

## Aromatic rice and factors affecting aroma in rice

### ABSTRACT

Aromatic rice is considered as an important value-added commodity with small variations in aroma determining preference and unacceptability by the consumers. Aroma is the most desired trait followed by taste and elongation. Indian sub-continent is home to aromatic rice diversity with north east being the richest reservoir of genetic variability of aromatic rice. Aromatic rice has two groups, the long-grained Basmati type and the small and medium-grained indigenous aromatic varieties. 2-acetyl-1-pyrroline (2AP) is the principal component responsible for aroma, produced through a single recessive allele (*fgr*) at a locus on chromosome 8 which corresponds to the gene that encodes betaine aldehyde dehydrogenase (*BADH2*). A premature stop codon upstream of key coding regions is introduced by an 8-bp deletion in exon 7 which makes *BADH2* non-functional. The mutant *BADH2* transcript leads to 2AP accumulation in aromatic rice. The flow of  $\gamma$ -aminobutyraldehyde (GAB-ald) to  $\gamma$ -aminobutyric acid (GABA) is inhibited and consequently, the accumulated GAB-ald is diverted to a flavour component, 2-acetyl-1-pyrroline (2AP) by a non-enzymatic reaction with methylglyoxal. Accumulation of 2-acetyl-1-pyrroline in aromatic rice depends on the interaction of various genetic and environmental factors. Aromatic rice is prone to biotic and abiotic stress. Grain quality of aromatic rice can be improved using existing aromatic genotypes by backcrossing, mutation breeding or conventional cross-breeding. There is an urgent need to document, evaluate and conserve underutilized germplasm for improvement of aromatic rice in the future.

**KEYWORDS:** 2-acetyl-1-pyrroline, betaine aldehyde dehydrogenase, aroma.

### ABBREVIATION

2AP- 2-acetyl-1-pyrroline, *fgr*, *BADH2*- Betaine aldehyde dehydrogenase, GAB-ald-  $\gamma$ -aminobutyraldehyde-, GABA  $\gamma$ -aminobutyric acid, GC- FID- Gas chromatography - flame ionization detector, GC-MS -Gas chromatography – mass spectrometry, SPME -Solid phase micro-extraction, TALEN -Transcription activator- like effector nucleases, CRISPR/Cas9- Clustered regularly interspaced short palindromic repeat-associated protein 9.

### 1. INTRODUCTION:

Aromatic rice is considered as an important value-added commodity with small variations in aroma determining preference and unacceptability by the consumers. *Oryza sativa* has two major varietal groups, *indica* (*Hsien*) and *japonica* (*Keng*) that have been recognized in China since ancient times. Conventional scented rice varieties consist of three isozyme groups viz., Group I (*indica*), Group V (*indica*), and Group VI (tropical *japonica*) [1]. Scented or aromatic rice is an important commodity worldwide and fetches premium prices over non-scented varieties both in local and international market due to their superior grain quality and pleasant aroma. Aroma is the most desired trait followed by taste and elongation by Indian consumers. Indian sub-continent is home to aromatic

rice diversity with north east being the richest reservoir of genetic variability of aromatic rice from the agro horticultural point of view. Aromatic rice bears special significance in the present globalized era due to its unique flavour and economic value. A diverse gene pool of aromatic rice is found in Assam which differs in intensity and durability of aroma, production potentialities and shape and size of grain. Aromatic rice landraces of Assam are a superior class under Sali or winter rice known as Joha rice [2].

Aromatic rice has two groups, the long-grained basmati type and the small and medium-grained indigenous aromatic varieties or landraces. Aromatic rice arising from diverse origins differs in aroma. Areas under cultivation of Basmati rice have stagnated as Basmati rice loses aroma and essential quality traits if grown beyond stipulated tracts. In addition to the traditional long-grain basmati which is confined to North India, several small and medium grain aromatic rice varieties are cultivated in India. There is a need to develop new aromatic rice varieties to maintain India's position in the global scented rice market to boost export. Only a few non-basmati scented rice varieties are traded in the international market while several are not, despite their eminent qualities such as elongation after cooking, aroma and agronomic characters [3]. Aromatic rice with variations in intensity of aroma, grain shape and size, durability, production potentialities etc. is found in Assam. Joha rice, an intermediate of typical Indica and Japonica class is medium slender or medium broad category. Joha rice cultivars are known for its unique aroma, good cooking qualities, superfine kernel and excellent palatability. Joha is popular due to its inherent scent and is high in demand for export. However, Joha rice is less favourable among the producers and area under Joha rice is nominal owing to its low productivity and poor yielding ability. Joha rice is also prone to lodging, pest attack and takes relatively longer time to mature (120-160 days). Joha grains are shorter (<4.7 mm) and have limited elongation (1.1 times) in comparison to other scented rice of India. Joha grains have crude protein content in the range of 9.17-11.67 % in comparison to that of Basmati. Variations in protein, amylase, amylopectin and mineral contents can be attributed to genetic factors and environmental influences. Joha rice has higher content of albumin, globulin and amylase and is nutritionally comparable to Basmati. High levels of albumin and globulin in aromatic rice play an important role in protein digestibility and also increase biological value [4].

