Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 1756-1760
Received: 26-05-2018
Accepted: 27-06-2018

Dr. Basabdatta Das
Division of Plant Biology, Bose Institute, Main Campus, 93/1APC Road, Kolkata, West Bengal, India

Dr. Samik Sengupta
Department of Horticulture (Fruit and Fruit Technology), Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Dr. Basudev Kole
Department of Statistics, Mathematics & Computer Application Bihar Agricultural University, Sabour, Bhagalpur Bihar, India

Tapas Kumar Ghose
Division of Plant Biology, Bose Institute, Main Campus, 93/1APC Road, Kolkata, West Bengal, India

Correspondence
Dr. Basabdatta Das
Division of Plant Biology, Bose Institute, Main Campus, 93/1APC Road, Kolkata, West Bengal, India

Elucidation of aroma levels within a set of rice landraces by means of aroma linked markers

Dr. Basabdatta Das, Dr. Samik Sengupta, Dr. Basudev Kole and Tapas Kumar Ghose

Abstract
Rice aroma is one of the most important quality traits that determine the price of rice in national and international market. Over 100 volatile aroma compounds have been identified in aromatic rice but the major aroma compound is 2-acetyl-1-pyrroline (2AP). The mutated *badh2* allele is responsible for the accumulation of 2AP and imparting fragrance to aromatic rice. This study has been designed to find out a correlation between the alleles of 8 aroma linked markers and aroma levels within a set of rice landraces.

Keywords: aromatic rice, aroma linked markers, *badh2* gene, 2AP

Introduction
Rice aroma is one of the most important quality traits that determine the price of rice in national and international market. Aromatic rice accessions have been identified in the genetic subpopulations of *indica*, *tropical japonica*, and Basmati and Sadri varieties1. Over 100 volatile aroma imparting compounds have been identified in aromatic rice but the major aroma compound is 2-acetyl-1-pyrroline (2AP) 2. This compound is produced in all parts of the rice plant except the roots. There are other traditional aromatic rice varieties that exhibit different volatile compounds other than 2AP such as acetaldehyde, propanol, 2-butanone, pentanal, hexanol etc.3 and hence, fragrance intensity of a variety may be associated with presence of multiple volatile compounds produced by different biochemical pathways. Using Restricted Fragment Length Polymorphism (RFPL) Ahn *et al*., [4] had identified a single locus on the long arm of chromosome 8 (*fgr* locus) associated with aroma in the rice cultivar Della. Later the location of the aroma gene was mapped using SSR markers RM210 and RM5155. Fine mapping of rice chromosomes following the rice genome-sequencing project revealed that a candidate gene homologous to betaine aldehyde dehydrogenase (*badh2*) on chromosome 8 was responsible for 2AP formation in aromatic rice6. It was also proposed that this *badh2* locus on the long arm of chromosome 8 of rice was the *fgr* gene, which is the major genetic determinant of fragrance. In this study, we have tried to elucidate the correlation between the aroma levels and alleles from aroma linked markers within a set of rice landraces.

Material and Methods
Plant material
The experimental set consisted of 46 rice landraces out of which 25 were aromatic and 19 were non aromatic (Table 1). The aromatic germplasms included three landraces from the North Eastern State of Assam, 4 basmati accessions, 18 landraces from West Bengal and two germplasms from United States Department of Agriculture (USDA) as outliers. The nonaromatic germplasms included 8 landraces from the North Eastern States, 10 landraces from West Bengal and 1 High Yielding Variety (HYV).

Measurement of rice kernel aroma
A rapid, microscale method for evaluation of aroma from grain and leaf material was developed in the laboratory following the principle of Sood and Siddiq7. For each genotype 10 kernels were ground into powder and transferred to a 1.5ml Eppendorf tube containing 500µl of 1.7% KOH solution and heated in a microwave oven (LG, model Intellogrill) for 30 seconds at setting 4 (medium). Positive and negative controls were included. A panel consisting of 3 trained evaluators made sensory evaluation of aroma and immediately classified the genotypes as being non-aromatic, faintly aromatic, moderately aromatic, fairly strongly aromatic, moderately strongly aromatic and highly strongly aromatic.

