Studies on Aroma Gene and its Application in Rice Genetics and Breeding

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Abstract

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, and the staple food for more than half of the world's population. Along with the sustained development of living standards, people's demand for high quality rice is increasing. As one of the types of cultivated rice, aromatic rice is preferred by most of the consumers because of its agreeable scent. With the rapid development of rice functional genomics and sequencing technology, great progress has been made in understanding the aroma gene in rice, and a series of functional markers has been developed for screening the aroma gene and breeding new rice varieties in recent years. This paper mainly reviews the progress in the genetic basis, gene function and regulation of the aroma gene, and the application of aroma gene functional markers in the genetic improvement and breeding of aromatic rice. Thus, it provides important references for the cultivation of new varieties of aromatic rice.

Keywords: Aroma gene, application, genetic breeding, aromatic rice

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, providing about 25% of energy to more than 3 billion people (Tian et al., 2009; Peng et al., 2017a). For most Southeast Asians, rice supplies more than 35% of their energy requirement (Peng et al, 2014a; Li et al., 2015). Fragrance in rice counts as one of its most important eating quality traits (Tang et al. 2009). These varieties are regarded as the rare types in the market (Bradbury et al., 2005a; Zhao et al, 2009; Myint et al., 2012; Daygon et al., 2016). The traditional rice varieties with poor resistance and low yield are difficult to be popularized in large areas (Bai et al, 2009; Du et al, 2009). However, the market price of aromatic rice is higher; and its demand has increased significantly in recent years (Shao et al., 2013; Shi et al., 2014; Peng et al, 2016a). Consequently the aroma gene research and its application in genetic breeding have been widely investigated by geneticists and rice breeders.

In the second half of the 20th century, Although, the rice yield was significantly increased, rice production has been stagnating since the 1980s. At present, different factors such as environmental pollution, water shortage and

frequent occurrence of extreme weather affect not only the growth and final grain yield of rice, but also affect seriously its quality (Stocker et al., 2013; Zhao and Fitzgerald, 2013; Goufo et al., 2014; Halford et al., 2014). With the continuous growth of the world population and the steady improvement of living standard, traditional rice breeding has been unable to meet the strong demand for high-quality and high-yield rice (Peng et al, 2016b). Finding new breeding strategies and methods (such as marker assisted selection, whole genome breeding, molecular design breeding and transgenic breeding) will make up for the shortage of traditional rice breeding so as to achieve breakthroughs in rice yield and synchronous improvement of rice quality. Great progresses have been made in the genetics and breeding of aromatic rice, including the isolation and cloning of aroma genes, and functional analysis (Shan et al., 2013, 2015; He and Park et al., 2015; Yan et al., 2015). At the same time, new technologies and breeding strategies have begun to be applied in the breeding of aromatic rice (Shan et al., 2015). Therefore, to clarify the molecular genetic mechanism of aroma gene, and new breeding strategies and methods such as molecular marker-assisted selection breeding, all of these will provide important information for the cultivation of new varieties of aromatic rice.

There are more than 200 kinds of volatile substances in rice (Mahattanatawee and Rouseff, 2014; He and Park, 2015), of which 2-acetyl-1-pyrroline (2-AP) is one of the main volatile substances in aromatic rice (Mathure et al., 2014; Mahattanatawee and Rouseff, 2014). At present, numerous methods have been used to detect the aroma of rice materials. Among them, the chewing method and KOH method are the most commonly used in the traditional rice breeding process (Bradbury et al., 2005b; Liu et al, 2014; Yan et al, 2015). 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. Therefore, how to identify scent in aromatic rice simply, accurately and quickly has always been one of the problems that rice breeders urgently need to solve (Yan et al., 2015). 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 rice genome, which is one gene closely related to the fragrance, and the *fgr* gene has been isolated and cloned at present (Bradbury et al., 2005a; Chen et al., 2006; Chen et al., 2008). Further studies have revealed that the *fgr* gene encoding betaine aldehyde dehydrogenase (Badh2). 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 (Niu et al., 2008; Shan et al., 2013). However, 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. In this paper, we have reviewed the recent progress made in the study of aroma gene in rice, and the development and application of functional markers of aroma gene, which will provide important references for the cultivation of new varieties of high quality aromatic rice.

