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SCREENING OF AROMATIC RICE LINES BY PHENOTYPIC AND MOLECULAR MARKERS

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Abstract

To improve the yield potential of local aromatic variety Kalizira, a segregating population was developed from a cross between Y-1281 (high yielding mutant variety) and Kalizira. Thirty two F_7 rice lines were used to evaluate agronomic characteristics, aroma detection through sensory test and genotypic analysis using microsatellite markers. Highly significant negative association was found between aroma and grain yield. Nine, 12 and 17 pedigree lines (PLs) having fragrance gene (*fgr*) locus were found using three SSR markers RM223, RM342A and RM515, respectively as homozygous condition in 32 rice lines. The marker RM515 detected highest number of *fgr* locus in PLs. Fourteen promising lines were identified with aroma genes having higher yield with good agronomic performance and other grain quality traits. These SSR markers could be utilized in marker-assisted selection (MAS) and would have a great impact on identifying *fgr* locus in rice genotypes.

Introduction

Rice (*Oryza sativa* L.) is the staple food of more than half of the world's population. Most of the world's rice is produced and consumed in Asia which constitutes more than half of the global population (Chakravarthi and Naravaneni 2006). In Bangladesh rice occupies about 70% of the total cropped area of about 13.9 million hectares. Out of this 70%, fine rice is cultivated in roughly 10% land. Grain quality in rice plays an important role in consumer acceptability. Juliano and Duff (1991) concluded that grain quality is second after yield as the major breeding objective for crop improvement. The quality in rice is considered based on milling quality, grain size, shape, appearance, aroma and other cooking characteristics (Dela Cruz and Khush 2000). Most of the scented rice varieties in Bangladesh are of traditional type, photoperiod sensitive, and cultivated during the Aman season. Majority of these indigenous aromatic rice cultivars are low yielding but its higher price and low cost of cultivation generate higher profit margins compared to other varieties. Aroma development in rice grain is influenced by both genetic and environmental factors. The biochemical basis of aroma was identified as 2-acetyl-1-pyrroline (Tanchotikul and Hsieh 1991). Most of the rice varieties have been developed traditionally by selection, hybridization and back crossing with locally adapted high-yielding lines. The conventional methods of plant selection for aroma are not easy because of the large effects of the environment and the low narrow sense heritability of aroma. More recently molecular markers, such as SNPs and simple sequence repeats (SSRs), which are genetically linked to fragrance and have the advantage of being inexpensive, simple, rapid and only requiring small amounts of tissue, have been developed for the selection of fragrant rice (Cordeiro *et al*. 2002). Moreover, an allele specific amplification (ASA) assay allows discrimination between fragrant and non-fragrant rice varieties and identifies homozygous fragrant, homozygous non-fragrant and heterozygous non-fragrant individuals in a population segregating for fragrance (Louis *et al*. 2005). SSR or microsatellite markers behave as a co-dominant marker which was used for this study to select rice lines having aroma with fine grain and good seed yield.

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Materials and Methods

Plant materials: A segregating population was developed by crossing Y-1281 (a yigh yielding variety) with Kalizira (a local aromatic variety) for developing superior quality of aromatic rice lines at the Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Thirty two F_7 pedigree lines along with their parents and a check variety, BRRIdhan 38 were used for phenotypic and genotypic study.

Phenotyping of rice germplasm: Five randomly selected plants of each genotype were used for agronomic data analysis. Data on plant height (cm), number of effective tillers/plant, panicle length (cm), number of filled grains/panicle, 1000 seed weight (g), days to maturity and grain yield/plant (g) were recorded and subjected to statistical analyses using MSTATC software. After harvesting the seeds of each genotype were dehulled for evaluation of the grain quality *viz.* grain size (grain length), grain shape (grain length-breadth ratio) and aroma. The grains were classified into different types based on their dimension according to Dela Cruz and Khush (1989). Forty grains of each genotype were soaked in 10 ml 1.7% KOH solution at room temperature in a covered conical flask 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.

Molecular marker analysis: DNA isolation was carried out using the mini preparation CTAB method (IRRI 1997). Three SSR markers RM223, RM342A and RM515 (linked to aroma) 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 PCR reaction mixture contained 2µl of 50 ng/µl template DNA, 8.25 µl ddH_2O, 1.5 µl 10X PCR buffer, 0.75 µl of 1 mM dNTPs, 1 µl of 5 µM forward and reverse primers and 0.5 µl Taq Polymerase(~ 2.5 units/µl). Template DNA was initially denatured at 94^{o}C for 5 min followed by 30 cycles of PCR amplification following: 30 sec of denaturation at 94^{o}C, 30 sec of primer annealing at 55^{o}C and 1 min of primer extension at 72^{o}C. Final 5 min incubation at 72^{o}C was allowed for complete of primer extension. The amplified products were electrophoretically resolved on a 1.5% agarose gel in 0.5xTBE 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 like aromatic type band (Kalizira), non-aromatic type (Y-1281) and heterozygotes type band (both).

