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# Characterization of Aroma and Aroma Containing Genes in Landraces and Improved Varieties of Rice (*Oryza sativa* L.)

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**Abstract**

Rice is one of the important food crops of the world. Fragrance is one of the important properties of rice which determines the market price. In the present study, 53 rice genotypes comprising landraces, advanced lines and improved varieties were characterized for their aroma based on sensory testing by KOH method, while molecular characterization of 48 lines was conducted using functional (gene specific) and simple sequence repeat (SSR) markers. The molecular analysis for 48 genotypes by SSR markers along with functional markers (Badex7-5, FMbadh2-E7) showed distinctive differentiation between the genotypes as aromatic or non-aromatic. The SSR markers also confirmed the existence of other QTL on chromosome 3 and 4 (*aro*3-1, *aro*4-1). The data obtained from the molecular studies was used to determine the genetic relationship and to construct the dendrogram. The genotypes were grouped into 3 clusters and 8 sub-clusters based on the presence of aroma. The molecular studies confirmed the sensory studies and dendrogram analysis grouped all these genotypes according to their similarities and relationships.

**Keywords: Rice; Aroma; Fragrance; Functional markers; Diversity.**

Rice (*Oryza sativa* L.) is the world's most important food crop and a primary source of food for more than one third of world's population. Rice shows a very diverse array of properties. Amongst all those properties fragrance is the most important constituent for high quality rice varieties. Aromatic rice are considered to be the best in quality even if they constitute a small but special group of rice varieties and landraces. The preference to fragrant rice often associated with high economic value has encouraged rice breeders to focus on the identification and discovery of fragrance associated genes and breeding for fragrant rice.

The aroma of rice is formed by a blend of various volatiles. Among the several volatile compounds 2-acetyl-1-pyrroline (2AP) has been regarded as principal flavour component with lower odour threshold. Non fragrant rice varieties also have the presence of 2AP but contains up to 100 times lower concentration than the fragrant rice varieties. 2AP in scented rice has a popcorn or butter-like odour (Buttery *et al*., 1983). Yajima *et al*., (1978) analyzed the volatile compounds from cooked rice. They identified over 100 compounds, which included 13 hydrocarbons, 13 alcohols, 16 aldehydes, 14 ketones, 14 acids, 8 esters, 5 phenols, 3 pyridines, 6 pyrazines and 8 other compounds.

The aroma of the aromatic rice has been normally described as being pandan-like by the orientals. In Asia, it is a common culinary practice to add Pandan (*Pandanus amaryllifolius*) leaves to a non-aromatic rice variety while cooking to make it aromatic. The genetic analysis has revealed that a recessive gene, *fgr* on chromosome 8 is responsible for rice fragrance. It was reported that the betaine aldehyde dehydrogenase (BADH2) gene is associated with rice fragrance which comprises of 15 exons and 14 introns on chromosome 8.

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The Badh2 allele of aromatic rice contains an 8-bp deletion and 3 SNPs in exon 7 (badh2-E7) compared to the Badh2 allele of non-fragrant rice, which leads to functional loss of the encoding BADH2 protein. Based on this locus, several PCR-based codominant markers are also developed. Moreover, based on the sequence variation in the *fgr* gene, some functional markers have also been developed (Amarawathi *et al*., 2008; Shi *et al*., 2008; Sakhtivel *et al*., 2009).

The prices of fragrant rice are increasing not only because of the demand for the aroma or its unique characteristics but also for the limited supply of them. Fragrant rice varieties generally show low yields which are caused by the environmental and genetic factors. Most non-traditional rice growing countries utilize intensive farming practices which may enhance yield of rice aroma. Because of their low yield, farmers are preferring disease resistant, fast growing, high yielding and non-fragrant varieties over local fragrant varieties. This ultimately is resulting in loss of genetic diversity in the fragrant rice.

However, there are many local rice landraces which are still under cultivation and show various quality traits and good aroma. Local varieties could be an important source of genes for aroma and other qualitative traits. Accurate genetic information of the important traits like aroma in the local adapted gene pool and use of this for improvement of yield and quality traits would help in evolving high yielding and location specific quality rice genotypes. There is need for valuable aromatic rice varieties to be conserved, characterized and researched to harness their potential to ensure better aroma quality and improved yields (Sakthivel *et al*., 2009). These developments could be useful for the improvement of the landraces and improved varieties of rice. In the present study, landraces and released varieties as well as breeding material were first characterized for presence of aroma using sensory test and later they were screened using known functional markers as well as SSR markers.