2. Genetic Basis of Rice Aroma Gene

Aromatic rice has been favored by the consumers, because it has a unique aroma in rice, at home and abroad (Kovach et al., 2009; Shan et al., 2015). In addition, 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, in 1992 by researchers and they found that the genetic distance between aroma gene and the molecular marker RG28 was 4.5 cM (Ahn et al., 1992). Since then, a number of research teams also detected the recessive gene controlling rice fragrance using different genetic populations and different types of molecular markers near the RG28 (Jin et al., 1995, 1996; Lorieux et al., 1996; Dong et al., 2001a, 2001b). For instance, Zhang Tao et al. (2008) used aromatic rice and *indica* non-fragrant rice as experimental materials, and located the aroma gene in the region of approximately 252 kb between 20 175 367 bp-20 386 172 bp on chromosome 8. Similarly, Wanchana et al (2005) had located the aroma gene *fgr* between the molecular markers RM223 and RM342. And further studies revealed that the difference between aromatic rice and non-aromatic rice was due to the two molecular markers on the gene encoding betaine dehydrogenase (BADH2).

There was a significant difference in the sequence of *Badh2* gene between the aromatic rice and the non-aromatic rice after sequencing the *fgr* region. Indeed, after map-based cloning and sequencing of the *fgr* region, it was found that there was a significant difference in the sequence of *Badh2* gene between aromatic rice and non-aromatic rice, and there was a mutation in aromatic rice in the 7th exon region of *Badh2* gene, which leading to the loss function of *Badh2* protein. Therefore, *Badh2* gene is likely linked to *fgr* gene, which control the rice fragrance (Bradbury et al., 2005a). In order to verify the function of the *Badh2* gene, 3 candidate genes in the *fgr* region were simultaneously transformed into a complementary transformation experiment. The results showed that the fragrance of the two candidate genes, *Cah* and *Mccc2,* did not change significantly in the rice before or

after the transgene, however, in the transgenic single plant of the *Badh2* gene, the fragrance was changed obviously. Though, when using 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., 2006, 2008, 2012; Niu et al., 2008).

The full length of *Badh2* gene is 1509 bp, which contains 15 exons and 14 introns, encoding 503 amino acids (Chen et al., 2008; Shan et al., 2015). Comparing the aromatic rice and non-aromatic rice, there were many kinds of mutations in *Badh2* gene among different rice varieties. For example, in aromatic rice varieties, it is common that there is an 8 bp deletion and 3 single nucleotide polymorphism sites (SNPs) in the 7th exon of *Badh2* gene (Bradbury et al., 2005a). In different varieties of aromatic rice, there is a 7 bp deletion in exon 2 of the *Badh2* gene and a 803 bp deletion between exon 4 and exon 5 (Shi et al., 2008; Kovach Et al., 2009; Shao et al., 2011), and there are variation sites in exons 1, 10, 13 and 14 (Shi et al., 2008; Amarawathi et al., 2008; Kovach et al., 2009). Further studies have found that the splice sites of the first exons and first introns of the *Badh2* gene, the promoter region, and the 5 'UTR region also have insertion, deletion, or single nucleotide mutation sites (Shao et al., 2011, 2013; Shi et al., 2014; Ootsuka et al., 2014). The difference in aroma of rice grain is largely determined by allelic variation of the *Badh2* gene. The aroma related rice varieties often include the deletion of seventh exon 8 bp and 3 SNPs, or the deletion of 7 bp in the second exon (Shi et al., 2008; Shao et al., 2013). Therefore, when *Badh2* gene is mutated in coding region or regulatory region, it could produce betaine dehydrogenase which has no biological activity, and cause rice to produce fragrance.