Table 1. SSR markers for analysis of fragrance in rice.

Primer name	Size range (bp)	Chrom. locus		Sequence	Annealing temp. (^{o}C)	Reference
RM223	139-163	8	Rev. Fwd.	GAAGGCAAGTCTTGGCACTG GAGTGAGCTTGGGCTGAAAC	55	Temnykh *et al*. 2000
RM342A	n.a.	8	Rev. Fwd.	ACTATGCAGTGGTGTCACCC CCATCCTCCTACTTCAATGAAG	55	Temnykh *et al*. 2000
RM515	205-231	8	Rev. Fwd.	TGGCCTGCTCTCTCTCTCTC TAGGACGACCAAAGGGTGAG	55	Temnykh *et al*. 2001

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

Results and Discussion

Phenotypic evaluation of aromatic rice genotypes: The results on agronomic performance (Table 2) showed that the majority of the genotypes were superior to the local Kalizira variety. The plant height of the aromatic rice genotypes ranged from 96.2 cm (Y-1281) to 154.2 cm (PL7). In comparison to Kalizira, there were considerable reduction in plant height in most of the rice

genotypes. The effective tillers/plant varied from 5.4 (PL32) to 15.6 (PL22). Eleven genotypes showed higher effective tiller number than that of Kalizira. Nine genotypes had longer panicles than both the parents. The number of filled grains/panicle was highest in PL26 (229.2) and lowest in PL17 (47.0). Twenty five genotypes had higher number of grains/panicle than the Kalizira parent. The 1000-grain weight was highest in PL11 (22.40 g) and lowest in PL23 (10.96 g). Days to maturity of the aromatic rice genotypes ranged from 108 (PL28, PL29) to 133 (Kalizira). Twelve genotypes showed higher grains/plant than both the parents.

Table 2. Agronomic, quality characteristics and genotypic analysis of 32 F_7 rice lines along with parents and check.

Lines	Agronomic and quality traits				Genotypic analysis [2]		
	Grain yield/plant (g)	Grain length (Grain size) mm	Grain length-width ratio (Grain shape) mm	Aroma[1]	RM223	RM342A	RM515
PL1	19.02	6.95	3.30	3	++	--	++
PL2	13.63	7.60	3.80	1	--	--	+-
PL3	13.91	6.85	3.04	3	++	++	+-
PL4	20.06	6.85	2.97	3	+-	--	+-
PL5	13.37	7.25	3.37	4	+-	--	++
PL6	11.32	7.25	3.29	4	++	++	++
PL7	11.64	7.10	3.38	3	--	--	++
PL8	23.27	7.00	3.25	3	--	--	+-
PL9	21.03	7.15	3.17	3	++	--	+-
PL10	9.98	6.75	3.14	3	+-	++	+-
PL11	17.49	6.75	2.81	4	+-	++	+-
PL12	12.08	7.55	3.59	2	--	+-	++
PL13	10.67	7.10	4.05	2	+-	+-	++
PL14	22.85	6.95	3.74	1	+-	+-	++
PL15	23.58	7.75	3.69	1	--	+-	++
PL16	9.31	7.20	3.78	3	++	+-	++
PL17	7.52	7.00	3.41	3	--	+-	--
PL18	6.52	7.40	3.61	3	--	++	--
PL19	17.63	7.20	3.42	4	--	++	++
PL20	11.50	7.00	3.11	3	+-	++	++
PL21	14.25	7.30	3.65	3	++	+-	++
PL22	24.08	7.35	3.41	3	++	--	++
PL23	11.40	6.70	3.35	3	--	+-	--
PL24	11.18	7.00	3.88	1	+-	+-	--
PL25	19.47	7.30	3.31	1	+-	--	++
PL26	32.61	7.55	3.35	1	+-	++	--
PL27	14.82	7.70	3.66	2	--	+-	--
PL28	12.00	7.00	3.68	1	--	+-	+-
PL29	11.36	7.00	3.68	1	+-	++	--
PL30	20.13	7.15	4.08	1	--	++	++
PL31	12.40	7.00	3.78	1	++	++	++
PL32	8.73	7.00	3.33	3	++	++	++
Y-1281(P)	15.36	7.75	3.69	1	--	--	--
Kalizira(P)	9.30	4.25	1.73	4	++	++	++
BRRIdhan-38	12.81	6.80	3.40	2	++	++	++
Range	6.21-32.61	4.25-7.75	1.73-4.08	1-4			
Mean	15.03	7.071	3.425	2.4			

[1]1 = None; 2 = Slight; 3 = Moderate and 4 = Strong. [2]++ = Present of aroma gene; -- = Absent of aroma gene and +- = Heterozygous condition.

