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Genetic Regulation of Aroma Gene in Rice

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Introduction

Fragrance in rice counts as one of its most important eating quality traits. There are more than 200 kinds of volatile substances in rice, of which **2-acetyl-1-pyrroline (2-AP)** is one of the main volatile substances in aromatic rice. In traditional rice breeding, chewing method and KOH method are the most commonly used for aroma test in rice. However, these two methods mainly rely on human senses to determine the fragrance, which is poor in accuracy and is difficult to be employed in the screening and breeding of new varieties of rice. Although the genetic basis of aroma in aromatic rice is complex, most researchers think that the fragrance is controlled by a **single recessive gene (*fgr*)**, on the eighth chromosome of the rice genome, which is one gene closely related to the fragrance, and the *fgr* gene has been isolated and cloned at present (Chen *et al.*, 2008). Further studies have revealed that the *fgr* gene encoding **betaine aldehyde dehydrogenase (*Badh2*)** and inhibits the expression of *fgr* gene, knockout *fgr* gene, or *fgr* gene mutation, which will cause the deletion of *Badh2* enzyme function, cause the increase of 2-AP precursor substance, and then accumulate the 2-AP to produce the fragrance in rice grain (Shan *et al.*, 2013). Although important advances have been made in the biochemical metabolic pathways of rice aroma gene, further research is needed on the allelic variation of aroma gene, the number of gene controlling aroma, and the application of aroma genes in the cultivation of new rice varieties.

Genetic Basis of Aroma Gene in Rice

The genetic basis of rice aroma gene has also made great progress indeed, the recessive gene that controls fragrance was located on the chromosome 8 in rice, found that

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the genetic distance between aroma gene and the molecular marker RG28 was 4.5 cM. Though RNAi technology to inhibit the expression of *Badh2* gene, that could also produce fragrance for non-fragrance in rice. These results might confirm that the *Badh2* gene is a recessive gene that controls grain fragrance in rice (Chen *et al.*, 2012). The full length of *Badh2* gene is 1509 bp, which contains 15 exons and 14 introns, encoding 503 amino acids (Shan *et al.*, 2015).

The genome of the aromatic rice showed that there are 2 quantitative trait loci (QTLs) that control the fragrance of rice grain in addition to the *Badh2* gene on the eighth chromosome. They were located on the third and fourth chromosome of rice. The quantitative trait loci on the fourth chromosome could be related to the *Badh1 gene*, while the *Badh1* and *Badh2* belong to the homologous gene, and the *Badh1* gene in rice was also highly homologous to the *Badh1* in the sorghum (*Sorghum Bicolor*) and the barley (*Hordeum Vulgare*) genome (Bradbury *et al.* 2008). Moreover, recently, it has been found that the *Badh1* gene has a significant positive correlation with salt tolerance in rice at the germination stage (He *et al.*, 2015).

Functions and Regulation of Aroma Genes in Rice

Although the aroma of rice grain is made up of a variety of volatile substances, 2-AP is currently the main aromatic compound and 2-AP has a lower odor threshold, easily soluble in ethanol and ether. Numerous previous studies showed that the *Badh2* gene mutation on the eighth chromosome could produce fragrant, while the *Badh2* protein encoded by *Badh2* in non-aromatic rice had the activity of betaine dehydrogenase. The activity of betaine dehydrogenase was lost in the mutation type, and the accumulation of 2-AP resulted in the production fragrant in rice grain (He and Park, 2015; Shan *et al.*, 2015).

In non-aromatic rice varieties, the activity of betaine aldehyde dehydrogenase encoded by dominant *Badh2* allele is highly inhibited, and 2-AP synthesis is inhibited. In aromatic rice varieties, two recessive alleles (*Badh2-E2* and *Badh2-E7*) of *Badh2* are not capable of producing active betaine aldehyde dehydrogenase, which could induce the formation of 2-AP (Chen *et al.*, 2008). The results of radioisotope tracer experiments show that proline is one of the precursors of 2-AP biosynthesis, which provides nitrogen source for 2-AP, and the formation of 2-AP has a significant positive correlation with the accumulation of proline (Yoshihashi *et al.*, 2002). The results also showed that the precursor of gamma aminobutyric

