and waxy rice is recommended to be this matrix in the AACC Method 61-03 (American Association of Cereal Chemists, 2000).

The difference in structure between the chains that constitute amylose and amylopectin molecules has allowed for their separation by size exclusion chromatography (SEC) after debranching the molecules to obtain the chains from each. Elution volumes of the debranched molecules of the two polymers have been identified: the longer amylose molecules elute first followed by the shorter amylopectin molecules (Ward et al., 2006). Through universal calibration and the use of pullulan standards, the elution volumes could be converted into chain lengths (or degree of polymerisation, $X$) (Ward et al., 2006). Thus, the proportion of amylose relative to amylopectin can be determined based on the area under the two peaks.

As shown in Figure 2, the waxy variety, Calmochi-101, does not show a peak in the chain-length area where amylose elutes. This is expected because GBSSI is not expressed in this variety (Figure 2). When SEC traces of varieties spanning the range of amylose classes are
compared (those in Figure 1), the area under the amylose peak ($X \geq 200 - 10000$) increases while the area of the amylopectin peak ($X < 200$) decreases with increasing amylose content and GBSSI expression (Figure 1). In non-waxy varieties, the amylose peak does not return to the baseline between the amylose and the amylopectin peaks (Figure 2). The distance from the baseline increased with increasing amylose content, indicating that GBSSI synthesises chains between the amylose and the amylopectin chain-length ranges.

Calamylow-201, the opaque mutant, was shown to express GBSSI, albeit weakly (Figure 1). The debranched chain-length distributions of this variety and of BL-2, another opaque mutant, confirm the presence of low amounts of amylose inside the grain (Figure 2) despite the resemblance in physical appearance with waxy grains. Moreover, these mutants appear to fall into haplotype 3 of the $Wx$ gene (Table 1), carrying the mutation on exon 4, based on their AC.

It has previously been reported that immature waxy rice grains contain amylose chains in the hilum, as indicated by distinctive colour reactions with iodine (Badenhuizen, 1956; Baker & Whelan, 1951; Juliano et al., 1987). The implication is that GBSSI is active at one point in grain-filling, and that waxy rice really does contain amylose, the product of that enzyme. However, genetics and SEC profiles indicate that these linear glucose chains could not be amylose, defined as the product of the $Wx$ gene, despite the blue reaction, because of the frame-shift mutation in exon 2 (Mikami et al., 2008; Wanchana et al., 2003) and the absence of a peak consistent with amylose in the mature varieties (Figure 2). To clarify whether amylose exists during grain-filling in a waxy variety, starch from immature rice grains of Thassano 1 (TSN1), a waxy rice variety from Lao PDR, was debranched and the molecules were separated by SEC (Cuevas, 2009). Results showed quite clearly that chains normally assigned as amylose were absent and only amylopectin chains were detected even in the immature stage (Figure 3).
The enzymes that are involved in starch synthesis are reported to have temporal patterns of expression. GBSSI is expressed quite late in the development of the grain, beginning to be detectable five days after flowering (Hirose & Terao, 2004). The iodine reaction then implies that some other enzyme synthesises glucose chains that mimic the response of amylose. There are many possible starch synthases that could produce such chains in immature grains. Early in grain-filling, genes coding enzymes involved in amylopectin synthesis are highly expressed. Starch synthases (SS) II-2 and III-1 are specifically expressed 1–5 days after flowering while SSI, II-1, IV-1 and IV-2 are expressed constantly during grain filling (Hirose & Terao, 2004).