Thirty two advanced lines along with their parents and check variety were subjected to quality analysis. Six lines including parent Y-1281 had a desirable rice gain length of more than 7.5 mm. Twenty eight lines were considered as long grain (6.61-7.50 mm). Kalizira had short grains of less than 5.50 mm. Rice with kernel length of 7 mm or more and breadth of less than 2 mm are highly remunerative in international trade. Seven genotypes with these characteristics were observed in this study. The L/B ratio was higher than 3 (slender type) except in three genotypes (PL4, PL11 and Kalizira). Sharma (2002) mentioned that the aromatic cultivars possessed a slender shape compared with the medium-slender shape of non-aromatic cultivars. Most of the studied genotypes were found to give moderate and strong type aroma. Variation was observed among all the genotypes for different quality traits (Table 2).

Trait correlation: The correlation between traits was estimated by regressing phenotypic values of one trait on those of another trait. Pair-wise trait correlations are presented in Table 3. The significant positively correlated traits ($p < 0.001$) included PH × PL (0.370), PH × DM (0.348), PH × Aroma (0.476), PL × 1000SW (0.384), FG × GY (0.612), 1000SW × GY (0.372) and DM × Aroma (0.295). A negative significant correlation was observed in FG × Aroma (-0.401) and GY × Aroma (– 0.256). Correlation studies between aroma and grain yield revealed that aroma is negatively correlated with grain yield. However, the usual yield of fine aromatic rice is low compared to high yielding varieties (Sarker 2002).

Table 3. Phenotypic correlations among yield related traits in Kalizira/Y-1281 derived F_7 population.

Traits	Panicle length (PL)	Effective tillers (ET)	Filled grains (FG)	1000-seed weight (1000-SW)	Days to maturity (DM)	Grain yield (GY)	Aroma
Plant height (PH)	0.370^{***}	-0.174^{*}	-0.053^{ns}	0.036^{ns}	0.348^{***}	-0.204^{**}	0.476^{***}
Panicle length (PL)		0.017^{ns}	0.118^{ns}	0.384^{***}	0.213^{**}	0.151^{*}	0.167^{*}
Effective tillers (ET)			-0.053^{ns}	0.034^{ns}	0.007^{ns}	0.173^{*}	0.118^{ns}
Filled grains (FG)				0.233^{**}	-0.063^{ns}	0.612^{***}	-0.401^{***}
1000-seed weight (1000-SW)					0.070^{ns}	0.372^{***}	0.020^{ns}
Days to maturity (DM)						-0.156^{*}	0.295^{***}
Grain yield (GY)							-0.256^{***}

ns indicates not significant, *, **, *** significant at < 0.05, 0.01 and 0.001, respectively.

Identification of fragrance (fgr) gene: Markers (RM223, RM342A and RM515) linked to aroma gene (*fgr*) selected on the basis of previous studies (Garland *et al*. 2000, Begum 2006) were found to be highly polymorphic between the parents in this study. Primer RM223 confirmed the presence of fragrance gene in 9 pedigree lines as parent Kalizira (aromatic type) at homozygous condition and 12 genotypes were identified as parent Y-1281 (non-aromatic type) as homozygous condition. Rest of the genotypes showed heterozygous condition (Table 2).

In case of RM342A, 12 lines gave aromatic banding pattern similar to Kalizira and the check variety, BRRIdhan-38 (Table 2). On the other hand, 9 lines had similar allele as non-aromatic parent Y-1281. Other genotypes showed heterozygous alleles. Eighteen lines showed aromatic alleles as Kalizira and check BRRIdhan-38 with RM515. Seven lines were found as non-aromatic allelic band like the parent Y-1281 and the rest of lines had heterozygous alleles (both Kalizira and Y-1281) (Fig. 1).

