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Assessment of Nepalese Aromatic Rice (*Oryza Sativa* L.) Landraces at Phenotypic and Genotypic Level

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Abstract

The aromatic rice harbour genes controlling and expressing peculiar characteristic of aroma. The presence and expression of aroma is highly affected by environmental variables indicating that phenotypic evaluation can misclassify aromatic rice and the genotypic evaluation is very limited. The aroma of rice is considered as special trait of enormous economic importance that affects consumers preference and pricing. Therefore, an investigation was carried out to characterize local rice landraces by combining both phenotypic and genotypic approaches at Center of Biotechnology, Agriculture and Forestry University during 2020. The 36 rice landraces were subjected to phenotypic test by 1.7% KOH and genotypic test using two functional markers BADEX7-5 and FME7. The Lalka Basmati and Sabitri were taken as positive and negative control, respectively. The sensory phenotypic test using KOH revealed that majority of landraces had some sort of aroma. On the other hand, genotypic evaluation detected only Basmati Kalo Tude, Jeerasari, Kalo Nuniya-10, Kalo Nuniya Thulo, Rato Basmati Thulo, Kalo Basmati Jhunge, Kalo Nuniya-1 and Rato Basmati Sano had the genes governing the distinctive aroma. This shows that Nepal has good amount of aromatic rice landraces that could fetch high value in market. A holistic approach is necessary for reliable classification of rice landraces for practical application.

Keywords: *Aroma, BADEX7-5, FME7, functional markers, rice landraces*

Introduction

Rice is the major staple food grains in the world as well as in Nepal. It plays pivotal role in the economics, nutrition and livelihoods of the farmers in Nepal. Both aromatic and non-aromatic rice are grown in diverse agro-climatic conditions from low altitude (Jhapa) to high altitude (Jumla) in the country. Even though the non-aromatic rice is more yielding, demonstrate good agronomic performances, and are highly adaptive to environmental conditions, farmers are still growing low-yielding, inferior agronomic characters posing aromatic rice due to its excellent aroma and superior grain quality (Prodhan *et al.,* 2017, Wakte *et al.,* 2017). Aromatic rice varieties are generally prone to insects and diseases attack, abiotic stress and highly responsive to photoperiodism (Nadaf *et al.,* 2014, Feng, 2019). Since the aroma is one of the important grain quality attributes of rice which significantly influence the consumer preference and pricing (Yang *et al.,* 2023), the farmers are planting the aromatic rice in their own farms continuously under different agro-ecology, crop management practices and post-harvest management.

Genetically, aroma of the rice is the phenotypic expression of spontaneous recessive mutation of the *Osbadh2* gene also called *fgr/osbadh2/OS2AP* (Courtois, 2007) which drives to presence and expression of 2-actyl-1-pyrolline (2AP) that is a major olfactory compound out of several volatile compounds identified from aromatic and non-aromatic rice (Yang *et al.,* 2008) contributing peculiar fragrance of aromatic rice (Bradburry *et al.,* 2005, Kongpun *et al.,* 2024). The synthesis of 2AP in fragrant rice is attributed to loss in function of BADH gene badh2. Bradbury *et al.,* (2005) reported that the betaine aldehyde dehydrogenase (BADH2) gene is associated with rice fragrance, and comprises 15 exons and 14 introns on chromosome 8. Study of Bradbury *et al.,* (2005) signaled that 8bp deletion and three SNP present in exon 7, whereas Amarawathi *et al.,* (2008) pointed out 5bp deletion in same location in exon 8. These studies shows that the variation in fragrance

(*fgr*) gene leading to non-functionality of BADH2 protein are related to fragrance in rice (Chen *et al.,* 2008).