The absence of amylose-length chains in immature grains of TSN1 (Figure 3) indicates that GBSSI is indeed absent from waxy grains. The blue core previously observed in the hilum of immature waxy grains (Baker & Whelan, 1951; Badenhuizen, 1956; Juliano et al., 1987) must have been caused by the presence of ‘atypical’ amylopectin molecules that form complexes with iodine (Buleon et al., 1998). Iodine forms a blue reaction with chains \( X \geq 45 \) (Bailey & Whelan, 1961); the steady expresser SSIV is associated with the synthesis of chain lengths in this range and is associated to the production of such chains in the hilum (Hirose & Terao, 2004; Roldan et al., 2007). Mutants of Arabidopsis that do not express SSIV do not synthesise starch (Roldan et al., 2007). This suggests therefore, that SSIV is implicated in the synthesis of the material that seeds the building of the amyloplast.

Fig. 3. Debranched chain-length distributions of immature TSN1 grains. The amylose peak is notably absent (Cuevas, 2009).

### 2.3 Effect of amylose on functional properties

The absence of the long linear chains of amylose in waxy rice affects its functional properties. Within the starch granule, amylose is believed to surround and to bind amylopectin blocklets together (Gallant et al., 1997; Morris, 2006; Ridout et al., 2006; Tang et al., 2006) which suppresses swelling of non-waxy starch granules (Lii et al., 1996). The effect on the swelling capacity of starch granules predictably affects the viscosity profiles of non-waxy and waxy starch (Allahgholtpour et al., 2006; Fitzgerald et al., 2003); hence, viscosity curves can be used to distinguish waxy from non-waxy rice.
Viscosity parameters (Figure 4a) are usually assessed for late generation material in breeding programs (Fitzgerald et al., 2003; Larkin et al., 2003). One instrument that measures these is the Rapid Visco Analyser (RVA); starch is suspended throughout the test by constant stirring (Lai et al., 2000) and viscosity is denoted by changes in the stirring torque (Miyoshi et al., 2000) during the temperature cycle. Parts of the viscosity curve have been associated with amylose content (Bao et al., 1999; Champagne et al., 1999; Juliano, 1985; Ong & Blanshard, 1995b). The RVA has been deemed an indirect method for determining quality of rice (Yan et al., 2005).

Fig. 4. Viscosity curves obtained by Rapid Visco-Analyser. The temperature profile is denoted by the black line. (a) An example of a viscosity curve, showing the different points: PV, peak viscosity; TV, trough viscosity; FV, final viscosity. (b) Comparison of curves of the different amylose classes. Error bars are placed at PV, TV, and FV.
As the temperature increases, starch granules and proteins begin to absorb water and to swell (Batey & Curtin, 2000; Matveev et al., 2000), causing an initial rise in viscosity. The temperature at this point, known as the pasting temperature, is associated with gelatinisation properties of the starch source. Viscosity continues to increase, peaking at the point at which the balance between granule swelling and bursting has been achieved (PV) (Fitzgerald et al., 2003). Amylose and some amyllopectin molecules, that leach while the granules are swelling (Tsai & Lii, 2000), form a continuous phase which contributes to the decrease in viscosity when the temperature plateaus (Lii et al., 1995); this is the lowest point in the curve and is known as trough (TV), or hot-paste, viscosity (Bergman et al., 2004; Bhattacharya & Sowbhagya, 1979; Onwulata & Konstance, 2006). As the starch slurry cools, viscosity starts to increase again because the leached amylose molecules form networks (Gidley, 1989; Nguyen et al., 1998). At the end of the test, the RVA records the final viscosity (FV) value as the temperature returns to a constant. From these three points, three additional parameters are derived: breakdown (BD, difference between PV and TV), consistency (CO, difference between FV and TV), and setback (SB, difference between FV and PV) (Bhattacharya & Sowbhagya, 1978; Fitzgerald et al., 2003; Juliano, 2007).