## **2. THE GENE FOR FRAGRANCE IN RICE:**

2-acetyl-1-pyrroline (2AP) is the principal component responsible for fragrance, produced through a single recessive allele (*fgr*) at a locus on chromosome 8 which corresponds to the gene that encodes betaine aldehyde dehydrogenase (*BADH2*). A premature stop codon upstream of key coding regions is introduced by an 8-bp deletion in exon 7 which makes this gene non-functional (*badh2*). The mutant *badh2* transcript leads to 2AP accumulation in aromatic rice [3]. 2AP is a colourless to yellow solid and is categorized as flavour and fragrance chemical compounds [5]. The major compound responsible for aroma in aromatic rice is 2-acetyl-1-pyrroline (2AP) which has a very low odour threshold making it possible for humans to detect it at minute concentrations. Oxidation of  $\gamma$ -aminobutyraldehyde (AB-ald), a 2AP precursor is catalyzed by BADH2 protein so that a nonfunctional allele results in the accumulation of both AB-ald and its cyclic form, delta-1 pyrroline, which results in increase in synthesis of 2AP [6]. The 2AP level in the aerial parts of plants is relatively higher in comparison to milled rice grains. The *badh2* alleles in aromatic rice varieties all have common insertions/deletions and single nucleotide

polymorphisms compared to non-aromatic rice varieties, demonstrating a common ancestor for all aromatic genotypes [7].

### **3. MAIN 2AP SYNTHESIS PATHWAY:**

In this pathway, polyamines (arginine, ornithine, putrescine etc.) are converted to GAB-ald (the immediate precursor of  $\gamma$ -aminobutyric acid, GABA) which spontaneously cyclises to delta-1-pyrroline. Delta-1-pyrroline is an immediate precursor of 2AP and a key factor for regulating the rate of biosynthesis of 2AP. In non-aromatic rice the functional BADH2 enzyme (coded by *OsBadh2*) converts GAB-ald to GABA which inhibits biosynthesis of 2AP. In aromatic rice, due to non-functional badh2 enzyme (coded by *osbadh2*) GAB-ald cannot be converted to GABA which results in accumulation of GAB-ald leading to the formation of 2AP [8].

### **4. DETECTION OF AROMA:**

Detection of aroma compounds in rice is done both quantitatively and qualitatively. Fragrance is measured on a scale of one to ten using sensory panelists scaling fragrance or by utilization of a combination of sensory panelists and gas chromatographic methods to measure the level of 2AP in plant samples. To categorize fragrance phenotype, binary system of fragrant/non-fragrant is used which is a simple method. Reaction of seeds and leaf tissue to Potassium hydroxide is used as a common and convenient method to evaluate aroma. Concentration of 2-AP is determined by gas chromatography - flame ionization detector (GC-FID) or gas chromatography – mass spectrometry (GC-MS). Volatile components are analysed by solid phase micro-extraction (SPME) fibers in combination with gas chromatography – mass spectrometry. SPME is useful in screening but not quantification of 2-acetyl-1-pyrroline in aromatic rice varieties [1].

## **5. FACTORS AFFECTING RICE AROMA AND FLAVOUR**

### **5.1. GENETICS**

2-acetyl-1-pyrroline (2AP) is believed to accumulate in rice due to an eight-base-pair (8-bp) deletion on chromosome 8 that encodes a putative betaine aldehyde dehydrogenase 2 (BAD2) leading to fragrance in rice [9]. 464 varieties of rice were evaluated to quantify 2AP and presence or absence of the fragrance allele (*fgr*). The 8-bp deletion was absent in several cultivars studied in spite of containing 2-AP suggesting that at least one other mutation influences accumulation of 2AP other than the 8-bp deletion in *fgr* causing aroma in rice. Identification of multiple mutations for 2AP will enable selection for multiple genetic sources of 2AP which will help rice breeding programmes to develop highly aromatic varieties of rice [10].