## Materials and Methods

**Plant material :** A total of 53 rice genotypes comprising landraces, local selections, advance breeding lines and improved varieties available at Agricultural Research Station (ARS), Radhanagari, District Kolhapur, Maharashtra State, India were used in the present study (Table 1). Many of these genotypes are known to contain aroma.

**Sensory test for fragrance/aroma:** The sensory test of all 53 rice genotypes was performed using a 1.7% KOH solution (Sood and Siddiq, 1978). The grains (seed) from individual plant were collected for the sensory evaluation. Ten rice grains with husks were placed into the petri plates with 10 ml of 1.7% KOH solution covered with lid at room temperature. After 15 minutes, the plates were opened and smelled immediately and were scored for aroma by a common panel of 10 members in a scale of 1-4. Score 1 was given for absence of aroma, score 2 was given for slight aroma, score 3 for moderate aroma, while score of 4 for highly aromatic fragrance (Rai *et al*., 2015). The scores given by the individual member of the panel were not disclosed to other members till the completion of the sensory test of all the genotypes. In case of different scores assigned by different members to a particular genotype, average of the score was calculated to place it in appropriate class of fragrance.In the present study Pusa Basmati-1 was used as a positive control and KOH was used as a negative control for the better comparison.

**Molecular markers:** Total of 15 different Simple sequence repeats (SSR) and functional markers which are specific to the aroma genes being characterized were selected and used in

the study. They were used to characterize rice genotypes for aroma containing genes (Table 2).

**Genomic DNA isolation and PCR amplification:** Of the 53 genotypes used, based on the sensory evaluation, five genotypes without aroma and agronomically not good as suggested by the Rice Breeder were excluded from further study. Genomic DNA from remaining 48 genotypes of rice were isolated from the young leaves of 3-4 weeks old seedlings following the modified CTAB method as per Gavhane *et al*. (2019). The quantity and quality of extracted genomic DNA was checked by agarose gel electrophoresis as well as using spectrophotometer with a NanoDrop system (ND-1000, Thermo Scientific, USA). PCR amplification were performed with a total volume of 20 µl for each PCR mixture containing 30 ng of genomic DNA, 2.0 µl 10 X Taq buffer, 1mM dNTPs and 1U/µl Taq DNA Polymerase and 10pmol of both forward and reverse primers (1.0 µl each primer). The PCR temperature cycles were programmed as initial denaturation at 94°C for 5 min, followed by 30 cycles of 30 sec at 94°C, 30 sec at 55°C annealing temperature and 1 min at 72°C with final extension of 5 minutes at 72°C. The amplified products were resolved on a 1.2% - 2% agarose gel stained with ethidium bromide and visualized under UV transilluminator. The gels were scored based on the banding pattern as fragrant and non-fragrant. Based on the marker data, haplotype of these 48 genotypes was also prepared.

Although the purpose of this study was to characterize the genes containing fragrance in the selected rice genotypes, based on the data of the molecular markers, we also attempted to group them to see whether the markers can group these genotypes according to presence or absence of aroma. Polymorphism information content (PIC) values were estimated as per standard formula.

