Chapter

Rice Aroma: Biochemical, Genetics and Molecular Aspects and Its Extraction and Quantification Methods

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Abstract

Aroma in rice is unique and a superior grain quality trait, varieties especially Basmati and Jasmine-type are fetching a high export price in the International markets. Among the identified volatile aroma compounds, 2AP (2 acetyl-1-pyrroline) is believed to be the distinctive biochemical compound contributing the flavor in rice. Genetically, aroma in rice arises by the phenotypic expression of spontaneous recessive mutations of the *OsBadh2* gene (also known as *fgr*/*badh2* /*osbadh2*/*os2AP* gene) which was mapped on chromosome 8. An 8-bp deletion in the exon 7 of this gene was reported to result in truncation of betaine aldehyde dehydrogenease enzyme whose loss-of-function lead to the accumulation of a major aromatic compound (2AP) in fragrant rice. Among the different sampling methods and analytical techniques for the extraction and quantification of scentedness, simultaneous distillation extraction (SDE) is traditional and normalized, whereas solid-phase micro extraction (SPME) and supercritical fluid extraction (SFE) are new, very simple, rapid, efficient and most importantly solvent-free methods. These methods are coupled with Gas Chromatography–Mass Spectrometry (GC–MS), Gas Chromatography-Flame Ionization Detector (GC-FID) and/or Gas chromatography olfactometry (GC-O) and also with sensory evaluation for readily examining 2AP compound found in rice. The major factor affecting the aroma in rice was their genetic makeup. However, the aroma quality may be differed due to different planting, pre-harvest and postharvest handling and storage. For a more extensive elucidation of all effective and fundamental factors contributing to fragrance, it is essential to explore target quantitative trait loci (QTLs) and their inheritance and locations.

Keywords: aromatic rice, 2-acetyl-1-pyrroline, *fgr*, *badh2*, evaluation methods, affecting factors

1. Introduction

Rice (*Oryza sativa* L.) is a dietary staple food crop and the grain being consumed by atleast 50 per cent of the world's population [1]. It had a decisive role in food, security and in improving the livelihood of people. With the continuous/marked

improvement in standard of living of the people, the ethnic preference of rice is under conversion which increases the demand for superior fine quality rice. Most of the scented rice are inferior in agronomic performances and highly prone to environmental variations [2] yet it paves much attention for their first-rated aroma. The origin and evolution of the aroma gene betaine aldehyde dehydrogenase (*BADH2*) remains unclear but the haplotype analysis firmly establishes a distinct origin of the *badh2.1* allele within the Japonica varietal group [3] and the centre of origin is considered to be the Himalayan foothills in the Indian subcontinent from where it spreads to various parts of the world [4].

It was reported that rice aroma was controlled by single recessive nuclear gene in rice [5, 6]. The biochemical analysis of rice grain reveals the presence of numerous volatiles in fragrant rice revealing the major aromatic compound, 2-acetyl 1-pyrroline (2AP). Recent advances had also discovered the biochemical pathway for biosynthesis of 2-Acetyl-1-pyrroline (2AP) from different types of amino acids and polyamines [7]. Based on the rice genome sequence information [8], the *OsBadh2* gene (present on chromosome 8) is identified as a candidate gene for aroma, which is the most important aroma gene till now. However, several other genes and locus had been reported to be the contributor for aroma [9, 10].

Rice aroma quality evaluation is quite complex due to the complex interaction of numerous volatile compounds, and many affecting factors during planting and processing. With the rapid advancement in the instrumentation and sampling methods for the isolation and determination of 2AP concentration levels it is possible to analyze compounds even at very low concentrations (ppb levels) [11]. This chapter gives insights into the flavor chemistry, the progresses pertaining to the genetic and molecular understanding of fragrance, various extraction, quantification methods and their interaction with genetic and non-genetic factors in rice.

2. Fragrant rice germplasm and varieties

Scented rices had been grouped into small, medium and long grained types based on grain length and could be categorized by their scentedness as mild and strong aromatic types. Broadly, the aromatic rice germplasm are grouped into three categories *i.e*. the Basmati, jasmine, and non-basmati/jasmine typed scented rice. The word 'Basmati' has its origin from two Sanskrit roots (vas = aroma) and (mayup = ingrained or present from the beginning) making the word vasmati and it has been pronounced as Basmati in course of time. Of the largest aromatic germplasm maintained at IRRI, about 86 had its root word as Basmati irrespective of grain dimensions and intensity of aroma [12].

A number of non-Basmati scented rices had been considered superior to Basmati in one or more characteristics like flavor, texture, linear elongation ratio on cooking, taste etc. Moreover, many of them can be cultivated under conditions and in areas where Basmati cannot be. Small-grain Bindli, for example, is superior to Basmati in aroma, grain elongation, taste and digestibility (as perceived by the farmers) and it performs well under water-logged conditions. A few such potential candidates could be Kalanamak, Tilakchandan, Sakar-chini and Dhania (U.P.), Ambemohar (Maharastra), Badshahbhog (Bihar and West Bengal), Bindli (waterlogged conditions of U.P.), Chakhao (Manipur), Madhumalti and Mushkan (H.P.), Kon-Joha - 1, Raja Joha and Krishna Joha (Assam), Randhuni Pagal (W.B.), Vishnubhog and Dhubraj (H.P.), Katarani and Sonachur (Bihar) [13]. The small and medium grain aromatic rices could be explored further and improved by selecting short stature, better yielding and early maturing plant types in order to develop varieties to be cultivated in non-traditional areas of basmati cultivation.

The scented rices are mainly cultivated and consumed in India, Pakistan, Thailand, Bangladesh, Afghanistan, Indonesia, Iran and United States. Of these, the major exporter of fine-grained fragrant rices includes India, Pakistan and Thailand Major aromatic rices of different states of India were presented in **Table 1** [12].

