PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.4625

HORIZON
e-Publishing Group
HePG

REVIEW ARTICLE

Enhancing rice aroma through innovative approaches

Jawahar Desigan[1], Madhan Mohan M[2*], Parameswari C[2], Juliet Hepziba S[1], Geetharani P[2], Kavitha M.P[2], Sudhakar G[2], Arumugam Pillai M[1]& Venkatesan K[3]

[1] Department of Genetics and Plant Breeding, V.O.C Agricultural college and Research Institute, Killikulam, TNAU, Tuticorin, Tamil Nadu-628 252, India

[2] Agricultural Research Station, Vaigai Dam, TNAU, Theni, Tamil Nadu-625 562, India

[3] Department of Floriculture and Landscape Architecture, Horticulture College and Research Institute, Periyakulam, TNAU, Theni, Tamil Nadu- 625 604, India

*Email: madhanmohan.m@tnau.ac.in

OPEN ACCESS

ARTICLE HISTORY
Received: 11 August 2024
Accepted: 19 September 2024
Available online
Version 1.0 : 16 October 2024

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Jawahar D, Madhan MM, Parameswari C, Juliet HS, Geetharani P, Kavitha MP, Sudhakar G, Arumugam PM, Venkatesan K. Enhancing rice aroma through innovative approaches. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.4625

Abstract

Aromatic rice is used extensively in many different cuisines around the world for its wonderful aroma and cooking qualities. Aromatic rice varieties such as Basmati and non-Basmati fragrant rice have gained popularity in both domestic and foreign markets, despite their origins being predominantly in Southeast Asia and the Indian subcontinent. The primary gene responsible for rice aroma is the *fgr/Badh2/Os2-AP*, situated on chromosome 8 and encodes betaine aldehyde dehydrogenase 2 (Badh2). Key aroma compounds are attributed to over 500 volatiles. The primary aromatic molecule in rice, 2-acetyl-1-pyrroline (2-AP), accumulates as a result of mutations in this gene and gives rice its distinctive scent. Aroma is not decided by single compound rather it is decided by volatile profile and also by environmental factors. The identification of Quantitative Trait Loci (QTLs) linked to fragrance features on different chromosomes has improved our comprehension of the genetic processes behind rice scent. Advances in genetic engineering, particularly CRISPR/Cas9 and TALEN have facilitated the manipulation of the Badh2 gene, enhancing aroma profiles in rice. Additionally, gene silencing and introgression techniques have also proven in increasing 2-AP content. The review explores the biochemical properties and advancement of aromatic rice, emphasizing its complex inheritance patterns and potential for breeding improvement.

Keywords

2-Acetyl-1-pyrolline; Badh2; rice aroma; gene editing; volatile compound

Introduction

Aromatic or scented rice is an exclusive small category of rice that is authoritative of better quality and holds a prominent place in the community for its aroma and cooking properties (1). Aromatic grain quality is the highest desired trait that boosts marketability and purchaser predilection over non-aromatic rice, both in domestic and international markets (2, 3). There are 2 classes of scented rice *viz*., long-grained basmati rice and small- to medium-grained (indigenous or landrace) fragrant non-basmati rice (4). The demand for aromatic rice varieties has experienced a significant increase due to the shift in global preferences towards higher quality rice as well as the potential health benefits for individuals with diabetes and obesity (5). Rice aroma positively affects human health by improving sensory experiences and emotional well-being. The compound 2-acetyl-1-pyrroline is integral to the unique fragrance of certain rice varieties,

which can elicit favorable emotions and alleviate stress (6). The ever-increasing demand for fragrant rice among consumers has therefore forced rice breeders to create new, high-yielding varieties of fragrant rice (7). The composition of several volatile compounds as well as the presence of a principal volatile compound known as 2-Acetyl-1-pyrroline (2-AP) decide rice's aroma (8, 9) and are also found in the leaves and tissues of the seed (10). Furthermore, the appealing scent of rice may promote healthier dietary choices, thus enhancing overall nutrition and health results. Traditional breeding techniques have been used to enhance the 2-AP contents of rice varieties to create aromatic ones, including hybridization, pure line selection and mutational breeding (11, 12). The aromatic group's genetic distinctiveness is made clear by its confined cross-compatibility with indica and japonica (13). Three isozyme patterns *viz*., Group I (indica), Group V (indica) and Group VI (tropical japonica) make up conventional scented rice varieties (14). A single locus on chromosome 8 (fgr) linked to fragrance was found as a result of research into the genetics of fragrance in rice and the expression of the *Badh2* gene serves to suppress the synthesis of 2-AP in non-aromatic rice varieties, whereas the *Badh2* gene loss function in aromatic rice cultivars (15, 16). Enhanced aroma in rice elevates market value and consumer appeal, thus rendering them favorable for culinary uses and cultural relevance (17, 18). Nevertheless, these aromatic varieties encounter agronomic issues, including reduced yields and storage complications, which may affect their market viability (19). Recent crop breeding programmes have shown encouraging results from the use of genome editing (GE) technology (CRISPR/Cas9, TALEN, ZFN etc.) which modifies plant genomes in a controlled setting (20). Targeted genetic modification using CRISPR system can accelerate the transition for crop enhancement through precision breeding(21). The application of genome editing technologies necessitates extensive information on the genetic makeup, arrangement and functionality of pertinent genes in addition to data on novel genes and QTLs (22). The summary of different types of approaches on aroma enhancement is given in Table. 1. This review, highlights the biochemical properties, genetic basis and biotechnological advancements in understanding and improving the aroma of scented rice.

Origin and Evolution of Aroma Rice:

According to ancient records, aromatic rice originated in the Indian subcontinent and some proof indicates that aroma rice is tilled in China and other South Asian countries (23). *Oryza rufipogan* and *Oryza longistaminata*, two wild perennials that are thought to have been independently domesticated in Southeast Asia and West Africa respectively, are the common ancestors of rice. *Oryza nivara* and *Oryza barthii*, are 2 wild annuals that later gave rise to 2 cultivated species, *Oryza sativa* and *Oryza glaberrima*. Chloroplast DNA analysis determined that *Oryza barthii* is more strongly related to *O. rufipogan* than to *O. longistaminata* (Fig.1) (24). However, the characteristics of aroma rice - phenol reaction, translucent kernel nature, intermediate gel consistency and amylose content that makes it between indica and japonica (25). A total of 1688 rice cultivars were systematically collected and categorized into 6 distinct groups; among these classifications, Group V is indicative of aromatic rice (Fig. 2)(14). The aromatic rice contains germplasm from Afghanistan, Bangladesh, China, India, Iran, Myanmar and Pakistan. The studies on genetic diversity identified that scented rice is more strongly associated with the japonica subgroup (27,28). A study investigated phylogenetic relationship of ten aromatic rice and 41 wild relatives and grasses, using chloroplast-encoded matK, which showed that scented rice is more related to the *Oryza sativa japonica* group (24). The Indian subcontinent's foothills of the Himalayas, which stretch across the states of Uttarakhand, Uttar Pradesh, Bihar and the Terai region of Nepal are the centre of origin and diversity for aromatic rices. Many aromatic rice landraces still exist there, despite the fact that their numbers are dropping alarmingly. From the foothills of the Himalayas, aromatic rices have spread to other areas: eastward to Bangladesh and Myanmnar as well as the Indian states of Assam, Bengal, Manipur and Odisha; north-west to Haryana, Punjab, Himachal Pradesh and Jammu-Kashmir in India and westward to Afghanistan, Pakistan, Iran and Iraq (19). The majority of aromatic rice accessions have acquired their cytoplasm as well as 29-47 % of their nuclear genome from the native Indian rice, which indicated that aromatic rice originated in the Indian subcontinent through hybridization between a local and wild population. This hybridization is thought to have happened between 4000 and 2400 years ago, not long after Japanese rice arrived in the area (29). Aromatic japonica and indica were linked to the presence of MITE at position 51 (30).

