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Research Article

Variation for Grain Morphology, Molecular Diversity and Aroma Analysis in Specialty Rice of Assam

Deepmoni Hazarika and Sharmila Dutta Deka

National Seed Project, Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat

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****Corresponding author:***
E-mail: sharmila9368@gmail.com

Abstract

The nature and magnitude of genetic diversity of aromatic rice accessions collected from different agro climatic zones of Assam were evaluated in the present study. Grain morphology and molecular diversity was investigated for a collection of fourteen genotypes. In molecular genetic analysis 32 random SSR markers and 1 gene- based marker (Aroma-1) were included, out of which 18 informative SSR were employed for genetic analysis on the basis of their amplification. Molecular taxonomy and genetic divergence analysis revealed considerable variation among the tested aromatic lines. Of the 18 SSR variation in 14 genotypes, the value of PIC ranged from 0.1326 (RM337) to 0.5408 (RM496). The phylogenetic relations indicate presence of diverse genetic origin of the aromatic rice collection. Aroma analysis with gene based marker (Aroma- 1) amplified a unique band in *Bokul Joha* which is different from rest of the genotypes indicating probable presence of alternative allelic constitution of BADH2 gene for aroma.

Keywords: Aroma analysis, aromatic rice, grain morphology, molecular diversity, phylogenic relationship

1. Introduction

Aromatic rice is a specialty rice group that possesses aroma, has been cultivated mostly in South and South-East Asian countries from ancient times and is highly prized in the world market and tied to folk customs (Itani *et al.,* 1983). Grain aroma is one of the key characteristic of quality rice whose importance is realized not only in Asian market but also widely recognized in Europe and other parts of the World (Wakte *et al.,* 2017). More than 100 volatile compounds have been identified in rice (Dudareva *et al.,* 2013; Mathure *et al.*, 2011; Mathure *et al.,* 2014.). Among these compounds 2-acetyl-1-pyrroline (2-AP) is identified as the principal aroma compound. The level of aroma expression generally associated with the increased levels of 2-acetyl-1-pyrroline (2AP) (Buttery *et al.,* 1983; Widjaja *et al.,* 1996; Yoshihashi, 2002). In order to assist in the rapid development of fragrant rice varieties suited to particular environmental conditions, rice breeders need a simple and inexpensive method for identification of fragrant rice genotypes. Chemical methods are available which involve smelling leaf tissue or grains after heating in water or reacting with solutions of KOH or I2-KI (Sood *et al.,* 1978) but these can not quantify the aroma. An objective method of 2AP identification using gas chromatography is available but the assay requires large tissue samples and is time consuming (Lorieux *et al.,* 1996). PCR-based DNA markers were developed to generate polymorphisms that can distinguish aromatic from non aromatic genotypes. Molecular markers closely linked to the aroma gene or "*fgr*" QTL can be used to facilitate early selection for the presence of aroma. Researcher defined the chromosomal location of the gene by mapping in segregating populations using simple sequence repeat (SSR) or microsatellite and single nucleotide polymorphism (SNP) markers (Cordeiro *et al.*, 2002; Jin

et al., 2010). A recessive gene, on chromosome 8 of rice, largely controlling the level of 2-acetyl-1-pyroline, has been identified in genetic studies (Bradbury *et al*., 2005). Based on positional cloning, the major aroma gene was identified as a defective or non-functional allele of the gene coding *betaine aldehyde dehydrogenase* (*BADH2 gene*) with an 8 base pair deletion and 3 SNPs observed in Exon 7 generating a pre-mature stop codon to the *BADH2* mRNA, resulting in its loss of function and accumulation of 2-AP is responsible for aroma (Bradbury *et al*., 2005).