Table 1: Details of the experimental set of rice germplasms

Name	Category	Source	Aroma level	Score
Rice landraces from West Bengal				
Badshah bhog	Aromatic	Rice Research Station, Chinsurah	Fairly Strong	1.66
Gandhamalati	Aromatic	Rice Research Station, Chinsurah	Moderate	1
Gobindobhog Fulia	Aromatic	Agricultural Training Centre, Fulia	Strong	3
Gobindobhog Chinsurah	Aromatic	Rice Research Station, Chinsurah	Strong	3
Kalindi	Aromatic	Agricultural Training Centre, Fulia	Moderate	1
Kalogobindobhog	Aromatic	Agricultural Training Centre, Fulia	Moderate	1
Kalobhog	Aromatic	Agricultural Training Centre, Fulia	Moderate	1
Kaminibhog	Aromatic	Agricultural Training Centre, Fulia	Fairly Strong	1.78
Kataribhog	Aromatic	Rice Research Station, Chinsurah	Fairly Strong	1.67
Lilabati	Aromatic	Rice Research Station, Chinsurah	Fairly Strong	1.52
Motibhog	Aromatic	Rice Research Station, Chinsurah	Moderate	1
Mohanbhog	Aromatic	Agricultural Training Centre, Fulia	Fairly Strong	1.43
Narayanpura	Aromatic	Agricultural Training Centre, Fulia	Fairly Strong	1.49
NC 365	Aromatic	Rice Research Station, Chinsurah	Fairly Strong	1.51
Radha tilak	Aromatic	Rice Research Station, Chinsurah	Fairly Strong	1.88
Radhuni pagol	Aromatic	Rice Research Station, Chinsurah	Fairly Strong	1.33
Tulsimanjari	Aromatic	Agricultural Training Centre, Fulia	Moderate	1
Sitabhog	Aromatic	Agricultural Training Centre, Fulia	Moderate	1
Bangladeshi Patani	Non aromatic	Agricultural Training Centre, Fulia	Nil	0
Bhasamanik	Non aromatic	Agricultural Training Centre, Fulia	Nil	0
FR43B	Non aromatic	Agricultural Training Centre, Fulia	Nil	0
Hatipanjra	Non aromatic	Rice Research Station, Chinsurah	Nil	0
Jhulee	Non aromatic	Agricultural Training Centre, Fulia	Nil	0
Kabirajsail	Non aromatic	Rice Research Station, Chinsurah	Nil	0
Kalopahar	Non aromatic	Rice Research Station, Chinsurah	Nil	0
Katki	Non aromatic	Agricultural Training Centre, Fulia	Nil	0
Latasail	Non aromatic	Rice Research Station, Chinsurah	Nil	0
Raghusail	Non aromatic	Rice Research Station, Chinsurah	Nil	0
Landraces from the North Eastern States				
Joha	Aromatic Assam	Assam Agricultural University	Moderately Strong	2.33
Lal binni	Aromatic Assam	Assam Agricultural University	Faint	0.67
Prasadbhog	Aromatic Assam	Assam Agricultural University	Fairly Strong	1.33
Biroi dhan	Non aromatic Assam	NBPGR, Umiam	Nil	0
Bhu	Non aromatic Mizoram	NBPGR, Umiam	Nil	0
IC524502	Non aromatic Nagaland	NBPGR, Umiam	Nil	0
IC524528	Non aromatic Nagaland	NBPGR, Umiam	Nil	0
IC524530	Non aromatic Nagaland	NBPGR, Umiam	Nil	0
Kalo Boro dhan	Non aromatic Assam	NBPGR, Umiam	Nil	0
Malsara dhan	Non aromatic Assam	NBPGR, Umiam	Nil	0
Morianghou	Non aromatic Manipur	NBPGR, Umiam	Nil	0
Basmatis				
Basmati Aman	Traditional Basmati	Agricultural Training Centre, Fulia	Strong	3
Basmati 370	Evolved Basmati	Agricultural Training Centre, Fulia	Strong	3
Basmati Type 3	Evolved Basmati	Agricultural Training Centre, Fulia	Strong	3
Taraori basmati	Traditional Basmati	Agricultural Training Centre, Fulia	Strong	3
Outliers and HYV				
Della	Outlier	United States Department of Agriculture	Faint	0.78
Domsiah	Outlier	United States Department of Agriculture	Moderate	1
IR 36	High Yielding Varieties	Rice Research Station, Chinsurah	Nil	0