Table 1. Different types of approches on aroma enhancement.

Approches	Contribution	Reference
Pure line selection	Basmati- 370, Jeeraksala, Improved Jeeraksala, Improved Kalanamak, C435, K441, DP33, Madhuri selection A, N-10B, N-12, Type-9, Type-1, Type-23, Sugandha.	(12)
Hybridization	Kusama(LS), PAU 29-295, GR101, PNR-546, Narendra Sugandha Dhan NDR-6093, Ketkijoha, Nua kalajeera, Nya Dhusara, Nua Chinikamini, CR Dhan 907, CR Suganth Dhan (908, 909, 910), Gangawati Ageti, HUBR-2-1.	(12)
Mutation breeding	Geetanjali, ADT41 (Mutant line of Basmati-370)	(12)
Molecular breeding	Improved PB-1, PB-1718	(12)
CRISPR and TALEN	Improved IR-96, aromatic ASD-16,	(77, 79)
Gene silencing	Transgenic IR-64 aromatic line	(83)
Gene pyramiding	R365, R403 Hybrid line	(84)

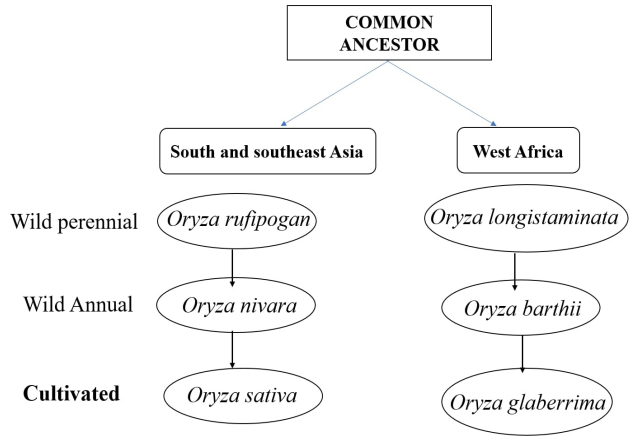


Fig. 1. Origin of rice (24).

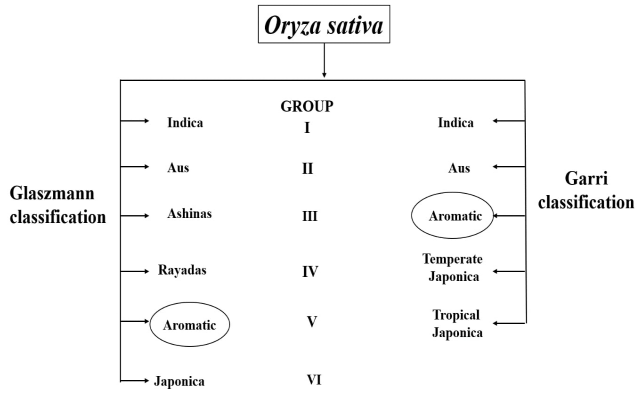


Fig. 2. Classification of *Oryza sativa* groups (14, 26).

Biochemical properties of aromatic rice:

Volatile compounds responsible for aroma:

Aroma is due to the chemicals present in the endosperm. Currently, rice has been found to contain over 500 volatile aroma influencing compounds. No single compound can be said to contribute a characteristic aroma, with the exception of 2-AP (31). Both the volatiles created during cooking and those already present in the rice are responsible for the distinctive scent of rice. Scented rice is enhanced by hydrocarbon volatile compounds like alcohol, aldehyde and ketone (32). More than a hundred volatile aromatic components, such as alcohols, ketones, esters, acids, pyridines, phenols, aldehydes, pyrazines, hydrocarbons and other materials, have been identified in cooked rice (33-36). The properties of major volatile compound are explained in Table. 3. 4-vinylphenol, (E)-2-nonenal, (E,E)-2,4-decadienal and 2-methoxy-4-vinylphenol are the 4 significant volatile components that were reported earlier. Besides the distinct flavor during cooking was produced by aldehydes, phenols and nitrogen (N_2) and sulfur (S) based volatile aromatic compounds (VACs) (37). Commercial Basmati rice consists of aldehyde (5952), alcohols (1869), hydrocarbons (548), ketones (234), heterocyclic compounds (1220), phenols (534), disulphides (79), terpenes (257) (38-41). Identifying the compounds responsible for the distinctive aroma of rice was the primary intent of the scientists and research teams. GC-MS, (gas chromatography-mass spectrometry) has made it simpler to detect and quantify organic volatile compounds in composites of sample materials and greatly enriched our understanding of the chemistry of rice fragrance (42). A study conducted a comparation of the volatile compounds between aromatic and non-aromatic rice and 70 aroma-causing components are listed with a description of fragrance (43). The main constituents were alkanals, alk-2-enals, alka(E)-2,4-denials, 2-pentyl-furan, 2-acetyl-l-pyrroline and 2-phenylethanol, also believe that several other components also responsible for the overall aroma profile. Comparatively, the concentration of n-hexanal, (E)-2-heptanal, 1-octen-3-ol, n-nonanal, (E)-2-octenal, (E)-2-€-4-decadienal, 2-pentylfuran, 4-vinylguaiacol, 4-vinylphenol is higher in non- scented rice. In Basmati rice, higher level of 2-phenylethanol and lower level of the n-hexanal was observed. The higher concentration of hexanal during rice storage indicates the higher possibility of rancidity and the development of oxidative off-flavors (44,45). A study revealed 16 hydrocarbon, 16 aldehyde, 15 alcohol, 4 acid, ketones and 10 other various components (46). In cooked rice, the main fragrance-producing compounds were n-butanol, n-hexanol decanal, octanal, hexanal, 2-acetyl-1-pyrroline, (E, E)-2, 4-decadienal, (E)-2-nonenal, 4-vinyl-guaiacol and 4-vinylphenol (46, 47). Also, 2-amino acetophenone and 4, 5-epoxy-(E)-2-decenal are 2 important scent compounds in rice (37). A significant volatile component of rice, hexanal is a derivative of linoleic acid and adds to the grain's green, fruity and grassy flavor with less smell (48). 4-vinyl guaiacol influences scent characteristics of Mailard- type systems and cooked odour (49). Undesirable, nutty, spicy and clove -like scents are characteristic of guaiacol derivatives (50-53).

Anabolic reaction of 2-acetyl-1-pyrroline:

The biochemical synthesis of 2-acetyl-1-pyrolline by polyamine degradation pathway reported (4). The initial discovery elucidated the biosynthetic pathway of 2-acetyl-1-pyrroline through the polyamine pathway (54). An instant precursor of 2-acetyl-1-pyrroline, δ-1-pyrroline is crucial for controlling the rate at which 2-acetyl-1-pyrroline is generated. The investigations found that proline, methylglyoxal, 1-pyrroline, glutamic acid and ornithine are the essential precursors of 2-AP (55-57). The pyrroline-5-carboxylate synthetase (P5CS) and it's genes are involved in the 2-AP biosynthesis pathway (58). The expression of *Badh2* gene associated with betaine aldehyde dehydrogenase (BADH) activity which suppress the production (15, 16). The illustration of the 2-AP biosynthesis pathway of fragrant rice was depicted in Fig. 3.