In order to further analyze the genetic characteristics of the rice aroma gene, the whole genome analysis of the aroma gene of the Barth Marty aromatic rice was analyzed. It was found that in the genome of the aromatic rice, there were 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 (Amarawathi et al., 2008). 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.2005a, 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). The mutation of the *Badh1* gene was also linked to the aroma in rice grain, but the effect is far less than the *Badh2* gene (Amarawathi et al., 2008). Therefore, the relationship between the *Badh1* gene and fragrance in rice grain need further study.

In different rice varieties, 18 bp deletion and 3 single nucleotide polymorphisms were found in the seventh exon of the *Badh2* gene, and the same mutation occurred in the seventh exon of the *Badh2* gene in the non-aromatic rice varieties (Fitzgerald et al., 2008). In another hand, the analysis of 280 wild rice varieties and 242 cultivated rice varieties from 38 countries and regions in Asia showed that there were 8 kinds of haplotypes without functional activity in *Badh2* gene. The *Badh2* gene derived from rice material similar to the genetic background of *japonica* rice. The aroma gene in the existing *indica* aromatic rice might be imported from *japonica* rice varieties and constantly selected by artificial selection (Kovach et al., 2009). Studying wild rice varieties, the research group found that there was also a type of *Badh2* gene with seventh exons in the common wild rice varieties in Asia, and the *Badh2* gene of these missing or mutant types were relatively less in wild rice varieties (Prathepha, 2009), and they could not be translated into normal translation, but they could lead to the *Badh2* gene. It performs efficient transcription and translation (Chen et al., 2008), and then inhibits the fragrance in rice grain. Previous studies showed that the aroma gene in the existing cultivated rice varieties originated from the earliest wild rice varieties, followed by a long period of artificial selection and domestication (Kovach et al., 2009; Prathepha, 2009; Shao et al., 2013; He et al., 2015). In the process of artificial selection and domestication, aroma gene is likely to produce a range of variations, leading to a large difference in the conclusion of different research groups. In regard of this fact, most researchers believed that the fragrance inheritance is controlled by a single recessive gene (Shao et al., 2013; He et al., 2015), while some others believed that the aroma of aromatic rice is inherited from a multi recessive basis. However, according to the decision resulting from studies such as (Tsuzuki and Shimokawa, 1990), some scholars believed that fragrant inheritance is controlled by some main-effective genes and micro-effective genes (Xu and Mo, 1995), and several QTLs controlling the fragrant in rice grain (Lorieux et al., 1996; Amarawathi et al., 2008). In conclusion, the genetic basis of rice aroma gene is complex, and the gene controlling rice fragrance are 1 pair, or 2 to 4 pairs or more, all of these may be due to: (1) The different genetic basis of different fragrant types (such as popcorn, jasmine, violet and hickory) varieties; (2) There are interactions between aroma genes and various environmental factors (light, temperature and soil fertility); However, in the present the diversity of the fragrant components in rice grain, the methods and techniques for identification of different types of fragrant still not perfect yet.

3. Function and Regulation of Rice Aroma Genes

Although the aroma of rice grain is made up of a variety of volatile substances, 2-AP is currently the main aromatic compound (Paule and Powers, 1989; Kovach et al., 2009), and 2-AP has a lower odor threshold, easily soluble in ethanol and ether. The aromatic rice from India and Thailand markets belong to *indica* aromatic rice varieties, and the 2-AP provides for its unique flavor. 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 (Kovach et al., 2009; He and Park, 2015; Shan et al., 2015). Further studies have found that there are multiple transcriptional starting sites in the *Badh2* gene. In non-aromatic rice, although the *Badh2* has 3 transcriptional transcripts with different lengths, only the *Badh2* gene transcriptional level with a total length of 1509 bp is higher than the other alleles. In the aromatic rice varieties, the main transcriptional starting site is located at downstream of the *Badh2* gene initiation codon, and the transcription of the *Badh2* gene is severely inhibited in the aromatic rice varieties (Chen et al., 2008). So far, although the loss of *Badh2* gene function has been proved to lead to the production of 2-AP, how does Badh2 protein regulate the synthesis process of 2-AP? 2-AP biosynthesis pathway and related regulatory network and regulatory mechanism are not clear in the current status.

