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Physico-chemical and Genetic Analysis of Aromatic Rice (*Oryza sativa* L.) Germplasm

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Abstract

For selection of aromatic rice lines, twenty six (26) rice genotypes were evaluated for agronomic characteristics and aroma detection through sensory test and genotypic analysis using SSR markers. Grain quality and yield attributes were analyzed after harvesting the grain. Aroma was detected by 1.7% KOH as a sensory test. Based on sensory test, six genotypes were detected for having strong aroma; 7 for moderate aroma; 10 for slight aroma and 3 for no aroma. Aroma had significant and positive association with grain length-width ratio; significant and negative association with grain width, significant and negative association with gelatinization temperature, and no significant association with grain length. Three SSR primers viz; RM223, RM515 and RM342 were used for identifying *fgr* gene locus in 26 rice genotypes. The primer RM223 identified the *fgr* locus as homozygous condition for 6 as strong aromatic, 7 moderate aromatic, 10 slight aromatic and the rest 3 as non aromatic. The primer RM515 detected 4 as strong aromatic, 6 as moderate aromatic, and 16 as slight to non aromatic. The primer RM342 detected 3 as strong aromatic and four as moderate aromatic, 19 as slight to non aromatic. Compared among the three markers, RM223 detected the highest number of *fgr* locus in aromatic rice genotypes. Among the three primers, RM223 responded best in all the 26 rice genotypes, because RM223 primer could be able to identify aromatic and non-aromatic genes having higher yield with good agronomic performance and other grain quality traits. These elite lines could be readily used in breeding programme for release aromatic rice variety with considerable yield.

Keywords: **Microsatellite markers, aromatic rice**

1. Introduction

Rice (*Oryza sativa* L.) is the staple food for more than two fifths (2.4 billion) of the world's population. Aromatic rice is preferred by consumers all over the world due to its flavor and palatability. Grain quality of rice plays an important role in consumer acceptability and it is the second after yield as the major breeding objectives. The quality of rice is considered from the view point of milling quality, grain size, shape, appearance and other cooking characteristics. Aroma development is influenced by both genetic and environmental factors (Juliano and Duff, 1991). It is known that aroma is best developed when aromatic rice is grown in areas where the weather is cooler during maturity (Dela Cruz *et al*., 1989). The scent aroma is due to presence of large number of compounds in endosperm in specific proportion. The biochemical basis of aroma was identified as 2-acetyl-1-pyrroline (Kadam and Patanker, 1938).

Molecular markers are important tools in the assessment of genetic variation and in the elucidation of genetic relationship within and among species. The concept of marker assisted selection (MAS) has provided an advantage for crop improvement over traditional methods based on phenotype. Molecular markers are being rapidly adopted for crop improvement research globally as an effective and appropriate tool for basic and applied research addressing biological components in agricultural production systems (Jones *et al*., 1997). Among PCR based marker microsatellites or SSR markers are excellent markers because of their locus identity, high polymorphism information content (PIC) value, multiallelism and more SSR markers are tandem repeats interspersed throughout the genome and can be amplified using primers that flank these regions. These markers are also termed simple sequence length polymorphism (SSLP) or sequence-tagged microsatellite site (STMS). These markers have been utilized for many purpose including genome mapping, gene tagging, estimation of genetic diversity, varietal differentiation and purity testing (McCouch *et al.*, 1997). Microsatellite marker analysis is also promising to identify major gene locus for grain quality traits that can be helpful for plant breeders to develop new cultivar and to use as a donor for future breeding programme. This experiment was conducted to: observe agronomic performance of 26 aromatic rice germplasm; evaluate physical and physico-chemical traits of aromatic rice genotypes; and measure the genetic variation among studied rice germplasm using microsattelite markers

2. Materials and Methods

2.1. Plant material

For selection of aromatic rice lines, twenty six rice genotypes were used to evaluate agronomic characteristics and aroma detection through sensory test and genotypic analysis using SSR markers. Grain quality and yield attribute data were obtained after harvesting the grains at the experimental field of Plant Breeding Division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. The seedlings of 26 rice germplasm were raised and 30 day old seedlings were transplanted in the field. Twenty cm distance was maintained between the rows and 15 cm between the plants. Recommended doses of fertilizers were used. Cultural practices were done as and when necessary.

