



Understanding the genetics of rice cooking quality

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in a nutshell

- Understanding the precise role of various starch synthesis genes and establishing detection tools for these genes will provide valuable genetic information to rice breeders to predict the precise quality traits and to identify different rice varieties by DNA finger printing.

Although the composition of developing rice grains is affected by cropping environment, it is the genetic make-up of a variety that dictates its specific cooking properties. To gain control over rice cooking quality attributes it is imperative to understand their genetic basis. Identification of different types of genes responsible for various quality traits will allow gene-specific markers to be developed to assist breeders at the early stages of crossbreed screenings and ensure the faster development of new designer varieties.

Rice cooking quality: what controls it?

Rice grain consists mainly of starch (90%) and small proportions of proteins (~6%), lipids (~2%) and minerals (~1%). Grain starch is comprised of two components: amylose and amylopectin, which differ in their amounts among various sub-species of rice. It is the amount and structure of these two starch components that play the key role in defining cooking properties of a rice variety. Grain lipids and proteins also influence the pasting properties of cooked rice through interaction with the amylose and amylopectin molecules.

Grain quality & amylose

The amount of amylose in the grain determines the texture and appearance of cooked rice. A gene known as the *Waxy* gene determines the levels of amylose in the grain. In glutinous rice, the *Waxy* gene is non-functional (mutated), resulting in very low amylose (<2%) and a cooked rice that is very sticky. Non-glutinous rices have a fully or partly functional *Waxy* gene. There are two types of *Waxy* genes – 'a' and 'b'.

Rice with the 'a' type *Waxy* gene, *indica* rice, has a fully functional *Waxy* gene and amylose levels can be up to 30%. When cooked, high amylose rices (>24%) are firm in texture and opaque in appearance. The *japonica* rices contain the 'b' type *Waxy* gene, which has lower amylose levels (16–18%) than the 'a' type. When cooked, these rices have softer,

translucent cooked grains, which is a unique characteristic of most Australian cultivars.

Predicting amylose content

In the course of this research project, we have developed genetic tests to detect the presence of the 'a' and 'b' types of *Waxy* gene and predict the amylose content of a crossbred. This test involves a simple technique of polymerase chain reaction (PCR), where DNA isolated from a rice line of interest is mixed with an enzyme and two small DNA fragments that are identical to a specific and relevant part or target region of the *Waxy* 'a' or 'b' type gene. The gene products from the PCR reaction can be separated and analysed on a gel matrix, as shown in Figure 1. The PCR product from the 'a'-type gene is smaller (290 bases) and can be easily distinguished from the 'b'-type (350 bases). Our genetic analysis (Figure 1) shows that most Australian rice varieties contain the 'b' type of *Waxy* gene, except Doongara, which has the 'a' type, and hence it is of firm cooking characteristic.

In addition to the presence of 'a' and 'b' gene types, the controlling region of the *Waxy* gene shows some differences in the number of repeated DNA sequences between varieties and can influence changes in amylose content. These differences can also be detected by the PCR technique. Such a genetic tool has already been implemented in the rice breeding program and plays an important role in predicting amylose content and tracking the pedigree of various crossbreeds.

Further research is currently in progress to understand other genetic factors that affect the amount and structure of amylose in different rice lines.

Grain quality & amylopectin

Amylopectin affects the cooking properties of rice due to its structure. It is synthesised by a suite of enzymes that interact to produce a highly branched structure unique to a specific rice cultivar. These enzymes are in turn controlled by various genes that differ between varieties and result in differences



in amylopectin architecture (ie number of branches, length of branches and branching patterns).

Starch synthase & amylopectin

One of the important genes involved in amylopectin synthesis, and which controls the cooking (gelatinisation) temperature of rice, is *SSIIa*. Researchers at Southern Cross University (SCU) at Lismore and in Japan, have identified minute differences in this gene between cultivars that have different cooking temperatures. Based on this information, new gene markers were developed at the Yanco laboratory to detect all types of the *SSIIa* alleles (form or type of gene)

in a single test and predict the cooking temperature of a rice crossbreed (Figure 2). Depending upon the type of the *SSIIa* allele present, the PCR test shows four different products of unique sizes (310, 240, 180 and 110 bases). A combination of 240 and 110 size bands corresponds to a rice line that will have a very high cooking temperature (~80°C), as observed for Pelde and Langi. A combination of 310 and 240 size bands will result in low gelatinisation temperature (~66°C, for Amaroo, Quest and Illabong) and the lines with a combination of 180 and 110 size bands will have intermediate cooking temperature (~70°C) as observed for Jarrah and Opus.

