MOLECULAR MARKER BASED GENETIC DIVERSITY ANALYSIS IN AROMATIC RICE GENOTYPES USING SSR AND RAPD MARKERS

K. KIBRIA, F. NUR, S. N. BEGUM[1], M. M. ISLAM[1], S. K. PAUL, K. S. RAHMAN, AND S. M. M. AZAM

Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, [1]Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh

Accepted for publication: December 03, 2008

ABSTRACT

Kibria K., Nur F., Begum S. N., Islam M. M., Paul S. K., Rahman K. S., and Azam S. M. M. 2009. *Molecular Marker based Genetic Diversity Analysis in Aromatic Rice Genotypes Using SSR and RAPD Markers.* Int. J. Sustain. Crop Prod. 4(1):23-34

The study was undertaken to assess the genetic diversity among aromatic rice genotypes using simple sequence repeat (SSR) and randomly amplified polymorphic DNA (RAPD) markers through marker aided selection (MAS). The research work was performed during July 2006 to April 2008 at Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. Three SSR primers (RM223, RM342A and RM515) exhibited fourty six bands among the genotypes, while the average number of effective allele ranged from 1.78 to 2.49. The marker RM223 showed the highest polymorphism (66.67%). Out of 15 tested RAPD primers, 3 markers (OPA-02, OPA-10 and 67AB10G7) produced 34 distinct bands of which 32 were polymorphic. OPA02 and 67AB10G7 primers performed 100% polymorphism. Based on Nei's genetic distance using the unweighted pair-group method with arithmetic means (UPGMA) dendrogram the SSR primers showed the highest genetic distance which was 2.306 and in case of RAPD marker it was 0.7634. Genotype pair (43-28-5-3-1 x 10-14-5-1-1) and (43-28-5-3-1 x 43-28-5-2-1) were clustered in a separate cluster as they have diverse genetic background. Considering the genetic distance values the genotypes was genetically different from each other which could be used in breeding programme to have potential genetic gains. The results of the genetic diversity will be useful for the selection of the parents for developing rice breeding variety.

Key words: Molecular markers, genetic diversity, aromatic rice

INTRODUCTION

Rice (*Oryza sativa* L.) belonging to the family *Graminae* is the staple food for one third of the world's population (Chakravarthi and Naraveni, 2006). The aromatic rice is preferred over non-aromatic rice during special occasions and for export, and thus they command a higher market price. Most of the scented rice varieties in Bangladesh are low yielding but its higher price and low cost of cultivation generate higher profit margins compared to the other rice varieties grown in the locality. 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. Molecular markers based on DNA sequence are found to be more reliable.

Molecular marker technology provides information that can help to define the distinctiveness of germplasm and their ranking according to the number of close relatives and their phylogenetic position. DNA marker is a new approach based on DNA polymorphism among tested genotypes, and thus applicable to biological research. Several molecular markers viz. RFLP, RAPD (Tingey and Deltufo, 1993), SSRs (McCouch *et al*., 1997), AFLP and SNPs are presently available to assess the variability and diversity at molecular level (Joshi *et al.,* 2000). Simple sequence repeat (SSR) markers or microsatellites are tandem repeats interspersed throughout the genome and can be amplified using primers that flank these regions (Giovannoni *et al*. 1991). More recently molecular markers, such as SNPs and SSRs, which are genetically linked to fragrance and to identify the nature of the locus (homozygous or heterozygous condition), and have the advantage of being inexpensive, simple, rapid and only requiring small amount of tissue, may also be useful for the rapid incorporation of the scent character into breeding lines (Cordeiro *et al.* 2002). On the other hand random amplified polymorphic DNA (RAPD) is the widely used molecular marker where DNA fragments are amplified by the Polymerase Chain Reaction (PCR) using short (usually 10 bases in length) synthetic primers of random sequence. RAPD markers tend to estimate intra or inter genetic distances more distantly related individuals. Inspite of many weaknesses, it is relatively easy, speedy, high degree of polymorphisms as well as virtually inexhaustible pool of possible genetic markers make the technique advantageous over other molecular techniques (Fristsch and Rieseberg, 1995). With the view, the present study was made to evaluate the genetic analysis and relatedness of 12 aromatic rice genotypes using SSR and RAPD markers.

MATERIALS AND METHODS

Plant materials

Twelve lines were developed in the background of Y-1281 (high variety) and Kalizira (aromatic) at Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Local aromatic rice variety

Kalizira was used as a donor for aroma. Y-1281 is an improved yigh yielding non-aromatic variety. Twelve genotypes along with their parents were evaluated for genotypic study during July 2006 to April 2008.