Waxy rice varieties used in this study exhibit distinctive pasting behaviours compared with varieties belonging to different amylose classes (Figure 4b). PV, TV, and FV are the lowest in waxy rice varieties (Figure 4b and Figure 5a-c), consistent with a previous report (Allahgholipur et al., 2006). The low FV in waxy varieties is likely to be explained by the absence of amylose chains. The linear nature of the amylose molecule allows it to retrograde as the RVA cools, forming a gel matrix in which amyllopectin molecules and starch ghosts are embedded (Gidley, 1989; Gidley & Bulpin, 1989; Miles et al., 1985). In contrast, because of the abundance of chains X 6–9, amyllopectin molecules retrograde more slowly (Kanae et al., 2004; Shi & Seib, 1992), taking up to several days or weeks (Colonna et al., 1992). Hence, the non-waxy varieties retrograde a lot faster, resulting in higher FV, than the waxy varieties (Figure 5c).

The effect of AC on PV is not as clear as its contribution to FV. It has previously been reported that AC is positively (Singh et al., 2006; Tran et al., 2001) and negatively (Chung et al., 2011; Juhász & Salgó, 2008; Tan & Corke, 2002) correlated with PV. Using data from IRRI’s quality evaluation program, Figure 5a shows that AC does affect PV for non-waxy varieties, in agreement with previous reports (Larkin et al., 2003; Yan et al., 2005). Previous studies all used much smaller sample sets than were available in IRRI’s quality evaluation database. While PV appears to be associated with the presence of amylose, it has been determined that varieties within the same amylose class are highly diverse in terms of PV (Figure 6a).

The heights of the viscosity curves appear to be limited by the height of the peak (Figure 6a). For instance, the low value of TV in waxy varieties (Figure 5b) can be attributed to the low PV in the waxy varieties. On the other hand, the other amylose classes had higher PV and had higher TV as well. Since TV is formed by disruption of starch structure due to shear, its level depends on the degree of swelling of the granules (which is associated with PV). Also, the associations of the derived values with amylose seem to depend on PV. BD did not associate with AC (Figure 5d), because both PV and TV did not. SB increased with amylose content (Figure 5f) not due to PV, but rather because FV is affected by AC. Meanwhile, since FV and TV of waxy varieties are lowest among the classes, it follows that the difference between the two parameters is the lowest among the classes as well (Figure 5e).
Fig. 5. Comparison of RVA parameters among the different amylose classes: (a) peak viscosity, (b) trough viscosity, (c) final viscosity, (d) breakdown, (e) consistency, (f) setback.
Due to the effect of PV (and of swelling as well) on the other RVA parameters, it could be meaningful to compare RVA curves of different samples if they have a fixed PV value (Bhattacharya & Sowbhagya, 1978). When the viscosities were recalculated so that all samples have the same PV, the samples could be classified into two main groups: one group contained the waxy varieties while the other group contained the non-waxy varieties (Figure 6b), which reaffirms the difference in pasting behaviour between waxy and non-waxy rice flour. Even with a fixed PV value, the varieties within each amylose class showed variability at other points in the RVA curves.

The variability in RVA curves within each amylose class indicates that the \textit{Wx} gene is not the lone predictor of cooking and eating properties of rice. Proteins are reported to affect the RVA curves, particularly because of their contribution to swelling (Baxter et al., 2004; Baxter et al., 2006; Derycke et al., 2005; Fitzgerald et al., 2003; Martin & Fitzgerald, 2002; Teo et al., 2000; Xie et al., 2008). Genes coding for starch branching enzymes (BE) I and III have been associated with variability in viscosity profiles (Han et al., 2004), along with other grain components such as phosphorus (Lin & Czuchajowska, 1998).

Aside from the RVA parameters, waxy rice varieties are distinguishable from non-waxy rice varieties based on the overall shape of the RVA curve (Figure 4 and Figure 6). The increase of viscosity from pasting to peak was much faster in waxy rice than in the non-waxy varieties; this was particularly evident when the RVA curves being compared had a fixed PV value (Figure 6b).