The aroma for each genotype was scored on a scale of 0 to 3 wherein 0 = non-aromatic, 0>1 = faintly aromatic, 1 = moderately aromatic, between 1 and 2 = strong aromatic, between 2 and 3 = moderately strongly aromatic and 3 = strongly aromatic (Table 1).

Isolation of genomic DNA and PCR amplification

Three day-old rice seedlings germinated from 10 rice grains were used for genomic DNA isolation according to the method of Walbot8. PCR amplification of this DNA was done with 4 pairs of SSR markers linked to aroma and 4 aroma specific markers designed from *badh2* locus by previous workers9. The details of the markers are given in Table 2. DNA amplification was carried out in 25 µl volumes in a MJR thermal cycler (USA). Each reaction mixture contained 1 µl of genomic DNA (100 ng), 0.5 µl of each primers (at a concentration of 10 pmole/µl), 2.5 µl of 10× PCR buffer, 0.75 µl of 50 mM MgCl2, 0.25 µl of 2.5 mM dNTP mixture, 0.2 µl (1 unit) of 5 unit/µl Taq DNA polymerase and 19.3 µl of PCR-grade water. The temperature profile of the first PCR cycle was 97 °C for 5 mins, followed by 35 cycles of 1 min at 95 °C, 1 min at 55-60 °C and 2 min at 72 °C. The final extension was at 72 °C for 10 min.

Table 2: Details of aroma linked markers used in this study

SSR markers used					
Name	**Motif**	**Chromosoma l location**	**Annealing temperature**		
RM 80	(CTT)20	8	65		
RM 207	(GA)25	2	65		
RM 210	(GA)23	8	55		
RM 337	(CTT)4-19-(CTT)8	8	59		
***badh2* markers used**					
Name	**Forward primer**	**Reverse Primer**		**Chromosomal location**	**Annealing temperature**
badh2 tm	GCAAGTGACGGAGTACGCCT	GCTAACTTCCGCTCACGCAA		8	62
FP1	CCATCTCCGTATCTCTCACC	AGTCACGGAAGCCAATTCAG		8	62
FP2	CTAGAGACGCTTGATTGTGG	AGTCACGGAAGCCAATTCAG		8	62
FP3	GATCTTGCAGAATCCTTGGA	AGTCACGGAAGCCAATTCAG		8	62

Polyacrylamide Gel electrophoresis

The PCR products were resolved in native polyacrylamide gel electrophoresis (PAGE) according to Sambrook *et al*. [10] in 6% gel in vertical electrophoresis tank (gel size of 16 cm × 14 cm, Biotech, India) with Tris-Acetate-EDTA buffer at 150 V. The gel was stained with ethidium bromide (5 µg of EtBr in 200 ml of Tris-Borate-EDTA buffer) washed twice with distilled water and analyzed in Gel Documentation System (Biorad, USA).

Allele scoring

A cluster of two to five discrete bands (stutter) was apparent in the stained gels for most of the markers. The size (in nucleotides) of the most intensely amplified band for each microsatellite marker was determined using the Quantity One software (Biorad, USA), based on the migration of the band relative to standard molecular weight size markers (100 bp DNA ladder SibEnzyme). The band with the lowest molecular weight for each SSR marker was assigned allele number 1 and the progressively heavier bands were assigned incrementally. For the individual markers, the presence of an allele in each of the germplasms was recorded as "1" and the absence of an allele was denoted as "0" 11.