In a previous study Begum (2006) reported that three markers RM223, RM342A and RM515 were located on chromosome 8, found to be strongly associated ($p < 0.0001$) with aroma and explained 22.46, 28.38 and 41.78% of the total phenotypic variation. The SSRs, RM342, RM42, and RM223 showed a high degree of polymorphism between Basmati and non-Basmati type

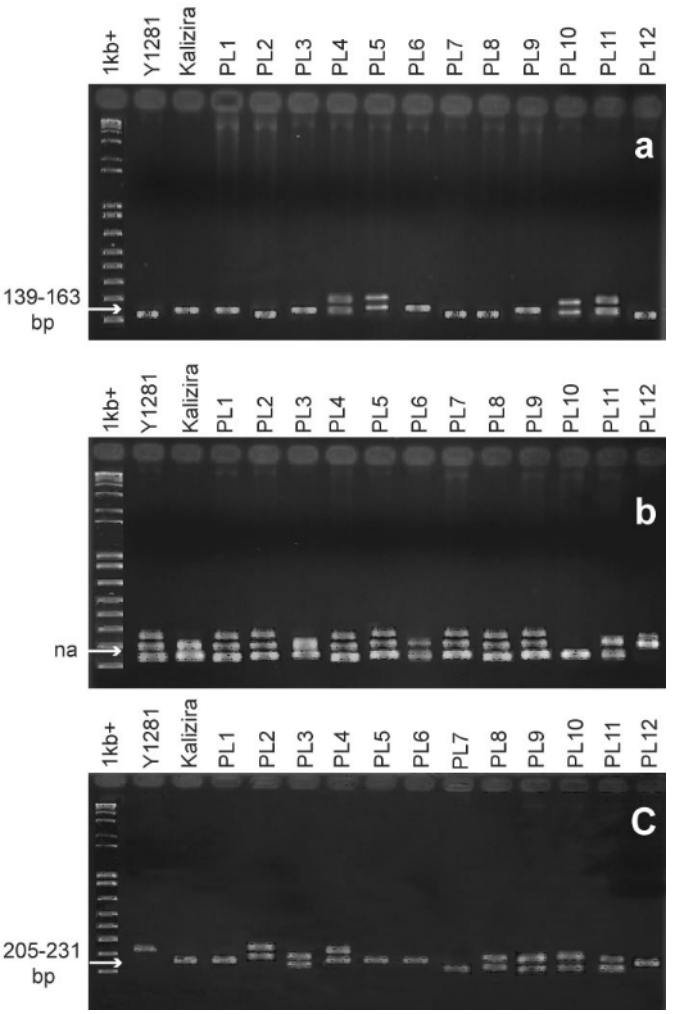

Fig.1. Banding pattern of some of the rice genotypes for a) RM223: single band like lane 2 non-aromatic (Y-1281); single band like lane 3 aromatic (Kalizira) and double band indicated heterozygous allele b) RM342A: triple band like lane 2 non-aromatic (Y-1281); double band like lane 3 aromatic (Kalizira) and single band indicated different allele of aroma gene than Kalizira c) RM515: same as "a", where Lane-1: 1 kb^+ ladder; Lane-2: Y-1281; Lane-3: Kalizira; Lane-4: PL1; Lane-5: PL2; Lane-6: PL3; Lane-7: PL4; Lane-8: PL5; Lane-9: PL6; Lane-10: PL7; Lane-11: PL8; Lane-12: PL9; Lane-13: PL10; Lane-14: PL11; Lane-15: PL12.

aromatic rice (Jain *et al*. 2004). Among 3 markers, RM223 could detect 9 pedigree lines having aroma while RM342A and RM515 identified 12 and 17 pedigree lines, respectively consisting aroma. RM223 and RM515 identified maximum lines (7 lines) having *fgr* locus that is responsible for aroma with RM515 detecting the highest number of lines. Bradbury *et al.* (2005) stated that

RM515 which is mapped at the same position as RM223 explained more variation for aroma than even RM223. Obviously the present results are in agreement with these findings. Considering phenotypic and genotypic observations, four lines, PL5, PL6, PL11 and PL19 having *fgr* gene also have strong aroma, higher grain yield, very long and slender grain. Eleven lines i.e. PL1, PL3, PL7, PL9, PL10, PL16, PL18, PL20, PL21, PL22 and PL32 were found to have moderate aroma, having *fgr* gene locus, different grain yield, and very long to slender grain. Rest of lines had slight to no aroma and absence of fragrance gene allele. PL6 and PL32 were identified as aromatic rice lines by three markers as well as phenotypic evaluation. On the other hand, PL31 was identified as aromatic rice line by using three markers but phenotypically aroma was not detected, although this line have considerable grain length, length-breadth ratio and yield. This might have happened due to error in scoring phenotype that cannot be avoided with the effect of environment, especially aroma (Lang and Buu, 2002). Based on the study, 14 lines (PL1, PL3, PL5, PL6, PL9, PL10, PL11, PL16, PL19, PL20, PL21, PL22, PL31 and PL32) having good aroma and superior agronomic performance have been selected in F_7 generation. The result having aroma in selected rice lines were confirmed by both phenotypic and genotypic analysis. These selected pedigree lines could be used as donors for future breeding programmes that aim to develop aromatic rice varieties.

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