**Table 1.** List of rice genotypes along with their pedigree

| Genotypes | Pedigree |
|---|---|
| Ambemohar 157 | Local selection from Pune district (M.S.) |
| Jaya | TN 1 x T 141 |
| Indrayani | Ambemohar 157 x IR 8 |
| Pawana | Pusa 33 x IR 28 |
| Bhogavati | Selection from Basmati Composite |
| Basmati 370 | Pure line selection from Basmati landraces |
| Ghansal | Local selection from Kolhapur dist. (M.S.) |
| Badshabhog | Local type from Orisa |
| Kothimbire | Landrace from Kolhapur district (M.S.) |
| BPT 5204 | (GEB 24 x TN 1) x Mahsuri |
| RDN 01-02 | N.A. |
| Diwani | Landrace from Uttar Pradesh |
| Shyamjeer | Landrace from Bihar |
| Juhibengal | Landrace from West Bengal |
| SD-1 | N.A. |
| SD-2 | N.A. |
| SD-3 | N.A. |
| SD-4 | N.A. |
| SD-7 | PR 109 x Pak Basmati |
| SD-8 | N.A. |
| SD-9 | N.A. |
| SD-10 | N.A. |
| SD-11 | N.A. |
| SD-17 | Pusa Basmati-1 x IET 12603 |
| SD-18 | N.A. |
| SD-19 | N.A. |
| MC-1 | N.A. |
| MC-2 | N.A. |
| MC-4 | Selection from Karnal Local |
| MC-10 | N.A. |
| MC-12 | N.A. |
| Khalibagh | Landrace from Sindhudurg dist. (M.S.) |
| Jagatpuri | Landrace from Sindhudurg dist. (M.S.) |
| Velkat | Landrace from Sindhudurg dist. (M.S.) |
| Tulshi tall | Landrace from Kolhapur district (M.S.) |
| Siddhagiri | Landrace from Kolhapur district (M.S.) |
| RDN 99-11 | Selection from Indrayani |
| RDN 99-14 | IET 13549 x RTN 24 |
| Taraori Basmati | Pure line selection from NBC 99 |
| RDN 99-16 | Indrayani x RTN 24 |
| RDN 99-17 | Indrayani x IR 36 |
| Ajara local | Landrace from Kolhapur district (M.S.) |
| IGT-13857 | Landrace from Nasik district (M.S.) |
| Pavsal | Landrace from Pune district (M.S.) |
| Kunchi | Landrace from Pune district (M.S.) |
| Antersal | Landrace from Pune district (M.S.) |
| Jaware | Local selection from Maval, dist. Pune |
| Phule Samruddhi | Indrayani x Sonsali |
| RDN 185-2 | Halvi Sal 17x TN 1 |
| Phule Radha | TN 1 x Kolamba 540 |
| Kundalika | RTN 24 x IET 3228 |
| RTN-1 | IR 8 x RTN 24 |
| Pusa Bamsati-1 | Pusa 150 x Karnal Local |

NA - not available; M.S. - Maharashtra state

**Cluster analysis:** Data obtained from all the polymorphic markers were used to determine genetic relationships. The amplified products were scored for the presence or absence of each marker allele. Data were entered into a binary matrix and scored as '1' for the presence and '0' for the absence of the allele. Similarity matrix was prepared using the software package TASSEL 4.0 (Bradbury *et al*. 2007), and dendrogram was constructed through neighbor joining method to group individual into different clusters making use of the archaeopteryx tree option as provided in TASSEL 4.0 and as described by Borse *et al*. (2017).

## Results and Discussion

**Sensory evaluation of aroma using KOH method:** A total of 53 genotypes were tested by 10 experts to determine the qualitative presence of aroma. The measured aroma was scored according to the scale given by Rai *et al*. (2015). This investigation allowed to qualitatively differentiate rice genotypes based on the type of aroma. Out of these 53 genotypes, 14 were found with absence of aroma (scored as 1), 10 as slightly aromatic (scored as 2), 9 moderately aromatic (scored as 3) and 20 strongly aromatic (scored as 4) (Table 3). The score in case of majority of the panel members were uniform for the given genotypes.

**Marker analysis:** The genomic DNA was isolated from 48 genotypes excluding SD-1, SD-2, SD-3, SD-4 and SD-9 and was further subjected to PCR amplification using 15 markers. Out of 15 markers all the markers were showing amplification with the alleles in the range of 2-5 per marker. All the 15 markers were found polymorphic with an average of 2.73 alleles per marker. The PIC range was 0.3 to 0.7 with an average of 0.551 (Table 2). Almost all the markers (except RM5474) amplified the alleles at expected size.

The functional marker Badex7-5 showed only 2 alleles in all the genotypes i.e. the aromatic genotypes amplified 95 bp (with 8 bp deletion) whereas a 103 bp pair was amplified in non-aromatic ones. The genotypes Basmati-370, Taraori Basmati, Pusa Basmati-1, Ambemohar-157, Badshabhog showed the allele of 95 bp i.e. the presence of aromatic gene and genotype Jaya showed the allele of 103 bp similar to the results by Sakhtivel *et al*. (2009).

