

Fragrance Gene Overview in Aromatic Rice and Strategies for Characterization

Ashu Singh¹ and R.S. Sengar²

Tissue Culture Lab, College of Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut-250110
E-mail: ashubiot25@gmail.com

Abstract—Rice (*Oryza sativa* L.) is the staple food of more than half of the world's population. Grain quality in rice plays an important role in consumer acceptability. The quality in rice is considered based on milling quality, grain size, shape, appearance, aroma and other cooking characteristics. Most of the scented rice varieties in India are of traditional type, photoperiod sensitive, and cultivated during the Aman/kharif season. Majority of these indigenous aromatic rice cultivars are low yielding but its higher price and low cost of cultivation generate higher profit margins compared to other varieties. Aroma development in rice grain is influenced by both genetic and environmental factors. The biochemical basis of aroma was identified as 2-acetyl-1-pyrroline. Most of the rice varieties have been developed traditionally by selection, hybridization and back crossing with locally adapted high-yielding lines. The conventional methods of plant selection for aroma are not easy because of the large effects of the environment and the low narrow sense heritability of aroma. More recently molecular markers, such as SNPs and simple sequence repeats (SSRs), which are genetically linked to fragrance and have the advantage of being inexpensive, simple, and rapid and only requiring small amounts of tissue, have been developed for the selection of fragrant rice.

Keywords: Rice, fragrance gene, aroma, molecular marker, grain, SNPs.

1. INTRODUCTION

Most of the world's rice is produced and consumed in Asia which constitutes more than half of the global population. In India rice occupies about 70% of the total cropped area of about 13.9 million hectares. Out of this 70%, fine rice is cultivated in roughly 10% land. Grain quality is second after yield as the major breeding objective for crop improvement. In Asian cultivated rice (*Oryza sativa* L.), aroma is one of the most valuable traits in grain quality and 2-ACP is the main volatile compound contributing to the characteristic popcorn-like odor of aromatic rice. Although the major locus for grain fragrance (*frg* gene) has been described recently in Basmati rice, this gene has not been characterized in true *japonica* varieties and molecular information available on the genetic diversity and evolutionary origin of this gene among the different varieties is still limited. The conventional methods of plant selection for aroma are not easy because of the large effects of the environment and the low narrow sense

heritability of aroma. More recently molecular markers, such as SNPs and simple sequence repeats (SSRs), which are genetically linked to fragrance and have the advantage of being inexpensive, simple, and rapid and only requiring small amounts of tissue, have been developed for the selection of fragrant rice.

The country's rice production declined to 89.13 million tonnes in 2009-10 crop year (July-June) from record 99.18 million tonnes in the previous year due to severe drought that affected almost half of the country. India could achieve a record rice production of 100 million tonnes in 2010-11 crop years on the back of better monsoon this year. Most of the scented rice varieties in India are of traditional type, photoperiod sensitive, and cultivated during the Aman/Kharif season. Majority of these indigenous aromatic rice cultivars are low yielding but its higher price and low cost of cultivation generate higher profit margins compared to other varieties. Aroma development in rice grain is influenced by both genetic and environmental factors. The biochemical basis of aroma was identified as 2-acetyl-1-pyrroline (Varietal diversification appeared to have occurred as a function of adaptation to diverse agro-climatic conditions coupled with conscious and continuous selection subsequently by man for his diverse quality preferences. There is another Aromatic rice which is a unique varietal group that has distinguished itself as a result of natural and human selection, which found wider acceptance all over the world as a specialty rice is called "Basmati rice". The unique features of Basmati rice such as extra long slender grain, lengthwise excessive elongation on cooking, soft and fluffy texture of cooked rice, and pleasant aroma which together determine uniqueness of Basmati rice. Aside their unique cooking qualities, Basmati rice are also reported to have low glycemic index and are micronutrient rich especially for iron and zinc.

The chemistry of rice fragrance: Chemical analysis of a wide range of rice varieties has revealed many compounds that differ in concentration between fragrant and non-fragrant rice varieties. (Yajima *et al.*, 1978; Buttery *et al.*, 1986; Paule and Powers, 1989; Petrov and Lorieux, 1996; Widjaja *et al.*, 1996;