2.2. Phenotypic characterization of rice germplasm

Five randomly selected plants of each genotype were used for agronomic characterization. Data were recorded for plant height, days to 50% flowering, days to maturity, number of filled grains plant^{-1}, number of effective tillers plant^{-1}, 1000 seed weight, grain yield plant^{-1} from 5 randomly selected plants of each genotype and were subjected to statistical analysis using MSTATC software. After harvesting, the seeds of each genotype were dehulled for evaluation of the grain length (grain size), grain shape (grain length-breadth ratio) and aroma. The grains were classified into different types based on their dimension according to shape (Dela Cruz and Khush, 1989). Twenty six freshly harvested milled rice grains from each of the genotypes were crushed or powdered. The powder was taken in conical flax. About 10 ml 1.7% KOH solution was added to each of the conical flax and the flaxes were covered immediately with aluminum foil and left at room temperature for about 1 h. The samples were scored on 1-4 scale with 1, 2, 3 and 4 corresponding to absence of aroma, slight aroma, moderate aroma and strong aroma, respectively. The score for each sample was recorded by a panel of five experts who have experience in aromatic rice breeding and quality evaluation.

Table 1. List of selected SSR markers used for grain quality traits

Primer name	Size range (bp)	Chrom locus		Sequence	Annealing Temp. (^{o}c)	Reference
RM223	139-163	8	Rev. Fwd.	GAAGGCAAGTCTTGGCACTG GAGTGAGCTTGGGCTGAAAC	55	Temnykhet al., 2000
RM342	n.a.	8	Rev. Fwd.	ACTATGCAGTGGTGTCACCC CCATCCTCCTACTTCAATGAAG	55	Temnykhet al., 2000
RM515	205-231	8	Rev. Fwd.	TGGCCTGCTCTCTCTCTCTC TAGGACGACCAAAGGGTGAG	55	Temnykhet al., 2001

n.a. indicate not available in Gramene DNA database website and Temnykh *et al.* 2000

2.3. Molecular marker analysis

DNA isolation from seed/plants was carried out using the mini preparation cetyltrimethyl ammonium bromide (CTAB) methods (IRRI, 1997). Three SSR markers such as RM223, RM342 and RM515 were used to confirm the presence of *fgr* gene as described by Garland *et al.* (2000) and Begum (2006). The details of the primers are given in Table 1. The Polymerase chain reaction (PCR) mixture contained 2 µl of 50 ng/ µl template DNA, 8.25 µl double distilled water (ddH_2O), 1.5 µl of 10 x buffer, 0.75 µl of 1 mM dNTPs, 1 µl of primer forward and 1 µl of reverse primer and 0.5 µl of *Taq* DNA polymerase. The template DNA was initially denatured at 94^0C for 5 min followed by 30 cycles of PCR amplification following: 30 s of denaturation at 94^0C, 30 s of primer annealing at 55^0C and 1 min of primer extension at 72^0C. Finally, 5 min incubation at 72^0C was allowed for completion of primer extension. The amplified products were electrophoretically resolved on a 1.5% agarose gel in 0.5 x TBE and visualized under UV light after staining with ethidium bromide. The bands representing particular alleles at the microsatellite loci were scored manually on the basis of parental bands.

3. Result and Discussion

3.1. Phenotypic evaluation of aromatic rice genotype

Regarding plant height, IR 50 had the minimum and Basmoti370 had the maximum height and ranged from 71.0 to 121.6 cm and the mean value for this trait was 93.00 cm. Regarding days to 50% flowering, the period ranged from 90 days to 145 days. PSB RC18 (IR51672-62-2-1-1-2-3) was found to be required more days whereas Basmati 370 took fewer days and the mean value was 127.07. The period of maturity of 26 germplasm ranged from 127 to 167 days. Basmati 370 was found to be the earliest maturing, whereas, YN96-5021 took maximum time to mature and the mean value for this trait was 155 days. Number of filled grains per plant ranged from 750 to 1217. IR73887-1-8-1-4 had the highest number of filled grains per plant, whereas IR72869-52-1-1-1 had the minimum number of grains per plant. Number of effective tillers per plant ranged from 9 to 20 and significant variation was observed among the genotypes. IR71144-393-2-2-3-1 had the maximum number of effective tillers and Binadhan7 had the lowest.