### **5.2. PRE HARVEST:**

#### **5.2.1. ENVIRONMENT:**

Absence of favourable condition during aromatic rice growth stage in different environments leads to difference in aroma expression. Further studies need to be carried out to reveal the involvement of enzymes responsible for aroma and environmental mitigation to connect the genetic, molecular and chemical aspect of aromatic rice [8].

#### **5.2.2. RESPONSE TO ABIOTIC STRESS:**

Aromatic rice is highly sensitive to abiotic stress mainly salinity, drought, extreme temperature, submergence and ultraviolet irradiation, which reduce crop productivity and grain quality [11]. A number of protective mechanisms involving proline, methylglyoxal, GABA and calmodulin are present in rice plants which act as signaling molecules and protective agents during stress conditions. Polyamine degradation pathway (functional *OsBadh2* gene) or GABA-shunt pathway (activity of *OsGAD* genes) produces GABA. During stress conditions GABA-shunt pathway becomes more active and GABA binds to the GABA-like receptor and releases  $Ca^{2+}$  from intracellular storage to increase  $Ca^{2+}$  accumulation in cytosol. As a result, the  $Ca^{2+}/CaM$  complex and stress response signal is amplified which induces stress response genes. Aromatic and non-aromatic rice with specifically inhibited *OsBadh1* and *OsBadh2* genes are more susceptible to salt stress than wild-type with normal gene expression. 2AP content increases due to drought stress during grain formation and salt stress during vegetative growth. The increase in 2AP content during stress conditions is due to emission of the starch-bound and free form of 2AP. GABA can be produced only by the GABA-shunt pathway in aromatic rice as the polyamines to GABA pathway remains inactive due to non-functional BADH enzyme. Therefore, elite aromatic rice varieties are susceptible to biotic and abiotic stresses. Factors such as soil, relative humidity, temperature, moisture content and pH during flowering to maturity stages highly affect aroma quality and survival of aromatic rice plant by regulating glycolysis, 2AP and GABA-shunt pathway [8].

##### **5.2.2.1. RESPONSE TO TEMPERATURE**

The quality of aroma is affected by high (> 30 °C), low (< 20 °C) and optimum temperatures (20 °C to

30 °C) temperature, with higher 2AP production at optimum temperature, environmental or suitable growing conditions[12]. Higher 2AP production occurs at optimum temperature because enzymes involved in the main 2AP biosynthesis pathway remain more active with low evaporation rate of 2AP. Additionally, methylglyoxal from glycolysis pathway contributes to produce 2AP by reacting non-enzymatically with delta-1-pyrroline by signaling for maintaining the physiological process. Polyamines such as glutamate, proline, ornithine, etc. are converted to delta-1-pyrroline content under optimum temperature condition which remains in equilibrium to the GAB-ald concentration. Moreover, proline produced from glutamate can be converted to GAB-ald which is further converted to 2AP instead of GABA in aromatic rice due to the non-functional *osbadh2* gene [8].

#### **5.2.2.2. SOIL:**

Aroma in rice decreases or is absent when soil pH is <4.70 or  $\geq 5.10$ , with optimum pH in range of 4.70 - 5.00 being suitable for aroma. Aroma is influenced by soil texture with highest aroma observed in sandy soil and loamy sand and lowest aroma in clayey soils. Aroma is more in sandy soil due to dehydration which makes it moderately saline and under drought stress. This results in a high level of proline synthesis for rice to adapt and survive under water stress which in turn increases synthesis of 2AP, leading to an increase in aroma. Therefore, sandy loam or loamy sand which is slightly acidic or a moderate salinity or slightly salty is suitable for higher aroma [13].

**5.2.3. CULTURAL PRACTICES:** Aroma and flavour of aromatic rice is influenced by cultural methods or cultivation practices [8].

#### **5.2.3.1. NUTRIENT APPLICATION**

Mo *et al.*, 2018 [14] studied the effect of time-specific nutrient application on 2-AP during the developmental phases of aromatic rice cultivar Yungengyou 14. The accumulation pattern of proline, 2-AP and nitrogen and the relationships among them regarding 2-AP accumulation were studied at three nitrogen levels (0, 30 and 60kg /ha) at the booting stage. The highest 2AP content was observed in ear axes and flag leaves at 17 and 30 DAF under all the nitrogen levels studied. High 2-AP and proline contents were observed in different plant tissues during the early grain filling stage on the application of nitrogen at the booting stage. Therefore, the grain aroma contents of aromatic rice can be improved by additional dose of nitrogen at the booting stage. Aroma in Basmati rice is determined by complex interactions among the recessive gene (*badh2*), soil conditions, climate and geographical location. Reduction in the quality of aroma in Basmati and other scented rice varieties has been observed which might be attributed to disturbance in rhizospheric microflora caused by the excessive use of inorganic fertilizers and adoption of modern agriculture practices. Beneficial microorganisms can be utilized which modify plant metabolomics to improve plant growth and health to enhance rice aroma [15]. The amylose and protein contents of rice are influenced by cultural practices and environment which affect the flavour and aroma of cooked rice. Rice samples of the same cultivar having low protein had more flavour than samples with high protein [16].

