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Competitive allele specific PCR molecular markers for rice [Oryza sativa] starch gelatinisation temperature

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Southern Cross University, through the Centre for Plant Conservation Genetics, is collaborating with Australian rice breeders to develop and implement molecular markers for the Australian rice breeding program. Markers within causal genes, perfect markers, are particularly attractive because they cannot become separated when recombination events occur between the marker and the gene during the course of breeding. Rice starch gelatinisation temperature (GT) is an important component of rice eating quality because it is associated with the cooking time and texture of cooked rice (Maningat and Juliano, 1978). Although GT is genetically determined, it is affected by environmental conditions and displays high year to year variability, making it a difficult trait to accurately assess within a breeding program and therefore a target for the application of molecular markers. Polymorphisms which determine whether rice has either high or low GT starch have been recently identified in the gene which encodes soluble starch synthase IIa (SSIIa) (Umemoto and Aoki 2005; Waters et al, 2006). Rice varieties with high-GT starch (average GT = 78 °C) have a combination of valine (SNP3, G) and leucine (SNP4, GC) at these residues. In contrast, rice varieties with low-GT starch (average GT = 70 °C) have a combination of either methionine (SNP3, A) and leucine (SNP4, GC), or valine (SNP3, G) and phenylalanine (SNP4, TT) at these same residues. We report here a competitive allele specific PCR assay for screening rice to determine its starch gelatinisation temperature status across a wide range of rice varieties and within segregating populations.

Competitive allele specific PCR molecular markers for rice (Oryza sativa) starch gelatinisation temperature

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Figure 1.

SNP3 assay, allele specific primer SSIIaSNP3F1 in combination with flanking primers SSIIaFF and SSIIaFR. Lane A, Roche 100 bp ladder. Odd numbered lanes are genotype “G” (variety Amaroo) and even numbered lanes are genotype “A” (variety Nipponbare). Annealing temperature varied in the following way, lanes 1 and 2, 58 °C, lanes 3 and 4, ~61 °C, lanes 5 and 6, ~64 °C and lanes 7 and 8, 68 °C.