States	Small grain	Medium grain	Long grain
Southern zone			
Andhra Pradesh	—	Jeeragasambha	—
Kerala	Jeerakasala, Gandhkasala	—	—
Karnataka	—	Kagasali	—
North eastern zone			
Assam	Bengoli Joha, Bhaboli Joha, Bhugui, Boga Joha, Bogamanikimadhuri, Boga Tulsi, Bogi Joha, Bokul Joha, Borjoha, Borsal, Cheniguti, Chufon, Goalporia Joha-1, Goalporia Joha-2, Govindbhog, Joha Bora, Kaljeera, Kamini Joha, Kataribhog, Khorika Joha, Kola Joha, Koli Joha, Kon Joha-1, Kon Joha-2, Krishna Joha, Kunkuni Joha, Manikimadhuri Joha, Ramphal Joha, Ranga Joha	—	—
Manipur	—	Chahao Amubi (black scented rice), Chahao Angangbi (pink/red scented rice)	—
Eastern zone			
Bihar	Badshahbhog, Deobhog, Karia Kamod, Katami, Shyam Jeera, Kanak Jeera, Kanakjeeri, Badshapasand, Mircha, Brahmabhusi, Ramjain, Kamina, Dewta Bhog, Tulsi Pasand, Chenaur, Sona Lari, Sataria, Tulsi Manjari.	Gopalbhog, Champaran Basmati (Lal), Champaran Basmati (Kali), Champaran Basmati (Bhuri), Bhilahi Basmati, Amod, Abdul, Bahami, Kalanamak, Kesar, Sonachur	Baikani
West Bengal	Badshabhog, Chinisakkar, Danaguri, Gandheshwari, Kalo Nunia, Kataribhog, Radhuni Pagal, Sitabhog, Tulai Panji, Tulsibhog	Kanakchur, Katanbhog	—
Northern zone			
Haryana	—	—	Basmati 370, Khalsa 7, Taraori Basmati, Pakistani Basmati
Punjab	—	—	Basmati 370, Basmati 385, Pakistani Basmati
Rajasthan	—	—	Basmati (local), Basmati 370

States	Small grain	Medium grain	Long grain
Himachal Pradesh	—	Achhu, Begmi, Panarsa local	Baldhar Basmati, Madhumalti, Chimbal Basmati, Mushkan, Seond Basmati
Central zone			
Madhya Pradesh	Chinore, Dubraj, Kalu Mooch, Vishnubhog, Tulsi Manjari, Badshabhog,	Chatri, Madhuri, Vishnu Parag	Laloo
Uttar Pradesh	Adamchini, Badshapasand, Bhanta Phool, Bindli, Chhoti Chinnawar, Dhania, Jeerabattis, Kanak Jeeri, Laungchoor, Moongphali, Rambhog, Ramjawain, Sakkarchini, Tinsukhia, Bengal Juhi, Thakurbhog, Yuvraj, Bhantaphool	Karmuhi, Kesar, Parsam, Sonachur, Tilak Chandan, Kesar, Kalanamak, Vishnuparag	Basmati 370, Dehraduni Basmati, Type 3, Hansraj, Nagina 12, Safeda, Vishun Parag, Kala Sukhdas, Lal Mati, Tapovan Basmati, T-9, Dubraj, Duniapat (T9), Ramjinwain (T1)
Western zone			
Maharastra	Ambemohor, Chinore,	Kagasali, Prabhavati, Sakoli-7	—

Table 1.
Zonal classification of scented Rices of different states of India [12].

3. Biochemical basis of fragrance

Generally, the aromatic rice cultivars are enriched with large volatile and semi volatile compounds *viz.,* alcohols, aliphatic aldehydes, alkane, alkene, aromatic aldehydes, aromatic hydrocarbon, carboxylic acid, ester, furan, ketone, N-heterocyclic, phenol, and terpenes [14–17].

3.1 Structure and chemistry of 2AP

Among the different volatile compounds, 2-acetyl-1-pyrroline (2AP) with popcorn-like aroma and lowest odor threshold is reported to be the potent biochemical compound to impart fragrance in rice [18]. The chemical structure of 2AP is an N-heterocyclic compound containing 1-pyrroline ring in which the hydrogen at position 2 is replaced by an acetyl group with a methyl ketone group. 2AP content in scented rice varieties include 0.04–0.09 ppm, whereas non-aromatic varieties have 10x less (<0.006–0.008 ppm) [19].

3.2 Biosynthetic pathway for 2AP

There are many contradictions and views regarding the biochemical pathway for 2AP synthesis and it is still being explored. It was reported that *L*-proline was the precursor for the production of 2AP [20]; and is involved in polyamine degradation pathway which is the main enzymatic pathway and there are some other non-enzymatic pathways reported having an influencing action on 2AP concentration. In the enzymatic polyamine pathway, arginine, ornithine, spermidine, putrescine, etc. are degraded into GAB-ald which spontaneously cyclises