Correlation of aroma levels with alleles

The correlations between traits were estimated by regression of the aroma level scores with the alleles of each marker in all possible pairwise combinations. Pair wise correlations are presented in Table 4.

Results

Aroma levels of different genotypes.

The category and score of the aroma levels of 22 aromatic rice landraces, 4 Basmatis and 2 outliers are tabulated in Table 1. The 4 Basmati accessions and the 2 Gobindobhog rice accessions were strongly aromatic with a score of 3. Joha, a landrace from Assam was moderately strongly aromatic with a score of 2.33. Nine landraces from West Bengal, Badshahbog, Kaminibhog, Kataribhog, Lilabati, Mohanbhog, Narayanpurna, NC 365, Radhatilak and Radhunipagol; and Prasadbhog an Assam landrace were all fairly strongly aromatic with scores ranging between 1 and 2. The West Bengal landraces Gandhamalati, Kalindi, Kalogobindobhog, Kalobhog, Motibhog, Tulsimanjari and Sitabhog; and the outlier Domsiah were moderately aromatic with a score of 1. Kalo Boro dhan and Lalbinni from Assam were faintly aromatic with scores of 0.51 and 0.67 respectively. The outlier Della was also faintly aromatic with a score of 0.78.

Allele scoring

The alleles identified by each of the markers are tabulated in Table 3. Eight alleles were identified by RM 80, fifteen by RM 207, seven by RM 210 and eleven by RM 337. Fifteen alleles were identified by badh2 tm, eleven alleles were identified by FP1, thirteen by FP2 and four by FP3. Allele 1 was prevalent among the aromatic landraces while screening with FP3

Table 3: Allele scoring with the markers

Name	**RM 80**	**RM 207**	**RM 210**	**RM 337**	**badh2 tm**	**FP1**	**FP2**	**FP3**
Badshah bhog	8	11	2	4	7	6	5	1
Gandhamalati	7	7	2	4	5	2	9	1
Gobindobhog Fulia	8	9	3	4	7	6	8	1
Gobindobhog Chinsurah	8	9	3	4	7	6	6	1
Kalindi	2	8	5	8	6	4	10	1
Kalogobindobhog	1	8	2	4	7	6	6	1
Kalobhog	1	6	3	8	7	6	7	1
Kaminibhog	7	7	3	8	6	4	4	1
Kataribhog	7	8	3	8	3	4	4	1
Lilabati	7	14	2	3	5	2	11	1
Motibhog	7	8	5	9	6	5	3	1
Mohanbhog	1	7	2	8	6	5	3	1
Narayanpurna	3	11	2	3	4	2	8	1
NC 365	9	11	3	9	5	1	9	1
Radhatilak	5	9	5	3	5	5	8	1
Radhunipagol	6	9	3	6	5	5	12	1
Tulsimanjari	4	10	6	7	6	5	12	1
Sitabhog	8	8	3	8	6	5	3	1
Bangladeshi Patnai	7	8	5	1	6	9	2	4
Bhasamanik	2	5	6	11	6	8	3	3

FR43B	2	6	5	11	6	8	4	4
Hatipanjra	2	6	5	11	6	10	6	3
Jhulee	2	6	5	11	9	9	7	4
Kabirajsail	8	5	5	2	4	7	4	3
Kalopahar	3	7	5	11	6	11	6	2
Katki	3	7	6	11	8	10	6	3
Latasail	6	7	5	9	4	9	4	3
Raghusail	6	10	2	7	2	4	12	1
Landraces from the North Eastern States								
Biroi dhan	2	9	7	8	10	3	8	1
Bhu	1	6	5	8	11	4	8	1
Joha	8	13	6	8	14	3	8	3
Kalo Boro dhan	7	1	5	8	12	4	8	1
Lal binni	8	13	7	9	11	4	8	1
Malsara dhan	3	13	5	9	10	4	4	3
Morianghou	2	7	6	11	15	11	6	3
Prasadbhog	6	4	5	1	11	3	8	1
IC524502	8	15	2	10	7	4	10	1
IC524528	2	6	5	11	13	9	7	2
IC524530	2	6	5	11	10	10	9	3
Basmatis								
Basmati Aman	3	8	3	7	13	4	2	1
Basmati 370	1	7	2	5	13	4	2	1
Basmati Type 3	6	12	2	9	11	2	2	1
Taraori basmati	7	2	5	8	14	2	1	1
Outliers and HYV								
Della	4	3	1	3	7	3	7	1
Domsiah	7	12	3	9	8	3	6	1
IR 36	6	10	2	3	1	4	13	2