Table 3 shows that the slope from TV to FV increased as the amount of amylose increased. The slope is significantly shallower in the waxy varieties than in the non-waxy varieties. On the other hand, the slope from pasting to PV was clearly significantly higher in waxy rice than in non-waxy rice (except for the very low amylose class) when the slope was computed.
based on temperature; when the slope was computed based on the time, the waxy varieties only had significant difference in slope when compared to the high amylose varieties. The results of these comparisons are in agreement with visual comparisons of viscosity profiles in previous reports (Allahgholipour et al., 2006; Horibata et al., 2004; Yan et al., 2005).

<table>
<thead>
<tr>
<th>Amylose class</th>
<th>N</th>
<th>Paste to PV (x-axis = time)</th>
<th>Paste to PV (x-axis = temp.)</th>
<th>TV to FV</th>
</tr>
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<tr>
<td>Waxy</td>
<td>54</td>
<td>1562.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Very Low</td>
<td>8</td>
<td>1317.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>323.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low</td>
<td>7</td>
<td>1424.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>70.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>489.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intermediate</td>
<td>18</td>
<td>1476.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>87.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>540.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>High</td>
<td>42</td>
<td>1304.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>588.5&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

Table 3. Means of slopes from pasting to PV and from TV to FV for the rice samples used in the study. Independent t-test was conducted to compare the slopes. Data analysed from Cuevas (2009). For each column, numbers followed by the same letters are not significantly different (α = 0.05).

Amylose content is affected by the activity of GBSSI, the enzyme encoded by the Wx gene. Polymorphisms within the gene have been identified and been linked with the different amylose classes. Rice functional properties, such as pasting behaviour, have also been previously associated with AC. However, within each amylose class, there is a high variability in pasting properties. Thus, other grain components besides amylose and other genes besides the Wx gene affect pasting behaviour. To understand the pasting properties of rice, these other factors must be taken into account as well.

### 3. Gelatinisation temperature

Gelatinisation temperature (GT) is used in varietal development as an indicator of the cooking time of rice samples (Cuevas et al., 2010a). It is an economically important indicator of quality because selecting for shorter cooking times leads to significant potential savings in fuel costs (Fitzgerald et al., 2009b); thus, GT is a significant component of the carbon footprint of rice.

Three classes of GT are recognised in rice breeding programs: high (>74 °C), intermediate (70 – 74 °C), and low (<70 °C) (Jennings et al., 1979; Juliano & Pascual, 1980; Juliano, 2003; Waters et al., 2006). In these programs, three methods are commonly being used to classify grains by GT: deducting 3 °C from the pasting temperature derived from the RVA (Juliano et al., 1965), the alkali spreading value (ASV) (Little et al., 1958), and by differential scanning calorimetry (DSC) (Normand & Marshall, 1989). However, various studies have shown conflicts regarding the relationship among these observations and the associations of these measurements with cooking time (Cuevas et al., 2010a).

Fortunately, the structure and the mechanism leading to high and low GT have already been discovered. Amylopectin, the other polymer of starch, is composed of linear glucose units linked by α-1,4 bonds, which are organised into clusters with α-1,6 bonds at the branch points (Ball & Morell, 2003). At the ultra-structural level, GT is affected by the debranched
chain-length distributions of amylopectin within the crystalline lamella; particularly, the proportions of chains with lengths (measured as X) 6 - 12 and X 12 - 24 (Nakamura et al., 2002). The synthesis of amylopectin chains has been associated with functionality of several enzymes. By re-plotting amylopectin chain-length distributions using a mechanistic approach originally developed to understand synthetic polymer synthesis, coupled with current biochemical knowledge, one could classify the process of amylopectin synthesis into four stages characterised by rates of chain elongation and of chain termination (Castro et al., 2005).