The biotic (2AP biosynthesis involving microorganisms), abiotic factors (water, temperature, light intensity, soil salinity and fertilizer management) and post-harvest management are prime variables ruling phenotypic expression of fragrance in aromatic rice. The presence and absence of the aroma in rice varieties is determined basically by sensory analysis (olfactory) and gas chromatography (Al-khalili *et al.,* 2025). Since the phenotypic expression of volatile compounds like 2AP is highly influenced by environmental variables, solely reliable on the olfactory or field based phenotypic evaluation may leads to misclassification of the rice genotypes and reduces the selection efficiency. Nanoukon *et al.,* (2024) tested 72 rice accessions collected across Benin using RM 7049, Aro 7 and RM 223 SSR markers linked to *fgr* aroma gene and identified 12 genotypes with *fgr* gene as aromatic rice. While sensory phenotypic test using KOH was carried out on rice accessions carrying *fgr* gene, only one has positive response to smell. This necessitates the incorporation of genotypic approaches for the classification of rice genotypes which can reliably identify aroma-related genes irrespective of environmental influence. Yet, many of the locally grown aromatic rice genotypes remain uncharacterized for the fragrance genes like BADH2. Besides that, limited genotypic information on presence of BADH2 gene restricts the application of marker-assisted selection and use of elite aromatic germplasms in breeding programs. In addition, dissimilarity between genetic approach and phenotypic level evaluation emphasize the integration of both olfactory and genetic approaches for accurate characterization of aroma in aromatic rice genotypes. Therefore, a study was carried out to characterize local aromatic rice landraces by integrating phenotypic and genotypic assessment methods. This will help in identification of stable aromatic rice genotypes and improve the reliability of selection of rice genotypes.

Materials and Methods

Experimental materials

A total of 36 rice landraces – maintained at Department of Genetics and Plant Breeding – were evaluated at the laboratory of Center for Biotechnology, Agriculture and Forestry University, Rampur during 2020. The variety served as positivecontrol was Lalka Basmati and another variety Sabitri was used as negative control for the study.

Phenotypic evaluation by KOH test

The leaf and grain aroma content were detected according to the method described by Sood and Siddiq (1978) using 1.7% KOH. For leaf aroma detection, 2-3 leaves from individual plant were collected from tillering stage and 2g fine cut green leaves was placed into petri dishes. For grain aroma detection, forty grains of each genotype were placed into the petri dishes. In both, 10ml of 1.7% KOH was added to the petri plates. The petri plates were kept at room temperature for about 10 minutes by covering them immediately. The plates were then opened and samples were smelled and rated for aroma by a panel of specialists in scale of 0 (non-aromatic) to 3 (strong aromatic).

DNA extraction and PCR amplification

The total genomic DNA of tested landraces and check varieties from leaves was isolated using modified CTAB isolation procedure (Bibha Rani *et al.,* 2016) from the plants at tillering stage grown in the field of Genetics and Plant Breeding following randomized complete block design (RCBD) procedure. The purity level of DNA for all the genotypes was assessed by measuring optical density at A260 and A280 by UV-Vis Spectrophotometer (NanoDropTM, Thermo Fisher). The DNA samples were checked on 0.8% agarose gel and then kept at 4ºC in laboratory. The analytical methods focused on PCR amplification with functional markers. For this study, two fragrance linked functional gene tagged markers were used to confirm the presence of the *fgr* gene: BADEX7-5 and FME7 located at chromosome 8. The forward and reverse sequence at 5’ to 3’ for BADEX7-5 were TGTTTTCTGTTAGGTTGCATT and ATCCACAGA AATTTGGAAAC, respectively. The band size 95bp indicates the presence of *fgr* gene and 103bp as absence. Similarly, the forward and reverse sequence at 5’ to 3’ for FME7 were TCCTGTAATCATGTATAC CC and AATTTGGAAACAAACCTT, respectively. The band size 143bp confirms the presence of *fgr* gene and 151bp as absence. The PCR amplification was carried out in a total 15µl reaction mixture consisting of 4µl nuclease free water, 7.5µl master mix, 1µl of each forward and reverse primer and 1.5µl of template DNA. The amplification for BADEX7-5 primer was carried out according to following program: an initial denaturation of 94ºC for 5 min followed by 35 cycles each consisting of a phase of denaturation at 94ºC for 30 sec, annealing of primer at 54ºC for 30 sec and

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extension to 72ºC for 2 min. Similarly, the amplification for FME7 primer was carried out according to following program: an initial denaturation of 95ºC for 3 min followed by 35 cycles each consisting of a phase of denaturation at 94ºC for 1 min, annealing of primer at 50ºC for 1 min and extension to 72ºC for 2 min. The program ends with a final extension phase at 72ºC for 10 min.

Agarose gel electrophoresis and visualization

The amplified products were migrated by gel electrophoresis on a 3.5% agarose gel in 100µl of 0.5x TBE buffer for 3hrs at 75V. The 7.5µl of PCR product of each sample along with 1.5µl of loading dye (6x) was loaded onto wells and gel image was visualized under UV light.

Data scoring

Band size and allele intensity of each functional marker was identified by using Image Lab 6.1 software. The presence of *fgr* allele was scored as "+" while the absence was as "-".