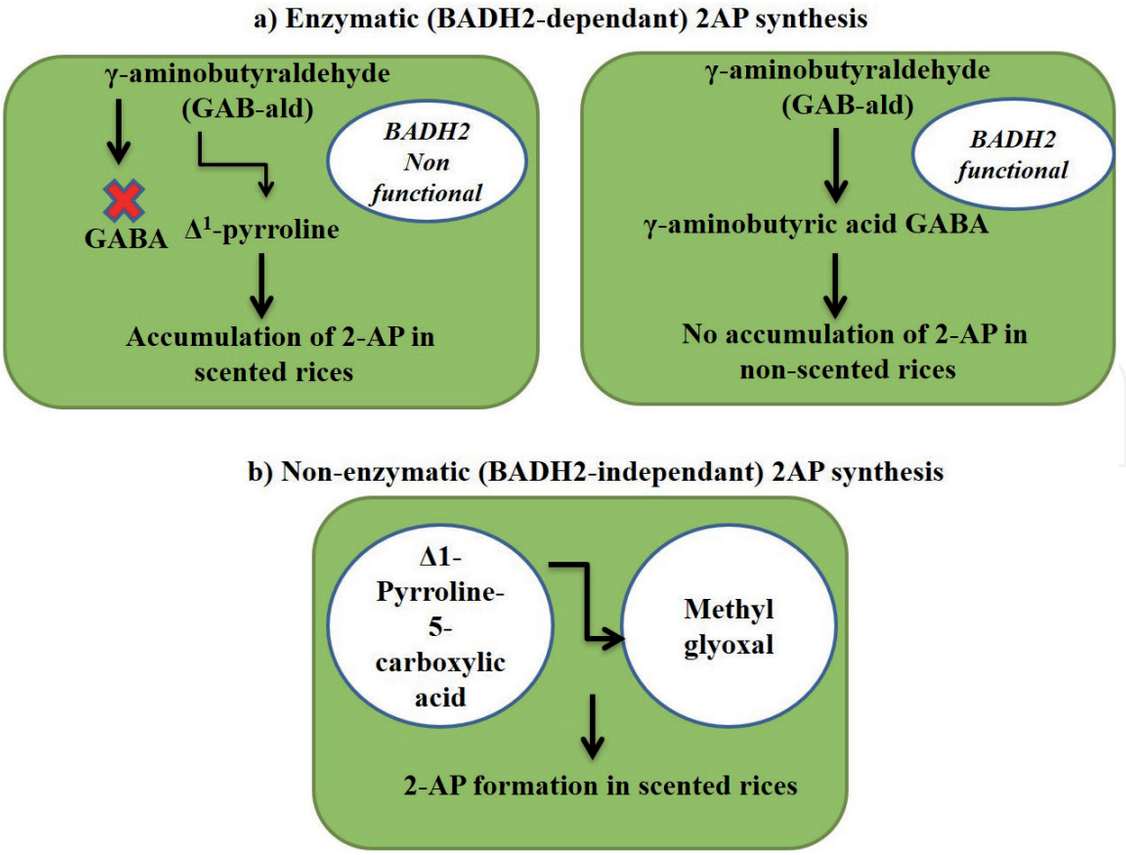

Figure 1.
2AP biosynthetic pathway in rice. (a) Enzymatic (BADH2-dependant) 2AP synthesis [21, 22] (b) non enzymatic (BADH2-independant) 2AP synthesis [23].

to Δ^1-pyrroline, an immediate precursor of 2AP biosynthesis [21]. The non-functional badh2 enzyme (encoded by *osbadh2* gene) inhibits the conversion of the γ-aminobutyraldehyde (GAB-ald) to γ-aminobutyric acid (GABA) thereby allowing the formation of Δ^1-pyrroline and ultimately the 2AP in scented rice whereas the reverse happens in non-scented rices (functional BADH2 enzyme coded by *OsBadh2* gene inhibits 2-AP formation) [22].

Some non-enzymatic direct pathways had also been described by many scientists and researchers. Glutamate produces proline and the proline accumulated during stress is converted to Δ^1-Pyrroline-5-carboxylic acid (P5C) by the enzyme Δ^1-Pyrroline-5- carboxylate synthase (P5CS). This P5C combines directly with methylglyoxal without involving any enzymes or might be converted to Δ^1-pyrroline and thereby enhancing the 2AP concentration [23]. In normal plants the methylglyoxal produced from glycolysis is detoxified by glyoxalase enzymes and their concentration was kept low. It was speculated that 2AP is a generative volatile compound to detoxify methylglyoxal in rice plant [24] (**Figure 1**).

2AP concentration differs in different plant parts of rice. The concentration is more in grains and flag leaf than in any parts of the plant [25–27]. Glutamate, ornithine and proline are important amino acids that serves as nitrogen (N_2) source in the ring of Δ^1-pyrroline [11]. The high aroma content in grains is mainly from the larger availability of N_2 from the soil. So, the aroma concentration may vary depending upon the nitrogen availability to the plants [28]. Advanced researches are essential in correlating the genetic and biochemical aspects of scented rice varieties, particularly with regard to the nitrogen and acetyl group donor in 2AP in order to reveal the key enzymes that are involved in the biosynthetic pathway of aroma in rice.

4. Genetic and molecular basis of fragrance

Inheritance of aroma is quite difficult to understand because it is controlled by number of unknown genes at different growth stages of rice and influenced by various concentrations of volatile and semi-volatile compounds. Although, plant breeders have reported the aroma inheritance by monogenic, digenic and polygenic pattern with recessive, dominant, complimentary and duplicate gene actions, indicating that complex genetic control of the trait. In majority of studies, the genetics of fragrance in rice is mainly due to single recessive gene [6, 9, 29–33] while other studies have also identified two, three or four genetic loci having influence on fragrance [9, 34–38]. Studies on the genetic control of aroma/fragrance/scent in rice have been presented in **Table 2**.

However, much of this conflicting information on the inheritance of aroma might have arisen due to (i) unreliable and cumbersome phenotyping methods used for fragrance determination [6], (ii) failure to consider the endosperm fragrance in rice seeds [29] and (iii) segregation distortion [9]. The nature of aroma inheritance appears to be cross/genotype specific due to the number of genes and the type of gene action varied with the genotype. However, the fragrance trait is a highly heritable as some of the lines derived from T142 (scented) x IR 20 (non-scented) cross, and some of the high yielding released aromatic rice varieties show strong scent.