In view of 2-AP biosynthesis pathway and its regulatory network, different research groups have carried out a number of related researches. 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 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) (Trossat et al., 1997; Chen et al., 2008; 2015). In rice, Badh2 protein could catalyze gamma aminobutyric acid to be gamma aminobutyric acid (gamma-Aminobutyric acid, GABA) (Shan et al., 2015), and the content of GABA in non-aromatic rice varieties is much higher than that in aromatic rice. In view of the biological function of betaine aldehyde dehydrogenase, there is a big discrepancy between the results obtained by different research groups. It is considered that betaine aldehyde dehydrogenase can inhibit the synthesis of 2-acetyl-1-pyrrolidine (Chen et al., 2008), and the results showed that betaine aldehyde dehydrogenase was involved in the decomposition of 2-acetyl-1-pyrrolidine (Trossat et al., 1997), and researchers believed that there was a competitive relationship between the precursor of betaine aldehyde dehydrogenase and 2-acetyl-1-pyrrolidine (Bradbury et al., 2005a). Betaine aldehyde dehydrogenase plays an important role in the biosynthesis of 2-acetyl-1-pyrrolidine, but how does betaine aldehyde dehydrogenase play its role in the synthesis of 2-acetyl-1-pyrrolidine, and whether other substances are involved in the regulation and control process are still unclear, thus further study is still needed.

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.
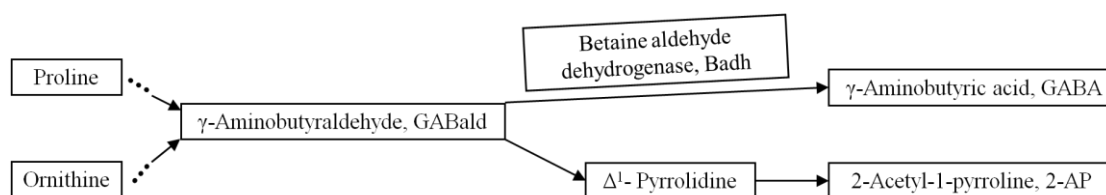
Figure 1. Possible synthesis pathway of 2-acetyl-1-pyrroline (2-AP) in rice

By comparison and analysis of the sequencing results of aroma gene in rice, most researchers believed that the loss of the second or seventh exon fragments of the *Badh2* gene leads to a large accumulation of 2-AP (Kovach et al., 2009), and the fragrant is produced. In fact, the 2-AP content of some rice varieties is higher, but there is no *Badh2* allele, which suggests that there may be other non *Badh2* alleles that could also cause fragrant (Amarawathi et al., 2008). Most researchers pay attention to the difference exons between aromatic and non-aromatic rice varieties, in addition to the exon of gene in eukaryote, intron also play an important role in gene expression and regulation (Fu et al., 1995; Jeon et al., 2000). By sequencing the *Badh2* in aromatic rice, the results showed that there was no mutation in the coding chain of the aromatic rice, neither of the seventh exon mutation nor the second exon mutation type, but the nucleotide sequence in the second and fourth intron region of the *Badh2* gene had been mutated (Xu et al., 2011). There were 86 aromatic rice among the 323 rice germplasm resources, of which 80 were mutational types of seventh exon (Zhang et al., 2015). Further, the *Badh2* gene of the other 6 aromatic rice germplasms was cloned and sequenced, the results showed that the *Badh2* gene sequence from the 6 aromatic rice varieties was complete. Therefore, the fragrant characters of rice grain may also be regulated by other genes or introns.