### **5.3. HARVEST:**

Concentration of 2-AP decreased with an increase in planting density and time of harvest. In order to select the optimum time of harvest, paddy yield and grain qualities has to be considered in addition to aroma content. 2-AP content decreased with time and was significantly affected by the temperature with higher 2-AP concentrations obtained with the shortest storage time of 3 months and the lowest storage temperature of – 4°C [17].

### **5.4. POST-HARVEST:**

#### **5.4.1. DRAIN AND HARVEST DATES**

Early drainage of fields may lead to moisture stress in grains before they attain physiological maturity. Moisture stress in grains will affect metabolic processes and in turn, volatile flavour compounds. Early harvest with higher moisture contents and delay in drying lead to microbial growth and off-flavour metabolites. Aroma and flavour declines with maturity [16]. Itani *et al.* (2004) [18] studied the effect of harvest time on the concentration of 2-AP in two cultivars. It was observed that concentration of 2-AP reached a peak at four or five weeks after heading (WAH) in early-heading cultivar and four WAH in a late-heading cultivar. The 2-AP concentration decreased rapidly to 20% and 40% of the maximum in early and late heading cultivar respectively. Champagne *et al.* (2005) [19] studied the effects of drain and harvest dates on sensory properties of rice and observed stable flavour with timing of field draining (14-day span) and harvesting (32-48 days after flowering).

#### **5.4.2. HARVEST MOISTURE CON**

Paddy may be held for more than 24 hours after harvest and before drying at moisture contents ranging from 16 to more than 26%. Growth of microbes may produce volatile compounds which affect the aroma or flavour of rice [16].

#### **5.4.3. DEGREE OF MILLING**

The aroma of milled rice varies with the degree of milling [16]. Increased milling from 8 to 14% led to higher sweet taste and lower hay-like flavour, raw rice flavour, wet cardboard flavour and bitter [20]. The effect of degree of milling on the flavour attribute intensities of three rice varieties were found to be dependent on moisture content and variety or location. Intensities of desirable and undesirable flavour attributes were higher in rice dried to 15% moisture compared to 12% [16].

#### **5.4.4. MILLED RICE STORAGE TEMPERATURE AND TIME**

Stale flavour is developed in milled rice during storage due to hydrolysis of surface lipids to form free fatty acids which are susceptible to oxidation. Free fatty acid formation also occurs due to the presence of lipase of residual bran on the surface of milled rice. Moreover, oxidation of unsaturated fatty acids and formation

of different secondary oxidation products contribute to development of odours and off flavours. The process is enhanced by milling by disruption of cells and release of lipoxygenase (Champagne, 2008). Muthikul *et al.* (2019) [21] studied the effect of drying and storage times on aroma quality of Khao Dawk Mali-105 (KDML). It was observed that drying time did not affect aroma characteristics of KDML fragrant brown rice (KBR) significantly. Longer storage time decreased the intensity of desirable aroma of uncooked and cooked KBR significantly while the intensity of undesirable increased significantly. It was observed that drying treatment and longer storage time decreased the 2-AP content of KBR. High intensity of desirable aroma and lower intensity of undesirable odour was observed in cooked KBR dried at 35°C for 2 and 6 hours than non-drying treatment.

#### **5.4.5. DRYING CONDITIONS OF ROUGH RICE, FINAL MOISTURE CONTENT AND STORAGE:**

The aroma and flavour of rice after milling is affected by the storage time and temperature. Concentration of 2-AP decreases if rice is dried at high temperature [16]. Wongpornchai *et al.* (2004) [22] studied the effect of storage time and drying methods on aroma of aromatic rice variety, Khao Dawk Mali 105. It was observed that concentration of 2-acetyl-1-pyrroline decreased with increase in storage time. Lower temperature provided higher 2-acetyl-1-pyrroline and lower concentration of off-flavour compounds.