The other functional marker used was FMbadh2-E7 also showed the presence of two alleles of 260 bp and 268 bp differentiating them as fragrant and non-fragrant genotypes, respectively. It designed on the basis of deletion in exon 7 on chromosome 8 of rice which shows the presence of fragrance. In the study the genotypes with 260 bp showed presence of aroma like Basmati 370 (Shi *et al*. 2008). This marker showed amplification in all the genotypes except Ghansal, RDN 99-17, Antersal and Phule Radha. The genotype Shyamjeer was unique which amplified at 255 bp.

**Table 2.** Details of amplification using different markers

| Marker | Marker type | Alleles amplified | Product size range (bp) | PIC |
|---|---|---|---|---|
| RM515 | SSR | 4 | 205-231 | 0.629 |
| RM342 | SSR | 3 | 132-150 | 0.583 |
| RM223 | SSR | 2 | 139-163 | 0.499 |
| BADEX7-5 | Functional | 2 | 95/103 | 0.500 |
| RM85 | SSR | 3 | 90-110 | 0.554 |
| RM42 | SSR | 2 | 160-170 | 0.607 |
| FMbadh2-E7 | Functional | 3 | 260-268 | 0.483 |
| RM5474 | SSR | 5 | 90-110 | 0.493 |
| RM282 | SSR | 3 | 124-138 | 0.353 |
| RM5633 | SSR | 2 | 203-225 | 0.570 |
| RM273 | SSR | 2 | 210-220 | 0.568 |
| RM80 | SSR | 3 | 115-137 | 0.547 |
| RM3459 | SSR | 2 | 180-197 | 0.707 |
| BO3127.8 | SSR | 2 | 122-134 | 0.518 |
| 10L03_FW | SSR | 3 | 186-200 | 0.656 |
| Total no. of alleles | 41 | - | | |
| Average | 2.73 | - | 0.551 | |

The SSR and functional markers together showed amplification in all the genotypes. The data derived from the sensory and from the molecular studies was correlated. Aromatic genotypes Basmati-370, Ambemohar-157, Pusa Basmati-1, Indrayani, Badshabhog and non-aromatic genotypes Jaya, Phule Radha, Jaware, Velkat, Tulshi Tall, Khalibagh, Jagatpuri, RDN 99-16, RDN 99-17 showed amplification of expected allele. The functional markers showed distinctive differentiation between the aromatic and non-aromatic genotypes than the SSR markers.

The markers which were located on chromosomes other than chromosome 8 such as on chromosome 3 (RM5474 and RM282) and 4 (RM5633 and RM273) linked to QTL *aro3*-1 and *aro*4-1 also showed amplification. The aro3-1 QTL linked marker RM282 showed better amplification than RM5474 hence, showing the presence of *aro*3-1 aroma QTL in some genotypes. Also the QTL *aro*4-1 both markers RM282 and RM5633 showed effective amplification determining the presence of other fragrance locus on chromosome 4.

**Genetic diversity analysis by molecular data:** To visualize the genetic relationship among the 48 rice genotypes, a dendrogram was constructed based on the neighbour joining method using the TASSEL 4.0 program (Fig. 1). The cluster information from the dendrogram is given in Table 4.

Based on the cluster analysis using the different markers used in the study, the genotypes were grouped in 3 major clusters namely I, II and III. The First cluster I was again sub clustered into 2 sub clusters namely IA and

**Table 3.** Distribution of rice genotypes in different fragrance types

| Sensory Scale | Fragrance type | Genotypes |
|---|---|---|
| 1 | Absence of aroma | Jaya, BPT 5204, RDN 01-02, Diwani, Shyamjeer, Juhibengal, Khalibagh,Jagatpuri, Velkat, Tulshi Tall, Siddhagiri, RDN 99-16, RDN 99-17, Antersal |
| 2 | Slightly aromatic | RDN 99-14, IGT-13857, Pavsal, Kunchi, Jaware, RDN 185-2, Phule Radha, Kundalika, RTN-1,Phule Samruddhi |
| 3 | Moderately aromatic | RDN 99-11, Indrayani, Pawana, Bhogavati, Ghansal, Kothimbire, Ajara local, SD-2, SD-4 |
| 4 | Strongly aromatic | Pusa Basmati-1,Ambemohar-157, Basmati 370, Badshabhog, Taraori Basmati , SD-1, SD-3, SD-7, SD-8, SD-9, SD-10, SD-11, SD-17, SD-18, SD-19, MC-1, MC-2, MC-4, MC-10, MC-12 |