4. Development of Functional Markers of Rice Aroma Genes

Since the *Badh2* gene was isolated and cloned, more than a dozen mutation sites have been found in *Badh2* (Kovach et al., 2009; Shao et al., 2013; He and Park, 2015, He et al., 2015), and a series of molecular markers are designed for these loci, which could be used for the identification of aroma gene, the selection of different aromatic rice varieties and the cultivation of new varieties of aromatic rice. Bradbury et al (2005b) used the *Badh2* gene to have 8 bp deletion and 3 SNPs in seventh exon, and primers were designed for PCR amplification to identify whether the *Badh2* allele is a type of aromatic rice. Then, new molecular markers YY5-YY8 were designed for the rapid detection and analysis of aromatic rice (Yan et al., 2015). By sequenced 24 aromatic rice varieties and 10 non-aromatic rice varieties, Shi et al (2008) found that there had 18 bp deletion and 3 SNPs mutation sites at seventh exon of the *Badh2* in 12 aromatic rice varieties. In addition, a new *Badh2* allele with no biological function was also found. The allele had the same mutation at exon 7, but a 7-bp deletion site was found at exon 2. For these variant regions of two exons, multiple pairs of molecular markers were designed for PCR detection analysis (Shi et al., 2008). Subsequently, in the detection of *Badh2* gene mutations at exon 7 and exon 2, the primer design was simplified, and only one pair of primers was needed for detection aroma gene by molecular marker (Wang F et al., 2008; Wang J et al., 2008). Through gene sequencing and sequence analysis, it was found that there might be a loss of 803 bp between the fourth and fifth exons of the *Badh2* gene, and one marker FMbadh2-E4-5 was developed to detect the deletion of the new site (Shao et al., 2011). In the study of aromatic rice varieties, the presence of a single-nucleotide mutation at exon 13 of the *Badh2* gene resulted in the generation of aromas in rice grain, and the mutation site was located at the splice site of the intron and exon. At this point, a new molecular marker was also designed (Ootsuka et al., 2014). According to the sequencing analysis of 295 different rice varieties, some aromatic rice varieties have a 3 bp deletion at the 12th exon and a 3 bp insertion at the 13th exon of *Badh2* gene. Thus the functional molecular marker FME12-3 was designed and verified using two F_2 genetic groups (He and Park, 2015). The development of a series of molecular markers of aroma gene has brought great convenience for the identification of rice aroma gene, screening of different aromatic rice varieties, and cultivation of new aromatic rice varieties. At the same time, these molecular markers will speed up the process of genetic breeding, popularization and application of aromatic rice.

Table 1. Development of functional markers for *Badh2* gene in rice (He and Park, 2015)

Functional markers	Primer sequence (5'-3')	Annealing temperature (℃)	PCR production size (bp) Non-fragrant/ fragrant
FMU1-2	F: TCCCACCACCACTCCACA	61	163/160
	R: ACGAAGAGCTGCCGCTGC		
FME2-7	F: ACGAAGAGCTGCCGCTGC	61	78/71
	R: GCGATTGCGCGGAGGTACT		
FME7	F: TCCTGTAATCATGTATACCC	50	151/143
	R: AATTTGGAAACAAACCTT		
FME12-3	F: TTGGTCCAGTGCTCTGTGTG	58	192/189
	R: GCACCAGCCAGACCATAAC		
FME13	F: TTGGTCCAGTGCTCTGTGTG	58	192/195
	R: GCACCAGCCAGACCATAAC		
FME14	F: TCGATGCCGGAATTATCTGGGTGA	61	60, 205/266
	R: TCCCCACGGCTCATCGGAGG		

