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Molecular characterization of short grain aromatic rice landraces of Odisha for detection of aroma

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ABSTRACT

Sensory method along with four molecular markers (ESP, EAP, INSP and IFAP) were used for evaluation of aroma in indigenous short grain aromatic rice genotypes collected from different regions of Odisha (India). Sensory method of aroma evaluation from kernel sample of these genotypes could detect mild, moderate and strong aroma in 8, 36 and 26 genotypes, respectively. The molecular level aroma detection could identify homozygous fragrance in 59 genotypes, whereas 11 genotypes were found to be with heterozygous nature for this locus indicating requirement of more efforts towards understanding their genetic base of aroma. Integration of sensory method and molecular level evaluation of aroma could help in rapid identification of aromatic genotypes within a large population.

Key words: *rice, aroma, sensory, landrace, Odisha*

India has a rich genetic diversity of aromatic rice landraces which constitutes a separate group, *i.e.* Group V (Glaszmann, 1987). India is the major exporter of Basmati rice, whose cultivation is limited to a specific geographic demarcation while majority of the indigenous aromatic rice genotypes cultivated throughout the country are small and medium grain and are known as non-Basmati aromatic rices (Singh *et al.,* 2000a) which are grown in localized pockets in all most all states of India (Shobharani and Singh, 2003). Moreover, these aromatic rice landraces enjoy premium market rate and are consumed locally as a delicacy for their better grain quality features including strong aroma. Explorations throughout India have resulted in a large assembly of diverse set of aromatic rice landraces maintained as working/active collections/base collections at various gene banks, but many of the accessions have not yet been characterized for important and valuable traits (Rana *et al.,* 2009).

Fragrance in rice is influenced by several chemical and volatile compounds (Cordeiro *et al.,* 2002), out of which elevated level of 2 acetyl-1-pyrroline (2AP) is predominantly found in majority of aromatic rice genotypes (Lorieux *et al.,* 1996; Yoshihashi, 2002). Further, Bradbury *et al.,* (2005a) reported that a recessive gene located on chromosome 8 contains 8bp deletion and 3 SNP's produces non-functional BADH2 enzyme, providing increase level of 2 acetyl-1-pyrroline (AP) accumulation and hence aroma in rice. Several molecular markers closely linked to aroma have been reported for the selection of aromatic rice genotypes (Lorieux *et al.,* 1996; Cordeiro *et al.,* 2002; Jin *et al.*, 2003). Moreover, the gene specific markers and the allele specific amplification (ASA) developed by Bradbury *et al.,* (2005a) is being widely used for discriminating aromatic rice genotypes from non-aromatic ones. The present study investigates characterization of native short grain rice landraces for their detection of aroma, both at sensory and molecular level which will pave way towards identification and improvement of suitable aromatic genotypes for crop improvement.

MATERIAL AND METHODS

A total of 70 aromatic short grain rice landraces from different regions of Odisha, India were collected and evaluated (Table 1). Seeds of individual accessions were germinated separately in sterile petridishes and two

weeks old seedlings were transplanted in experimental pots. Aroma phenotype was evaluated with three replications per individual genotypes according to the modified protocol of Ahmadikhah *et al.*, (2010). Three de-hulled rice grains of individual genotypes were put in a 200ìl PCR tube and 150 ìl distilled water was added to each tube. Cap-closed tubes were incubated in 95ºC for 20 min in a thermal cycler PCR (Applied Bio Systems) for uniform distribution of temperature to each of the tube. The vapor was smelled after one minute of cooling by three individuals and samples were scored in comparison to controls *i.e.* Basmati (aromatic) and Swarna (non-aromatic).

The genomic DNA was extracted from 10 days old rice seedlings as per Dellaporta *et al.,* (1983). PCR analysis (single tube allele specific amplification) was performed with 0.2 ìl Taq DNA Polymerase (5U $ìl^{-1}$) (Biotools), 1 ìl of genomic DNA 10 ng $ìl^{-1}$, 1 ìl of 10X buffer (Biotools), 0.5 ìl of dNTPs (2.5 mM), 1.25ìl of each primer reported by Bradbury *et al.,* (2005a) *i.e.* ESP (ttgtttggagcttgctgatg), IFAP (cataggagcagct gaaatatatacc), INSP (ctggtaaaaagattatggcttca) and EAP (agtgctttacaaagtcccgc) in a total volume of 10 ìl. PCR cycling conditions performed using thermal cycler

Table 1. List of aromatic short grain rice landraces and their nature of aroma based on sensory and molecular evaluation