Genetic Basis of Aromatic Rice:

Genes Responsible for Aroma:

Identification of aroma gene and its role in biosynthesis was suggested (58).Rice's flavor and fragrance are regulated by a single recessive gene (*fgr* gene) on chromosome 8 (59, 60), and encoding betaine aldehyde dehydrogenase (*Badh2*). Any changes or mutation in *fgr* gene caused the function loss of Badh2 enzyme, thereby raising the concentration of 2-AP precursor, accretion of 2-AP (principal component of aroma), producing scent in aromatic rice (61-63). *Badh2* gene 1509 bp long containing 14 intron and 15 exon that encodes a protein with 503 amino acids (64). It was

identified the main alterations in the badh2 gene, notably an 8 bp deletion and 3 SNPs in exon 7 (59) and reported 7 bp deletion in exon 2 and 803 bp loss between exons 4 and 5 (65). Both the non-functional *Badh2-E2* and *Badh2-E7* alleles had very low transcription levels in comparison to the functional *Badh2* allele, as shown by real-time RT-PCR and RNA gel plot analysis. This suggests that mRNA transcription is significantly suppressed by a loss of functional mutation in *Badh2* (64) and 2-AP level rise in non-scented rice when the *Badh2* transcript is suppressed (66). Furthermore, studies revealed single nucleotide deletions in *Badh2* gene loci, intron 1, exon 1 splice sites, promoter and 5' untranslated regions (67, 68). A few more candidate genes, including *Osbadh1*, *OsGly* and *OsP5CS*, have been discovered through integration mapping and map-based cloning, aside from the *Osbadh2* gene and it is present on various loci, could be responsible for rice's high concentration of 2-AP and fragrance (4). Apart from the identified *Badh2* gene, there is another *Badh1* (Os04 g39020; 92 % homology), which is a homolog of *Badh2* (30), that is delineated on rice 4th chromosome. The monogenic, digenic and polygenic patterns of fragrance inheritance in rice, revealed complementary, dominant, recessive and duplicate gene interaction. Inheritance of fragrance can be challenging as it depends on the amounts of different volatile and semi volatile substances at different phases of rice growth and it is likely regulated by an unknown number of genes (inheritance). Presence of diverse aroma rice varieties shows various alleles of *Badh2* gene (3). The *Badh2* gene locus has been found to have various mutations (Table 2). A variety development program aimed at developing high-yielding scented rice through marker-aided selection of useful genes for aroma is made easier with the assistance of QTL analysis, which is one of the best ways to figure out the underlying genetics of aroma and other traits in the rice variety.

Table 2. Various mutation in Badh gene.

Allele	Location	Sequence Variation	Reference
Badh-5' UTR-1	5' UTR	8 bp insertion	(68)
Badh-5' UTR-2	5' UTR	3 bp deletion	(68)
Badh-5' UTR-3	5' UTR	5bp deletion	(85)
BAdh-5' UTR-4	5' UTR	253 bp deletion	(85)
Badh-5' UTR-5	5' UTR	MITE absent	(30)
Badh1.1	Exon 1	2bp deletion	(34)
Badh1.2	Exon 1 and Intron 1 junction	G/A SNP	(67)
Badh2.1	Exon 2	7 bp deletion	(86)
Badh2.2(1)	Exon 2	7 bp deletion	(87)
Badh2.2(2)	Exon 2	75 bp deletion	(87)
Badh2.4-5	Exon 4-5	806 bp deletion	(87)
Badh2-E7	Exon 7	13 bp deletion	(62)
Badh2.7	Exon 7	8 bp deletion	(87)
Badh2.10	Exon 7	G/A SNP	(87)
Badh2.13	Exon 13	C/T SNP	(87)
Badh4.1	Exon 4 to Exon 5	803 bp deletion	(65)
Badh4.2	Exon 4 to Exon 5	806 bp deletion	(87)
Badh7.1	Exon 7	8 bp deletion and 3 SNP	(59)
Badh8.1	Exon 8	7 bp deletion	(33)
Badh10.1	Exon 10	1 bp deletion	(34)
Badh10.2	Exon 10	1bp deletion	(34)
Badh10.3	Exon 10	G/T SNP	(34)
Badh10.4	Exon 10	G/A SNP	(87)
Badh12	Exon 12	3 bp deletion	(88)
Badh13.1	Exon 13	3 bp insertion	(34)
Badh13.2	Exon 13	C/T SNP	(34)
Badh14.1	Exon 14	1bp insertion	(34)
Badh14.2	Exon 14	G/T SNP	(34)
Badh2-p	5' UTR	8 bp insertion	(89)

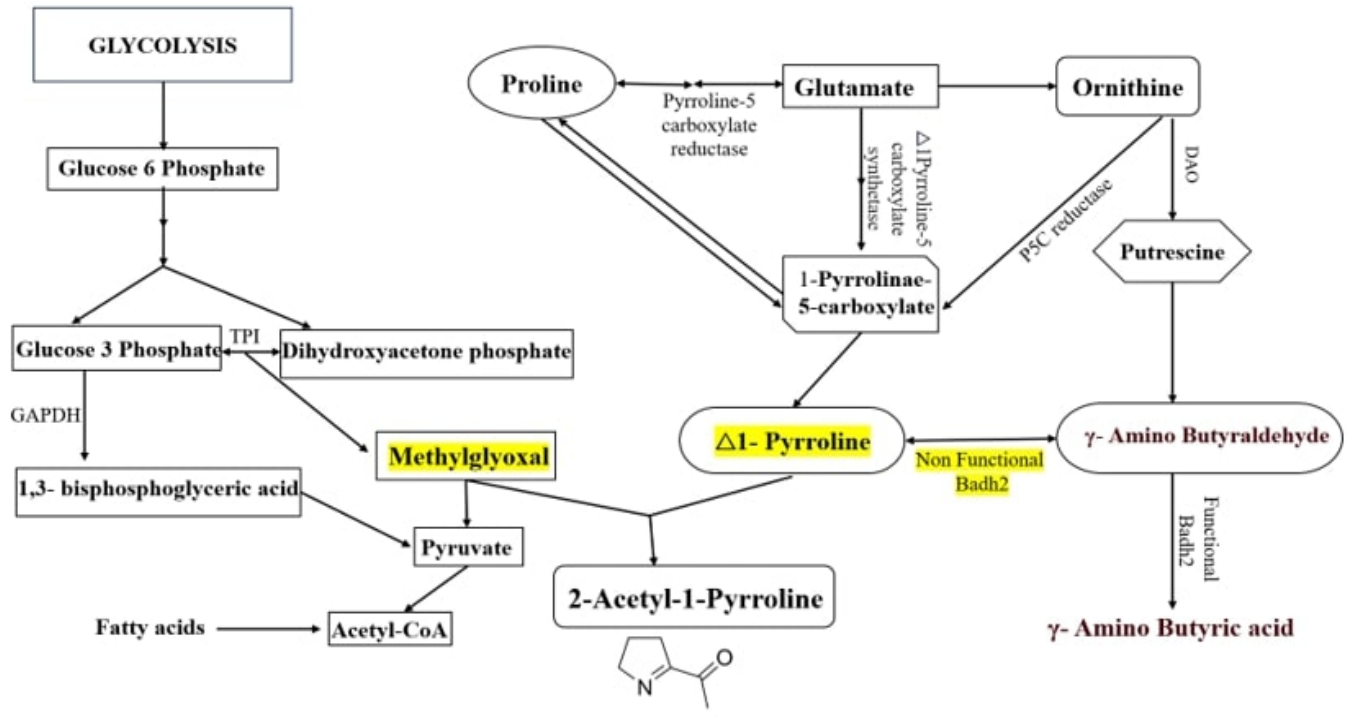
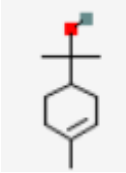
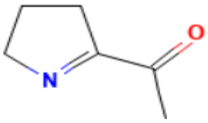
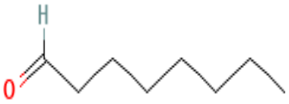
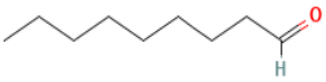
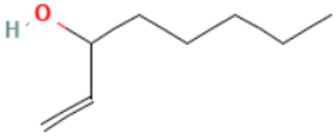
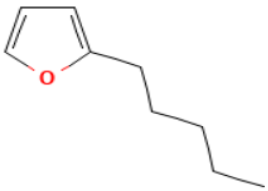
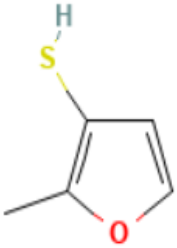


Fig. 3. Anabolic reaction of 2-acetyl-1-pyrroline in aromatic rice (15).