Correlation of aroma level scores and alleles

Pair wise correlation among the alleles derived using primers designed from the aroma linked markers and the aroma level scores are presented in Table 4. From the table it can be seen that the alleles of FP1 and FP3 a significantly correlated with the aroma scores at 1% level of significance whereas alleles of RM337 is significantly correlated with the aroma score at 5% level of significance. The correlation of the alleles of other markers with the aroma level score is insignificant. The alleles of FP1 are also significantly correlated with the alleles derived using FP3, RM80, RM210 and RM337 at 1% level of significance. The correlation of alleles of FP1 with the alleles of RM207 is at 5% level of significance. Significant correlations at 1% level of significance are also observed between alleles of FP3 and RM210 and between alleles of RM210 and RM337. Alleles of badh2 tm are significantly correlated with alleles of FP2, RM210 and RM337 at 5% level of significance. Similar correlations are observed between alleles of FP2 and RM207, FP3 and RM337, RM80 and RM207 and between RM80 and RM337. The other correlations are insignificant.

Table 4

	Badh 2 tm	**FP1**	**FP2**	**FP3**	**RM.80**	**RM.207**	**RM.210**	**RM.337**	**Score**
badh2 tm	1	0.007767	-0.34566*	0.034812NS	-0.26278 NS	-0.17702 NS	0.328725*	0.300055*	0.191121 NS
FP1		1	-0.1825 NS	0.700635**	0.39655**	-0.32327*	0.426594**	0.39598**	-0.60636**
FP2			1	-0.21495 NS	0.083649 NS	0.299507*	-0.06295 NS	-0.14939 NS	-0.03325 NS
FP3				1	-0.24122 NS	-0.16501 NS	0.490632**	0.317047*	-0.65924**
RM.80					1	0.328033*	-0.17539 NS	-0.32274*	0.275809 NS
RM.20 7						1	-0.20671 NS	-0.04126 NS	0.211614 NS
RM.21 0							1	0.421049**	-0.2705 NS
RM.33 7								1	-0.35585*
Score									1

Discussion

This study was designed to find out the aroma levels of a set of rice landraces and to correlate the aroma levels with alleles generated by PCR based screening of those rice landraces with aroma linked markers. As was expected the Basmati landraces were the most fragrant. Gobindobhog a landrace from West Bengal also had the same aroma score as the Basmati landraces. However, the grains of Gobindobhog are not long and slender and have a tendency to disintegrate and become sticky on slight overcooking. For these reasons, Gobindobhog does not fetch the same price as the Basmati in both national and international market. Both Domsiah and Della are very fragrant rice varieties. Domsiah is an aromatic long-grained rice from Iran. It is related to Basmati rice and is often more aromatic than other Basmati-type rice. Della was developed by the USDA. It is long-grained aromatic rice. Ideally, both Domsiah and Della should have high aroma score, but in this experiment, their scores were considerably lower than that of Basmati or other Indian aromatic rice landraces. The seed of these varieties were procured from USDA and was grown in the agro-climatic conditions of southern West Bengal, India, that has a hot and humid tropical environment. Earlier Mo *et al*., [11] have proposed that intensity of aroma is affected by environmental changes. The main aroma compound 2-acetyl-