The implementation of marker assisted selection is a significant supplement to traditional approaches, altering the selection process directly or indirectly from

S.No.	Gene action	References
1.	Monogenic dominant	[39, 40]
2.	Monogenic recessive	[29–32, 38, 41–63]
3.	Monogenic recessive with an inhibitor	[41, 64]
4.	Digenic or trigenic dominant	[34]
5.	Monogenic or digenic recessive	[37]
6.	Digenic recessive	[38, 65, 66]
7.	Three recessive genes	[67]
8.	Two dominant complimentary genes	[68]
9.	Three dominant complimentary genes	[69, 70]
10.	Four dominant complimentary genes	[35]
11.	Monogenic or digenic recessive or dominant, complimentary	[71]
12.	Monogenic or digenic dominant, duplicate	[72]
13.	Digenic dominant suppression epistasis interaction	[73]
14.	Polygenic	[9, 10, 74]

Table 2.
Inheritance pattern of aroma in rice.

phenotype to genes [75]. A novel compound namely 2AP (2-acetyl-1-pyrroline) plays a major role in most of the aromatic rice cultivars for the presence and absence of unique popcorn like characteristic aroma. Several attempts have been made at molecular level for genetic mapping the fragrance gene governing the 2AP synthesis in different aromatic rice varieties such as Della [30], Azucena [9, 76], Suyunuo [77, 78] and Wuxianjing [77]. Quantitative trait locus (QTL) mapping was also performed in indica aromatic rice KDML105 (Jasmine) [52, 79], Kyeema [80] and Wuxiangxian [77] (**Table 3**).

By using RFLP technique, a single recessive gene (*fgr*) that controls fragrance was mapped on chromosome 8 tightly linked with a single-copy marker RG28 and found that genetic distance between aroma gene and RG28 was 4.5 cM [30]. The close linkage between RG28 and *fgr* (5.8 cM) was confirmed by [9], also identified two quantitative trait loci for fragrance, one on chromosome 4 and the other on chromosome 12.

Further, a gene responsible for 2AP synthesis was mapped in a Jasmine rice variety KDML105 between the flanking regions of RG1 and RG28 [86]. The original region (1.13 Mb) flanking between RG1 and RG28 was narrowed down to 82.2 Kb in segregating population, within this region three KDML BACs were cloned and identified three new candidate genes. Among them, a single recessive gene (Os2AP) was identified which majorly contributing the 2AP synthesis in rice. The comparative analysis between aromatic KDML105 and Nipponbare for Os2AP gene sequences revealed two important mutational events within the exon 7 of Os2AP of KDML105, at positions 730 (A to T) and 732 (T to A), followed by the 8-bp deletion "GATTAGGC" starting at position 734 [87]. A similar mutational event was also reported by [79] within the flanking regions of RM515 and SSRJ07, a gene responsible for 2AP in Kyeema fragrant rice cultivar.

Number of Genes	Type of markers	Chromosome location	References
1	RFLP	8	[30]
1	RFLP	8	[81]
1	RAPD	—	[52]
1 major gene and 2 QTLs	RFLP, STS	8, 4 and 12	[9]
1	SSR	8	[56]
1	SSR	8	[32]
1	SNP	8	[33]
1	SSR	8	[80]
1	EST, SSR	8	[82]
1	SSR	8	[77]
1	SSR	—	[66]
1	SSR	8	[83]
3 QTLs	SSR	QTLs on 3, 4 and 8	[10]
1	SSR, RFLP	8	[58]
1	SSR	8	[60]
1	SSR	8	[84]
1	SSR	8	[85]
2	—	—	[73]

Table 3.
Molecular mapping of fragrance gene in rice.

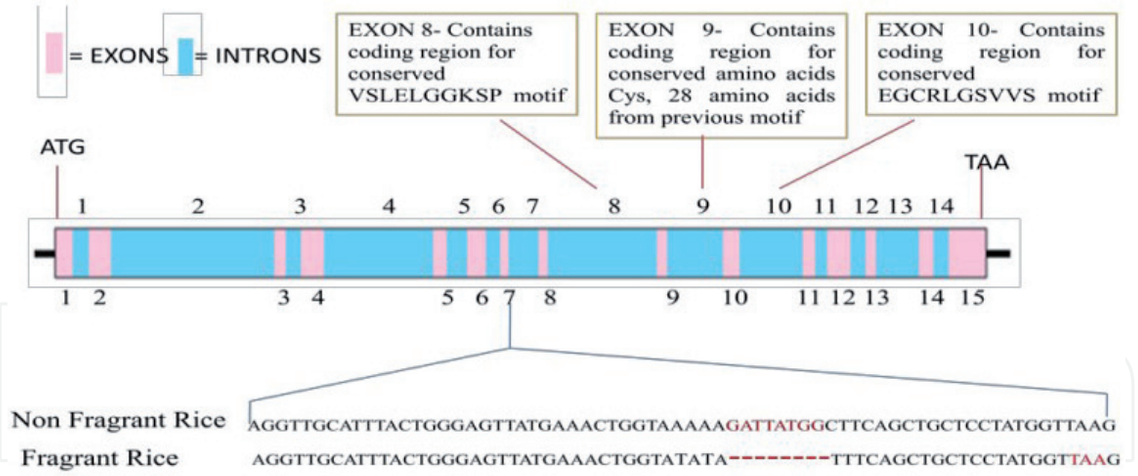

Figure 2.
Structure of the fgr gene showing ATG, initiation codon, exons (15), introns (14) and the termination site (TAA).

