Genotyping of the fragrance allele in rice

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Abstract

We have previously determined that fragrance in rice, a recessive trait, is due to a large deletion (8bp) and 3 SNP’s in a gene on chromosome 8 which encodes a putative betaine aldehyde dehydrogenase 2 (BAD2). This mutation results in the formation of a truncated BAD2 enzyme because of the creation of an in-frame termination signal 800bp before that of the wild type. Because this truncated BAD2 is missing key binding domains, it is unlikely that it is capable of acting upon the target substrate and this leads to an accumulation of the principal fragrant molecule, 2-acetyl-1-pyrroline. Here we utilise single tube allele specific amplification (STASA) as a simple, low-cost, perfect assay to discriminate between fragrant and non-fragrant rice varieties in addition to homozygous fragrant, homozygous non-fragrant and heterozygous non-fragrant individuals in a population segregating for fragrance. Two external primers generate a 580bp fragment as a positive control for each sample. Internal primers in conjunction with their corresponding external primer pair produce a 355bp fragment from a non-fragrant allele and a 257bp fragment from a fragrant allele, allowing analysis on agarose gels.

Figure 1. Structure of the fragrance gene (fgr) (RICE ID: J023088C02) showing initiation codon (ATG), 15 exons, 14 introns and the ATT termination site. The nucleotide sequence of exon 7 is shown for both non-fragrant and fragrant rice varieties. The fragrant variety shows a large deletion and 3 SNP’s which then terminates prematurely (stop codon in red), within this exon.

Figure 2. Relative positions of PCR primers used in fragrance PCR (ESP - External Sense Primer, INSP - Internal Non-fragrant Sense Primer, IFAP - Internal Fragrant Anti-sense Primer, EAP - External Anti-sense Primer.)

Figure 3. Agarose Gel showing (lane 1-4) a non-fragrant variety (Nipponbare), a fragrant variety (Kyeema), a heterozygous individual (Kyeema/Gulfmont) and a negative control (water) flanked by Roche DNA Ladder 14.

Figure 4. Agarose gel showing 96 individuals from a population segregating for fgr analysed using competitive PCR assay. Lanes with non-fragrant and fragrant bands are heterozygous for fgr.