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Understanding the Fragrance in Rice

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Introduction

Aroma in plants is a result of numerous volatile and semi volatile compounds present within Fragrance in grain rice is the most attractive trait that provides a premium price in the market. Continuously increasing demand for fragrant rice in global market gained a special attraction of rice breeders and forced them for considering rice grain aroma among major objectives for commercially improved rice varieties. Since, aromatic varieties are very rare these may be considered among most precious treasure of India and can also be considered as national asset and pride. The fragrance of rice plays an important role in affecting the market value and consumers' preference [1-3]. These characteristic aromatic rice have been reported in three different genetic sub populations of rice viz. Group V (Sadri and Basmati), *indica* (Jasmine), and tropical *japonica* [4] of more than 250 volatile and non-volatile compounds reported in both fragrant and non-fragrant rice varieties, a novel compound namely 2-acetyl-pyrrolin (2AP) has been significantly found as primary contributor in imparting the unique pop-corn like aroma in rice [5-8]. It has been reported that both aromatic and non-aromatic rice varieties have the characteristic aroma compound 2AP and the only difference lies in concentration between them [9]. In a comparative study, compounds alkanals, alk-2-enals, alka(E)-2,4-dienals, 2-pentylfuran, 2-acetyl-1-pyrroline and 2-phenylethanol have been reported as major contributors in total aroma profiling of rice. Besides, 2-acetyl-1-pyrroline and other previously known aroma imparting volatile compounds, recently few more novel compounds viz. 2-amino acetophenone and 3-hydroxy-4,5-dimethyl-2(5H)-furanone (found in high levels in Basmati 370) [10], and guaiacol, indole and p-xylene in Black rice [11], were found to be mainly responsible for its unique flavour. The strength of aroma greatly depends upon the concentration of 2AP in rice grain tissues, however the odour threshold level of rice is 0.1 ppb, its range of concentration in aromatic rice varieties varies from 6 ppb to 90 ppb for the milled rice and 100 ppb to 200 ppb in brown rice, depending upon the variety such as in Basmati rice (0.34 ppm), Jasmine rice (0.81 ppm) and Texmati (0.53 ppm).

Genetics and molecular basis of rice aroma

Inheritance of aroma is quite complicated to understand as this trait is influenced by concentrations of various volatile and semi-volatile compounds and probably controlled by unknown number of genes at various stages of rice growth. Although, plant breeders have reported the monogenic, digenic and polygenic pattern of inheritance of aroma in rice with recessive, dominant, complimentary and duplicate gene actions, in various studies [12-23]. Recent advancements in plant science and availability of high density linkage maps and fully sequenced rice genome have provided better opportunities for plant scientists to look inside the secrets of aroma in rice. Since, 2AP was found as a key role player behind the presence and absence of desired popcorn like characteristic aroma in most of the aromatic rice cultivars, plant scientists were more dedicated in understanding the regulation and molecular basis of 2AP resulting in a number of appreciable attempts in the array of mapping the gene governing the 2AP synthesis in several varieties of aromatic rice such as Della, Azucena [24-27], Suyunuo [28,29], KDML105 [30], Kyeema [31] and Wuxiangxian. But, the varying level of 2AP among different aromatic rice verities and its expensive assay limited the mapping experiments. Ahn et al. [25] with the help of RFLP technique mapped a gene which was responsible aroma, on chromosome number 8 tightly linked with clone RG28 and proposed that aroma of rice is controlled by a single recessive gene *fgr*. Later a gene responsible for the 2AP synthesis was identified and mapped between the flanking regions of RG1 and RG28, in a Jasmin rice variety KDML105 [32]. In the segregating generation the original region of 1.13 Mb flanking between RG1 and RG28 was narrowed down to 82.2 Kb. Within this narrowed region of 82.2 kb three KDML BACs were cloned including the identification of three new candidate genes [33]. Among a single recessive gene namely Os2AP was identified as a major gene determining the 2AP synthesis in the rice. The comparative analysis for Os2AP gene sequences between KDML105 and Nipponbare revealed two important mutational events within the exon 7 of Os2AP of aromatic KDML105, at positions 730 (A to T) and 732 (T to A), followed by the 8-bp deletion "GATTAGGC" starting at position 734 [33].

A similar mutational event was also reported by [30] in an aromatic rice variety Kayeema, in a gene responsible for 2AP within the flanking regions of RM515 and SSRJ07 on the longer arm of chromosome 8. The development of an *in silico* physical map using four BAC of Nipponbare spanning within a region of 386 bp from RM515 to SSRJ07 suggested one BAC clone (clone AP004463) as most likely to be having the gene. Further, resequencing of all 17 genes lying within the BACs helped in identification of a novel gene with 3 SNPs along with the 8-bp deletion in the exon 7 [34]. The newly identified gene was showing a significant homologue with BAD1 locus of chromosome 4 and hence named as BAD2 [30]. A comparative study between sequences and amino acids of Os2AP and BAD2 suggested them as one gene with two different names.

In addition of major aromatic gene Os2AP or BAD2 it has been found that three minor QTLs located on chromosome 3, 4 and 12 significantly play role in imparting the aroma, however the direct role of these three minor QTLs are completely not known [27,33].