Breeding rice varieties with preferred grain quality features has become second most important objective next to yield. Therefore, assessment of diversity of available germplasm is important pre-breeding exercise. There are reports on wide variability for pheno-genomic traits including grain quality characters and its importance in aromatic rice breeding programmes (Islam *et al.,* 2018). India is home to wide varieties of rice cultivars, landraces and many lesser known varieties. The landraces maintained by farmers are endowed with tremendous genetic variability and valuable genes for unique aroma (Prodhan *et al.,* 2020). Varietal diversity in indigenous rice is found to suit varying growing conditions, ethnic preferences and diverse uses (Mo *et al.,* 2015; Thakur *et al.,* 2020).Rice diversity is also well explained using molecular markers(Dwivedi *et al*., 2019; Kaur *et al.,* 2020) The present study is formulated looking at future prospects and demand of the aromatic rice breeding projects. Understanding the dynamics of expression pattern of *BADH2* gene in different aromatic rice varieties grown in different parts of Assam will help in characterization of trait of aroma in the cultivated aromatic rice of Assam. This study also describes nature and extent of genetic variation in aromatic rice collected from six different agro-climatic regions of Assam in regards to grain quality. Further a simple PCR based method targeting the candidate gene for fragrance, will be amenable for routine genetic purity testing of aromatic rice collection.

2. Materials and Methods

2.1 Experimental materials

Experimental material consists of 14 native aromatic rice germplasms including traditional and improved varieties (Table 1) collected from different agro climatic regions of Assam. The pre-germinated seeds were sown in the field following randomized block design with three replications in July, 2018 in the Instructional cum Research farm of Assam Agricultural University.

Table 1. Rice varieties with accession numbers

S. No.	Genotypes
1	Badshah bhog *(J2)*
2	Bhoboli joha *(J4)*
3	Boga joha *(J6)*
4	Maniki madhuri joha *(J10)*
5	Boga tulsi *(J11)*
6	Borshal joha *(J12)*
7	Chuban joha *(J14)*
8	Gualporiya joha-1 *(J15)*
9	Joha bora *(J19)*
10	Kon joha-2 *(J22)*
11	Kola joha-1 *(J24)*
12	Kaljira *(J29)*
13	Keteki joha (*KJ*)
14	Bokul joha (*BJ*)

2.2 Grain characteristics

The grain morphology was studied with five taxonomic traits along with grain Amylose content. Ten competitive plants were randomly selected from each genotype in each replication to record the data following test guidelines for the conduct of test for Distinctiveness, Uniformity and Stability for rice (2007).

2.3 Molecular characterization

The genomic DNA was extracted from young seedlings following CTAB method developed by Murray and Thompson (1980) with slight modification.A total of 32 SSR primers were used in the present study and the informative primers are listed in the Table 2**.** PCR was performed in 20 µl reaction volumes containing 25 ng of DNA, 50 pmol each forward and reverse primer for SSR, 25 µM of dNTPs (Fermentas, Bangalore Genei) and 0.6 U of Taq Polymerase (Bangalore Genei). PCR program was followed as, initial denaturation at 94 °C for 3 min followed by 35 cycles, each consisting of denaturation at 94 °C for 1 min, annealing at appropriate temperature for 1 min and elongation at 72 °C for 2 min for SSR primers. A final extension step included 72 °C incubation for 7 min followed by hold at 4 °C. Perkin Elmer (model 9600) thermo-cycler was used to carry out the reaction.

The amplified DNA fragments were resolved on ethidium bromide stained agarose gel (2.5%) in 1X TAE buffer at 50 V. The gels were visualized on trans-UV and photographed in Bio-red Gel Doc XR 2.0.

Table 2. Informative SSR primers with PIC values

Marker	Annealing temp. (°C)	Product size (bp)	PIC
RM 1	55	130 & 110	0.1378
RM 9	55	180 & 130	0.1734
RM 10	55	170 & 175	0.4688
RM 21	55	180 &140	0.1528
RM 55	55	230 & 240	0.4260
RM 164	55	310 & 290	0.2602
RM 206	55	180 & 140	0.2449
RM307	55	130 & 140	0.3368
RM 316	55	210, 200 & 180	0.4971
RM 336	55	180 & 160	0.1860
RM 337	55	180 & 150	0.1326
RM 480	55	220 & 200	0.3109
RM 496	55	310, 290 & 270	0.5408
RM 541	55	190 & 170	0.3295
RM 548	55	290 & 270	0.1357
RM 556	55	260 & 240	0.2449
RM 11	55	130 & 110	0.1378
RM 12	55	180 & 130	0.1734