Table 2. Different grain quality traits of the 26 rice genotypes

Genotypes	Phenotypic data				
	Yield/ Plant (g)	Aroma	Grain Length (mm)	Grain wide (mm)	Length-Wide Ratio(L/W) (Grain shape)
IR72860-98-3-2-1	23.3	4	6.9	2.0	3.4
PARASSANA	28.4	3	6.8	2.0	3.4
IR72860-74-1-2-1	26.8	2	7.2	1.9	3.7
IR77743-39-3-2-5	24.5	3	8.0	2.0	4.0
IR77512-111-2-1-2	25.3	2	7.6	1.9	4.0
IR77512-128-2-1-2	21.1	4	7.0	2.0	3.5
CNTLR85033-93-1-1	21.5	4	7.3	2.0	3.6
DIANSHAO1	21.0	2	7.7	2.1	3.6
IR77512-89-3-2-3	24.4	1	7.4	2.2	3.3
IR71144-393-2-2-3-1	25.2	3	6.4	2.0	3.2
OMFI-1	26.1	1	6.6	2.2	3.0
IR69710-7-2-1-2-2	20.4	2	5.9	2.5	2.3
TOX1889-22-103-1	21.0	2	6.9	2.0	3.4
IR73887-1-8-1-4	30.9	3	6.8	2.0	3.4
IR69726-95-3-2-2-3	21.3	4	7.6	1.6	4.7
IR73719-23-3-3-1	29.2	2	7.3	2.1	3.4
TOX3440-171-1-1-1-1(WITA 7)	28.0	2	7.0	2.0	3.5
YN96-5021	22.2	4	7.1	1.9	3.7
IR72869-52-1-1-1	21.4	3	6.4	2.0	3.2
IR50	28.3	2	6.4	1.9	3.3
IR 72	23.6	2	6.9	2.2	3.1
PSB RC2(IR32809-26-3-3)	26.2	3	7.0	2.1	3.3
PSB RC18(IR51672-62-2-1-1-2-3)	25.3	3	7.1	2.1	3.4
IR59552-21-3-2-2(PSB RC64)	22.9	2	7.0	1.9	3.6
BINA dhan 7	31.7	1	6.9	2.4	2..9
Basmati370	17.6	4	7.4	1.8	4.1
Mean	23.75	2.615	7.023	2..03	3.461
Range	20.4-31.7	1-4	5.9-8.0	1.6-2.5	2.3-47
SD	4.41	0.963	0.452	0.1749	0.436

The result showed that maximum 21 genotypes had grain length between 6.65-7.75 mm and 5 genotypes had a grain length more than 7.5 mm (Table 2). Twenty four rice genotypes had higher grain length-width ratio over 3.0 mm and only two rice genotypes had grain length-width ratio below 3.0 mm. The result also showed that most of the germplasms were found to give strong to moderate type aroma. Only ten genotypes had slight aroma and three had no aroma. With respect to alkali spreading value, ten genotypes having a score of 1 to 2. In general, long grains are preferred in the Indian subcontinent, but in Southeast Asia, the demand is for medium long rice. Tomar and Nanda (1985) observed that slender kernel was dominant over the medium and bold, whereas medium kernel was dominant over bold. Sharma (2002**)** noted that the aromatic cultivars possessed a slender shape compared with the medium-slender shape of non-aromatic cultivars.