Using four BAC of Nipponbare spanning within a region of 386 bp from RM515 to SSRJ07, an in silico physical map was developed and suggested that 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 single nucleotide polymorphisms (SNPs) with the 8 bp deletion in the 7th exon of the gene, which resulted in a premature stop codon [10]. The newly identified gene was showing homolog with BAD1 (betaine aldehyde dehydrogenase 1) locus of chromosome 4 and hence named as BAD2 [79]. A comparative study between amino acids and sequences of Os2AP and BAD2 suggested them as one gene with two different names. Recent surveys of diverse fragrant germplasm support the association of *badh2* with fragrance [76, 78, 88], and transformation of a fragrant variety with the dominant non-fragrant allele has been proved to abolish aroma [21], confirming that *badh2* is the major and effective genetic determinant of aroma in rice (**Figure 2**).

The nucleotide sequences of 7 exon are shown for both the rice varieties. The fragrant variety shows a deletion of 8 bp with 3 SNPs that terminates prematurely, within this exon. Thus, fragrant varieties truncated protein might lack the conserved sequences which is encoded by 8, 9 and 10 exons and that are believed to be important for correct protein function [84].

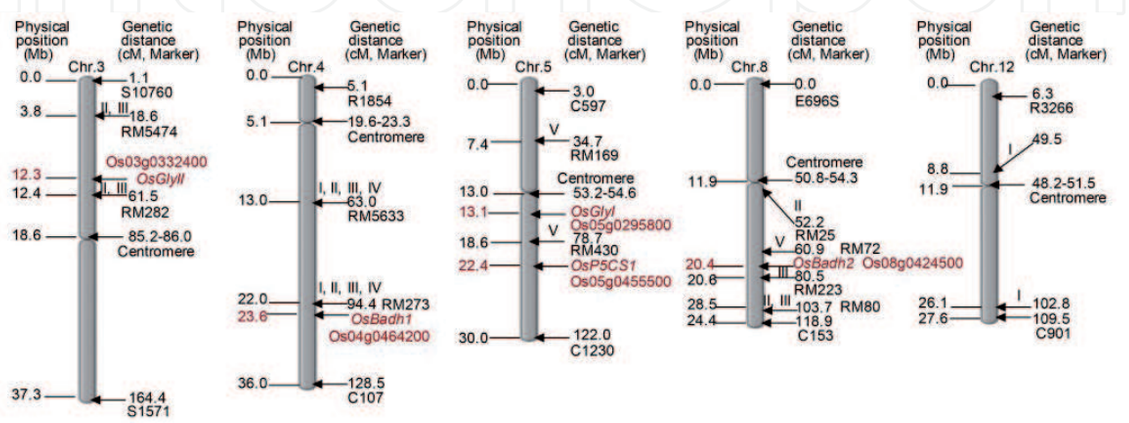

Figure 3.
QTLs for aroma with respective candidate genes in rice.

Since the *Badh2* gene was isolated and cloned, more than a dozen mutation sites have been found in *Badh2* [3, 89–91] and a series of molecular markers were designed for these loci, which could be used for the identification of gene responsible for aroma, selection of different aromatic rice varieties and cultivation of new varieties of aromatic rice.

4.1 QTLs for aroma

A number of QTLs for aroma have been identified on chromosomes 4, 8 and 12, at least three QTLs have been located on chromosomes 3, 4 and 8 in Pusa 1121 [9, 10, 92]. Recently, three QTLs were detected for rice grain aroma on chromosome 5 (one QTL) and chromosome 8 (two QTLs) [93]. However, until now only a few QTLs and associated markers have been confirmed (**Figure 3**).

5. Aroma extraction, identification and quantification methods

The extraction process is influenced by various criteria viz., type of matrix, volatility of the analyte, concentration of constituents in the sample and extraction conditions; therefore, efficient methods of extraction is needed [14]. However, so far there is no single method that will prove ideal for aroma extraction, identification, and quantification of rice. Although, several traditional and modern methods are available for extraction and isolation of rice aroma chemicals which are coupled with analytical methods (**Figure 4**).