Single Sequence Repeat (SSR) analysis

Mini preparation CTAB method was followed to extract DNA from leaf sample. Three SSR markers RM223, RM342A and RM515 (linked to aroma) were used to confirm the presence of *fgr* gene. The PCR reaction was performed in 13 µl reaction volume containing 1.5 µl 10X PCR buffer, 8.25 µl ddH_2O, 0.75 µl dNTPs, 1 µl each of forward and reverse primer, 0.5 µl Taq Polymerase and 2µl template DNA. Profile was used as follows: an initial denaturing step at 94^{o}C for 5 min followed by 30 cycles at 94^{o}C for 30 seconds, appropriate annealing temperature at 55^{o}C for 30 sec and primer elongation at 72^{o}C for 1 min. Final 5 min incubation at 72^{o}C was allowed for complete of primer extension.

Random Amplified polymorphic DNA (RAPD) analysis

The PCR reactions mixture contained 2 µl of total genomic DNA, 2.5 µl primer, 1.0 µl dNTPs, 1.0 µl Ampli *Taq* polymerase buffer, 3.3 µl ddH_2O and 0.2 µl Ampli *Taq* DNA polymerase. Out of fifteen (OPA-01, OPA-02**,** OPA-10, OPA-11, OPA-12, OPB-01, OPB-02, OPB-06, OPB-07, OPB-18, OPC-01, OPC-02, 66AB10G6, 67AB10G7 and 69AB10G9), 3 oligonucleotide random primers (OPA-02, OPA-10, 67AB10G7) were selected for analysis. Amplification was performed in a thermal cycler with the following profile: 94^{0}C for 3 min (initial denaturation), 94°C for 1 min, annealing at 36°C for 1 min, extension at 72^{o}C for 2 min for 40 cycles with a final extension at 72°C for 7 min.

Electrophoresis

The SSR and RAPD-PCR products were analyzed directly on 1.5% agarose gels in TBE buffer, visualized by staining with ethidium bromide and transillumination under short-wave UV light.

Data analysis

Only clear and unambiguous SSR and RAPD markers were scored to the presence and absence of the corresponding band among the genotypes. The scores '1' and '0' indicated the presence and absence of bands, respectively. Both markers data subjected to cluster analysis using the software POPGENE (version 1.31). The unweighted pair-group method with arithmetic means (UPGMA) dendrogram was drawn by using the software TREEVIEW. Nei's (1972) genetic distance value was computed using the formula as described in the POPGENE (version 1.31) software user manual.

RESULTS AND DISCUSSION

SSR analysis

Three primers (RM223, RM342A, and RM515) linked to aroma gene (*FGR* gene) were found to be highly polymorphic between the parents in this study. Fourty six bands were scored from three SSR markers among the genotypes (Figure 1a-c). The number of alleles ranged from one to two per locus, while the average number of effective allele ranged from 1.78 to 2.49 (Table 1). Thanh *et al*. (1999) detected 41 alleles with an average of 2.9 alleles per locus using 14 primers pair with 13 upland rice lines from Vietnam. Saker *et al.,* (2005) observed that the six SSR primer sets which were distributed through 6 different rice chromosomes revealed 25 alleles, eight alleles with RM10 primer, six with T92, five alleles with RM13 primer, four alleles with RM8 and two with RM3 and RM14 primer sets.

The genotypes 37-4-1-2, 41-12-3-1-2 and 37-1-3-1 showed the highest polymorphism. The highest polymorphism was 66.67%) and the lowest was 33.33% using RM-223 and RM-342A, respectively. According to Nei's (1972) the highest level of gene diversity value was 0.99 (RM-223) and the lowest level of gene diversity value was 0.75 (RM-515); average gene flow for all microsatellite loci in 14 rice genotypes is 0.0717 (Table 1). The values of pair-wise comparisons of Nei's (1972) genetic distance (D) between genotypes were computed from combined data for the 3 primers, ranged from 0.0000 to 2.3026 (Table 2). Comparatively higher genetic distance was observed between 43-28-5-3-1 vs 10-14-5-1-3 genotypes pairs than the other combinations. Nagaraju *et al*. (2002) stated that the lowest genetic distance was among the traditional Basmati varieties, whereas average genetic distance was 0.675 for the *indica* and 0.484 for *japonica*.