Starch synthase (SS) I elongates chains of X 6 - 12 (Fujita et al., 2006) while SSIIa is reported in various botanical sources to extend the lengths of these chains further to X 12 - 24 (Craig et al., 1998; Konik-Rose et al., 2007; Morell et al., 2003; Umemoto et al., 2004; Yamamori et al., 2000). Branching enzyme (BE) IIb, on the other hand, cleaves the elongating chain and transfers the short amylopectin segments (Nishi et al., 2001; Tanaka et al., 2004). Disruptions to activity of any one of these starch synthase and branching enzymes are believed to have pleiotropic effects by altering the dynamics of the processes. In wheat and maize, the enzymes of starch synthesis are organised into complexes (Hennen-Bierwagen et al., 2008; Tetlow et al., 2004, 2008), so a change to one enzyme in the complex alters the composition of the complex and thereby the functionality (Tetlow et al., 2008). Changes in enzyme functionality could lead to changes in the amylopectin chain-length distribution; such changes can be observed by comparing conventional chain-length distribution plots (Hanashiro et al., 1996; Jane et al., 1999) or re-plotting those distributions using the mechanistic approach (Cuevas et al., 2010b). Observed changes in chain-length distributions suggest mutations in the genes coding for the enzymes involved in amylopectin synthesis.

Studies addressing GT have focused on the chain elongation aspect of amylopectin synthesis. Differences in proportions of chains with X 6 - 12 and X 12 - 24 have been associated with mutations in the gene coding for SSIIa (SSIIa), located on chromosome 6 (Umemoto et al., 2004). Four haplotypes have been defined based on two SNPs and a functional nucleotide polymorphism (FNP) in SSIIa. These haplotypes generally group rice varieties into high and low GT classes (Cuevas et al., 2010a; Umemoto & Aoki, 2005; Waters et al., 2006), with a few exceptions (Figure 7).

The four haplotypes of SSIIa are grouped into two classes, each spanning 10 °C in GT. Moreover, the distribution of GT by SSIIa haplotype does not show a clear population of varieties with intermediate GT (Figure 7) (Cuevas et al., 2010a). It has been reported that any of the four SSIIa haplotypes could lead to GT between 70 and 74 °C and improved varieties with intermediate GT by ASV could be classified as high GT by DSC (Cuevas et al., 2010a). Clearly, SSIIa does not code for intermediate GT.

Intermediate GT is commonly found in improved varieties cultivated and consumed in Asia (Juliano, 2003). In most programs at the International Rice Research Institute (IRRI), breeders select for lines with intermediate GT (Kaosa-ard & Juliano, 1991). Figure 8 shows that this is still the case in this century. Since intermediate GT has such a high importance in the selection process, the genetic basis for this trait has yet to be identified.

The inability of SSIIa to distinguish the intermediate GT class presents an opportunity to understand this functional property more deeply. Other enzymes involved in amylopectin synthesis may be involved. Minor quantitative trait loci (QTLs) that could possibly lower GT
have been mapped to chromosomes 1 and 7 (Bao et al., 2004). Another gene on chromosome 6, called \textit{alk2(t)}, was associated with differences in thermal properties and amylopectin chain-length distributions in varieties with the same \textit{SSIIa} haplotype (Shu et al., 2006b). Other enzymes such as starch synthase or branching enzymes (Butardo et al., 2011), could also be involved in changing the amylopectin structure (Ryoo et al., 2007) leading to intermediate GT.

Fig. 7. Comparison of kernel density plots among the four \textit{SSIIa} haplotypes (as represented by the SNP combinations) in 1500 rice varieties. Data analysed from Cuevas et al. (2010a).

Fig. 8. Distribution of GT classes based on ASV in 29,690 IRRI breeding materials analysed in 2004 – 2007.
Other studies have investigated the role of chain termination on GT. Changes in functionality of BEIIb have been associated with variations in GT by affecting the chain-length distribution of amylpectin in amyllose-extender mutants (Jiang et al., 2003; Nishi et al., 2001; Tanaka et al., 2004; Yamakawa et al., 2007). Comparisons of amylpectin chain-length distributions between IR36ae, an established BEIIb mutant (Asaoka et al., 1986; Juliano et al., 1990), and its wildtype IR36, and between Goami 2, a mutant variety with functional properties similar to those of BEIIb mutants (Kang et al., 2003), and its wildtype Ilpumbyeo, suggest that BEIIb functions in complex with SSIa (Cuevas et al. 2010b). A number of gene interactions have been reported to affect GT in an inter-subspecific doubled haploid population (He et al., 2006). However, there is still genetic work to be done to discover the basis of intermediate gelatinisation temperature.