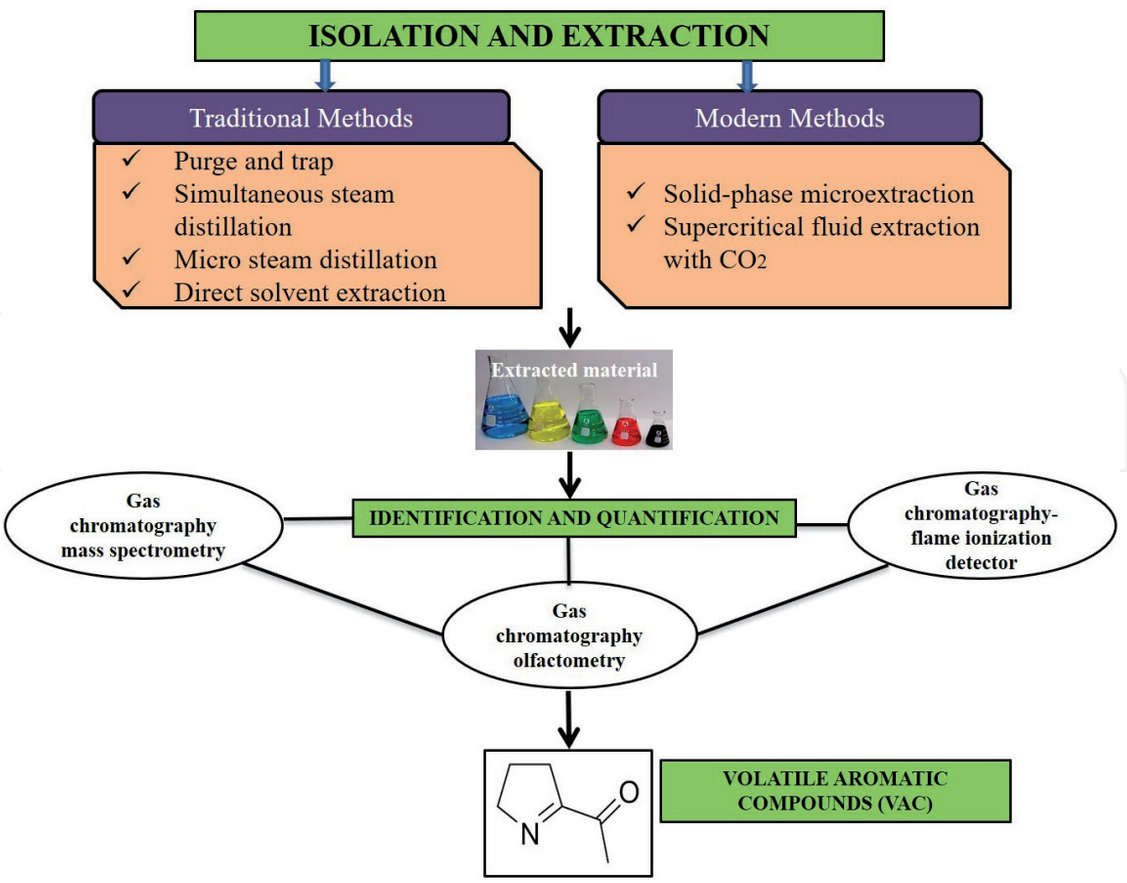

Figure 4.
Extraction and isolation methods of rice aroma.

5.1 Isolation and extraction methods

Several extraction methods are available for extraction of VACs (volatile aromatic compounds) in rice. While considering the extraction efficiency, it differs dramatically for each method. Based on that, the selection of extraction technique can efficiently extract volatile compounds viz., alcohols, aldehydes, hydrocarbons, carboxylic acids, esters, furans, ketones, N_2-containing, phenols and terpenes [94]. Most of the volatile compounds are insoluble in water so conventional extraction methods need non polar solvent as a medium.

The isolation techniques such as vacuum SDE apparatus; PTM, SDE, SEfbDI, SPME, SFE, HAS, and HSSE have their own distinguished characteristic feature. For extraction and isolation of 2-AP concentration from rice sample a desired efficient technique is a prerequisite. From the above methods, simultaneous distillation extraction (SDE), solid-phase microextraction (SFME) and supercritical fluid extraction (SFE) are the most widely used method for extraction of volatile compounds.

5.1.1 Simultaneous distillation extraction (SDE)

Simultaneous distillation extraction is a combination of vapor distillation and solvent extraction method for extraction of VACs [95]. SDE is also known as the Likens–Nickerson steam distillation, it is one of the most popular method for rice aroma chemical analysis. Solvent extract is the final product of these method. Many researchers have used this method for extraction of 2AP volatile compounds and it showed to be the most effective approach for a quantitative evaluation of 2AP. A laborious concentration step is still needed for traditional SDE procedure. Therefore, a modified version of so called micro-SDE device was proposed to overcome the problem [96].

The main advantage of this method is only a small amount of solvent is used for extraction and it also shortens the extraction time and improves the extraction efficiency. The solvents, such as: hexane, dichloromethane (DCM) and n-pentane, can be used and the amount of solvent required for SDE has been dramatically reduced compared to that of the conventional LLE. However, the major disadvantage of this method is the atmospheric pressure SDE was also obvious. Due to its high temperature, there is a possible occurrences of undesirable ester hydrolysis, Maillard reaction and sugar degradation [95].

5.1.2 Solid-phase microextraction (SPME)

This method was first introduced in early 1990s by Arthur and Pawliszyn [97]. Solid-phase microextraction is a newly emerging extraction technique for extraction of rice aroma compared with other method. It was applied in both laboratories as well as on-site [98]. Because of its persistence over other method of extraction, many results have been reported on 2AP aroma compound from rice grains [99].

Solid-phase microextraction has been used for the extraction of volatiles due to certain advantages, such as low-cost, simple, solvent-free, rapid and time-saving technique when compared with SDE method. This method can eliminate contamination, prolong the fiber lifetime and lead to reproducible results [98]. The chemistry of volatiles is decided with the help of desorption and adsorption behavior. If such extracted analytes are varied in their polarities that requires a different chemistry of SPME fiber [99].

5.1.3 Supercritical fluid extraction (SFE)

Supercritical fluid extraction is a separation and extraction process and it uses the supercritical fluids (SCFs) as the extraction solvent. It is a type of solvent which is clean and pure. The supercritical fluid is considered as 'Green Chemistry', because it is less toxic in compression compared to the organic solvents. The carbon dioxide (CO_2) is an extensively used SCF; sometimes it is modified by ethanol or methanol as such co-solvents [100].

Nowadays, Supercritical fluid extraction (SFE) is widely used for extraction of volatile compounds from rice and also in other plant samples such as vegetables, fruits and so on [101].

It is a quick technique and it can recover the majority of the VACs [102]. Within 10–60 minutes the whole process would be completed and it produces the pure extract by releasing the pressure. One of the main disadvantage of SFE is less effective, than solvent extraction [101].

5.2 Identification and quantification methods

The identification and quantification of volatile aromatic compounds from various types of rice is a tedious process. The research on aroma in rice is conducted by the researchers, scientists and industry groups for more than four centuries but still now it is not possible to identify all aroma compounds presented in rice. To determine the sensory quality of foods, need more concentration/efforts towards the application of modern and recently developed technologies.

There are several analytical methods for identification and quantification of rice aroma *viz.,* GC–MS, GC–MS-FID, GC–MS-AFID, GC–MS-FTD, GC–MS-SIM, Capillary GC–MS, GC-O, GC-FID, GC-PFPD, GC-TOF-MS, GLC, GLC-Capillary or GLC-Packed column. From the above methods, Gas Chromatography–Mass Spectrometry (GC–MS), Gas chromatography-olfactometry (GC-O), Gas Chromatography-Flame Ionization Detector (GC-FID) these three methods are widely used for identification and quantification of aroma in rice sample.