2.4 Aroma analysis

Gene based primers (Table 3) *viz.,* Aroma 1, designed based on the 8 bp deletions and 3 SNPs in the Exon 7 of the defective allele of *Betain Aldehyde Dehydrogenase* 2 (BADH 2) gene (Bradbury *et al.,* 2005) and was used for analysis of aroma among the tested genotypes

Table 3. Aroma 1 primer

Primer name	Sequence (5' - 3')
External Sense Primer (ESP)	TTGTTGGAGCTTGCTGATG
Internal Fragrant Antisense Primer (IFAP)	CATAGGAGCAGCTGAAATATATACC
Internal Non-fragrant Sense Primer (INSP)	CTGGTAAAAAGATTATGGCTTCA
External Antisense Primer (EAP)	AGTGCTTTACAAGTCCCGC

2.5 Data analysis

The amplicons were scored as 1 for presence and 0 for absence for each genotype. Polymorphic information content (PIC) was calculated based on bands per primer (Botstein *et al.,* 1980) Cluster analysis was performed using NTSYSpc version 2.1 (Rohlf 1998). Pair-wise combinations of genotypes were employed to calculate Jacquard's similarity coefficient (Jaccard 1908). The similarity matrix was used to generate an "Unweighted Pair Group Method with Arithmetic Average" (UPGMA)-based dendrogram (Sneath and Sokal 1973) using the SIMQUAL module of NTSYSpc.

3. Results and Discussion

3.1 Genetic variation and comparative performance of genotypes for grain characteristics

The analysis of variance revealed significant differences among the genotypes for all quantitative grain characters under study. The mean performance of genotypes is presented in Table 4. The groupings of the grain characteristics were done following DUS guideline (2007). Based on grain type, genotypes can be classified into three groups. Eight genotypes belongs to long-slender type, four genotypes with long-bold grain and rest three

are medium-slender type. Pair wise similarity for 14 aromatic rice cultivars based on grain characteristics using euclidean distance coefficient was also calculated. The distance coefficient ranged from 0.713 to 6.890, indicating no true duplicates. The maximum genetic divergence was observed between Boga joha (J6) and Kaljira (J29), which is 6.890. The lowest diversity (0.713) has been observed between Borshal joha (J12) and Keteki joha. The dendrogram based on grain characteristics, separated the 14 aromatic rice genotypes into two major clusters (Fig. 1). The cluster I with 11 and II consisted of 3 genotype. In the cluster-I, Borshal Joha and Keteki Joha was indicated as similar varieties. However, there is significant difference for Amylose content and few other grain characters are different among them. Variability in aromatic rice cultivation is reported in many other studies also (Talukdar *et al.,* 2017; Mahajan *et al.,* 2018). The exhibited variability in the present study has immense importance selection of parents for varietal improvement program. The grain morphology data will also help in genetic identification of varieties in maintenance breeding.