Twelve genotypes with their parents were used to make dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA). In this study, 14 aromatic rice genotypes have been differentiated into two main clusters; Y-1281, 40-1-2-1, 48-8-1-2-6, 37-4-1-2, 43-28-5-3-1, 37-1-3-1 and 48-1-2-3 in cluster 1 and Kalizira, 43-28-5-3-1, 43-28-5-2-2, 10-14-5-1-1, 41-12-3-1-2, 33-144-1-1, 10-14-5-1-3 formed cluster 2 (Figure 2). Cluster 1 was grouped in to two sub clusters. In sub-cluster I, 37-4-1-2 alone was

grouped in a sub cluster and 48-8-1-2-6 alone and Y-1281 and Kalizira grouped in sub-sub-cluster. Genotypes 48-1-2-3 alone grouped in a sub-cluster II and 43-28-5-3-1, 37-1-3-1 had been grouped in different sub cluster. In cluster 2, 10-14-5-1-3 alone grouped in a sub-cluster and Kalizira, 43-28-5-2-1, 43-28-5-2-2, 10-14-5-1-1, 41-12-3-1-2 and 33-144-1-1 were grouped in a sub-cluster while Kalizira, 43-28-5-2-1 and 10-14-5-1-1, 41-12-3-1-2 were grouped in a sub-sub-cluster. Thanh *et al.* (1999) found that the SSR-based dendrogram resolved the 31 Vietnamese upland rice accessions into two major groups. The rice genotypes were classified and grouped into 11 distinct groups (Chakravarthi and Naraveni, 2006). Yu *et al.* (2004) studied cluster analysis of the 223 accessions parental lines of rice and showed three major groups and nine sub groups. The dendrogram revealed that the genotypes that are derivatives of genetically similar type clustered together.

RAPD analysis

Genetic relationship among the twelve aromatic rice genotypes has been carried out using RAPD markers. Among the fifteen primers, three primers (OPA02, OPA10 and 67 AB10G7) generated reproducible, and informative RAPD profiles were selected. These primers produced multiple band profiles (Figure 3 a-c) with a number of amplified DNA fragments where average 11.33 scorable bands and 10.66 polymorphic bands were scored by per primer. The selected three primers generated 34 distinct bands of which 32 bands were polymorphic. The primers OPA02 and 67AB10G7 produced maximum number of polymorphic bands (12) and another primer OPA10 produced 8 polymorphic bands. The highest polymorphism was 100% (OPA02 and 67AB10G7) and the lowest was 80% (OPA10). This proportion of polymorphism was higher compared to some previous RAPD analysis in rice e.g., 53.85% in six different rice cultivars (Rahman *et al.,* 2007), 67.5% in some Indian aromatic rice (Choudhury *et al.*, 2001), 69.2% in 111 glutinous rice collected from 7 countries (Kim *et al.,* 2005).

Among the RAPD markers OPA10-6 marker revealed low level of differentiation (0.4384) in the studied genotypes of aromatic rice. According to Rahman *et al.* (2007), when gene flow is lower than gene diversity is higher. In this experiment, 43-28-5-2-1 and 43-28-5-3-1 showed higher genetic distance (0.7634) than others. So it can be commented that lower gene flow might be occurred in these genotypes. Genetic diversity values of 12 genotypes with their parents for 3 primers are given in Table 3. Average gene diversity **(**h) and Shannon's Information index (I) were found 0.3149 and 0.4788, respectively. 67AB10G7-1 and 67AB10G7-7 locus showed high gene diversity value and Shannon's Information index (0.5000 and 0.6931) and the lowest values were found in locus OPA10-2 and OPA10-3. Over all gene diversity across all populations for all the loci were 0.208, reported by Rahman *et al.,* 2007, 0.42 was reported by Qian *et al.,* 2006. Large number of pair-wise differences (low genetic similarity value) was observed among those genotypes developed from genetically distant parental line. Comparatively higher genetic distance (0.7634) was found between 43-28-5-3-1 and 43-28-5-2-1 pair lines than other pair combination. The lowest genetic distance (0.1634) was revealed between pedigree line Y-1281(P) and 48-8-1-2-6 pedigree line. Considering the genetic distance values, the result indicated that the genotypes were genetically different from each other which could be used in breeding programme to have potential genetic gains.

