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Review

The genetics and biosynthesis of 2-acetyl-1-pyrroline in fragrant rice

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ABSTRACT

Rice (*Oryza sativa* L.) is a staple food for the majority of the world's population. Rice fragrance, aroma, or scent an eating quality of rice. Rice fragrance is a trait that is widely desired among rice consumers. Consequently, rice producers are sorting for rice cultivars with strong fragrance. High demand for fragrant rice cultivars has prompted rice breeders and researchers to investigate the genetics and the ways to improve fragrance in rice.

It has been established by many researches that *fgr* gene on the chromosome 8 of rice controls its fragrance. As with other plants, rice contains BADH but because rice does not accumulate GB, a catalyst for BADH coding, BADH1 on chromosome 4 of rice and BADH2 on chromosome 8 of rice have been widely reported to be responsible for encoding BADH. badh2, a recessive allele of BADH2 has been confirmed to be responsible for fragrance in rice. badh2 and its alleles have been associated with the accumulation and synthesis of 2AP. Proline, ornithine, glutamate, methylglyoxal, Δ1-pyrroline-5-carboxylate synthetase and glyceraldehyde-3-phosphate dehydrogenase have all been identified as the precursors for the synthesis and accumulation of 2AP.

By reviewing and summarising the main results of various researchers, we have been able to elucidate how various genes and metabolites influence 2AP accumulation in fragrant rice. It is our hope that this paper will be beneficial to researchers, who are working on the improvement of rice fragrance.

1. Background

Globally, rice consumption is increasing and as more people are lifted out of poverty in the emerging economies of the world, the demand for rice with high quality has also increased (Calingacion et al., 2014). Rice is a staple food for over half of the world's population (Hu et al., 2014). However, the cooking and eating qualities of rice have been a major problem facing rice consumers around the world (Bagchi et al., 2015; Fan et al., 2005). Fragrance, aroma or scent is a very unique quality that represents one of the eating qualities of rice. Rice consumers are known to have a strong affinity for fragrant cultivars.

Kovach et al. (2009) stated that fragrance is one of the most highly valued grain quality traits in rice. He and Park (2015) noted that introducing fragrance into elite rice accessions will result in rice with a high market value, but the sensory approach to improving fragrance is often time-consuming, expensive, and unreliable. Rice fragrance plays a significant role in rice marketing (Hashemi et al., 2015). The high market value of fragrant rice cultivars is attributed to their desirability and higher popularity in Asia, Europe, America, and the Middle East (Huang et al., 2008; Pinson, 1994; Bourgis et al., 2008).

It is therefore not surprising, that fragrant rice types such as Basmati, produced in India and Pakistan, and Jasmine which is widely cultivated in South East Asia, are specifically favoured not only locally, but also outside their production regions, where they find major and increasing markets, particularly in Europe, North America, and Australia (Mumm et al., 2016). However, the popularity of these fragrant rice cultivars is also extending to West Africa and China. This review aims to explain in detail the genetics and the biosynthesis of 2-acetyl-1-pyrroline (2AP), a major compound associated with fragrance in rice.

2. The genetics and biosynthesis of 2AP in fragrant rice

There are large genetic diversities displayed among rice varieties (Huang, 2012; Khush, 1997). Rice fragrance is a highly heritable trait that is controlled by genes; however, it has been proven that there are genetic differences among aromatic rice varieties (Huang et al., 2008). In other to understand the genetics and biosynthesis of 2AP, this section will be divided into several sub-sections.

Abbreviations: 2AP, 2-acetyl-1-pyrroline; BADH, Betaine aldehyde dehydrogenase; GB, Glycine betaine