Table 4: Mean performances of grain characteristics

Genotypes	Grain length (cm)	Grain width (cm)	Decorticated grain length (cm)	Decorticated grain width (cm)	Grain L/B ratio	Amylose content (%)
Badshah Bhog (J2)	6.00	2.17	4.25	1.50	2.46	28.70
Bhoboli Joha (J4)	8.25	2.00	6.33	2.00	4.34	21.30
Boga Joha (J6)	9.25	2.17	7.00	2.08	4.27	18.60
Maniki madhuri joha (J 10)	6.58	2.00	4.75	1.75	3.13	22.90
Boga Tulshi (J 11)	6.50	2.00	4.58	2.00	3.23	24.50
Borshal Joha (J 12)	8.08	2.17	6.00	2.00	3.77	19.10
Chuban Joha (J 14)	6.08	2.42	4.75	2.08	2.53	18.20
Gualporia Joha -1 (J 15)	7.33	2.00	5.83	2.00	3.65	18.00
Joha Bora (J 19)	7.25	3.08	5.92	2.83	2.33	12.80
Kon Joha-2 (J 22)	7.58	2.33	5.17	2.00	3.23	17.60
Kola Joha-1 (J 24)	8.58	2.83	6.08	2.50	3.03	19.70
Kaljira (J 29)	6.67	2.58	4.67	2.58	2.60	24.20
Keteki Joha	8.58	2.25	6.33	2.08	3.87	24.70
Bokul Joha	5.92	2.50	4.75	2.42	2.40	22.10
Mean	7.3333	2.3214	5.4583	2.131	3.2038	20.8857
S.E.	0.1418	0.1017	0.1598	0.0674	0.1708	-
C.V.	3.3493	7.5871	5.0699	5.477	9.2312	-
C.D. 5%	0.4122	0.2956	0.4644	0.1959	0.4964	-

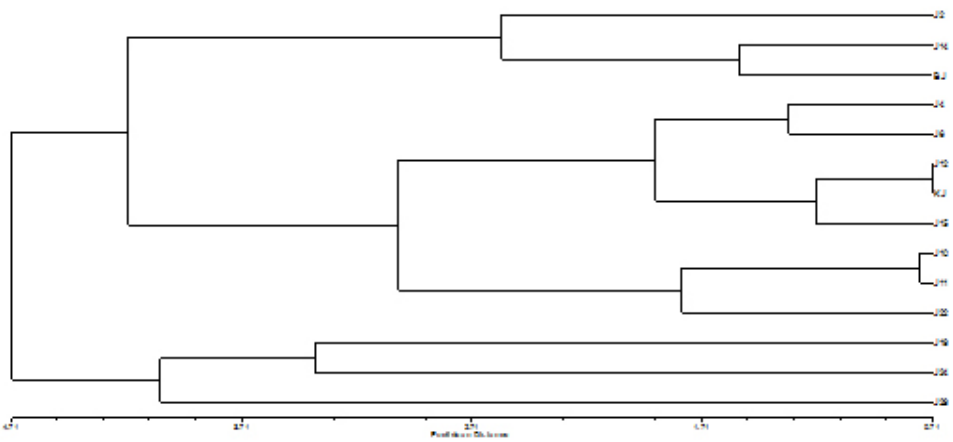

Fig. 1. Dendrogram of rice varieties based on grain morphology

3.2 Molecular phylogeny and genetic divergence

The importance of molecular markers and its application on aromatic rice breeding programmes has been of prime importance for precise and effective breeding programme (Peng *et al.,* 2018). 32 SSR primers were used to study the molecular genetic diversity of the aromatic rice genotypes, out of which 18 primers (Table 2) were polymorphic and were used for genetic analysis. The primers RM496 and RM316 produced three alleles each and the rest have produced two alleles. RM496 had the highest PIC value of 0.5408 and the PIC value was found to be lowest in the marker RM337 (0.1326), indicating RM496 is much more informative in comparison with all the other SSR markers used under study. Allelic variation for few SSR marker is depicted in Fig. 3. The Dendrogram based on UPGMA separated the 14 aromatic rice genotypes into two major clusters (Fig. 2). The cluster I and consisted of 12 genotypes cluster II with 2 genotypes. The majority of genotypes in cluster-I, indicates the close phylogenetic relationship among the cultivated aromatic rice. The clear separation of the two genotypes Bokul Joha and Joha Bora in cluster II from the rest indicates their genetic dissimilarity. The present set of SSR markers could not distinguish between Gualporia joha-1 (J15), Kon joha-2 (J22) and Kola joha-1 (J24) as limited number of SSR markers in the study and their random selection might have amplified the same alleles in all the genotypes warranting the use of more markers to get a better picture of diversity among them. However, the genotypes included in the present study are mostly farmer's varieties and the present result indicates sharing of common parentage among them. Hence, there is a possibility of cultivating the same variety in different names in different locations. Only two varieties in the Cluster II, *Joha bora* (J19) and *Bokul joha*, defines similar ancestral origin, hence close phylogenic relationship among them. Short grain aromatic class of rice is locally termed as *joha* and semi glutinous and glutinous are commonly known as *bora* in Assam. Local name *joha bora* indicates the presence of glutinous character in this variety. These 2 genotypes may have ancestral similarities with the glutinous group of rice, some of which have japonica origin .Aromatic rice varieties of NE India represent distinct genetic stock. Global classification places them into both *Indica* and *Japonica* types (Jain *et al.,* 2004; Talukdar *et al.,* 2017). Accessions from Assam, Manipur and Sikkim are assigned to *indica* group, while those from Nagaland exhibit close association with *Japonica* (Roy *et al.,* 2015). The present study on molecular phylogeny explains the genetic diversity and relationship can be well exploited in future breeding programs.