The UPGMA dendrogram based on Nei's (1972) genetic distance between different pairs was correlated with their sources of origin (Figure 4). The dendrogram indicated segregation of 12 aromatic rice genotypes with their parents were grouped into three distinct clusters, 33-144-1-1-1, Y-1281(P), 48-8-1-2-6, 40-1-2-1, 43-28-5-2-2, 48-1-2-3 and 37-4-1-2 were grouped in cluster I, while 37-1-3-1, 41-12-3-1-2, 10-14-5-1-3 and 43-28-5-3-1 were grouped in cluster II and Kalizira (P), 10-14-5-1-1-1 and 43-28-5-2-1 in cluster III. Three distinct clusters of rice cultivars were also observed by Rahman *et al.* (2007). From the study it was found that Y-1281 (P) was closed 48-8-1-2-6 with the lowest genetic distance (0.1634). Y-1281 and 48-8-1-2-6 were grouped in sub-sub cluster II under the cluster I. On the other hand, 43-28-5-3-1 showed the highest genetic distance (0.7634) with 43-28-5-2-1. The genotypes 43-28-5-3-1 and 43-28-5-2-1 were distantly located and also presented in different cluster II and III. In this study RAPD markers have been proved to be powerful tools for molecular genetic analysis of aromatic rice genotypes for plant breeding programme to assess allelic variation available to allow for the production of new variation that are aimed towards the improvement of crop productivity and able to withstand from biotic and abiotic factors.

In the present investigation, both SSR and RAPD markers were employed to assess the genetic diversity among 14 rice genotypes. The employment of SSR and RAPD markers in genetic diversity analysis helped in grouping the genotypes according to their subspecies level. Among the SSR primers, RM223 and the RAPD primers, OPAO2 and 67AB10G7 showed higher polymorphism 66.67% and 100%, respectively. The RAPD markers gave more clusters with fewer genotypes in each clusters, while the SSR markers gave fewer clusters with more genotypes in each and therefore, more variation within each cluster, Based on the study SSR and RAPD-based dendrogram genotype pair (43-28-5-3-1 x 10-14-5-1-1) and (43-28-5-3-1 x 43-28-5-2-1) are clustered in a

separate cluster as they have far genetic background from each other and from the rest of the investigated genotypes. Thus microsatellite markers (SSR) and random amplified polymorphic DNA markers (RAPD) could identify some of the aromatic rice genotypes under investigation have probably originated from closely related ancestors and possess high degree of genetic similarity.

Table 1. Summary of heterozygosity and genetic variation statistics for all loci

Locus	Sample Size	E_{Het}	A_{Het}	Nei	N_m	N_a	N_e	*I
RM-223	28	0.6217	0.1786	0.5995	0.1061	3.0000	2.4968	0.9999
RM-342A	28	0.5847	0.1071	0.5638	0.0587	3.0000	2.2924	0.9010
RM-515	28	0.4550	0.0714	0.4388	0.0486	3.0000	1.7818	0.7589
Mean	28	0.5538	0.1190	0.5340	0.0717	3.0000	2.1903	0.8866
Standard Deviation	-	0.0875	0.0546	0.0844	-	0.0000	0.3683	0.1211

E_{Het}=Expected Heterozygosity [Expected heterozygosity was computed using Levene (1949]
Ave_{Het}=Average Heterozygosity, Nei=Nei's (1973) expected heterozygosity
N_m= Gene flow estimated from F_{st} or F_{cs}. e.g., $N_m=0.25(1-F_{st})/F_{st}$
N_a = Observed number of alleles, N_e = Effective number of alleles
I = Shannon's Information Index

Table 2. Nei's genetic distance (below diagonal) values among studied aromatic genotypes

Genotypes	Y-281(P)	Kalizira (P)	40-1-2-1	10-14-5-1-3	43-28-5-3-1	43-28-5-2-2	10-14-5-1-1	43-28-5-2-1	48-1-2-3	37-4-1-2	41-12-3-1-2	33-144-1-1	37-1-3-1	48-8-1-2-6
Y-1281(P)	****													
Kalizira (P)	1.4525	****												
40-1-2-1	1.0986	0.4055	****											
10-14-5-1-3	0.0912	1.7006	0.6020	****										
43-28-5-3-1	1.7006	0.3143	1.0075	2.3026	****									
43-28-5-2-2	1.7006	0.3143	0.0912	0.9163	0.9163	****								
10-14-5-1-1	0.6020	1.0075	0.6020	0.9163	0.5108	0.5108	****							
43-28-5-2-1	1.0986	0.4055	0.0000	0.6020	1.0075	0.0912	0.6020	****						
48-1-2-3	1.0986	0.4055	1.0986	1.7006	0.0912	1.0075	0.3143	1.0986	****					
37-4-1-2	0.2027	0.0000	1.5890	0.3993	1.4979	1.4979	0.3993	1.5890	0.8959	****				
41-12-3-1-2	0.8959	0.8959	0.4904	1.0924	0.3993	0.3993	0.1116	0.4904	0.4904	0.6931	****			
33-144-1-1	0.4055	1.0986	0.4055	0.6020	0.6020	0.6020	0.0912	0.4055	0.4055	0.4904	0.2027	****		
37-1-3-1	0.8959	0.8959	0.4904	0.3993	0.0000	0.3993	1.4979	0.4904	0.0000	0.6931	1.3863	1.5890	****	
48-8-1-2-6	0.0000	0.0000	0.4055	1.7006	0.3143	0.3143	1.0075	0.4055	0.4055	0.0000	0.8959	1.0986	0.8959	****