Results

Phenotypic evaluation of fragrance

The olfactory test performed with the 36 rice landraces along with check varieties are presented in the Table (1). The test revealed that only 29 landraces had slight to strong aroma when tested with leaf taken from field. The grain-based results shows that 32 landraces had slight to strong aroma. The Kalo Basmati Jhunge only had strong smell of aroma after comparison with the smell experienced in the aromatic control Lalka Basmati using leaf. But in grain-based aroma, Kalo Nuniya-10, Aanga and Seto Basmati showed strong smell of aroma equivalent to aromatic control Lalka Basmati.

Table 1. Grain and leaf aroma of aromatic rice landraces using KOH

S.N.	Aromatic Rice Landraces	Leaf aroma	Grain aroma
1.	Basmati Rato Tude	1	1
2.	Jaghad	0	1
3.	Basmati Kalo Tude	2	2
4.	Jeerasari	2	2
5.	Chhatraj	0	1
6.	Kariya Khera	1	1
7.	Gasulochan	1	2
8.	Chameli	0	1
9.	Kalo Nuniya-10	2	3
10.	Lalka Jesariya	0	0
11.	Aanga	1	3
12.	Laghi	1	1
13.	Dudhraj	0	0
14.	Kasturi	1	1
15.	Kalo Nuniya Thulo	2	2
16.	Shyangeera	2	1
17.	Ramaywine	2	1
18.	Rato Basmati Thulo	2	2
19.	Gajargawl	1	1
20.	Bhabhhi	1	0
21.	Rato Basmati Sano	1	2
22.	Seto Basmati	1	3
23.	Bagar Anadi	0	1
24.	Mansara	1	1
25.	Bangalia	2	2
26.	Balamsar	1	0
27.	Jhorpal Basmati	1	2
28.	Karangi	1	2
29.	Aanp Ghuthe	1	1
30.	Ghusara	1	2
31.	Kalo Basmati Jhunge	3	2
32.	Birimphool	2	2
33.	Kalo Nuniya-1	1	2
34.	Andi Dhan	0	1
35.	Parewapawankh	1	1
36.	Champasari	1	2
37.	Sabitri	0	0
38.	Lalka Basmati	3	3

Genotypic evaluation of fragrance

The PCR amplification applying BADEX7-5 and FME7 produced the bands of 95bp and 143bp, respectively flagship the presence of the gene encoding desired aroma (Figure 1 & 2 and Table 2). Out of 36 landraces screened with BADEX7-5, we obtained that only 7 landraces produced 95bp of band as a positive Lalka basmati controlling the characteristic of the *fgr* gene. Concerned to FME7 marker, 8 landraces presented 143bp bands peculiar to the desired gene of aroma. Thus, only the landraces producing 95bp and 143bp were taken as aromatic rice. Basmati Kalo Tude, Jeerasari, Kalo Nuniya-10, Kalo Nuniya Thulo, Rato

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Basmati Thulo, Kalo Basmati Jhunge and Kalo Nuniya-1 have produced the recessively mutated bands of both functional markers as of control Lalka Basmati. This shows that these seven landraces have both kinds of allele involved in the expression of aromatic characteristic of rice. The Rato Basmati Sano only produced 143bp bands of FME7 marker while it fails to produced 95bp band of BADEX7-5. It indicates that the fragrance characteristics of Rato Basmati Sano is only governed by a single allele of *fgr* gene harbour in chromosome 8.

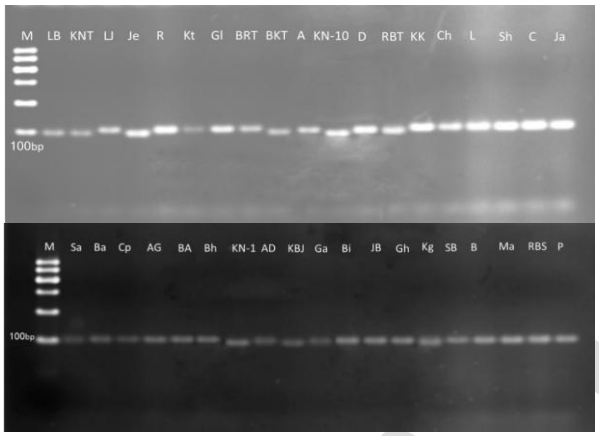

Fig. 1: Agarose gel profile of functional marker BADEX7-5 analyzed on 36 aromatic rice landraces and 2 check variety