4. Gel consistency

Gel consistency (GC) is a measure of firmness of the rice after cooking and is performed to classify rice varieties of the same AC, particularly in the high AC class, into hard, medium, or soft texture (Cagampang et al., 1973; Kohlwey, 1994). GC is commonly measured by determining the length of a cooled gel made from flour previously cooked in 0.2 M KOH (Cagampang et al., 1973). Variations in the method exist and are used depending on the AC of the samples. For waxy varieties, which form pastes instead of gels (Li et al., 1995; Tsai et al., 1997), the amount of flour used is higher (Bean et al., 1984) or neutral solutions are used instead of the alkaline solution (Perdon & Juliano, 1975). However, GC is not generally used in rice improvement programs focusing on rice varieties of intermediate and lower AC classes.

GC is a measure of the strength of the gel. The range of GC values to classify rice varieties according to this property is wide. Samples are grouped into arbitrarily set classes based on the length of the gel: hard (length of gel < 40 mm), medium (length of gel 41 – 60 mm), and soft (length of gel > 61 mm) (Graham, 2002). Weak and rigid gels depend on the association of starch polymers in the aqueous phase (Dea, 1989). Since amylose is the main polymer that leaches as starch granules are heated (Tsai & Lii, 2000) and amylose forms networks as the gel starts to cool (Gidley, 1989; Nguyen et al., 1998), GC could well be related to AC. Indeed, correlations between the two properties have been reported in many populations and landraces (Tan & Corke, 2002; Septiningsih et al., 2003; Zheng et al., 2007). In addition, the decrease in AC in irradiated rice was attributed to the softening of the rice gel (Yu & Wang, 2007).

The established correlations support the associations between GC and QTLs mapped to the Wx locus (He et al., 2006; Lanceras et al., 2000; Zheng et al., 2007). Studies have even reported that the gene coding for GC is located within the Wx locus (i.e., Tang et al., 1991; Tian et al., 2005). It was recently shown that the major gene for GC is in fact the Wx gene, and the mutation is a SNP on exon 10, a C → T polymorphism, which groups high AC rice varieties into hard and soft GC classes only (Tran et al., 2011). In the same way that a major gene separates high and low GT, as reviewed above, the extremes of GC are explained by biallelic variability at a single locus, but the intermediate class was not accounted for by that locus (Tran et al., 2011).

The relationship between AC and GC in IRRI breeding materials, however, is not as clear as those previously reported (Figure 9). These results show that GC is spread across all the AC classes in the breeding materials. Varieties from waxy and very low AC tended to have soft
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GC (higher GC values) while varieties from the other AC classes had GC readings from hard to soft. Thus, other factors aside from amylose must be contributing to the strength of the cooling gel, and understanding these will assist breeding programs to select more accurately for traits of texture.

Fig. 9. Relationship of amylose content and gel consistency in IRRI breeding materials analysed in 2004 – 2007 ($r = -0.169$).

In the IRRI rice breeding programs, materials with soft GC appear to be preferred (Tran et al., 2011). Since the SNP in exon 10 of the $Wx$ gene explains extreme variations in GC, it can be used as a tool in selecting breeding lines, particularly in programs working on varieties with high AC.