Table 3. List of major volatile compound and their properties.

Volatile compound	Formula	Molecular weight (g/mol)	Structure	Nature of Aroma	Reference
α-terpineol	$C_{10}H_{18}O$	154.25		Floral, lilac	(90)
2-acetyl-1-pyrroline	C_6H_9NO	111.14		Fishy	(91)
1-hexanol	$C_6H_{14}O$	102.17		Sweet alcohol	(92)
Octanol	$C_8H_{16}O$	128.21		Strong, Fruity	(93)
Nonanal	$C_9H_{18}O$	142.24		Orange-rose, green	(94)
1-octen-3-ol	$C_8H_{16}O$	128.21		Powerful sweet earthy odour	(95)
2-pentylfuran	$C_9H_{14}O$	138.21		Fruity aroma	(96)
2-methyl-3-furanthiol	C_5H_6OS	114.17		Roasted meat aroma	(97)

QTLs Responsible for Aroma in Rice:

The accessibility of the whole rice genome sequence creates new opportunities for QTL mapping and identification that confer aroma traits. The aromatic traits of scented rice may also be regulated by several Quantitative Trait Loci and a recessive gene (Badh2) (4,69). It was first used 4 markers (RFLPs, RAPDs, STSs, isozymes) and mapped 1 major QTL (Chromosome 8) and minor QTL on 4 and 12 chromosomes (70).There have been a few QTLs related specifically to aroma. The number of candidate genes linked to aroma was determined through genetic mapping and map-based cloning (4). It was found 3 QTLs on chromosomes 8 (2 QTLs) and 5(1 QTL) (71) (Fig. 4). A study disclosed QTL one on each of 3,4 and 8 chromosomes (32) (Fig.4). It was revealed QTL associated to aroma on chromosome 3's short arm (aro3.1), chromosome 4's long arm (aro4.1) and chromosome 8's long arm (aro8.1) (72).

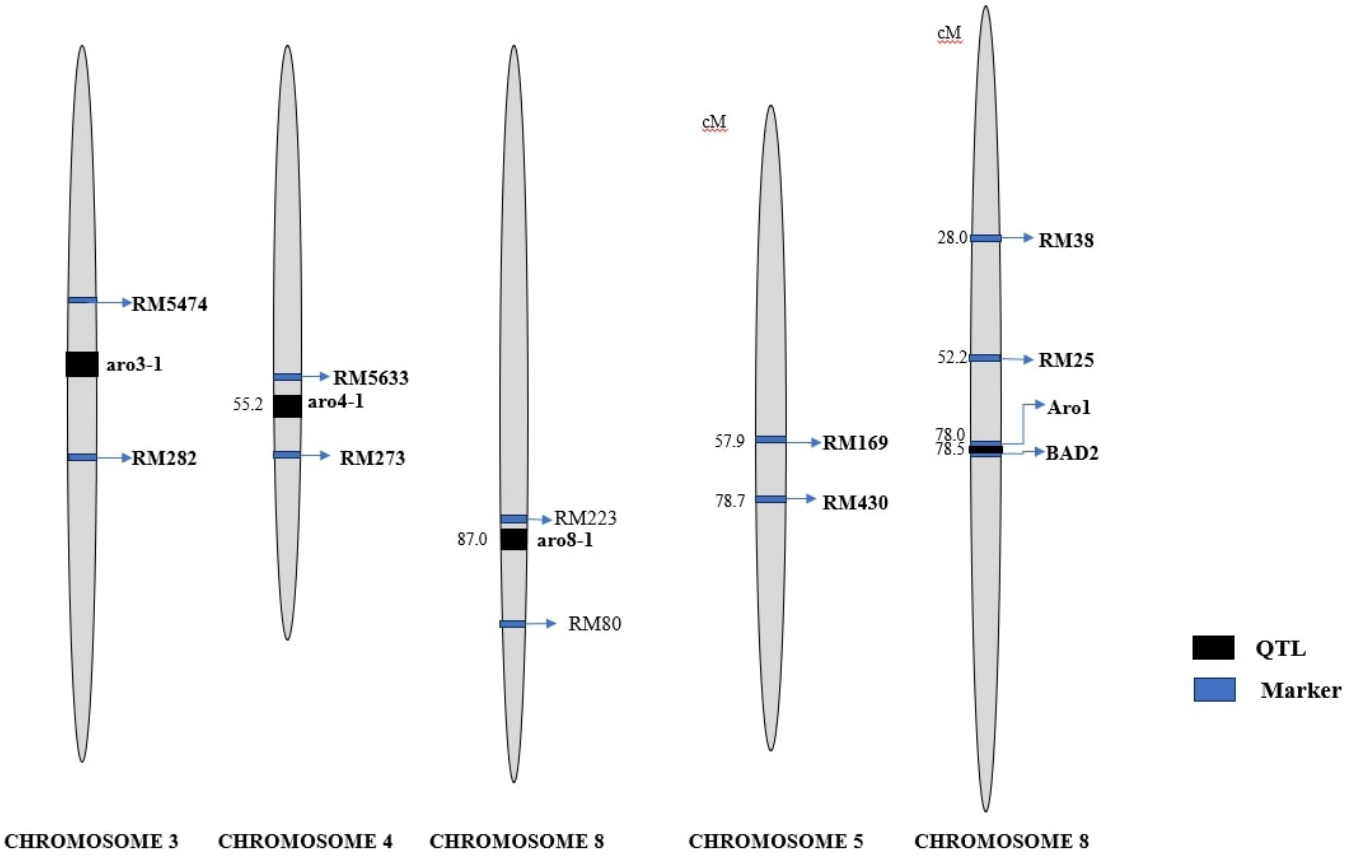


Fig. 4. Quantitative traits loci of rice aroma (32, 70).