Sl. No.	Local Name	AS	AG
Control (+ve)	Basmati 370	3	DD
Control (-ve)	Swarna	0	NN
1	Kalajeera	5	DD
2	Kalazeera	5	DD
3	Kalajira	5	DD
4	Acharmati-1	5	ND
5	Acharmati-2	5	DD
6	Basayabhog	1	DD
7	Badsabhog-1	3	DD
8	Badsabhog-2	3	DD
9	Laxmibilas-4	3	ND
10	Samudrabali	3	DD
11	Chatianaki	3	DD
12	Nadiakata	1	ND
13	Bhadrakabasumati	5	DD
14	Lilabati	3	DD
15	Bhatagundi	1	ND
16	Basumati-1	5	DD
17	Kendragali	1	DD
18	Pipulabasa	3	DD
19	Karpurakanta	5	DD
20	Kalagiri	1	DD
21	Nadiarasa	3	DD
22	Saragadhuli	3	DD
23	Dhusara	5	DD
24	Basumati-2	3	DD
25	Kukudajata	3	DD
26	Koiamba	5	DD
27	Kalajiri-2	5	DD
28	Gopbasmati	3	ND
29	Krisnabhog	3	DD
30	Thakurbhog	3	DD
31	Garmatia	3	DD
32	Krishnabhog	5	DD
33	Ganjam local-1	5	DD
34	Ganjam local-2	3	DD
35	Karpurkali	3	ND
36	Kalajiri-1	3	DD
37	Mahulkuchi	3	ND
38	Atmassital	3	DD
39	Laxmibilas-1	5	DD
40	Laxmibilas-2	3	DD
41	Benugopal	3	ND
42	Pipalbasa	5	DD
43	Benubhog	3	DD
44	Laxmibilas-3	5	DD
45	Badaguda	5	DD
46	Bhuinsasal	3	DD
47	Durgabhog	1	DD
48	Dhurabahila	3	DD
49	Phulbanilocal-1	1	ND
50	Phulbanilocal-2	3	DD
51	Basanapuri	5	DD
52	Basubhog	3	DD
53	Kalikati-1	5	DD
54	Basanaphula	3	DD
55	Morllu	3	DD
56	Ganjeikali	3	DD
57	Deulabhog	1	ND
58	Jubaraj	3	DD
59	Kalakrushna	3	DD
60	Basnadhan	3	ND
61	Leelabati	5	DD
62	Parbatjira	3	DD
63	Manikagunda	3	DD
64	Basanaparijata	5	DD
65	Batakarua	5	DD
66	Laxmikajol	3	DD
67	Baiganamanji	5	DD
68	Basaparijata	5	DD
69	Kalikati-2	5	DD
70	Kalakrishna	5	DD

AS: Aroma score (0: absent; 1: mild; 3: moderate; 5: strong); AG: Aroma genotype (DD: 8bp deletion; NN: non-deletion; ND: heterozygote)

(Applied Biosystems) was 94^{0}C for 2 min, followed by 30 cycles of 94^{0}C for 5 sec, 58 for 5 sec, 72^{0}C for 5 and a final extension of 72^{0}C for 7 min. The amplified products were separated in 3% agarose gel and under UV light and photographed using a Typhoon FLA 7000 fluorescent image analyzer (GE Healthcare Bio-Sciences AB, Uppasala, Sweden). The rice varieties *i.e.* Basmati 370 and Swarna were used as positive and negative control respectively and the genotypes used in this experiment were compared for fragrant, non-fragrant and heterozygotes. The genotypic and allelic frequencies were computed based on Hardy-Weinberg formula.

RESULTS AND DISCUSSION

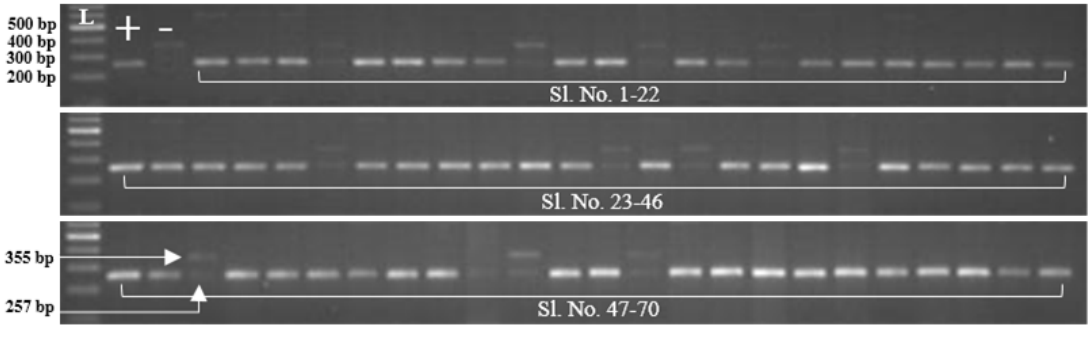
The aroma score of the 70 genotypes ranged from 1 to 5 and these genotypes were grouped into 3 distinct classes, *i.e.* mild aroma, moderate aroma and strong aroma according to their aroma scores (Table 1). Basmati 370 (positive control) was detected to be with moderate aroma from sensory method of aroma evaluation.