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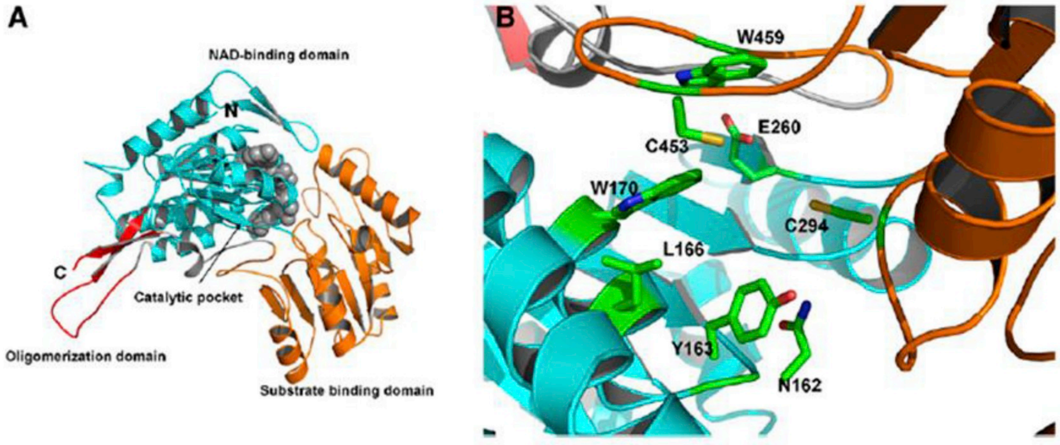

Fig. 1. The Three-Dimensional Structure of BADH2 and the Annotated Active Sites (Chen et al., 2008). (A) Representation of BADH2. Domain colouring is as follows: the oligomerization domain is shown in red, the substrate binding domain is shown in orange, and the NAD binding domain is shown in green. 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, sulphur. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.1. 2AP: a major compound associated with rice fragrance

Rice fragrance involves a very complex sensory attributes with a variety of chemical compounds. As a result, over 100 compounds have been associated with the fragrance of cooked rice (Yajima et al., 1978; Widjaja et al., 1996). The roles, genetics, and biosynthesis of all the compounds associated with rice fragrance are not yet well understood. However, Buttery et al. (1982) reported that all fragrant rice varieties contain 2AP (C_6H_9NO). Hinge et al. (2016) demonstrated that 2AP had no significant correlation with other odour active compounds in fragrant rice during growth and development, which shows that 2AP expression pattern, was unique and specific throughout rice development. In contrast, Daygon et al. (2017) have shown that four other amine heterocycles: 6-methyl, 5-oxo-2,3,4,5-tetrahydropyridine (6M5OTP), 2-acetylpyrrole, pyrrole and 1-pyrroline, correlate strongly with the production of 2AP. It has been reported by various authors that 2AP possesses popcorn-like aroma (Bourgis et al., 2008; Huang et al., 2008; Hinge et al., 2016; Hashemi et al., 2015).

2AP is the only component that rice breeders can select to modify the phenotype and genotype of rice cultivars (Bryant and McClung, 2011). The desirability of rice fragrance has resulted in strong human preference and selection of the trait (Bradbury et al., 2005a). 2AP has been reported in all parts of aromatic rice (Lorieux et al., 1996), including in the callus and other vegetative organs of rice (Yoshihashi et al., 2002), but they are absent in the roots of rice (Lorieux et al., 1996). However, lower levels 2AP have also been detected in non-aromatic rice (Widjaja et al., 1996; Maraval et al., 2010). The quantity of 2AP found in fragrant rice was reported by Buttery et al. (1983) to be about ten times more than the quantity that is found in non-fragrant rice.

The high demand for fragrant rice cultivars by consumers, have led rice producers to sort for cultivars with strong fragrance. Human tasting of individual rice grains has been identified by Reinke et al. (1991) as one of the methods for the quality selection in breeding fragrant rice varieties. However, Garland et al. (2000) noted that the problems with the sensory detection of fragrance are the considerable variation between analysts in their ability to detect fragrance or the associated flavour. The chemical detection of 2AP is a more reliable method, but the major challenge with this method as noted by Lorieux et al. (1996) and Widjaja et al. (1996) is that it requires longer time and larger samples. According to Garland et al. (2000) PCR-based molecular marker is one method with many advantages over sensory dedication by human and chemical detection methods for 2AP, because many plants can be processed and analysed with sample sizes of 0.1 g or less. Bradbury et al. (2005b) noted that the PCR products can be analysed easily and inexpensively on agarose gel or by using more sophisticated high throughput equipment, making the assay a very versatile tool.

2.2. The function of fgr gene

A single recessive gene, *fgr*, on chromosome 8 of rice is associated with rice fragrance (Bourgis et al., 2008; Bradbury et al. 2005; Ahn et al., 1992), and determines the quantity of 2AP in rice (Lorieux et al., 1996; Hashemi et al., 2015).