Badh2 Manupulation Through Genetic Engneering:

CRISPR/Cas9 and TALEN:

The production of desirable characteristics through conventional breeding is a laborious process. Utilizing molecular techniques, genome manipulation is a regulated, site-specific procedure that modifies DNA sequences using base editing, prime editing, zinc finger nucleases (ZFN), transcriptional activator-like effector nucleases (TALENs) and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) System (73). *Badh2* was disrupted via TALEN-based genome editing, which raised 2-AP levels from 0.35 to 0.75 mg/kg. This level of 2-AP is nearly identical to that of the positive control variety of aromatic rice (74, 75). It was given evidence that the aroma of IR-96 was improved by genome editing (76). 20 T_1 individuals were genotyped after Zhonghua 11's fragrant gene *Badh2* was altered using CRISPR-Cas9 and a transgenic was produced that had an additional T base in the first exon of *Badh2*. The mutant had higher levels of 2-acetyl-1-pyrroline (0.9 mg/kg) and lower levels of *Badh2* mRNA than the wild-type. In addition, the mutant varies significantly from the control in 5 yield-related attributes, 3 cooking and eating-quality traits and tiller numbers and seed-setting rate. This offered a wealth of theoretical direction to quicken the fragrant rice breeding process (77). A CRISPR/Cas9 vector containing the rice U6 promoter and a single-guide RNA (sgRNA) intended for targeting the second exon of the *Badh2* gene was created in order to produce new alleles of *Badh2* (66). In the non-aromatic rice variety ASD16, they observed allelic variation in the *Badh2* gene's exon 7 that contributed to aroma. Furthermore, during sequence analysis, 22 distinct mutations in the sgRNA region (from ~17 to +15 bp) were discovered in aromatic T_0 lines. A mutant with 2 or 5 bp deletion produced a strong aroma, which was steadily passed down to the T_1 generation. As a result, 13 novel alleles of the aroma gene might be employed for future breeding purposes (31). Using the CRISPR/Cas9 system medicated by Agrobacterium-mediated editing at the splicing site of a plant gene resulted in exon skipping and the development of 2 mutants, *viz*., Rbadh2△G and Rbadh2△AAG, in rice culture R317. A premature termination codon (PTC) was found in exon 3 as a result of the deletion of exon 2 during splicing, according to an analysis of the processed mRNA from the Rbadh2ΔG and Rbadh2ΔAAG mutants. They gazed into how OsBADH2 exon 2 skipping affected the concentration of 2-AP in the grains of homozygous transgene-free plants of Rbadh2ΔG and Rbadh2ΔAAG. They discovered that 2-AP accumulation is increased due to the loss of OsBadh2 function. Two mutants show a comparatively higher 2-AP than the positive control, but there is no other significant variation in mutants (78).

Gene Silencing:

Gene silencing constitutes a molecular mechanism that suppresses or inhibits the gene expression, serving an essential function in a multitude of biological processes across diverse organisms (19). The production of GABA gradient panicles, which could diminish the yield in transgenic plants, is facilitated by *Badh2* (79). Although *Badh2* expression can be suppressed by gene silencing to produce steadily fragrant lines quickly, conventional breeding yields simpler and likely higher 2-AP content. Historically, hp-RNA technology has been used in attempts to silence *Badh2* gene. In addition, comparing wild type with

japonica rice variety's development delayed when subjected to salinity stress due to silencing of *Badh2* (66). The cDNA of *Badh2* was joined in the opposite direction to produce a hairpin RNAi, which was then driven by the 35S promoter. In transgenic Nippon bare rice, the levels of 2-AP rose to 20-fold. Introduction of RNAi technology made recognition for micro-RNA, which offers a potent tool for gene knockdown in wide range of eukaryotic species, including rice (80). GABA's contribution to yield is supported by the transgenic IR-64 aromatic line. The elevation of 2-pentylfuran and octanal in response to 2-AP induction suggests that their pathways coexist with 2-AP biosynthesis. Likewise, upregulating BADH2 also resulted in an expression of Badh2 gene dropped up to eight-fold in RNAi callus, up to 14-fold in the leaves of transgenic IR-64 seedling and also enzyme activity is reduced by 40 %, thereby validating their function in 2-AP biosynthesis (81). A similar increase in 2-AP production was observed when hpRNA disrupted *OsBadh2*, indicating that different *OsBadh2* gene expression levels affect scent accumulation. The 3 domains that the Badh2 enzyme is expected to have been the oligomerization, substrate, and NAD binding domains. It is anticipated that Badh2 will catalyse the oxidation of 3-amino propionaldehyde, 4-amino butyraldehyde (ABald), and betaine aldehyde. Badh2 was found throughout the cytoplasm. Non-Functional Badh2 alleles led to an increase in 2-AP biosynthesis and an accumulation of AB-ald (64). Artificial microRNA (amiRNA) technology is more specific and effective method for gene silencing compared to RNAi using hp-RNA (82).

Gene Pyramiding:

Gene pyramiding is a breeding technique that combines multiple advantageous genes into a single genotype to enhance traits such as yield and resistance and its objectives include improving trait effectiveness, increasing resilience to biotic stressors, facilitating selection through molecular markers and optimizing breeding practices (83). Pyramiding of fragrance *Badh2* gene (Wenxiang-1) and rice blast resistance Pi2 gene (R1179) with help of whole-genome SNP genotyping Marker assisted selection on the Wenxiang-1/R1179 F_2 segregation population with the functional markers Pi2-1 and Badh2-1, plants homozygous for both Pi2 and badh2 were selected. An analysis of the genetic composition of R365 revealed that 40.67 % of its entire genome was inherited from Wenxiang-1 and 59.33 % came from R1179. As a result, obtained R365 and R403 are two elite hybrid line as the male parent with high productivity (84).

Conclusion

Aroma development in scented rice, a quality trait is controlled by both genetic and environment factors. Among the 500 identified aroma compounds, 2-Acetyl-1-pyrroline (2-AP) is the principal aroma compound. Using CRISPR-Cas9, TALEN, gene silencing and gene pyramiding fragrance efficiency of the rice is possible through the engineered BADH2 gene. On the whole, the integration of traditional breeding methods with modern genetic tools holds great potential for the development of high-yielding, aromatic rice varieties. Further research in the biochemical pathways of aroma in rice will enable to manipulate the scented traits, preservation and improvement of aromatic rice.

Acknowledgements

The authors wish to heartfelt thanks to Abarna Thanushkodi for her support in writing assistance and general support given to prepare the manuscript.

Authors' contributions

JD and MMM conceived the concept and wrote the manuscript. MMM, PC, JHS and APM gave idea for design the diagram and tables. JD designed the diagram and tables. PC, APM, JHS, GP, KMP, SG and VK revised and finalized the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used none of AI Technology. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

1. Ahuja S, Panwar D, Uma A, Gupta K. Basmati rice: the scented pearl. Basmati rice: the scented pearl. 1995. Directorate of Publication, CCS Haryana Agricultural University, Hisar, India. 11-24.
2. Hashemi FG, Rafii M, Ismail M, Mahmud T, Rahim H, Asfaliza R, et al. Biochemical, genetic and molecular advances of fragrance characteristics in rice. Critical Reviews in Plant Sciences. 2013;32 (6):445-57. https://doi.org/10.1080/07352689.2013.807716
3. Wakte K, Zanan R, Hinge V, Khandagale K, Nadaf A, Henry R. Thirty -three years of 2-acetyl-1-pyrroline, a principal basmati aroma compound in scented rice (*Oryza sativa* L.): a status review. Journal of the Science of Food and Agriculture. 2017;97(2):384-95. https://doi.org/10.1080/07352689.2013.807716
4. Prodhan ZH, Qingyao S. Rice aroma: A natural gift comes with price and the way forward. Rice Science. 2020;27(2):86-100.
5. Hu EA, Pan A, Malik V, Sun Q. White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review. Bmj. 2012;344. https://doi.org/10.1016/j.tifs.2020.01.003
6. Yang X, Zandstra EH, Boesveldt S. How sweet odors affect healthy food choice: An eye-tracking study. Food Quality and Preference. 2023;109:104922.
7. Juliano BO, Villareal C. Grain quality evaluation of world rices. Int Rice Res Inst.; 1993.
8. Fitzgerald MA, McCouch SR, Hall RD. Not just a grain of rice: the quest for quality. Trends in Plant Science. 2009;14(3):133-39.