At present, there are at least 17 mutation sites have been discovered in the *Badh2* gene, and these sites are located in the 5'-UTR region, exon 1, exon 1 and intron 1 junction, exons 2, exons 4 and 5, exons 7, 8, 10, 12, 13 and 14 of the *Badh2* gene (He and Park, 2015). For these mutant loci, many pairs of molecular markers have been designed at the first exon, intron 1, exon 2, 4, 5, 7, 12, 13 and 14 of the *Badh2* gene, which could also be used to identify the mutation type of the *Badh2* gene (Bradbury et al., 2005a, 2005b; Shi et al., 2008; Kovach et al., 2009; Shao et al., 2011; Ootsuka et al., 2014; He and Park, 2015). There are also multiple variant sites in the exons 1, 2 and 10 of the *Badh2* gene, but there is no direct correlation between them and the aroma of rice, this indicates that most of these mutations are synonymous mutations, or the mutations that change the sequence of Badh2 protein do not affect the function of Badh2 protein. Based on the above molecular markers, six functional markers of rice aroma gene were further developed (Table 1) and verified by segregating populations (Shi et al., 2008; Kovach et al., 2009; Ootsuka et al., 2014; He and Park, 2015; Peng et al., 2017b). Among them, the functional marker FMU1-2 could not only detect the 8 bp deletion mutation of *Badh2* gene in the 7th exon, but also could detect whether there is an 8 bp deletion in the 5'-UTR region of *Badh2* gene. The functional markers FME2-7, FME7, FME12-3, FME13, and FME14 were used to identify the mutation types of the fragrant gene in the exons 2, 7, 12, 13, and 14 of *Badh2*, respectively. The functional marker FME14 belongs to the cleaved amplified polymorphic sequence, and the detection of PCR products requires the use of restriction endonuclease *Bsl* I. Therefore, the development of these functional markers of rice aroma gene provides an accurate, rapid and effective method for molecular breeding of aromatic rice.

Application of Aroma Genes in Rice Genetics and Breeding

At present, the major techniques in rice breeding are still conventional cross-breeding, and for aromatic rice, it is time-consuming and laborious to cultivate new varieties using the traditional breeding methods (Shan et al., 2015). Since the aroma gene was mainly controlled by a recessive gene, the traditional breeding must first introduce the *Badh2* mutant gene into the existing elite varieties through hybridization and backcrossing, and then screen the individuals in the offspring population, and it is difficult to quickly identify the process at the seedling stage. However, rice breeders in China have done a lot of researches on the cultivation of high-quality, high-yield, multi-resistance fragrant rice varieties, and screened a series of aromatic rice restorer lines, sterile lines, and new hybrid combinations (Huang et al., 2006 Hou et al., 2007; Liu et al., 2008; Jiang et al., 2008). With the development of molecular markers, especially the functional markers of *Badh2* gene, molecular marker assisted selection has been widely used in rice genetics and breeding, which has 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 (Garland et al., 2000; Cordeiro et al., 2002; Jin et al., 2003). With the further study of the function of the *Badh2* gene, a series of specific primers (functional markers) were designed for the interior of the *Badh2* gene for the screening of fragrant gene and the breeding restoration lines of aromatic rice (Bradbury et al., 2005b; Shi et al., 2008; Wang F et al., 2008; He and Park, 2015; Yan et al., 2015; Xu et al., 2015; Du et al., 2009). In addition, transgenic technology is also applied and promoted in the cultivation of new aromatic rice varieties (Niu et al., 2008; Chen et al., 2012). The utilization of

RNAi mediated *Badh2* gene silencing could also transform non-aromatic into aromatic rice varieties (Chen et al., 2012; Peng et al., 2017a), 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. At the same time, the use of RNAi mediated transgenic technology to cultivate new aromatic rice varieties also faced with the risk assessment of late transgenic and strict supervision, and the promotion and application of new varieties of transgenic rice are still faced with many problems.