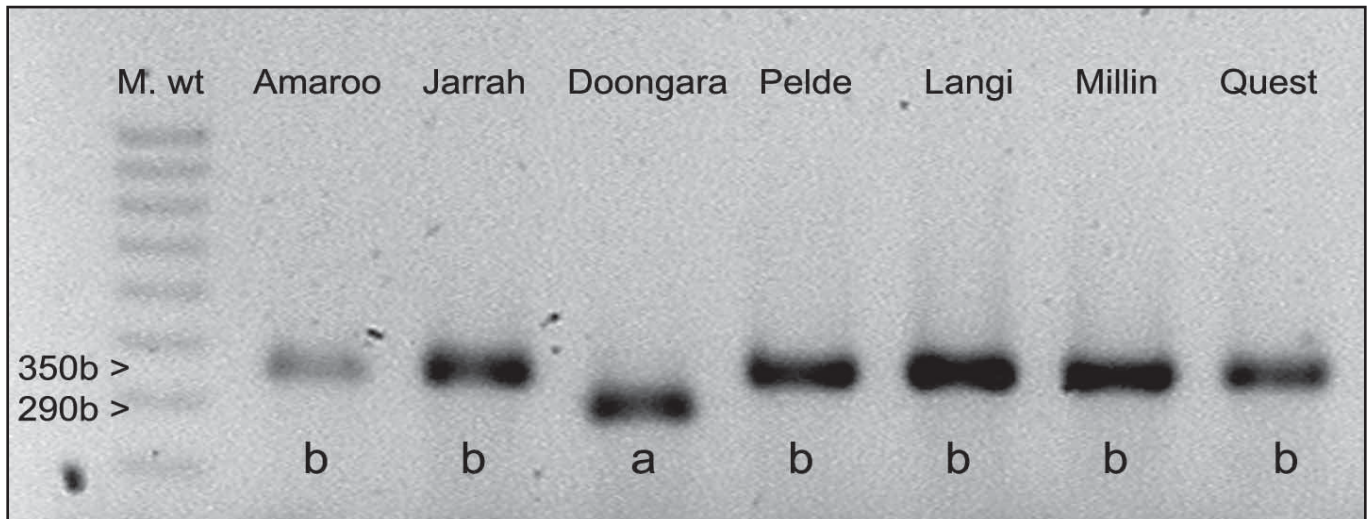


Figure 1: Detection of Waxy 'a' and 'b' gene types (alleles) in different rice cultivars