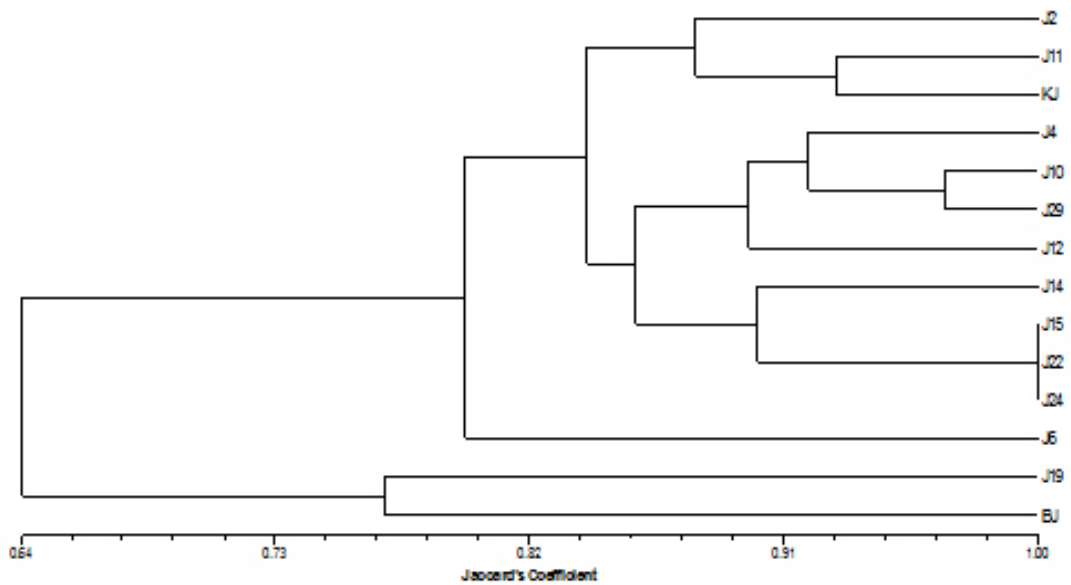

Fig. 2. Dendrogram of rice varieties based on SSR banding pattern

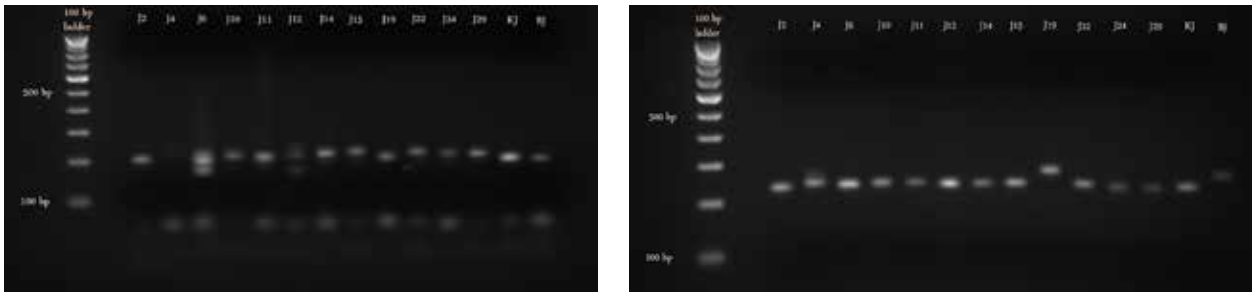

Fig. 3. Polymorphism for SSR RM556 and RM316 among the genotypes

3.3 Aroma analysis based on gene based primer

Gene based primer Aroma 1 consists of two external primers *viz.,* ESP and EAP, and two internal primers *viz.* INSP and IFAP, which is reported to be amplified at a 355 bp band in non-fragrant rice and a band of 258 bp in fragrant rice (Bradbury *et al.,* 2005). All the genotypes revealed the presence of 258 bp band except Bakul Joha and Badshah bhog, confirming the presence of *BADH-2* gene for aromatic expression (Fig. 4). A peculiar band produced by Bokul *Joha* with the primers Aroma 1 is of 355 bp (Fig. 4). This indicates that *BADH2* gene is fully functional and the aroma in Bakul *Joha* may be due to the presence of additional allele. The genotypes collected from different farmer's field and amplification of aroma allele in Bokul *Joha* might have due to differential expression in varied environmental condition and due to differential selection pressure. Similar reports have been indicated in several studies about differential expression of aroma in varying environmental conditions. (Pachauri *et al*., 2014; Hinge *et al*., 2016). Badshah bhog did not amplify the aroma allele implying the absence of *BADH2*. However the name of