Mutations in BADH2

Due to progress in robust and high throughput sequencing methods

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along with the availability of reference genomes and finely mapped *fgr* gene, sequence based allelic diversity study for the variants in the *fgr* gene present in the large available gene pool of aromatic rice had gained a world-wide interest [34]. Any mutation event in functional BADH2 leads to a premature termination in the gene producing a truncated protein that results in nullification of the function of the enzyme BADH2 and led to the synthesis of 2AP in fragrant rice verities. In addition of the mutations reported by [31] and [33] several other mutations including a 7-bp insertion in exon 8 [34]; a 7-bp deletion in exon 2 [29]; absence of MITE (miniature interspersed transposable element) in promoter [25]; two new SNPs in the central section of intron 8 [35]; a TT deletion in intron 2 and a repeated $(AT)_n$ insert in intron 4 [28]; a 3 bp deletion in exon 12 [36] have been reported during the desecration of allelic variants of BADH2 in different studies. of badh2 were reported in various fragrant varieties. Several other mutations in BADH2 causing in aroma had also been reported in many aromatic rice verities [8,34,37-39] indicating the existence of allelic diversity in fragrant gene.

Biosynthesis and regulation of 2AP in rice

2AP is synthesized via polyamine pathway [32]. 1-pyrroline (1P) which is supposed to be formed from an immediate precursor of 4-aminobutyric acid (GABA) namely 4-aminobutyraldehyde (AB-ald), is presumed as an immediate precursor of 2AP. 4-aminobutyraldehyde (AB-ald) is found to be maintained in an equimolar ratio with an immediate 2AP precursor, Δ^1-pyrroline, and the AB-ald levels appears to be an important factor regulating the rate of 2AP biosynthesis [40,41]. In contrast to aromatic rice verities with characteristic 2AP aroma the dominant encodes for a functional BADH2 enzyme inhibits the 2AP biosynthesis by converting AB-ald to GABA in no-aromatic rice. The non-functional badh2 (encoded by recessive bad2) result in AB-ald accumulation followed with the increment in the concentration of 2AP in scented rice. Bradbury et al. [42] suggested that γ-aminobutyraldehyde (GABald) is an effective substrate for BADH2 and that accumulation and spontaneous cyclisation of GABald to form Δ^1-pyrroline due to a non-functional BADH2 enzyme as the likely cause of 2AP accumulation in rice. However, in another study, increased expression of Δ^1-pyrroline-5-carboxylate synthetase in fragrant varieties compared with non-fragrant varieties, as well as associated elevated concentrations of its product, led to the conclusion that Δ^1-pyrroline-5-carboxylate, usually the immediate precursor of proline synthesized from glutamate, reacts directly with methylglyoxal to form 2AP [43], with no direct role proposed for BADH2.

The biosynthesis of 2AP in rice is controlled by mutated non-functional BADH2 gene. The mutational events led to production of premature stop codon causing in the loss of function of the aroma gene. Adifferential levels of aroma gene depending upon the age and part of the plant have been reported. The gene starts expressing in the early seedling stage of the rice palnt and continues even after the grain harvest. Except root, biosynthesis of 2AP has been reported in all plant parts. But Vanavichit et al. [32] reported a very low level of transcript of gene in roots.

The aroma gene shows a suppressive expression due to a premature stop codon at position 753, which shortens the full-length peptide to 252 amino acids in aromatic rice [30,32] and triggers nonsense-mediated decay (NMD) in several cases [43].

The role of suppressive expression of BADH2 in 2AP synthesis was confirmed with RNAi, constructed from the genomic sequence spanning exon 6 to exon 8 in the opposite direction from the corresponding cDNA. This allowed the transcript to create double-stranded RNA, resulting in NMD and aromatic Nipponbare that could accumulate 2AP in a range of 0.05–0.20 ppm [44]. In this experiment, the strongest RNAi expression gave the strongest suppression and the highest accumulation of 2AP, comparable to the 2AP content in Jasmine rice [44]. In an independent study, transgenic rice containing RNAi by an inverted repeat of cDNA encoding Os2AP accumulated 2AP in considerable amounts [45].

Environmental effect on fragrance

The quantity of aroma of rice is based upon genetic as well as environmental elements [46,47]. The evidence is supported by Taba and Bocchi, reported that due to differences in genotype, environment and interaction between genotype and environment, 2AP highly exist in Italian and Basmati rice. The fragrance may controlled by major gene, but the environmental conditions and cultivations practices could easily influence the intensity of fragrance or the concentration of 2AP [48]. There are lots of environmental factors which influence the aroma quality. For example, storage time and temperature, effect of planting density, harvesting time etc. [49,50] reported that planting at low density and early harvesting could improve aroma content and other seed qualities. In other studies showed that, Basmati variety seems to contain stronger aroma if they day temperature remains cold between 25 to 32°C whereas overnight temperature should be between 20 to 25°C. The humidity should be 70-80% in grain-filling and primordial phases [48]. Bradbury et al. reported that, level of 2AP is higher in plants exposed to water stress due to contribution of BADH with stress tolerance. There are lots of other environmental factors which affect the quantity of aroma like soil type [48], abiotic stress [51], conditions of storage and time of harvest and flowering time [48,51]. Mo et al. [52] reported that shading during the grain filling period increases 2AP content in fragrant rice. Another factor affecting quality of aroma is milling process of rice. The extent to which bran is removed from rice kernels during the milling process is referred to as the degree of milling (DOM). Recently, Rodriguez-Arzuaga et al. [53] reported that, DOM affects raw-rice appearance and aroma-related attributes. Likewise, the study conducted by Griglione et al. [54] reported that the storage temperature (5°C vs 25°C) does not significantly influence aroma preservation and they also reported that, heptanal/1-octen-3-ol and heptanal/octanal were act as indices of aroma quality for the Italian cultivars investigated and more in general, of rice aroma quality. In this way, lots of environmental attributes affects the aroma of rice. Hence aroma is rice is a significant feature and is a vital objective for breeding rice cultivars for their commercial importance for global market.

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