5.2.1 Gas chromatography-mass spectrometry (GC-MS)

Gas Chromatography–Mass Spectrometry (GC–MS) method was the most common instrumental analysis method for rice aroma analysis and effective method for analyzing volatiles, and widely used for qualitative and quantitative analysis of volatiles in rice [103]. In this method, the volatile compounds present in rice are determined and separated by GC and then identified by GC–MS [12]. This method can separate VACs having a molecular weight of less than 1,000 Dalton [104]. To date, the identification and quantification of volatile components from rice depends on advanced technologies and improved GC with multidimensional use. The qualitative and quantitative analysis of VACs is proved to be very sensitive in this method. The performance of MS is based on generated charged particle (ions) from molecules of analyses.

5.2.2 Gas chromatography-olfactometry (GC-O)

Gas chromatography-olfactometry (GC-O) is considered as one of the most advanced analytical method for the identification and quantification of VACs in sample matrix of rice. In GC-olfactometry (GC-O) system, human nose was applied to detect the odor intensity of volatiles. Two detectors which perceived the odor-active

compounds (hexanal, longifolene, 2-methoxyphenol and so on) eluted from the chromatographic column [105]. Although this method was very much useful for identification of aroma-active compounds from food samples. However, it is not suitable method for quantitative and qualitative analysis of VACs. As a result, the GC–O analytical method is not only an instrumental but also a sensorial analysis [106].

5.2.3 Gas chromatography-flame ionization detector (GC-FID)

Gas Chromatography-Flame Ionization Detector is the combination of FID with GC. This method is considered as very effective and crucial GC method because of its excellency [97]. It enables the separation, identification, and quantification of volatile compounds with their existing levels of concentrations from different food sample [98]. By comparing the retention time (RT) in GC-FID, the identification of volatile aromatic compounds of rice is completed and the retention times are converted into system-independent constant known as Kovatx retention index [99].

6. Factors affecting rice aroma

6.1 Genetic factors

The genes controlling aroma was found to be a highly heritable and also relatively complex in nature. In chromosome 8 the main candidate gene was *fgr*/*badh2*/*Os2AP* homologous to betaine aldehyde dehydrogenase (BADH), whereas many other genes were also reported [103]. Deletion of 8 base pair in exon 7 or deletion of 7 base pair in exon 2 of BADH2 gene on chromosome 8 results in a loss of function of BADH2, which catalyzes the oxidation of 4-aminobutanal to 4-aminobutanoic acid. It was reported that 4-aminobutanal existed in solution equilibrium with its cyclic form 1-pyrroline which was a precursor for 2AP [107].

According to [88], the gene *fgr*/*badh2*/*Os2AP* was not the only aromatic gene in rice. The aromatic compound 2AP was identified in a number of rice varieties not carrying the 8-bp deletion. The aromatic landraces in Japan consists of six clades, none of which had the 8-bp deletion in exon 7 of BADH2 and also Japanese aromatic and non-aromatic landraces were found genetically different [108]. About 84 Subsp. indica rice landraces were investigated with respect to 8-bp deletion in BADH2 gene [109]. The results showed that aroma traits were genetically controlled by recessive monogenes, independent of cytoplasmic genes, however, aroma was also studied as a quantitative trait, and many genes were included in the expression [110].

6.2 Planting and harvesting factors

The maximum down regulation of BADH2 gene was reported in temperature of 25°C, highest 2AP content and excellent phenotypic aroma score, indicating the function of temperature on regulating phenotypic expression of aroma and final rice aroma quality. BADH2 gene expression is influenced by the temperature, phenotypic aroma score and 2AP content were investigated in three different temperatures (ambient or 28.29 ± 0.91°C, 25°C and 20°C) [2].

There is a significant positive effect on 2AP content in rice grains (Meixiangzhan and Nongxiang 18) by the application of manganese, which esults in probably improvement of enzyme activities involved in 2AP formation. Higher total soil nitrogen plays a major role in producing rice aroma. During flowering stage, it was found that Si contents in leaves were positively related with 2AP contents.

Thus, indicating that Si application to some amount will improve 2AP contents in grains [111].

An increase of 2AP content in grains with salinity was observed for three improved aromatic rice varieties and salinity was thought to have a positive effect on rice aroma quality [112]. NaCl stress enhanced aroma production in Tulaipanji, Radhunipagal and Gobindobhog rice varieties while weaken that in Kalonunia [113]. Shading treatments during grain filling significantly increased 2AP content in both Yuxiangyouzhan and Nongxiang rice varieties, and had a selective effect on the metabolism of other volatiles [114].

6.2.1 Effect of planting density on 2-acetyl-1-pyrroline content

2AP content decreases with an increase in planting density. The content of 2-AP in rice grains obtained during the early season will be stored for 6 months. However, other seed quality attributes at the exception of head rice yield and grain vitreosity were not affected by planting density [115].

6.2.2 Effect of harvesting time on 2-acetyl-1-pyrroline content

Reduction in 2AP was observed with increasing harvest date during the early season. During the late season, however, the concentration of 2AP is gradually decreased from 10 DAH and seemed to stabilize at 40 DAH, a reduction rate of 60%. However, it is well compensated for by the high level of 2AP in both brown and white rices, which remains significant even after a storage period of 3 months at ambient temperature [116].