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aldehyde (gamma-Aminobutyraldehyde, GABald) was synthesized by proline and ornithine, through the intermediate 1-pyrroline (delta 1-P), then the 2-acetyl-1-pyrrolinewas synthesized finally (Schieberle, 1990). Betaine aldehyde dehydrogenase not only catalyzed 3-aminopropionic (A-Pald), two methyl sulphur propionic (DMSPald) and sugar beet (Betald), but also catalyzed the synthesis of 2-AP precursors gamma aminobutyral (GABald) (Chen *et al.*, 2015). The content of GABA in non-aromatic rice varieties is much higher than that in aromatic rice. Gamma aminobutyral as the precursor of 2-acetyl-1-pyrroline, may play a key role in the biosynthesis of 2-acetyl-1-pyrroline. In the fragrant rice varieties, the loss of the function of *Badh2* protein may lead to the accumulation of gamma aminobutyral in the body and the transformation of gamma aminobutyral to 1-pyrroline, and the final synthesis of a large number of 2-acetyl-1-pyrrolines (Shan *et al.*, 2015). In non-aromatic rice varieties, *Badh2* protein has the catalytic activity of betaine aldehyde dehydrogenase, which may convert gamma aminobutyric aldehyde into gamma aminobutyric acid in rice grain, while inhibiting the synthesis of 1-pyrrolidine (2-AP precursor), resulting in the failure to synthesize 2-AP (Peng *et al.*, 2017a) (Figure 1). In rice, ornithine and proline could be used as precursors for the synthesis of gamma aminobutyral. The regulatory network of this synthesis process and the regulatory relationship between them and betaine aldehyde dehydrogenase are not yet clear. The complex regulatory relationship between ornithine, proline, gamma aminobutyral and 1-pyrrolidine still needs further research.

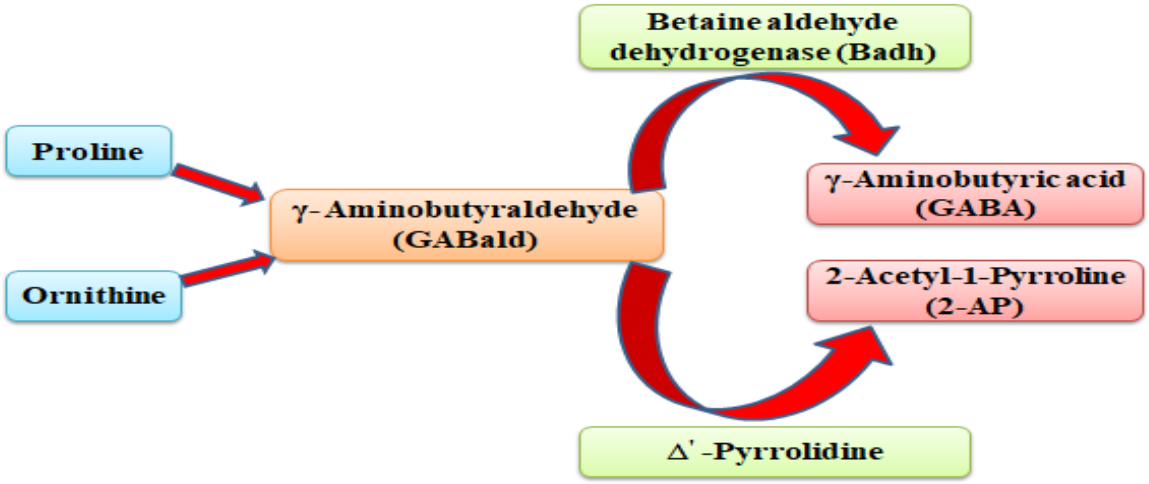

Figure 1. Possible synthesis pathway of 2-acetyl-1-pyrroline (2-AP) in rice (Peng *et al.*,2018)

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Application of Aroma Gene in Rice Breeding

Since the aroma gene was mainly controlled by a recessive gene, the transfer of this gene by traditional breeding is time-consuming and laborious to develop new varieties with a unique aroma. The development of molecular markers, especially the functional markers of *Badh2* gene, greatly accelerated the process of screening and breeding of new varieties of aromatic rice, and many varieties of aromatic rice have been approved and have been widely used in production. Early studies found that molecular markers closely linked to the *frg* gene (eg, RG28, SCU015RM, and RSP04) could distinguish between aromatic rice and non-aromatic rice.

Transgenic technology is also applied and promoted in the cultivation of new aromatic rice varieties. The utilization of RNAi mediated *Badh2* gene silencing could also transform non-aromatic into aromatic rice varieties (Peng *et al*., 2017), but this technique often does not completely inhibit the expression of *Badh2* gene and needs to be screened in a large number of transgenic progeny plants. Genome editing technology in the recent era brings wide opportunities through transcriptional activator effect factor nuclease technology, zinc finger nuclease technology and cluster law interval short palindromic repetition technology for crop genetics and breeding. Any insertion, deletion or replacement of *Badh2* gene will lead to premature termination codon or encoded amino acid change or even non coding corresponding *Badh2* protein in rice, which could make non-aromatic rice produce fragrance. Therefore, the loss function of *Badh2* gene could promote the synthesis and accumulation of 2-AP, and it is conceivable that any mutation that causes the loss of *Badh2* gene function will lead to the emergence of one new aroma gene. Then, using TALEN technology to knock out the *Badh2* gene, one non-aromatic rice variety could be transformed into aromatic rice varieties (Shan *et al*., 2013), and the TALEN technology could also be used to create a genetically homozygous mutant of aromatic rice plant by knock out the *Badh2* gene in non-aromatic rice and quickly produce the corresponding aromatic rice varieties of high quality and high yield rice.

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