In recent years, genome editing technology has developed rapidly, including transcriptional activator effect factor nuclease technology, zinc finger nuclease technology and cluster law interval short palindromic repetition technology have been applied to crop genetics and breeding (Voytas and Gao, 2014). 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 (Bradbury et al., 2005a; Shi et al., 2008; Kovach et al., 2009). 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 (Shan et al., 2015). Thus, the TALEN technology could be used to knock out the *Badh2* gene in non-aromatic rice (Shan et al., 2015; Birla et al., 2017), and quickly produce the corresponding aromatic rice, which greatly accelerates the breeding process of the new varieties of high quality and high yield rice.

5. Prospect of the Application of Aroma Gene in Rice Breeding

Fragrance is one of the most important traits in rice genetics and breeding, and the consumption demand of high-quality rice will continue to increase. Therefore, the breeding of new aromatic rice varieties and the research of aroma genes are attracting more and more attention from rice genetics and breeding scientists and plant scientists. With the rapid development of rice functional genome, it will become clear for the genetic basis, function and regulation of aroma genes in rice. In the last decade, the gene sequencing technology has developed rapidly, and a large number of rice varieties and aromatic rice germplasm resources from different sources have been sequenced, thus a series of mutation sites and alleles have been identified (He and Park, 2015, He et al., 2015). According to these mutation loci, the functional markers of aroma gene have also been developed, and have been widely applied in aroma gene screening and breeding of elite parents of hybrid rice. Although the aroma gene functional markers have been used for the breeding of aromatic rice restorer lines (Du Xueshu et al., 2009), and many aromatic rice varieties have been approved and widely used in production, the main problem in the process of aromatic rice genetics and breeding is still very few restorer lines with good scent type, which slows down the genetic breeding process of aromatic rice.

At present, not only the aroma gene is used to cultivate new aromatic rice varieties, but also the corresponding aroma genes are discovered in other crops and applied to the genetic breeding of major grain crops. For example, a 2 bp deletion in exon 10 of the *GmBADH2* gene was located on chromosome 6 will cause the gene to prematurely terminate the translation process in *Glycine max*, thereby producing a fragrance (Juwattanasomran et al., 2011, 2012). 2-AP could be synthesized in large quantities if the *GmBADH2* gene was silenced in soybean, and fragrant soybeans could be produced immediately (Arikit et al., 2011). At the same time, studies in sorghum found that the *SbBADH2* gene is a recessive gene that controlled the fragrant in *Sorghum* (Yundaeng et al., 2013).The history of domestication of sorghum, rice, and soybeans is not the same, but the fragrant of the three crops is dominated by a single recessive gene (Murty et al., 1982; He and Park, 2015), in which the aroma gene is mutated and the production of these crop aromas is closely related, and the recessive gene controlling the fragrant have a certain degree of homology (Arikit et al., 2011). Although the loss of aroma gene in rice, sorghum and soybeans does not affect their survival, it will play an active role in drought conditions.

With the rapid development of genome editing technology, sequence-specific nucleases could be widely used for gene knockout of various plants, including the important crops such as rice, maize, wheat, barley and soybean (Li et al., 2012; Wendt et. al., 2013; Haun et al., 2014; Liang et al., 2014; Wang et al., 2014). Among them, TALEN technology has also been applied to the genetics and breeding of aromatic rice (Shan et al., 2013, 2015; Birla et al., 2017). These new techniques provide a new strategy for rice breeders to cultivate aromatic rice varieties. The utilization of SSNs (including TALEN, ZFN and CRISPR) for molecular design breeding is superior to the traditional breeding and genetically modified (RNAi or genetic engineering) breeding：(1) The target genes could be accurate edited. (2) No need for hybridization and backcross process, which will save a lot of time and convenience. (3) A single plant without screening markers can be obtained (Shan et al., 2015; Peng et al., 2017a). Therefore, there will be more and more new technologies applied to the genetic breeding of

aromatic rice, such as TALEN technology, ZFN technology, and CRISPR technology, and these new technologies will accelerate the cultivation of new aromatic rice varieties with high-quality, high-yield and multiple resistance.

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