**Table 4.** Distribution of rice genotypes into different clusters

| Cluster | Sub-cluster | Genotypes | Remarks |
|---|---|---|---|
| I | IA<br>IB | SD-10, Indrayani, Pawana, Bhogavati<br>SD-18, Pusa Basmati-1, SD-7, Ambemohar-157, SD-8 | Highly aromatic genotypes |
| II | IIA<br>IIB<br>IIC | RDN 01-02<br>Diwani, Kothimbire, Ghansal, Basmati-370, Badshabhog,<br>SD-19, MC-4, MC-1, SD-11, SD-17 | Strong and moderately aromatic genotypes |
| | IID | BPT 5204, MC-12, Juhibengal, Shyamjeer, Jaya, MC-2, Khalibagh, IGT-13857, Tulshi tall, Jagatpuri, Velkat, Siddhagiri, RDN 99-17, RDN 99-16, Antersal, Kunchi, Pavsal, RTN-1, Jaware, Phule Radha, Kundalika, Phule Samruddhi, RDN 185-2 | Slightly or absence of aroma |
| III | IIIA | Ajara local | Absence of aroma genotypes |
| | IIIB | MC-10, RDN 99-11, RDN 99-14, Taraori Basmati | |

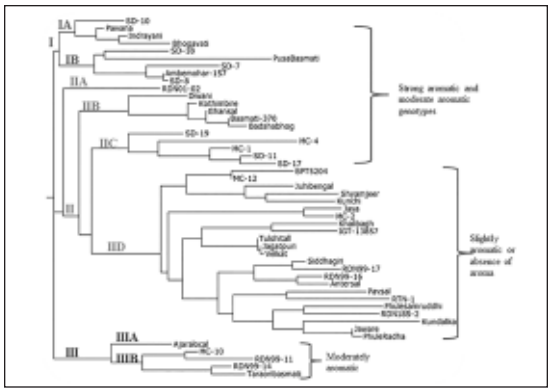


**Fig. 1.** Consensus tree showing clustering of rice genotypes using marker data

IB. The second cluster was divided into its subtypes that are IIA, IIB, IIC and IID sub clusters. Then the third cluster was sub clustered into 2 sub groups namely IIIA and IIIB. All the genotypes in cluster IA and IB were highly aromatic which include SD-10, Indrayani, Pawana, Bhogavati, SD-18, Pusa Basmati-1, SD-7, Ambemohar-157 and SD-8. The genotypes in cluster IIA, IIB and IIC are strong or moderately aromatic which include RDN 01-02, Diwani, Kothimbire, Ghansal, Basmati-370, Badshabhog, SD-19, MC-4, MC-1, SD-11, SD-17. The sub cluster IID show slightly aromatic genotypes which are BPT 5204, MC-12, Juhibengal, Shyamjeer, Jaya, MC-2, Khalibagh, IGT-13857, Tulshi tall, Jagatpuri, Velkat, Siddhagiri, RDN99-17, RDN99-16, Antersal, Kunchi, Pavsal, RTN-1, Jaware, Phule Radha, Kundalika, Phule Samruddhi, RDN185-2. Cluster III comprised of all the genotypes which have no aroma including Ajara local, MC-10, RDN99-11, RDN99-14 and Taraori Basmati.

Result from above analyses including sensory evaluation, molecular studies and dendrogram analysis could characterize genes responsible for aroma in various landraces and improved varieties of the rice genotypes. By comparing all the data, highly aromatic varieties (Basmati-370, Ambemohar-157, Pusa Basmati-1, Indrayani), absence of aroma varieties (Jaya, RDN 99-16, RDN 99-17, Jaware, Jagatpuri, Velkat, Tulshi tall) and other slightly and moderately aromatic varieties were determined. The molecular studies confirmed the sensory study and the dendrogram analysis grouped all these genotypes according to their similarities and relationships. The results are useful for the rice breeders to undertake further improvement programmes.

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