https://doi.org/10.1016/j.plantsci.2008.06.005

9. Jewel Z, Patwary A, Maniruzzaman S, Barua R, Begum S. Physico-chemical and genetic analysis of aromatic rice (*Oryza sativa* L.) germplasm. The Agriculturists. 2011;9(1-2):82-88. https://doi.org/10.3329/agric.v9i1-2.9482
10. Kadaru SB. Identification of molecular markers and association mapping of selected loci associated with agronomic traits in rice. 2007. Ph.D, Louisiana State University and Agricultural and Mechanical college, Baton Rouge. LA, USA.
11. Bordoloi D, Sarma D, Sarma Barua N, Das BK. Mutation induction in aromatic Joha rice of Assam for improvement of morpho-agronomic traits through M1 to M3 generation. International Journal of Radiation Biology. 2023;99(11):1760-77.
12. Sharma A, Srivastava A, Singh S, Mishra S, Mohan S, Singh A, et al. Aromatic rice of India: It's types and breeding strategies. Integrative Advances in Rice Research. 2021;59.
13. Engle LM. The cytogentics of sterility in F1 hybrids of Indica x Indica and Indica x Javanica varieties of rice, *Oryza sativa* L. Philippine Agriculturist. 1969;53(5-6):289-307. https://doi.org/10.1508/cytologia.34.572
14. Glaszmann J-C. Isozymes and classification of Asian rice varieties. Theoretical and Applied Genetics. 1987;74:21-30. https://doi.org/10.1007/BF00290078
15. Luo H, Duan M, Kong L, He L, Chen Y, Wang Z, et al. The regulatory mechanism of 2-acetyl-1-pyrroline biosynthesis in fragrant Rice (*Oryza sativa* L.) under different soil moisture contents. Frontiers in Plant Science. 2021;12:772-28. https://doi.org/10.1016/j.tibtech.2013.04.004
16. Luo H, Zhang T, Zheng A, He L, Lai R, Liu J, et al. Exogenous proline induces regulation in 2-acetyl-1-pyrroline (2-AP) biosynthesis and quality characters in fragrant rice (*Oryza sativa* L.). Scientific Reports. 2020;10(1):139-71. https://doi.org/10.1038/s41598-020-70984-1
17. Patra PS, Saha R, Ahmed AS, Kanjilal B, Debnath MK, Paramanik B, et al. Enhancing aromatic rice production through agronomic and nutritional management for improved yield and quality. Scientific Reports. 2024;14(1):15555. https://doi.org/10.1038/s41598-024-65476-5
18. Ramtekey V, Cherukuri S, Modha KG, Kumar A, Kethineni UB, Pal G, et al. Extraction, characterization, quantification and application of volatile aromatic compounds from Asian rice cultivars. Reviews in Analytical Chemistry. 2021;40(1):272-92. https://doi.org/10.1515/revac-2021-0137
19. Singh U. Aromatic rices: Int Rice Res Inst.; 2000.
20. Gaj T, Gersbach CA, Barbas CF. ZFN, TALEN and CRISPR/Cas-based methods for genome engineering. 2013;31(7):397-405. https://doi.org/10.1016/j.tibtech.2013.04.004
21. Zaidi SS-e-A, Vanderschuren H, Qaim M, Mahfouz MM, Kohli A, Mansoor S, et al. New plant breeding 2019;363(6434):1390-91. https://doi.org/10.1126/science.aav6316
22. Jiang Y, Cai Z, Xie W, Long T, Yu H, Zhang Q. Rice functional genomics research: progress and implications for crop genetic improvement. Biotechnology Advances. 2012;30(5):1059-70. https://doi.org/10.1016/j.biotechadv.2011.08.013
23. Singh S, Singh M, Kumar S, Sravan US. Cultivation of aromatic rice: A review. Agronomic Crops: Volume 1: Production Technologies. 2019;175-98. https://doi.org/10.1007/978-981-32-9151-5_10
24. Wambugu PW, Brozynska M, Furtado A, Waters DL, Henry RJ. Relationships of wild and domesticated rices (Oryza AA genome species) based upon whole chloroplast genome sequences. Scientific Reports. 2015;5(1):13957. https://doi.org/10.1038/srep13957
25. Bhattacharjee P, Singhal RS, Kulkarni PR. Basmati rice: a review. International Journal of Food Science and Technology. 2002;37(1):1-12. https://doi.org/10.1046/j.1365-2621.2002.00541.x
26. Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S. Genetic structure and diversity in *Oryza sativa* L. Genetics. 2005;169(3):1631-38.
27. RGP K. The 3000 rice genomes project. GigaScience. 2014;3(1):2047-175.
28. Zhao K, Tung C-W, Eizenga GC, Wright MH, Ali ML, Price AH, et al. Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. Nature Communications. 2011;2(1):467. https://doi.org/10.1038/ncomms1467
29. Civáň P, Ali S, Batista-Navarro R, Drosou K, Ihejieto C, Chakraborty D, et al. Origin of the aromatic group of cultivated rice (*Oryza sativa* L.) traced to the Indian subcontinent. Genome Biology and Evolution. 2019;11(3):832-43. https://doi.org/10.1093/gbe/evz039
30. Bourgis F, Guyot R, Gherbi H, Tailliez E, Amabile I, Salse J, et al. Characterization of the major fragance gene from an aromatic japonica rice and analysis of its diversity in Asian cultivated rice. Theoretical and Applied Genetics. 2008;117:353-68. https://doi.org/10.1007/s00122-008-0780-9
31. Champagne ET. Rice aroma and flavor: a literature review. Cereal Chemistry. 2008;85(4):445-54. https://doi.org/10.1094/cchem-85-4-0445
32. Ashokkumar S, Jaganathan D, Ramanathan V, Rahman H, Palaniswamy R, Kambale R, et al. Creation of novel alleles of fragrance gene OsBADH2 in rice through CRISPR/Cas9 mediated gene editing. PloS One. 2020;15(8):e0237018. https://doi.org/10.1371/journal.pone.0237018
33. Amarawathi Y, Singh R, Singh AK, Singh VP, Mohapatra T, Sharma TR, et al. Mapping of quantitative trait loci for basmati quality traits in rice (*Oryza sativa* L.). Molecular Breeding. 2008;21:49-65. https://doi.org/10.1007/s11032-007-9108-8
34. Kovach MJ, Calingacion MN, Fitzgerald MA, McCouch SR. The origin and evolution of fragrance in rice (*Oryza sativa* L.). Proceedings of the National Academy of Sciences. 2009;106(34):p.14444-49. https://doi.org/10.1073/pnas.0904077106
35. Lang NT, Buu BC. Identification and fine mapping of SSR marker linked to fgr gene of rice. Omonrice. 2002;10:16-22.
36. Lang NT, Buu BC. Development of PCR-based markers for aroma (fgr) gene in rice (*Oryza sativa* L.). Omonrice. 2008;16:p.16-23. https://doi.org/10.1111/j.1601-5223.1999.00121.x
37. Jezussek M, Juliano BO, Schieberle P. Comparison of key aroma compounds in cooked brown rice varieties based on aroma extract dilution analyses. Journal of Agricultural and Food Chemistry. 2002;50(5):1101-05. https://doi.org/10.1021/jf0108720
38. Hussain A, Mujtaba Naqvi S, Hammerschmidt F, editors. Isolation and identification of volatile components from basmati rice (*Oryza sativa* L.). Flavour Science and Technology: Proceedings of the 5th Weurman Flavour Research Symposium, held at the Sara Hotel, Voksenasen, Oslo, 23rd-25th March, 1987/edited by M Martens, GA Dalen and H Russwurm, Jr; 1987: Chichester [West Sussex]: Wiley, c1987.
39. Lin CF, Hsieh TCY, Hoff BJ. Identification and quantification of the "Popcorn"-like aroma in Louisiana aromatic Delia rice (*Oryza sativa* L.). Journal of Food Science. 1990;55(5):p.1466-67. https://doi.org/10.1111/j.1365-2621.1990.tb03961.x
40. Tanchotikul U, Hsieh TC. An improved method for quantification of 2-acetyl-1-pyrroline, a" popcorn"-like aroma, in aromatic rice by high-resolution gas chromatography/mass spectrometry/selected ion monitoring. Journal of Agricultural and Food Chemistry. 1991;39(5):944-47. https://doi.org/10.1021/jf00005a029