3.2. Trait correlation

The correlation between traits was estimated by regressing the phenotypic values of one those of another trait. Pair-wise correlations are presented in Table 3. In this study, aroma had significant and positive association with grain length width ratio; significant and negative association with grain width and gelatinization temperature and no significant relation with grain length. Gelatinization temperature had non-significant and negative correlation with grain length, significant and negative association with grain length width ratio, significant and positive association grain width. Grain length had significant and negative correlation with grain width; significant and positive correlation with length width ratio. Grain width had significant and negative correlation with length width ratio. Begum (2006) supported this finding. Chauhan (1998) also found similar result. He estimated the gene effects for grain weight, grain length, breath and shape (L/B ratio) in rainfed rice. He observed that dominance gene effects (h) and dominance × dominance (i) interaction were important for grain weight and grain shape. In contrast, Tomar and Nanda (1985) did not find any association between kernel size and shape. It had significant negative association between grain width and grain length width ratio. It had significant positive correlation between grain length and grain width. Begum (2006) found highly significant and negative correlation.

3.3. Identification of fragrance (fgr) gene

Markers such as RM223, RM342, RM515 linked to aroma gene *(fgr)* were found to be highly polymorphic between the parents in this study (Fig. 1). Primer RM223 conformed the primer RM223 identified the *fgr* locus as homozygous condition for 6 as strong aromatic, 7 moderate aromatic, 10 slight aromatic and the rest 3 as non aromatic. In case of the primer RM515 detected 4 as strong aromatic, 6 as moderate aromatic, and 16 as slight to non aromatic. The primer RM342 detected 3 as strong aromatic, 4 as moderate aromatic, 19 were slight to non aromatic.

Table 3. Phenotypic correlations among aroma, gelatinization temperature, grain length, grain width and grain length width ratio of the twenty six rice germplasms

Traits	Gelatinization temperature (ASS)	Grain length (mm)	Grain width (mm)	Grain length width ratio
Aroma	-0.613 **	0.161 ^{NS}	-0.569* *	0.487*
Gelatinization temperature (ASS)		-0.231 ^{NS}	0.515 * *	-0.450 *
Grain length			-0.422*	0.776**
Grain width				-0.877 **