The *fgr* locus is on the long arm of chromosome 8 between the SSR markers RM223 and RM515 (2.34 cM) (Hashemi et al., 2015). Lorieux et al. (1996) have shown a close linkage between RG28 and *fgr* (5.8 cM) and have also identified two quantitative trait loci (QTL) for fragrance, one on chromosome 4 and the other on chromosome 12. Ahn et al. (1992) have found that the *fgr* gene was linked to the RFLP clone RG28 on chromosome 8, at a genetic distance of 4.5 cM. Bradbury et al. (2005a) demonstrated that the markers RM515 and SSRJ07 flanked *fgr* with the physical distance of 386 591 bp between them. In a similar investigation by Chen et al. (2006), the *fgr* locus was discovered on chromosome 8 between RM8264 and RM3459, and a physical distance of 800 kb.

2.3. The role BADH2 in rice fragrance

Betaine aldehyde dehydrogenase (BADH) is an enzyme found in a large number of plant species, with glycine betaine (GB) as its catalyst (Singh et al., 2010). Baicharoen et al. (2018) performed two 0.5 μs simulations to explore the nature of BADH2 dimer structurally and dynamically. They reported that each monomer comprises of 3 domains: substrate-binding, NAD + -binding, and oligomerization domains. (with the NAD + -binding domain as the most mobile). BADH is also found in rice; however, unlike some other plants rice does not accumulate GB (Fitzgerald et al., 2008; Singh et al., 2010; Bradbury et al., 2008). Therefore, rice uses a different mechanism to encode BADH. Fine mapping of the *fgr* locus on chromosome 8 and extensive sequence analysis have identified two isozymes, BADH1 and BADH2, encoding BADH (Fitzgerald et al., 2008). According to Bradbury et al.

(2008), BADH1 and BADH2 found in rice are encoded on chromosome four and chromosome eight respectively, but only BADH2 is responsible for fragrance in rice. Fig. 1 shows the three-dimensional structure of BADH2 and the annotated active sites.

Bradbury et al. (2005a) reported that BADH2 comprises of 15 exons and 14 introns on chromosome 8. During growth and development in fragrant rice, changes in BADH2 transcript levels are independent of BADH1 (Fitzgerald et al., 2008). BADH2 has been suggested to be domesticated during rice evolution (He and Park, 2015). However, the absence of BADH2 in fragrant rice did not negatively affect normal growth (Chen et al., 2008).

2.4. Understanding the functions of badh2 and its recessive alleles

Badh2 is a dominant allele that encodes BADH2 gene, while its non-functional recessive allele badh2 is responsible for fragrance in rice (Fitzgerald et al., 2008; Bradbury et al., 2008; He and Park, 2015). Chen et al. (2008) have also demonstrated that badh2 locus of rice constitutes the *fgr* gene that determines fragrance, while Badh2 is a dominant allele that encodes the BADH2 and inhibits fragrance in rice. It is thought that any mutation rendering the Badh2 gene non-functional would lead to a new functional badh2 allele (He and Park, 2015). badh2 codes for a substantially truncated BADH2 enzyme which is responsible for the elevated level of 2AP (Fitzgerald et al., 2008; Chen et al., 2008). Bourgis et al. (2008) found that badh2 gene mutation is exactly the same in different rice varieties.

In fragrant rice badh2 was found to be 1.7–5.56 fold lower transcript at booting stage and in matured seeds when compared to seedling stage, tillering stage, flowering stage and dough grains, however, higher levels of 2AP were accumulated in the booting stage and matured grains, indicating that badh2 expression was negatively associated with 2AP accumulation in fragrant rice (Hinge et al., 2016). In all tissue types, transcript levels of the non-functional badh2 allele in the fragrant varieties were significantly lower than those of the functional BADH2 allele in the non-fragrant varieties (Fitzgerald et al., 2008). Hinge et al. (2016) demonstrated that at all growth and developmental stages, the badh2 expression was 9–30 fold reduced in fragrant rice cultivars than non-fragrant rice cultivars.