The diversity in GC in each amylose class indicates that GC may be controlled by several minor genes besides the $Wx$ (He et al., 2006; Tang et al., 1991). In backcross populations derived from hard/medium and medium/soft parents, medium GC appeared to be recessive to hard GC while soft GC is recessive to medium GC, indicating three alleles for this trait (Tang et al., 1991). However, the exon 10 SNP in the $Wx$ gene does not distinguish the medium type. The appearance of the medium GC trait might be contributed by other genes. For instance, various minor QTLs in chromosomes 1, 2, 6, and 7 have been associated with GC (He et al., 1999, 2006; Lanceras et al., 2000; Bao et al., 2002; Septiningsih et al., 2003; Zheng et al., 2007). Aside from minor genes, pleiotropic effects might be influencing GC as well. Interactions between $Wx$ and $BEIII$ and between $Wx$ and $Pul$ have been suggested to contribute to GC (He et al., 2006). With enzymes functioning in complexes, it may be possible that inactivity in one enzyme could affect the functionality of others within the complex, leading to medium GC, if complexes exist in rice.
5. Future directions

Though useful in predicting quality, AC, GT, and GC do not paint the whole picture of rice quality. Varieties identical in these three traits may be grouped into one quality class based on these parameters but consumers easily distinguish a premium variety from a lower quality one (Champagne et al., 2010). This could lead to low rates of adoption of newly developed improved varieties by farmers. Unfortunately, consumers are rarely able to describe the difference when they eat supposedly identical rice varieties (based on AC, GT, and GC if applicable). Hence, the next steps in discovering genes for sensory quality include finding descriptors for the sensory experience and developing phenotyping tools that can be used to quantify these descriptors. Once the phenotype is known, association mapping can begin, using appropriate populations. Such an approach will then lead to the delivery of validated genotyping tools to breeding programs.

An example of the value and need for phenotyping tools is the trait of aroma. Aroma is easy to define as present or absent in cooked rice. It was therefore possible to develop a phenotyping tool, in this case gas chromatography (Bergman et al., 2000), and then use mapping populations, genome-wide genotyping and sequencing of candidate loci to find genes and allelic variation (Kovach et al., 2009). Unfortunately, other sensory properties of rice are more abstruse because they are not as easily described by consumers. To find adjectives for these other sensory properties, descriptive sensory profiling is employed; a trained sensory panel assesses food for aroma, texture, and flavour (Champagne et al., 2010). Comparisons by a trained panel between similar varieties (in terms of AC, GT) but classed as premium and second-best showed that slickness, roughness, and springiness were textural attributes that separated the two classes while sweet taste, popcorn flavour, and metallic mouthfeel were the flavour attributes (Champagne et al., 2010). Without phenotyping tools, these traits could not be associated with genetic loci. Thus, discovering novel sensory quality genes goes in tandem with developing phenotyping tools.

6. Conclusion

Genetics has advanced people’s understanding of rice grain quality. The identification of the genes controlling the different quality traits has certainly helped breeders in improving rice varieties, especially with the capacity to deliver those outcomes using genome-wide genotyping tools. In the case of AC, GT, and GC, knowledge of the genetic factors has already translated into molecular markers; these tools are being applied in breeding programs with a caveat: the genetics of the extreme classes of GT and GC are now understood; for programs aimed at targeting for the intermediate phenotype, the gene(s) have yet to be identified. For AC, the SNPs coding for all classes are likely to be known; but there are other factors that contribute to pasting properties. The genetics behind these other factors have yet to be fully understood.

Waxy rice varieties do not have amylose and thus could not be grouped into quality classes based on this property. Within the waxy class, rice varieties are very diverse and new approaches in characterising and classifying them are being developed. The RVA is a promising tool for screening eating and cooking properties of waxy rice and in defining the quality classes. However, since the viscosity curves within each amylose class are different, it is important to understand how the other factors affecting pasting properties interact with AC.
The challenge now is to find new genes for rice quality beyond AC, GC, and GT. Development of high-throughput genotyping technologies is progressing at a hectic pace. The progress, then, of finding novel sensory quality genes hinges on the pace of development of phenotyping tools that target traits that rice consumers find difficult to characterise.

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Genetic Diversity in Plants


Genetic Diversity in Plants


Genetic Diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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