1-pyrroline (2AP) has the propensity to decrease with increasing sunlight and temperature. Hence the decreased aroma levels of Domsiah and Della in this case are, in all probability, attributed to the hot and humid tropical conditions. The correlation analysis highlighted significant correlations, mostly at 1% level of significance; between alleles derived from the markers FP1, FP3, RM80, RM210, RM337 and the aroma score levels in various pairing combinations. All the five markers mentioned above are derived from DNA sequences of chromosome 8 of rice. In an earlier study, Bradbury *et al*. [12] reported that an 8 bp deletion in the 7th exon of *badh2* gene on the 8th rice chromosome is responsible for 2AP production. The wild type *badh2* gene encodes the betaine aldehyde dehydrogenase enzyme (BADH2) which is supposed to be associated with stress tolerance especially salt tolerance in rice13. Indeed the aromatic rice varieties are more susceptible to stress both abiotic and biotic. Kovac *et al*. [6] reported that there are various other mutations scattered from 1st to 14th exon of the *badh2* gene that can render BADH2 enzyme non-functional and lead to aroma production in rice.

Conclusion

From the results of this study, we can see that the presence of aroma cannot be fully explained by the alleles derived from the *badh2* locus (badh2 tm, FP1, FP2 and FP3). As previously reported, this study also reiterates the fact that rice fragrance is a complex trait that involves a number of volatile compounds and the interaction of multiple genes with the internal environment of the rice plant and the microclimate of rice cultivation. Hopefully, future dissertations will be able to elucidate these interactions for a better understanding of rice aroma.

Acknowledgement

The authors wish to thank Bose Institute for providing the facilities for conducting this research.

Conflict of interest: the authors declare that there is no conflict of interest

References

1. Fitzgerald MA, Sackville HNR, Calingacion MN, Verhoeven HA, Butardo VM. Is there a second fragrance gene in rice? Journal of Plant Biotechnology. 2008; 6:416-423.
2. Buttery RG, Lilng LC, Juliano BO. 2-Acety-1-pyrroline: An important aroma component of cooked rice. Chem Ind, 1982, 958-959
3. Yang DS, Shewfelt RL, Lee KS, Kays SJ. Comparison of odor-active compounds from six distinctly different rice flavor types Journal of Agriculture and Food Chemistry. 2008; 56(8):2780-2787
4. Ahn SH, Bollich CN, Tanksley SD. RFLP tagging of a gene for aroma in rice. Theoretical and Applied Genetics. 1992; 84:825-828.
5. Ren JS, Xiao PC, Chen Y, Huang X, Wu XJ. Study on heredity of aroma genes in several maintainer lines of aromatic rice. Seed. 2004, 12
6. 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 Science USA. 2009; 106:14444-14449.
7. Sood BC, Siddiq EA. A rapid technique for scent determination in rice. Indian, Journal of Genetics and Plant Breeding. 1978; 38:268-271.
8. Walbot V. Preparation of DNA from single rice seedling. Rice Genetics Newsletter. 1988; 5:149-151.
9. Chen SH, Yang Y, Shi WW, Ji Q, He F, Zhang ZD *et al*. Badh2, encoding betaines aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. Plant Cell. 2008; 20:1850-1861.
10. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual. 2nd edition. New York: Cold Spring Harbour Laboratory Press, 1989.
11. Mo Z, Li W, Pan S, Fitzgerald TL, Xiao F, Tang Y *et al*. Shading during the grain filling period increases 2-acetyl-1-pyrroline content in fragrant rice. Rice. 2015; 8:1-10.
12. Bradbury LMT, Henry RJ, Jin Q, Reinke RF, Waters DLE. A perfect marker for fragrance genotyping in rice Molecular Breeding. 2005; 16:279-283.
13. Niu X, Tang W, Huang W, Ren G, Wang Q, Luo D *et al*. RNAi-directed downregulation of OsBADH2 results in aroma (2-acetyle-1-pyrroline) production in rice (*Oryza sativa* L.). BMC Plant Biology. 2008; 8:100.