Several fragrant alleles have been reported on the badh2 loci that were responsible for fragrance in rice; they include badh2-E2 (Shi et al., 2008), badh2-E7 (Shi et al., 2014; Chen et al., 2008; Xu et al., 2011), badh2-p-50UTR (Shi et al., 2014). However, Kovach et al. (2009) showed evidence that a single allele, badh2.1, is the predominant allele in virtually all fragrant rice varieties, including the widely popular Basmati and Jasmine types. They demonstrated that badh2.1, was selected as a de novo mutation in *O. sativa* after domestication from its wild progenitor, and presumably after the divergence of the Japonica subpopulations. And showed that fragrant accessions carrying the badh2.1 allele exhibited a dramatic reduction in nucleotide diversity (97%) and elevated linkage disequilibrium around the gene compared to non-fragrant accessions, this is consistent with strong positive selection for the badh2.1 allele. According to the data published by Shi et al. (2014), badh2 allele cannot complement the defect of badh2-E7 and badh2-E2 alleles, but Badh2 allele of non-fragrant rice complemented the defect of the badh2 allele.

The presence of rice varieties exhibiting elevated 2AP levels, but lacking any known non-functional allele of BADH2, raised the possibility that there might be additional fragrance-causing alleles of BADH2 (Fitzgerald et al., 2008). It has been speculated that BADH2 is not the only gene associated with fragrance in rice (Sakthivel et al., 2009).

2.5. Brief genetics of non-fragrance in rice

While recessive allele *fgr* has been demonstrated by various authors mentioned above as been responsible for fragrance in rice, its dominant allele *Fgr* has been identified by Huang et al. (1994), Jin et al. (2003), Chen et al. (2008) to be responsible for inhibiting fragrance in rice. Chen et al. (2008) found that out of the three *Fgr* candidates in the rice genomic region to which *Fgr* was mapped, only Badh2 significantly reduced 2AP content and thus inhibited fragrance in rice.

2.6. Accumulation of 2AP in fragrant rice

It has been revealed that in rice, odour active compounds follow the same accumulation trend throughout plant growth and development, however, only 2AP has different accumulation pattern (Hinge et al., 2016). The genetics of 2AP accumulation are already well understood (Kovach et al., 2009; Bradbury et al. 2005a, 2005b). However, different authors have reported different mechanisms of 2AP synthesis and accumulation in rice. The finding by Hinge et al. (2016), suggests that 2AP was synthesized in leaves and stem sheaths and transported to mature grains. But reports by Mo et al. (2015), Poonlaphdecha et al. (2016), Poonlaphdecha et al. (2012), suggest that 2AP synthesis and accumulation in rice grains happens through the translocation of proline from rice into grains.

In rice, the synthesis and availability of 2AP, as well as other volatile organic compounds are highly variable in different distinct growth phases (Hinge et al., 2016). However, as already explained above, 2AP synthesis has a polygenic aspect that involves a biochemical pathway in which badh2 is supposed to play a critical role since only its predicted non-functionality is associated with fragrance in rice.

It has been demonstrated that in fragrant rice the failure to convert 4-aminobutyraldehyde (AB-ald) into 4-aminobutyric acid (GABA) due to the absence of BADH2 enzymatic activity results in AB-ald accumulation, thereby activating 2AP biosynthesis (Chen et al., 2008). The relative abundance of BADH2 transcript detected in non-fragrant rice varieties supports the notion that BADH2 is physiologically active in non-fragrant rice and a loss of function of this enzyme leads to an increase in 2AP accumulation (Fitzgerald et al., 2008).

Hinge et al. (2016) reported that 2AP content increased during seedling, tillering and booting stages, but it decreased during flowering, only to increase again in milky, dough, and mature grains (with mature grains having the highest level of 2AP accumulation). The authors speculated that the enhancement of 2AP content at or after booting stage could be the reason for the increased level of 2AP contents found in mature grains. Fig. 2 shows the probable biosynthetic pathway of 2AP in fragrant rice cultivars.

2.7. The role of metabolites in 2AP accumulation

Proline, ornithine, and glutamate have all been associated with 2AP biosynthesis in *Bacillus cereus* (Romanczyk et al., 1995). Yoshihashi et al. (2002) also reported that proline, glutamic acid, and ornithine are the precursors of 2AP biosynthesis in rice seedling and callus. 2-AP synthesis has been reported to be positively correlated with an accumulation of Pro (Yoshihashi et al., 1999). L-proline has also been found to enhance the in vitro synthesis of 2-AP in a semi-differentiated callus culture of *Pandanus amaryllifolius* (Thimmaraju et al., 2005). L-proline and hydroxyproline are reported by Suprasanna et al. (1998) as the possible precursors of 2AP formation in Basmati rice callus. At all developmental stages, fragrant rice have higher proline content compared to non-fragrant rice (Hinge et al., 2016). However, the authors demonstrated that their proline content remained constant during vegetative development and increased significantly at flowering and reduced at grain filling and maturity stages.