Grosch and Schieberle, 1997; Wilkie and Wootton, 2004) (Table 1.1). Using a combination of sensory panels and gas chromatogram techniques Buttery *et al.* (1983) determined 2-acetyl-1-pyrroline (2AP), although only present in fragrant rice at low concentrations, was the primary chemical responsible for the characteristic aroma of Jasmine and Basmati rice. 2AP is also present in non-fragrant rice varieties but at a concentration in the range of 10 to 100 times lower than that of fragrant rices (Buttery *et al.*, 1983; Buttery *et al.*, 1986; Widjaja *et al.*, 1996; Wilkie and Wootton, 2004). The threshold concentration at which 2AP can be detected by the human nose is around 0.1ppb when diluted in water (Buttery *et al.*, 1983), but is probably somewhat higher in the complex rice grain. Fragrant rice grain has 2AP concentrations from about 3000 times this level and upwards, while non-fragrant rice has concentrations of 2AP of only about 30 times this threshold level of 2AP in water (Table 1.1). The aroma of fragrant rice is often described as Pandanus like and in some Asian cultures dried Pandanus leaf is added to non-fragrant rice while cooking to impart a characteristic Basmati/Jasmine scent. Since the discovery that 2AP is the major chemical compound involved in fragrance in both rice and Pandanus, it has been found the flavor of a range of foods, including, popcorn (Schieberle, 1995), corn tortillas (Buttery and Ling, 1995), baguettes (Zehentbauer and Grosch, 1998), ham (Carrapiso *et al.*, 2002), cheese (Zehentbauer and Reineccius, 2002), mung bean (Brahmachary and Ghosh, 2002), green tea (Kumazawa and Masuda, 2002) and wine (Herderich *et al.*, 1995) is associated with the presence of 2AP.

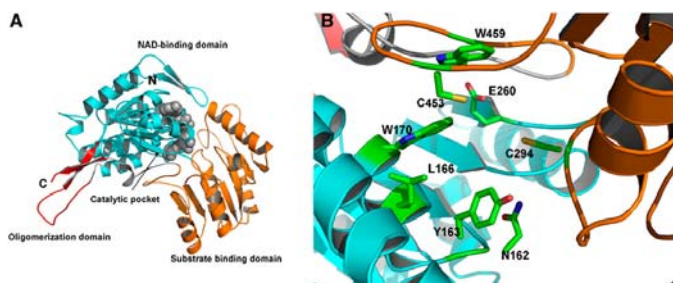


Fig. 1: The Three-Dimensional Structure of BADH2 and the Annotated Active Sites.

(A) Representation of BADH2. Domain coloring is as follows: the oligomerization domain is shown in red, the substrate binding domain is shown in orange, and the NAD binding domain is shown in green. To position the NAD cofactor in the predicted model, the NAD cofactor from the crystal structure of ALDH2 is superimposed into the predicted BADH2 model and is indicated by gray spheres.

(B) The annotated active sites of BADH2. Asn-162 and Cys-294 are catalytic residues that are predicted to interact with the substrate oxygen. Tyr-163, Leu-166, Trp-170, Glu-260, Cys-453, and Trp-459 are predicted to form the substrate binding pocket. The side chain atoms in these highlighted residues are

represented as sticks and are colored as follows: green, carbon; blue, nitrogen; red, oxygen; orange, sulfur. Images in both panels were prepared using PyMol (<http://pymol.sourceforge.net>).

The genetics of rice fragrance: Molecular markers are used widely as a selection tool for specific traits in breeding programs in many species including rice. If the genetic cause of a trait is known or the genomic region that controls a trait is sufficiently narrow, genotypic tests using molecular markers offer many advantages relative to many direct phenotypic tests. The advantages include a requirement for small quantities of tissue, independence from the confounding effects of the environment and a single platform test for multiple traits. The majority of studies which have focused on the genetics of fragrance in rice determined fragrance is due to a single recessive gene (Sood and Sidiq, 1978; Berner and Hoff, 1986; Ahn *et al.*, 1992; Bollich *et al.*, 1992; Lorieux *et al.*, 1996; Cordeiro *et al.*, 2002; Jin *et al.*, 2003) while other studies have identified two, three or four genetic loci as having an influence on fragrance (Kadam and Patankar, 1938; Dhulappanavar, 1976; Geetha and Nadu, 1994; Pinson, 1994; Vivekanandan and Giridharan, 1994; Lorieux *et al.*, 1996). Cordeiro *et al.* (2002) utilized rice genome sequence data to identify a SSR marker which was approximately 4 cM from *fgr*. This loci was highly polymorphic, 13 alleles will be identified, and eight will be found in the fragrant plants and eight in the non-fragrant plants, three of which will be common to both fragrant and non-fragrant varieties. DNA sequence analysis of approximately 500bp of the 5' ends of 14 genes chosen based on their proximity to RG28 revealed only one SNP (RSP04) between Kyeema (fragrant cultivar) and Doongara (non-fragrant cultivar) (Jin *et al.*, 2003). This SNP was assessed for linkage to *fgr* by pyrosequence analysis of the SNP in 164 phenotyped F2 individuals from a Gulfmont/Doongara cross, placing RSP04 approximately 2 cM from *fgr*, making RSP04 the closest known marker to *fgr*. This study was undertaken to identify the gene responsible for the generation and accumulation of the subtle yet potent fragrance compound 2-acetyl-1-pyrroline in Jasmine and Basmati style rice. A combined molecular, enzymatic and metabolomics investigation of this fragrance pathway was undertaken to aid in the breeding of fragrant rice varieties and to gain insights into the potential enhancement of 2-acetyl-1-pyrroline levels in a variety of foodstuffs.