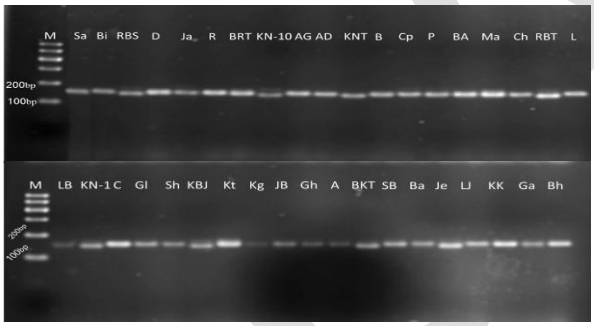

Fig. 2: Agarose gel profile of functional marker FME7 analyzed on 36 aromatic rice landraces and 2 check variety

Table 2. Scoring of genotypic data obtained from BADEX7-5 and FME7 markers

S.N.	Aromatic Rice landraces	BADEX7-5	FME7
1.	Basmati Rato Tude	-	-
2.	Jaghad	-	-
3.	Basmati Kalo Tude	+	+
4.	Jeerasari	+	+
5.	Chhatraj	-	-
6.	Kariya Khera	-	-
7.	Gasulochan	-	-
8.	Chameli	-	-
9.	Kalo Nuniya-10	+	+
10.	Lalka Jesariya	-	-
11.	Aanga	-	-
12.	Laghi	-	-
13.	Dudhraj	-	-
14.	Kasturi	-	-
15.	Kalo Nuniya Thulo	+	+
16.	Shyangeera	-	-
17.	Ramaywine	-	-
18.	Rato Basmati Thulo	+	+
19.	Gajargawl	-	-
20.	Bhabhhi	-	-
21.	Rato Basmati Sano	-	+
22.	Seto Basmati	-	-
23.	Bagar Anadi	-	-
24.	Mansara	-	-
25.	Bangalia	-	-
26.	Balamsar	-	-
27.	Jhorpal Basmati	-	-
28.	Karangi	-	-
29.	Aanp Ghuthe	-	-
30.	Ghusara	-	-
31.	Kalo Basmati Jhunge	+	+
32.	Birimphool	-	-
33.	Kalo Nuniya-1	+	+
34.	Andi Dhan	-	-
35.	Parewapawankh	-	-
36.	Champasari	-	-
37.	Sabitri	-	-
38.	Lalka Basmati	+	+

(+) indicates presence of 8-bp deletion and (-) indicates absence of 8-bp deletion.

Discussion

The studied landraces show variation in fragrance while leaf and grain-based scoring was made by sensory method. The differences might be due to differences in the sensory capacity of the specialist panels since the olfactory techniques relies on human nose that detects the aroma from the chromatography column (Al-khalili *et al.,* 2025). According to Itani et al (2004) and Mo *et al.,* (2015) environmental variables like temperature, light, water, salinity,

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submergence and UV irradiation can reduce the grain quality and affect rice aroma quality. Thus, the variation obtained in aroma expression of our tested landraces shall be strongly affected by the environmental variables. In molecular genotyping, about 22.22% of the total landraces tested produced both 95bp and 143bp bands of BADEX7-5 and FME7, respectively suggesting that they reside peculiar alleles of *fgr* gene. Nanoukon *et al.,* (2024) also concluded that there is presence of different allele or QTL involved in expression of fragrance of the rice while testing 72 rice accession using SSR markers RM 7049, RM23 and Aro 7. Similarly, Peng *et al.,* (2018) conclusively stated that fragrant characteristic of rice grain can also be controlled by other genes or introns. In addition, these two functional markers were found to be polymorphic and discriminating.

In the present study, the phenotypic evaluation of fragrance was not consistent with the genotypic evaluation. Landraces with some sort of aroma detected phenotypically did not contains the allele governing the aroma at genetic level. Therefore, classification of rice landraces solely based on phenotypic evaluation might misguided and biased on selection. It insists to couple both approached for reliable classification and selection of rice landraces. In this study the only two functional markers were used which might limited its results application in field. Therefore, it is better to characterize rice landraces for aroma using several other tightly linked or functional markers and gas chromatography-based evaluation of the rice landraces for wider application in breeding programs.

Conclusion

The aromatic rice landraces hold different alleles for expression of characteristic aroma predominately. The sensory based evaluation resulted in a greater number of landraces as aromatic rice. Limited number of landraces are confirmed with *fgr* allele responsible for aroma producing in genotypic evaluation. These results concluded that solely depending either on phenotypic or genotypic approach can result in wrongly assigning rice landraces in aromatic group. Thus, integration of both approaches in rice landraces characterization is better alternative for its reliability and wider application in selection.

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