41. Tava A, Bocchi S. Aroma of cooked rice (*Oryza sativa*): Comparison between commercial Basmati. 1999;76(4):526-29. https://doi.org/10.1094/cchem.1999.76.4.526

42. Verma DK, Srivastav PP. Extraction technology for rice volatile aroma compounds. Food Engineering: Apple Academic Press. 2016;281-328. https://dx.doi.org/10.13140/RG.2.1.5172.9364

43. Widjaja R, Craske JD, Wootton M. Comparative studies on volatile components of non-fragrant and fragrant rices. Journal of the Science of Food and Agriculture. 1996;70(2):151-61. https://doi.org/10.1002/(sici)1097-0010(199602)70:2%3C151::aid-jsfa478%3E3.0.co;2-u

44. Bergman C, Delgado J, Bryant R, Grimm C, Cadwallader K, Webb B. Rapid gas chromatographic technique for quantifying 2-acetyl-1-pyrroline and hexanal in rice (*Oryza sativa* L.). Cereal Chemistry. 2000;77(4):454-58. https://doi.org/10.1094/CCHEM.2000.77.4.454

45. Wongpornchai S, Dumri K, Jongkaewwattana S, Siri B. Effects of drying methods and storage time on the aroma and milling quality of rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105. Food Chemistry. 2004;87(3):407-14.https://doi.org/10.1016/j.foodchem.2003.12.014

46. Kim C-Y, Lee J-C, Kim Y-H, Pyon J-Y, Lee S-G. Volatile flavor components of scent, colored and common rice cultivars in Korea. Korean Journal of Crop Science. 1999;44(3):181-85.

47. Buttery RG, Turnbaugh JG, Ling LC. Contribution of volatiles to rice aroma. Journal of Agricultural and Food Chemistry. 1988;36 (5):1006-09.

48. Hu X, Lu L, Guo Z, Zhu Z. Volatile compounds, affecting factors and evaluation methods for rice aroma. 2020;97:136-46. https://doi.org/10.1016/j.tifs.2020.01.003

49. McGorrin RJ. Character impact compounds: Flavors and off-flavors in foods. Flavor, Fragrance and Odor Analysis: CRC Press. 2001;p. 391-430. https://doi.org/10.1201/9780203908273.ch14

50. Lam H, Proctor A. Milled rice oxidation volatiles and odor development. Journal of Food Science. 2003;68(9):2676-81. https://doi.org/10.1111/j.1365-2621.2003.tb05788.x

51. Tananuwong K, Lertsiri S. Changes in volatile aroma compounds of organic fragrant rice during storage under different conditions. Journal of the Science of Food and Agriculture. 2010;90(10):1590-96. https://doi.org/10.1002/jsfa.3976

52. Weber D, Rohilla R, Singh U. Chemistry and biochemistry of aroma in scented rice. Singh, Singh and Khush (eds), Aromatic Rices, Oxford and IBH Publ. 2000;29-46.

53. Yang DS, Lee K-S, Jeong O-Y, Kim K-J, Kays SJ. Characterization of volatile aroma compounds. 2008; 56(1): 235-40. https://doi.org/10.1021/jf072360c

54. Vanavichit A, Yoshihashi T. Molecular aspects of fragrance and aroma in rice. Advances in Botanical Research. 56: Elsevier. 2010;49-73. https://doi.org/10.1016/B978-0-12-381518-7.00002-9

55. Hinge VR, Patil HB, Nadaf AB. Aroma volatile analyses and 2AP characterization at various developmental stages in Basmati and Non-Basmati scented rice (*Oryza sativa* L.) cultivars. Rice. 2016;9 (1):1-22. https://doi.org/10.1186/s12284-016-0113-6

56. Poonlaphdecha J, Gantet P, Maraval I, Sauvage F-X, Menut C, Morère A, et al. Biosynthesis of 2-acetyl-1-pyrroline in rice calli cultures: Demonstration of 1-pyrroline as a limiting substrate. Food Chemistry. 2016;197:965-71. https://doi.org/10.1016/j.foodchem.2015.11.060

57. Yoshihashi T, Huong NTT, Inatomi H. Precursors of 2-acetyl-1-pyrroline, a potent flavor compound of an aromatic rice variety. Journal of Agricultural and Food Chemistry. 2002;50(7):2001-04. https://doi.org/10.1021/jf011268s

58. Kaikavoosi K, Kad TD, Zanan RL, Nadaf AB. 2-acetyl-1-pyrroline augmentation in scented indica rice (*Oryza sativa* L.) varieties through Δ 1-pyrroline-5-carboxylate synthetase (P5CS) gene transformation. Applied Biochemistry and Biotechnology. 2015;177:1466-79. https://doi.org/10.1007/s12010-015-1827-4

59. Bradbury LM, Fitzgerald TL, Henry RJ, Jin Q, Waters DL. The gene for fragrance in rice. Plant Biotechnology Journal. 2005;3(3):363-70. https://doi.org/10.1111/j.1467-7652.2005.00131.x

60. Peng B, Kong D, Song X, Li H, He L, Gong A, et al. A method for detection of main metabolites in aromatic rice seeds. Agricultural Biotechnology. 2018;7(1):112-16.

61. Chakraborty D, Deb D, Ray A. An analysis of variation of the aroma gene in rice (*Oryza sativa* L. subsp. *indica* Kato) landraces. Genetic Resources and Crop Evolution. 2016;63(6):953-59. https://doi.org/10.1007/s10722-016-0414-z

62. Ky H, Tung NCT, Hien NL, van Manh N, Thanh NN, Thanh VC, et al. Novel deletion in exon 7 of betaine aldehyde dehydrogenase 2 (BADH2). Rice Science. 2023;30(2):104-12. https://doi.org/10.1016/j.rsci.2023.01.003

63. Shan Q, Wang Y, Chen K, Liang Z, Li J, Zhang Y, et al. Rapid and efficient gene modification in rice and Brachypodium using TALENs. Molecular Plant. 2013;6(4):1365-68. https://doi.org/10.1093/mp/sss162

64. Chen S, Yang Y, Shi W, Ji Q, He F, Zhang Z, et al. Badh2, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. The Plant Cell.2008;20(7):1850-61. https://doi.org/10.1105/tpc.108.058917

65. Shao G, Tang A, Tang S, Luo J, Jiao G, Wu J, et al. A new deletion mutation of fragrant gene and the development of three molecular markers for fragrance in rice. Plant Breeding. 2011;130 (2):172-76. https://doi.org/10.1111/j.1439-0523.2009.01764.x

66. Niu X, Tang W, Huang W, Ren G, Wang Q, Luo D, et al. RNAi-directed downregulation of OsBADH2 results in aroma (2-acetyl-1-pyrroline) production in rice (*Oryza sativa* L.). BMC Plant Biology. 2008;8(1):1-10. https://doi.org/10.1186/1471-2229-8-100