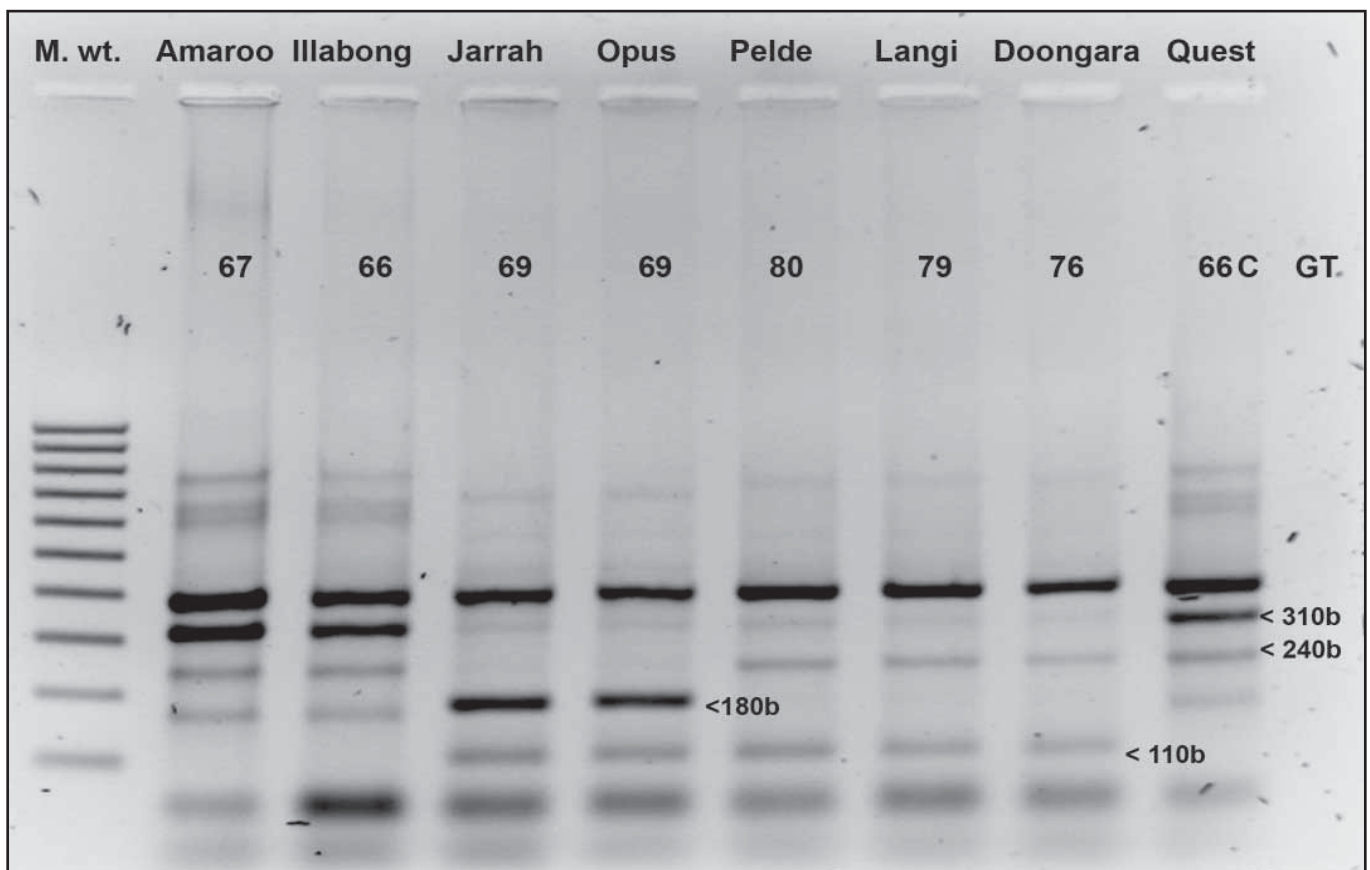


Figure 2: Detection of the *SSIIa* alleles in rice lines to predict gelatinisation temperature (GT)



Branching enzyme & amylopectin

Among the various branching enzymes (BE), BE-I plays a major role in introducing branches on the long chains of amylopectin. Genetic differences occur between the *Be-I* gene of *japonica* and *indica* rice that show differences in their amylopectin structure and cooking properties. We have developed markers to distinguish the two types of *Be-I* alleles by PCR analysis (Figure 3). In *japonica* rice, the PCR product is larger (540 bases) than the one in *indica* rice (220 bases). Our genetic analyses indicate that most of Australian rice varieties contain the *japonica* type *Be-I* gene, except 'Doongara' that has an *indica* type. Although the precise effects of these two *Be-I* alleles on amylopectin structure is still under investigation, quality evaluation studies show that Doongara has cooking properties similar to Basmati type *indica* rice.

De-branching enzymes & amylopectin

During the synthesis of amylopectin, debranching enzymes (DBE) play an important role in final pruning of amylopectin chains, to produce a final structure unique to a cultivar. Our recent studies showed that one of the key debranching enzyme genes exists at least in two different forms. Molecular markers have been developed to detect these alleles and studies on its effects on amylopectin and cooking properties of rice are currently underway.