#### **6. DISAPPEARANCE OF AROMATIC RICE VARIETIES:**

Introduction of high yielding and better-quality varieties has shifted research focus from aromatic to modern varieties of non-aromatic rice which has led to subsistence farming of aromatic varieties. The decrease in area under cultivation of aromatic rice in addition to low productivity, pest susceptibility, unavailability of quality seeds as a result of increasing erosion of genetic diversity discourage farmers from cultivation of aromatic rice. Economic constraints such as limited financial support from financial institutions, lack of better infrastructure and transportation facilities for processed rice are faced by the farmers. Erosion of aromatic rice diversity is a huge problem which is mainly due to small farm sizes as a result of which farmers are able to cultivate only a small quantity of a particular aromatic rice variety. Inability by the farmers to retain the produce for seeds further contributes to the continuous disappearance of aromatic rice varieties. Due to non-replacement of seeds for years together, seed quality gets deteriorated due to which several valuable aromatic rice varieties in the past 20 years have either become extinct or are on the verge of extinction [23].

#### **7. CONCLUSION:**

Aromatic rice is generally grown for subsistence agriculture and leads to nonexistence of well-developed market. With the utilization of modern agricultural methods, several cultivars have been replaced by lesser number of varieties. Varieties with high quality can be developed using traditional varieties. The intensity and type of aroma determines the uniqueness of aromatic genotypes which is dependent on genetic architecture and the agro-climatic factors. Protection of high value aromatic genotypes requires active participation of farmers [24]. India is home to several indigenous aromatic short grain cultivars and land races with most of them being area specific with states having their own aromatic rice varieties. Many states

particularly Uttar Pradesh, Madhya Pradesh, Bihar, Orissa, West Bengal and Assam have a wealth of genetic diversity of short grain aromatic types which with the exception of shorter grain length and shape, have specific adaptation, excellent cooking and eating quality traits similar to Basmati rice. The short grained aromatic rice varieties are mostly used by local consumers due to lack of export potential. Several aromatic rice varieties have been lost and are on the verge of extinction especially the small and medium-grained non-Basmati aromatic rice. There is an urgent need to document, evaluate, conserve and utilize underutilized germplasm for improvement of aromatic rice in the future [25].

Aromatic rice contains superior nutritional values and better amino acid profiles than non-aromatic varieties. Few short grain aromatic varieties/landraces have better aroma and other quality traits in comparison to long grain type but are susceptible to various diseases with low yield due to lack of planned breeding programme for their genetic manipulation. Therefore, there is a need to identify and promote genetic improvement of such short grain varieties for cultivation and marketing globally. Knowledge gained by characterization and quantification of genetic diversity within and among closely related crop varieties will help in the development of a suitable breeding program and management of plant genetic [1]. With the increase in demand and consumption of high-quality aromatic rice there is a need for research on aroma genes and also produces new aromatic varieties of rice. Grain quality of aromatic rice can be enhanced by backcrossing, mutation breeding or conventional cross-breeding of existing aromatic genotypes which is time-consuming and laborious. Transfer of desirable genes to aromatic rice genotypes will assist to overcome limitations of existing aromatic rice varieties [8]. With consumer preferences playing a huge role, it is necessary to maintain quality checks from farm to table level. In order to maintain quality, safety factors such as pesticides residues, irradiation doses should be considered. India plays a huge role in trade of aromatic rice globally and therefore it is essential to understand the pattern of domestic trade of aromatic rice at the micro level to promoting their cultivation and export. Moreover, there is a need for conservation and utilization of available germplasm for breeding new varieties while retaining the aroma quality. Multi-location trials can be carried out to understand the bio physical socio economic and cultural dimensions of aromatic rice. Aromatic varieties must be registered to prevent biopiracy. Active participation of research organizations and all stakeholders in cultivation is necessary especially in different agro-climatic zones dominated with aromatic rice cultivation to improve and maintain quality of aromatic rice as well as improve domestic trade [26].

Non-aromatic rice can be used to produce aromatic rice by manipulating the aroma genes. Premature termination codon or encoded amino acid change corresponding Badh2 protein in rice is caused by insertion, deletion or replacement of *OsBadh2* gene. Synthesis and accumulation of 2AP function will lead to the emergence of new aroma gene(s) due to loss of function of *OsBadh2* gene by RNAi and transcription activator-like effector nucleases (TALEN) technology [8]. Clustered regularly interspaced short palindromic repeat-associated protein 9 (CRISPR/Cas9) was used to edit the *Badh2* gene in rice which will help to accelerate the breeding process of fragrant rice [27]. Introduction of fragrance into elite rice accessions will result in increase in market value of rice. Out of 17 reported *badh2* alleles, 7 have been used in MAS [28]. Keeping in view the demand for aromatic rice in the global market, aroma has been considered by the breeders for developing commercially improved rice varieties. Aroma plays an important role in consumers' preference and market

value [29].

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### **Author's contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.