67. Ootsuka K, Takahashi I, Tanaka K, Itani T, Tabuchi H, Yoshihashi T, et al. Genetic polymorphisms in Japanese fragrant landraces and novel fragrant allele domesticated in northern Japan. Breeding Science.2014;64(2):115-24. https://doi.org/10.1270/jsbbs.64.115

68. Shi Y, Zhao G, Xu X, Li J. Discovery of a new fragrance allele and development of functional markers for identifying diverse fragrant genotypes in rice. Molecular Breeding. 2014;33:p.701-08. https://doi.org/10.1007/s11032-013-9986-x

69. Sakthivel K, Sundaram R, Rani NS, et al. Genetic and molecular basis of fragrance in rice.Biotechnology Advances. 2009;27(4):468-73. https://doi.org/10.1016/j.biotechadv.2009.04.001

70. Lorieux M, Petrov M, Huang N, Guiderdoni E, Ghesquière A. Aroma in rice: genetic analysis of a quantitative trait. Theoretical and Applied Genetics. 1996;93:1145-51. https://doi.org/10.1007/BF00230138

71. Talukdar PR, Rathi S, Pathak K, Chetia SK, Sarma RN. Population structure and marker-trait association in indigenous aromatic rice. Rice Science. 2017;24(3):145-54. https://doi.org/10.1016/j.rsci.2016.08.009

72. Pachauri V, Mishra V, Mishra P, Singh AK, Singh S, Singh R, et al. Identification of candidate genes for rice grain aroma by combining QTL mapping and transcriptome profiling approaches. Cereal Research Communications. 2014;42:376-88. https://doi.org/10.1556/crc.42.2014.3.2

73. Aqib Z, Ahmad S, Tabbasum J, Sheng Z, Peisong H. Rice grain yield and quality improvement via crispr/cas9 system: an updated review. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2022;50(3):12388.

74. Hui S, Li H, Mawia AM, Zhou L, Cai J, Ahmad S, et al. Production of aromatic three-line hybrid rice using novel alleles of BADH2. Plant Biotechnology Journal. 2022;20(1):59-74. https://doi.org/10.1111/pbi.13695

75. Shan Q, Zhang Y, Chen K, Zhang K, Gao C. Creation of fragrant rice by targeted knockout of the Os BADH 2 gene using TALEN technology. Plant Biotechnology Journal. 2015;13(6):791-800. https://doi.org/10.1111/pbi.12312

76. Usman B, Nawaz G, Zhao N, Liu Y, Li R. Generation of high yielding and fragrant rice (*Oryza sativa* L.) lines by CRISPR/Cas9 targeted mutagenesis of three homoeologs of cytochrome P450 gene family and OsBADH2 and transcriptome and proteome profiling of revealed changes triggered by mutations. Plants. 2020;9(6):788. https://doi.org/10.3390/plants9060788

77. Shao GaoNeng SG, Xie LiHong XL, Jiao Guiai JG, Wei XiangJin WX, Sheng ZhongHua SZ, Tang ShaoQing TS, et al. CRISPR/CAS9-mediated editing of the fragrant gene Badh2 in rice. 2017;31(2): 216-22. https://doi.org/10.16819/j.1001-7216.2017.6098

78. Tang Y, Abdelrahman M, Li J, Wang F, Ji Z, Qi H, et al. CRISPR/Cas9 induces exon skipping that facilitates development of fragrant rice. Plant Biotechnology Journal. 2021;19(4):642. https://doi.org/10.1111/pbi.13514

79. Bradbury LM, Gillies SA, Brushett DJ, Waters DL, Henry RJ. Inactivation of an aminoaldehyde dehydrogenase is responsible for fragrance in rice. Plant Molecular Biology. 2008;68:439-49. https://doi.org/10.1007/s11103-008-9381-x

80. Warthmann N, Chen H, Ossowski S, Weigel D, Hervé P. Highly specific gene silencing by artificial miRNAs in rice. PLoS One. 2008;3(3):e1829. https://doi.org/10.1371/journal.pone.0001829

81. Khandagale KS, Chavhan R, Nadaf AB. RNAi-mediated down regulation of BADH2 gene for expression of 2-acetyl-1-pyrroline in non-scented indica rice IR-64 (*Oryza sativa* L.). 3 Biotech. 2020;10 (4):145. https://doi.org/10.1007/s13205-020-2131-8

82. Ossowski S, Schwab R, Weigel D. Gene silencing in plants using artificial microRNAs and other small RNAs.The Plant Journal. 2008;53(4):674-90. https://doi.org/10.1111/j.1365-313x.2007.03328.x

83. Dormatey R, Sun C, Ali K, Coulter JA, et al. Gene pyramiding for sustainable crop improvement against biotic and abiotic stresses. Agronomy. 2020;10(9):1255.

84. Wang Y, Tang S, Guo N, An R, Ren Z, Hu S, et al. Pyramiding rice blast resistance gene Pi2 and fragrance gene badh2. Agronomy. 2023;13(2):589. https://doi.org/10.3390/agronomy13020589

85. Khandagale KS, Chavan R, Nadaf AB. RNAi mediated down regulation of BADH2 gene for expression of 2-acetyl-1-pyrroline in non-scented indica rice IR-64 (*Oryza sativa* L.). Can J Biotechnol. 2017;1:169. https://doi.org/10.24870/cjb.2017-a155

86. Shi W, Yang Y, Chen S, Xu M. Discovery of a new fragrance allele and the development of functional markers for the breeding of fragrant rice varieties. Molecular Breeding. 2008;22:185-92. https://doi.org/10.1007/s11032-008-9165-7

87. Shao G, Tang S, Chen M, Wei X, He J, Luo J, et al. Haplotype variation at Badh2, the gene determining fragrance in rice. Genomics. 2013;101(2):157-62. https://doi.org/10.1016/j.ygeno.2012.11.010

88. He Q, Park Y-J. Discovery of a novel fragrant allele and development of functional markers for fragrance in rice. Molecular Breeding. 2015;35:1-10. https://doi.org/10.1007/s11032-015-0412-4

89. Ganigara Bindusree GB, Purushothaman Natarajan PN, Sukesh Kalva SK, Parani Madasamy PM. Whole genome sequencing of *Oryza sativa* L. cv. seeragasamba identifies a new fragrance allele in rice. 2017;12(11);e0188920. https://doi.org/10.1371/journal.pone.0188920

90. Information NCfB. PubChem compound summary for CID 17100, alpha-terpineol: Pubchem [updated 07-09-2024.Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Alpha-Terpineol.

91. Information NCfB. PubChem compound summary for CID 522834, 2-acetyl-1-pyrroline 2024 [updated 07-09-2024. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/2-Acetyl-1-pyrroline.

92. Information NCfB. PubChem compound summary for CID 8103, 1-hexanol 2024 [updated 07-09-2024. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/1-Hexanol.

93. Information NCfB. PubChem compound summary for CID 454, octanal. 2024 [updated 09-07-2024. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Octanal.

94. Information NCfB. PubChem compound summary for CID 31289, nonanal. 2024 [pdated 07-09-2024. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Nonanal.

95. Information NCfB. PubChem compound summary for CID 18827, 1-octen-3-OL. 2024 [updated 07-09-2024. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/1-Octen-3-OL.

96. Information NCfB. PubChem compound summary for CID 19602, 2-pentylfuran. 2024 [updated 07-09-2024. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/2-Pentylfuran.

97. Information NCfB. PubChem compound summary for CID 34286, 2-methyl-3-furanthiol. 2024 [updated 07-09-2024. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/2-Methyl-3-