Hinge et al. (2016), further demonstrated that Δ1-pyrroline-5-carboxylate synthetase (P5CS) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) play a role in 2AP accumulation and reported methylglyoxal as another precursor of 2AP biosynthesis. By speculating that Δ1-pyrolline-5-carboxylic acid could react with methylglyoxal to form 2AP, Huang et al. (2007) also suggested that Δ1-pyrroline-5-carboxylic acid and methylglyoxal might be precursors for 2AP

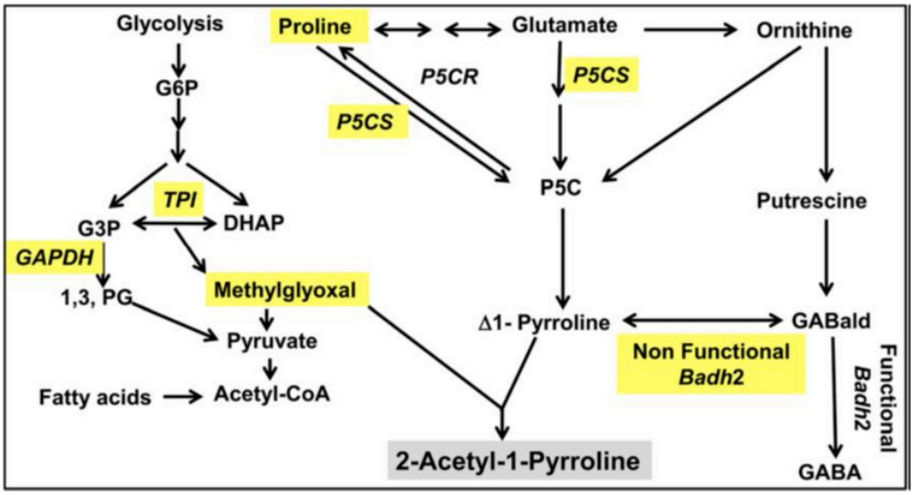

Fig. 2. Biosynthesis pathway of 2AP in fragrant rice cultivars (Hinge et al., 2016). (G6P: Glucose 6 Phosphate; P5CS: Δ1Pyrroline-5 carboxylate synthetase; P5CR: Pyrroline-5 carboxylate reductase; GA-Bald: γ- Amino Butyraldehyde; GABA: γ- Amino Butyric acid. TPI: triose phosphate isomerase; badh2: betaine aldehyde dehydrogenase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; 1,3, PG: 1,3-bisphosphoglyceric acid; DHAP: Dihydroxyacetone phosphate; Acetyl CoA: Acetyl coenzyme A).

biosynthesis. Strong positive correlations have been reported between Methylglyoxal and 2AP accumulation.

Methylglyoxal was accumulated at significantly higher level in fragrant rice compared to non-fragrant rice and were found to be positively correlated with 2AP accumulation in fragrant rice (Hinge et al., 2016). Huang et al. (2008) found that the differential levels of Δ1-pyrolline-5-carboxylic acid measured in fragrant and non-fragrant rice varieties showed positive correlation with the levels of 2AP.

3. Conclusion

As of today, 2AP is the only compound that rice breeders can select to adjust fragrance in rice. Shan et al. (2015) reported the creation of fragrant rice (with high 2AP content) from a non-fragrant variety by using gene editing technologies, sequence-specific nucleases (SSNs) and transcription activator-like effector nucleases (TALENs).

In this review paper, we have attempted to examine most of the published articles on the genetics and biosynthesis of 2AP accumulation in rice. By reviewing and summarising the main results of various researchers, we have been able to elucidate how various genes and metabolites influence 2AP accumulation in fragrant rice. It is our hope that this paper will be of immense benefit to the researchers, who are working on the improvement of rice fragrance.

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Ethics approval and consent to participant

Not applicable.

Consent for publication

The authors whose figures were used in this manuscript granted us the permission to use them.

Conflicts of interest

The authors declare no competing interest.

Availability of data and materials

Not applicable.

CRediT authorship contribution statement

Nnaemeka Emmanuel Okpala: Writing - original draft.

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