A maximum of 51.4% of the genotypes were identified to be moderately aromatic, whereas 37.14% were having strong aroma. Further, only 8 genotypes were found to be mild for their nature of aroma (Table 2). The different accessions of the genotypes like Phulbanilocal, Laxmibilas, Basumati, Kalajiri and Ganjamlocal collected from different farmers field varied in their nature of aroma which might have due to differential expression of aroma in varied environmental condition (Singh, 2000; Golam *et al.,* 2010).

Table 2. Phenotyping of aromatic short grain rice landraces based on their degree of aroma

Phenotype	Genotypes
Mild aroma	Basayabhog, Nadiakata, Bhatagundi, Kalagiri, Durgabhog, Phulbanilocal-1, Deulabhog, Kendragali
Moderate aroma	Badsabhog-1&2, Laxmibilas-2&4, Samudrabali, Chatianaki, Lilabati, Pipulabasa, Nadiarasa, Saragadhuli, Basumati-2, Kukudajata, Gopbasmati, Krisnabhog, Thakurbhog, Garmatia, Ganjamlocal-2, Karpurkali, Kalajiri-1, Mahulkuchi, Atmasital, Benugopal, Benubhog, Bhuinsasal, Dhurabahila, Phulbanilocal-2, Basanaphula, Basubhog, Morllu, Ganjeikali, Jubaraj, Kalakrushna, Basnadhan, Manikagunda, Parbatjira, Laxmikajol
Strong aroma	Kalajeera, Badaguda, Kalazeera, Kalajira, Acharmati-1&2, Bhadrak Basumati, Basumati-1, Karpurakanta, Dhusara, Koiamba, Kalajiri-2, Ganjamlocal-1, Laxmibilas-1&3, Krishnabhog, Pipalbasa, Basanapuri, Kalikati-1&2, Basanaparijata, Leelabati, Batakarua, Baiganamanji, Basaparijata, Kalakrishna

The markers employed in the present study could discriminate clearly between the homozygous fragrant control (Basmati 370) and homozygous non-fragrant control (Swarna) with the products of 257bp (deletion/DD type) and 355bp (non-deletion /NN type) bands, respectively (Fig. 1). Absence of the common band of 580bp in the PCR products is due to the low concentration of Taq polymerase used in the study. The amplicon size resulted in our study was similar to the earlier reports by (Bradbury *et al.,* 2005a; Sarhadi *et*



L: 100 bp ladder; +: Basmati 370; -: Swarna

Fig. 1. Gel representing 70 genotypes of aromatic short grains analyzed using single tube ASA

al., 2008) discriminating between aromatic and non-aromatic genotype groups.

The combined results suggest that 100% of the genotypes were homozygous fragrant and heterozygotes which is in higher proportion in comparison to the results of Prathepha, (2008). Fifty nine, out of 70 genotypes were identified for DD (8bp deletion type) and 11 were ND type (heterozygote), while none of the genotypes were detected to be with non-deletion type (NN) (Table 1 and 3).

Table 3. Genotype and allelic frequencies of fragrance gene in 70 aromatic short grain rice landraces along with controls

Genotype frequencies			
DD	NN	ND	Total
59	0	11	72
Allelic frequencies			
D allele	N allele		
0.921	0.079		

The molecular approach by allele specific amplification detected homozygous fragrant or heterozygote alleles among these genotypes. The overall allelic frequency was 0.921 for D allele and 0.079 for N allele, suggesting that the 8bp deletion is predominant among Indian native aromatic rice cultivars. The genotypes with strong level of aroma might be due to involvement of multiple genes and/or factors as reported in the earlier studies conducted by Pinson, (1994) and Lorieux *et al.,* (1996). All the genotypes evaluated in the present study were detected to be aromatic but their degree of aroma varied from mild, moderate and strongly aromatic.

Many conventional methods have been utilized by plant breeders for detection of grain aroma in the field level for varietal selection, which are time consuming, labor intensive and more over unreliable due to saturation of sensory organs. Since past few decades, with the availability of molecular markers to discriminate between aromatic and non-aromatic genotypes, marker assisted selection approach is being widely employed to separate aromatic lines/genotypes from the non-aromatic ones. However use of markers could not resolve the intensity of aroma in these genotypes which may be due to varied level of different physico-chemical factors (Buttery *et al.,* 1983; Yoshihashi *et al.,* 2002; Bradbury *et al.,* 2005b).

The study validates the practical utility of the PCR based assays which allows determination of the genotypic status of an individual rice accessions for a specific trait like aroma which will greatly help the breeders worldwide to analyze the breeding material at the early stage of the crop growth, preferably in the nursery itself. Moreover the 26 individuals identified to be strongly aromatic can be useful for introgression of their fragment allele in the back ground of high yielding cultivars for genetic enhancement and improvement of aromatic short grain rices there by providing higher economic gain to the farmers. The eleven genotypes which showed a heterozygous nature at molecular level and are still aromatic, will be useful towards understanding their genetic basis of aroma.

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