**=1% level of significant,*= 5% level of significant, N S = Not significant

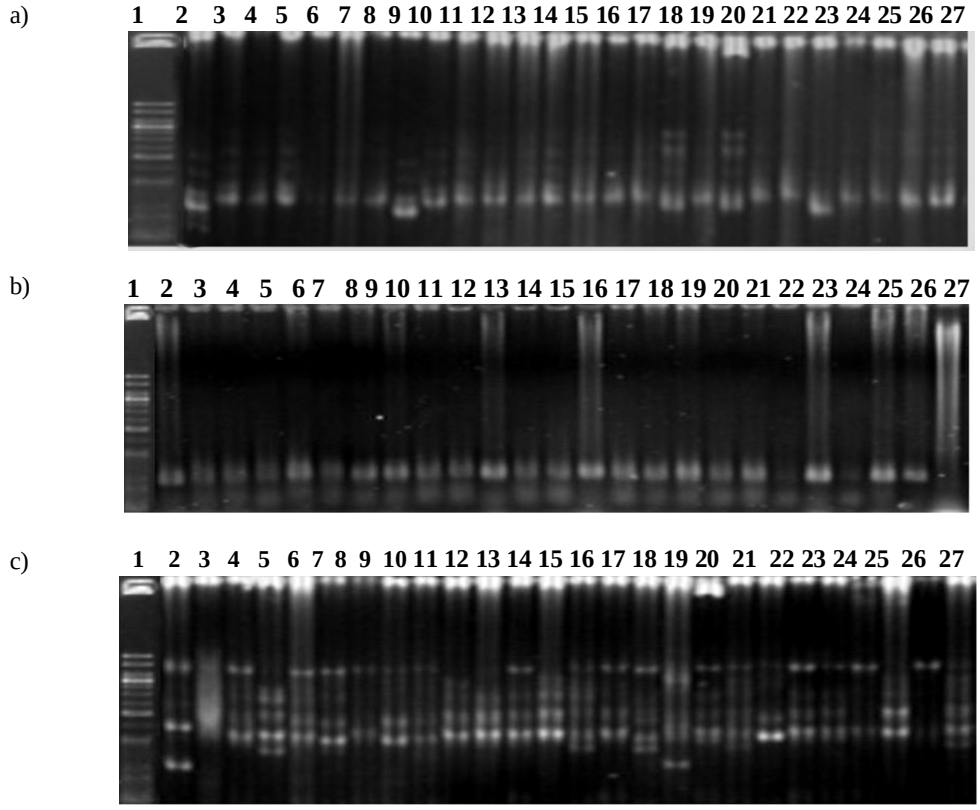

Fig.1. Banding pattern of some of the rice genotype for a) RM223; single band like lane 2 non aromatic; single band like lane aromatic and double band indicated heterozygous allele, b) RM515 same as "a" where where Lane-1: 20bp ladder; Lane-2: Basmoti370, Lane-3: BINA dhan 7, Lane-4: IR72860-98-3-2-1, Lane PARASSANA, Lane-6:IR72860-74-1-2-1, Lane-7: IR77743-39-3-2-5, Lane-8: IR77512-111-2-1-2, Lane 9:IR77512-128-2-1-2, Lane-10: CNTLR85033-93-1-1, Lane-11: DIANSHAO1, Lane-12: IR77512-89-3-2- 3, Lane-13IR71144- 393-2-2-3-1, Lane-14: OMFI-1, Lane-15: IR69710-7-2-1-2-2, Lane-16:TOX1889-22- 103-1, Lane-17: IR73887-1-8-1-4, Lane-18: IR69726-95-3-2-2-3, Lane-19: IR73719-23-3-3-1 Lane-20: TOX3440-171-1-1-1-1(WITA 7),Lane-21: YN96-5021, Lane-22: IR72869-52-1-1-1, Lane-23: IR50, Lane- 24: IR 72, Lane-25: PSB RC2(IR32809-26-3-3), Lane-26: PSB RC18(IR51672-62-2-1-1-2-3), Lane-27: IR59552-21-3-2-2(PSB RC64)26 rice germplasm using RM342, c) RM342; triple band like lane 2 aromatic; double band like lane 4 non aromatic and single band indicated different allele of aroma gene

In a previous study Begum (2006) reported that three markers RM223, RM342 and RM515 located on chromosome 8, were found to be strongly associated (P<0.0001) with aroma and explained 22.46, 28.38 and 41.78 of the total phenotypic variations. The RM223, RM342 and RM515 showed a high degree of polymorphism between Basmati and non-Basmati type of aromatic rice (Jain *et al*. 2004). Compared among the three markers, RM223 detected the highest number of *fgr* locus in aromatic rice genotypes. After phenotypic and genotypic observation, it was found that four genotypes (Basmati 370, CNTLR85033-93-1-1, IR69726-95-3-2-2-3 and IR77512-128-2-1-2) having *fgr* gene, that indicate strong aroma. Three genotypes (IR72860-74-1-2-1, YN96-5021 and IR77512-111-2-1-2) were identified moderate aroma with *fgr* gene in accordance to primer RM223 and RM515. The Primer RM342 identified Basmati 370, IR69726-95-3-2-2-3 and CNTLR85033-93-1-1 with strong aroma but BINA dhan 7, IR77512-111-2-1-2, CNTLR85033-93-1-1, TOX3440-171-1-1-1-1(WITA7) with moderate aroma while PSB RC18(IR51672-62-2-1-1-2-3) was found with slight to no aroma. The banding pattern for the primer RM342 were different in rest of the germplasms. The different banding patterns were found due to polymorphism.

4. Conclusions

Finally, it can be concluded that among the three primers, RM223 responded best in all the 26 rice genotypes, because RM223 primer could be able to identify aromatic and non-aromatic germplasm effectively which supported the phenotypic results. It can also be said that among the three primers, RM223 responded best in all the 26 rice lines which could be readily used in breeding programme for releasing aromatic rice variety with considerable yield.

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