the variety Badshah bhog indicates its ancestral origin may not from local aromatic rice although it contains other grain characters similar to local *Joha* type. The present study implies, apart from Badshah bhog (J2) and Bokul *joha,* the aroma in rest of the cultivars among the tested genotypes, may be due to defective BADH 2 allele which is similar to the earlier reports (Bradbury *et al.,* 2005; Yi *et al*., 2009; Fitzgerald *et al.,* 2010; Talukdar *et al.,* 2017).

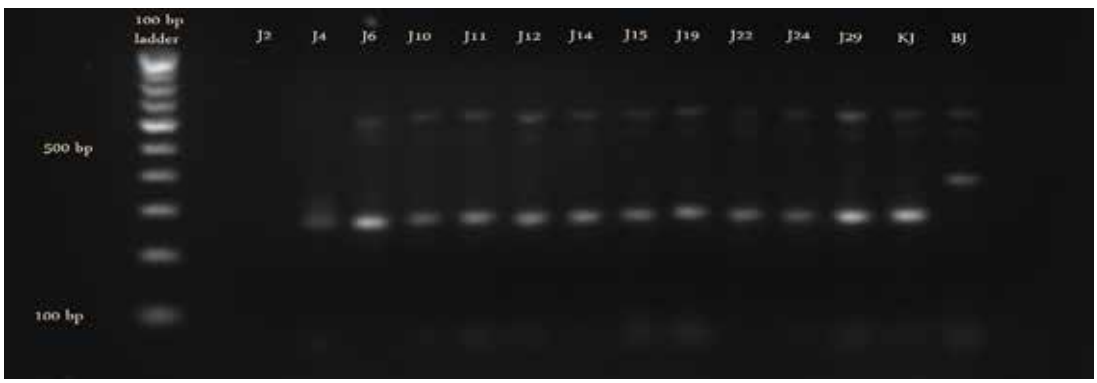

Fig. 4. Aroma analysis: allelic distribution of Marker Aroma 1 (ESP + EAP + IFAP + INSP)

4. Conclusion

Aroma of Assam aromatic rice collection may be due to *BADH2* gene and with more than one allelic constitution. The phylogenic relationship indicates the ancestral origin of the aromatic rice may be of diverse. The present study also indicates probable presence of both *indica* and *japonica* type in Assam aromatic rice collection which needs further verifications.

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Conflict of Interest

Authors declare that they have no conflict of interest.

Ethical Compliance Statement

NA

Author's Contribution

Conceptualization of research (SDD); Designing of the experiments (SDD); Contribution of experimental materials (SDD, DH); Execution of field/lab experiments and data collection (DH)

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