The gene for fragrance in rice: The flavor or fragrance of basmati and jasmine rice is associated with the presence of 2-acetyl-1-pyrroline. A recessive gene (*Fgr*) on chromosome 8 of rice has been linked to this important trait. Here, we show that a gene with homology to the gene that encodes betaine aldehyde dehydrogenase (BAD) has significant polymorphisms in the coding region of fragrant genotypes relative to non-fragrant genotypes. The accumulation of 2-acetyl-1-pyrroline in fragrant rice genotypes may be explained by the presence of mutations resulting in a loss of function of

the *Fgr* gene product. The allele in fragrant genotypes has a mutation introducing a stop codon upstream of key amino acid sequences conserved in other BADs. The *fgr* gene corresponds to the gene encoding BAD2 in rice, while BAD1 is encoded by a gene on chromosome 4. BAD has been linked to stress tolerance in plants. However, the apparent loss of function of BAD2 does not seem to limit the growth of fragrant rice genotypes. Fragrance in domesticated rice has apparently originated from a common ancestor and may have evolved in a genetically isolated population, or may be the outcome of a separate domestication event. This is an example of effective human selection for a recessive trait during domestication.

Molecular marker analysis: DNA marker is a new approach based on DNA polymorphism among tested genotypes, and thus applicable to biological research. Several molecular markers viz. RFLP, RAPD (Tingey and Delfino, 1993), SSRs (McCouch et al., 1997), AFLP and SNPs are presently available to assess the variability and diversity at molecular level (Joshi et al., 2000). Simple sequence repeat (SSR) markers or microsatellites are tandem repeats interspersed throughout the genome and can be amplified using primers that flank these regions (Giovannoni et al. 1991). More recently molecular markers, such as SNPs and SSRs, which are genetically linked to fragrance and to identify the nature of the locus (homozygous or heterozygous condition), and have the advantage of being inexpensive, simple, rapid and only requiring small amount of tissue, may also be useful for the rapid incorporation of the scent character into breeding lines (Cordeiro et al. 2002). On the other hand random amplified polymorphic DNA (RAPD) is the widely used molecular marker where DNA fragments are amplified by the Polymerase Chain Reaction (PCR) using short (usually 10 bases in length) synthetic primers of random sequence. RAPD markers tend to estimate intra or inter genetic distances more distantly related individuals. In spite of many weaknesses, it is relatively easy, speedy, high degree of polymorphisms as well as virtually inexhaustible pool of possible genetic markers make the technique advantageous over other molecular techniques (Fritsch and Rieseberg, 1995). With the view, study should be conducted to evaluate the genetic analysis and relatedness to create future data.

Genetic mapping: Fragrance can be evaluated according to Berner and Hoff (1986). The phenotypes of F2 individuals be classified as fragrant, segregating or non-fragrant by tasting dehulled F3 seed. At least 12 F3 seeds from individual F2 plants will be chewed individually. F2 plants will be rated homozygous fragrant or non-fragrant if all 12 F3 seeds will be fragrant or nonfragrant, respectively. F3 seeds from heterozygous F2 plants will be expected to contain both fragrant and non-fragrant seeds, therefore if the sample from a single F2 plant will be a mixture of fragrant and non-fragrant, the F2 plant will be considered to be heterozygous. The observed segregation ratio of fragrant: segregating: non-fragrant was tested by χ^2 analysis against the expected ratio

for a single gene. Following identification, SSRs will be assessed for polymorphism by comparison of parental alleles. Polymorphic SSRs will be genotyped in F2 individuals from the mapping population. The genetic distances between *fgr* and the polymorphic SSRs will be estimated using MAPMAKER V.3.0 and determined as the percentage of recombinant chromosomes (cM).

2. FUTURE PROSPECT

Fragrance in Basmati and Jasmine style rice is a recessive trait which results principally from the presence of elevated levels of the compound 2-acetyl-1-pyrroline (2AP) in the aerial parts of the plant. A deletion in the gene encoding AAD2 (BAD2) on chromosome 8 which disables the AAD2 (BAD2) enzyme is the most likely cause of fragrance. Earliest reference to the existence of aromatic rices in India can be traced to the documents of Susrutha (circa 400 BC), the great Indian pioneer in medicine and surgery. For directed improvement of Basmati rices, knowledge of genetics of the key traits of Basmati quality is a prerequisite. Early efforts to understand the genetics and breeding behavior of the key indices of Basmati quality have revealed that all except aroma are polygenically controlled. Knowledge of the most likely genetic cause of fragrance has allowed the development a perfect assay for fragrance in rice. A single tube allele specific PCR which allows determination of the genotypic status of an individual rice plant, either homozygous fragrant, homozygous non-fragrant or heterozygous non-fragrant, has practical utility for rice breeders worldwide. The assay is a simple robust method for screening rice to determine its fragrance status across a wide range of rice varieties and within segregating populations using DNA isolated from rice following simple, inexpensive and rapid extraction protocols. The PCR products can be analyzed easily and inexpensively on agarose gel or alternatively using more sophisticated high throughput equipment, making the assay a very versatile tool.

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