6.3 Processing factors

6.3.1 Cooking

Presoaking is a traditional pretreatment before cooking. It would result in uniform cooking and less cooking time. Presoaking for 30 min before cooking resulted in significant increase in sewer/animal flavor and summed negative flavor attributes, and significant decrease in sweet taste and summed positive flavor attributes, mainly as a result of an increase in sulfur-containing free amino acids and their breakdown products [19].

According to [117], divided the cooking process into four stages and identified the major compounds of Japanese rice cultivar Akitakomachi. In stage I (25 min, from the start of heating to start of steam coming out of rice cooker) were aldehydes such as n-nonanal, n-decanal, and (E)-4-nonenal. The dominating compounds identified at cooking stage II (13 min, from the start of steam coming out of rice cooker to the end of steam coming out of rice cooker) were hexadecanoic acid and tetradecanoic acid. The major compounds identified at cooking stage III (10 min, from the end of steam coming out of rice cooker to automatic stop of heating) and IV (keeping the rice warm for another 30 min) were aldehydes and fatty acids.

6.3.2 High hydrostatic pressure and superheated processing (HHP)

High hydrostatic pressure (HHP) had stabilized effects on low molecular weight volatiles [118], and it is one of the effective processing to improve products flavor. HHP was thought to be a good pretreatment option to enhance aroma quality of cooked rice. HHP process enhanced the formation of aldehydes, alcohols and

ketones in germinated brown rice [119]. The volatile compounds in rice were cooked by superheated steam rice cooking machine were compared -with those of ordinary cooked rice [105].

6.3.3 Roasting and parboiling

While roasting there is a change in volatiles by the Maillard and caramelization reactions, and consequently form unique flavor, and usually increase the popularity of consumers. Increases the content of heterocycle compounds and decreases the content of hydrocarbons and benzene derivatives by roasting process [120]. Parboiling cause concomitant changes in the physical, chemical, and nutritional properties of grains, and consequently greatly affect organoleptic and other qualities. Hydrothermal treatment during parboiling would inactivate lipases, and inhibit the development of off flavors [121]. Hence, it was a good method to keep rice aroma during storage.

6.3.4 Milling

Un-milled black rice contained significantly larger amounts of total volatiles than milled black rice [122]. That is, the volatile compounds were mainly distributed in the bran layer of black rice (624 ± 17.7 ng g − 1), and significantly decreased by milling, especially the contents of acids, esters, and alcohols. When milling aromatic rice (Cheonjihyang-1-se), hexan-3-one, benzene, 2-pentylfuran, and pentanal decreased to 79%, 70%, 54%, 78% with milling time from 10s to 140 s, while (E)-non-2-enal, pentadecanal, (5E)-6,10-dimethylundeca5,9-dien- 2-one, and menthol increased 252%, 185%, 172% and 159% [123].

6.4 Storage factors

It was reported that, proteins, lipids and carbohydrates were decomposed into volatiles contributing rice odor during storage [124]. Enzyme catalyzed reactions were drastically inhibited at low temperature. This was one reason for slower deterioration of rice aroma. In general, lower storage temperature and better packaging materials would be more appropriate for aromatic rice to better maintain desirable rice aroma. OPP/Al/LLDPE package was superior to Nylon/LLDPE package, and storage at lower temperature (15°C) was better than ambient temperature, since they better retarded the formation of lipid oxidation products and other characteristic odorants in organic red aromatic rice [125]. Some paper assumed that lower temperature during storage would minimize volatilization of 2AP from rice [126, 127].

6.4.1 Effect of storage time and temperature on 2-acetyl-1-pyrroline content

Fragrant rice harvested in June and kept for 6 months at – 4°C contained up to four times 2-AP in all forms (brown and white), compared to those kept at 30°C. High losses of 2-AP occurred under a very warm condition of 30°C. There were also significant differences in the concentration of 2-AP between samples collected in November with losses of 25 to 35% occurring after storage of 3 months at 20°C compared to 8°C [115].

Therefore, insights into extraction and quantification methods and various factors affecting the quality of aroma are essential, and also modern biotechnological advances like Transcription Activator Like Effector Nuclease (TALENs), Zinc Finger Nuclease (ZFNs) and Clustered Regularly Interspaced Short Palindromic

Repeats (CRISPR) associated endonuclease Cas9 (CRISPR/Cas9) are being entrusted in improving the rice aroma content and quality. Researchers succeeded in editing *Badh2* gene and generating high yielding fragrance rice varieties by using TALENs [128] and CRISPR/CAS9 [129–131] technologies, which led to increased accumulation of 2AP.

7. Conclusion and future prospects

Aroma in rice is a key quality trait determining its acceptability and marketability. 2AP has gained major importance among other volatiles as the primary compound for aroma. Aroma compound is encoded by betaine aldehyde dehydrogenase 2 (*badh2*) gene also called fragrance (*fgr*) gene which is located on chromosome 8 and the level of aroma depends on this gene caused by mutation in *badh2* of 8 bp deletion and 3 SNPs. Apart from Basmati genotypes possessing long slender grains, only few other medium/short slender grain rice varieties possessing aroma are in the market. Those short/medium slender aromatic genotypes are not high yielding and possess several other disadvantages. Development of aromatic rice varieties possessing superior grain qualities through conventional or molecular breeding approaches takes considerable number of years and in some cases retaining the superior grain qualities of elite genotypes still remains a challenge. In recent years, genome editing technologies like TALENs, ZFNs and CRISPR/Cas9 has been employed in developing superior quality rice grains and it has opened new avenues for accelerated improvement of rice varieties thereby gaining competitive advantage in improving economy on national and global scale. These new technologies seems to be an attractive strategy to overcome the number of years required for developing desired genotypes and also to overcome the problems due to linkage drag. It will accelerate the cultivation of new aromatic rice varieties with high quality, yield and multiple resistance.

Conflict of interest

The authors declare no conflict of interest towards this chapter.

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