Further gene identification

In addition to the work described in the previous sections, identification of other genes that influence grain quality is in progress. The entire suite of starch synthesis genes and their precise detection tools (perfect markers) will allow us to conduct studies on the effects of environmental factors in relation to grain quality. Importantly, these gene markers will provide valuable tools to assist breeders in accurate screening of their crossbreeds. At present, breeders have to wait three to four years until they have sufficient seed quantity of crossbreeds (more than 150 g) to allow quality evaluation tests. Even then, the genetic make-up/purity of these lines is not assured. With the development of new genetic marker tests, which can be done on a single grain, effective and precise screening will be feasible at the initial stages of crossing. In addition, gene markers for specific traits will

allow breeders to combine unique alleles in one cultivar and keep track of them in subsequent generations, thus expediting the rice varietal development and improvement program.

Gene markers & diagnostics

Molecular markers/flags developed for grain quality traits will have additional application in accurate identification of rice cultivars. In January 2006, there was confusion among growers as to whether the seed provided to them was Amaroo or Jarrah, and since the two cultivars have similar appearance, visual identification proved difficult. However, as their genetic make-up is different, DNA markers for some of the starch enzyme genes were utilised to verify the variety of the crops planted. Thus, a spin-off from our research on grain quality proved useful in diagnostic tests.

Future work

Our next goal is to establish unique DNA profiles (finger prints) for each Australian cultivar, to ensure that seed purity and identity can be maintained. Although another diagnostic approach, DArT, reported in *Farmers' Newsletter* No. 171, pp 40–41, 'Diversity array technology (DArT) for the rice breeding program', has been out-sourced by rice breeders at times, DNA finger-printing is quicker and can be performed at our Yanco laboratory. With the possible de-regulation of the rice industry, it is anticipated that DNA-based diagnostic tools will play a vital role in variety identifications. ☀

Acknowledgments

Many thanks to the rice breeding team for providing various seed material. Information on the *SSIIa* gene sequences from the SCU group is duly acknowledged. Special thanks to Laurie Lewin, Russell Ford and Daryl Gibbs for helpful discussions on emerging importance of DNA markers to the rice industry.

RIRDC DAN-238A

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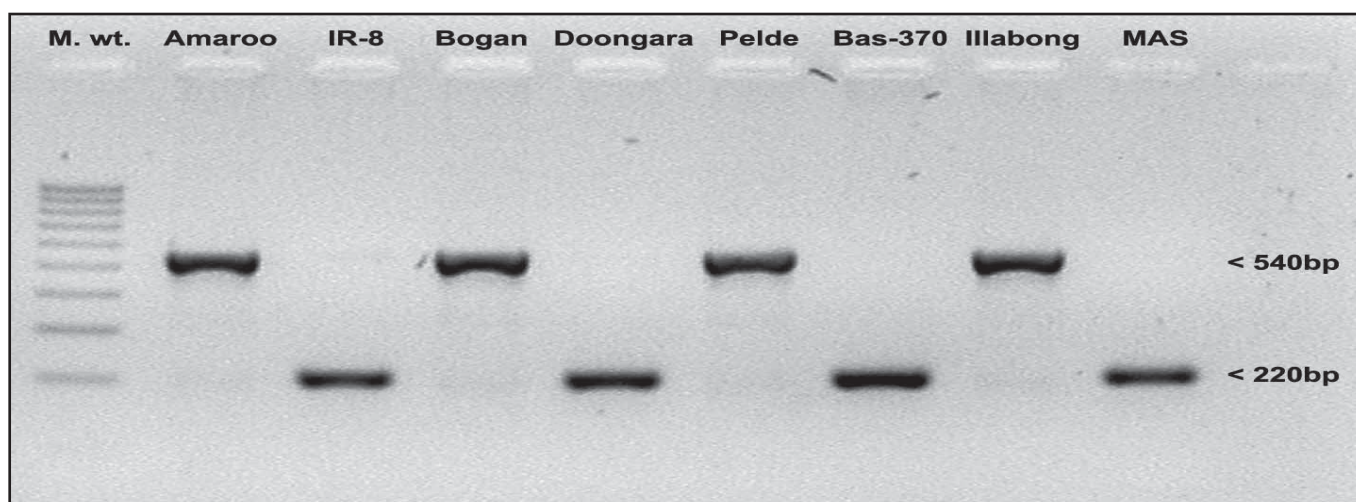


Figure 3: Genetic analysis of rice line for two types of the *Be-I* gene