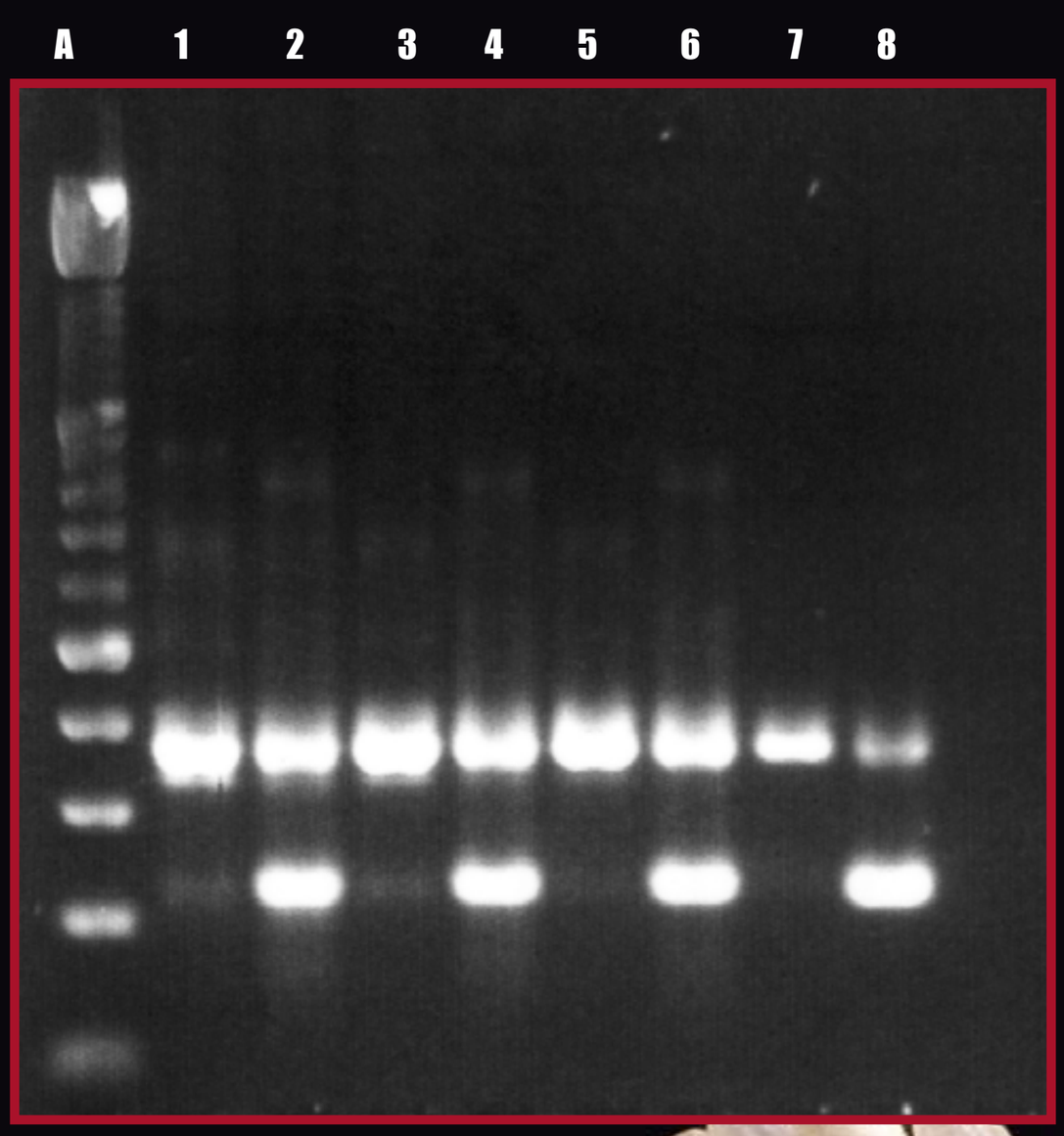
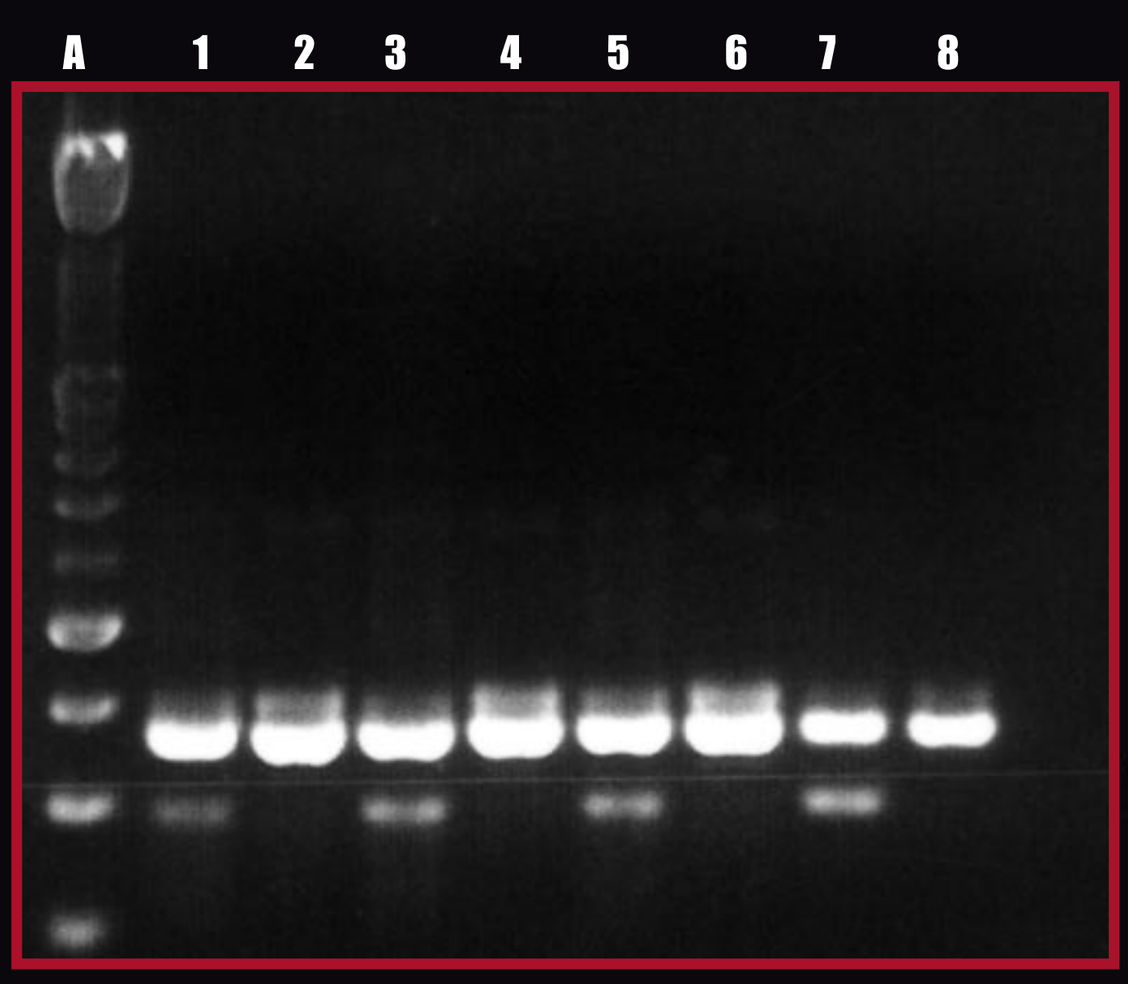


Figure 2.

SNP4 assay, allele specific primer SSIIaSNP4R3 in combination with flanking primers SSIIaFF and SSIIaFR. Lane A, Roche 100 bp ladder. Odd numbered lanes are genotype “TT” (variety Amaroo) and even numbered lanes are genotype “GC” (variety Nipponbare). Annealing temperature varied in the following way, lanes 1 and 2, 58 °C, lanes 3 and 4, ~61 °C, lanes 5 and 6, ~64 °C and lanes 7 and 8, 68 °C.



At an annealing temperature of 68 °C, the allele specific primers SSIIaSNP3F1 and SSIIaSNP4R3 can be used in duplex.

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