Table 3. Genetic diversity, Gene flow (Nm) and gene differentiation (G_{ST}) across 3 primers in 12 aromatic rice lines with their parents

Locus	Sample size	na*	ne*	h*	I*	G_{ST}*	N_m*
OPA02-1	42	2.0000	1.6897	0.4082	0.5983	1.0000	0.0000
OPA02-2	42	2.0000	1.6897	0.4082	0.5983	1.0000	0.0000
OPA02-3	42	2.0000	1.2499	0.1999	0.3521	0.8257	0.1056
OPA02-4	42	2.0000	1.4867	0.3274	0.5089	0.4676	0.5694
OPA02-5	42	2.0000	1.2769	0.2168	0.3743	0.5177	0.4659
OPA02-6	42	2.0000	1.2769	0.2168	0.3743	0.5177	0.4659
OPA02-7	42	2.0000	1.6897	0.4082	0.5983	1.0000	0.0000
OPA02-8	42	2.0000	1.3243	0.2449	0.4101	1.0000	0.0000
OPA02-9	42	2.0000	1.8491	0.4592	0.6518	1.0000	0.0000
OPA02-10	42	2.0000	1.3243	0.2449	0.4101	1.0000	0.0000
OPA02-11	42	2.0000	1.5077	0.3367	0.5196	1.0000	0.0000
OPA02-12	42	2.0000	1.8491	0.4592	0.6518	1.0000	0.0000
OPA10-1	42	2.0000	1.5077	0.3367	0.5196	1.0000	0.0000
OPA10-2	42	1.0000	1.0000	0.0000	0.0000	****	****
OPA10-3	42	1.0000	1.0000	0.0000	0.0000	****	****
OPA10-4	42	2.0000	1.5858	0.3694	0.5562	0.9056	0.0521
OPA10-5	42	2.0000	1.0859	0.0791	0.1719	0.5592	0.3942
OPA10-6	42	2.0000	1.5934	0.3724	0.5595	0.4384	0.6405
OPA10-7	42	2.0000	1.1783	0.1514	0.2848	0.5394	0.4270
OPA10-8	42	2.0000	1.7115	0.4157	0.6063	0.6131	0.3155
OPA10-9	42	2.0000	1.9365	0.4836	0.6767	0.7837	0.1380
OPA10-10	42	2.0000	1.9986	0.4997	0.6928	0.9572	0.0224
67AB10G7-1	42	2.0000	2.0000	0.5000	0.6931	1.0000	0.0000
67AB10G7-2	42	2.0000	1.3243	0.2449	0.4101	1.0000	0.0000
67AB10G7-3	42	2.0000	1.9865	0.4966	0.6897	0.9298	0.0377
67AB10G7-4	42	2.0000	1.9365	0.4836	0.6767	0.7837	0.1380
67AB10G7-5	42	2.0000	1.5077	0.3367	0.5196	1.0000	0.0000
67AB10G7-6	42	2.0000	1.3243	0.2449	0.4101	1.0000	0.0000
67AB10G7-7	42	2.0000	2.0000	0.5000	0.6931	1.0000	0.0000
67AB10G7-8	42	2.0000	1.2968	0.2289	0.3898	0.6954	0.2190
67AB10G7-9	42	2.0000	1.2968	0.2289	0.3898	0.6954	0.2190
67AB10G7-10	42	2.0000	1.5858	0.3694	0.5562	0.9056	0.0521
67AB10G7-11	42	2.0000	1.4294	0.3004	0.4775	0.8840	0.0656
67AB10G7-12	42	2.0000	1.1529	0.1327	0.2573	1.0000	0.0000
Mean	42	1.9412	1.5192	0.3149	0.4788	**0.8690**	**0.0754**
St. Dev		0.2388	0.3036	0.1417	0.1839	-	-

* na = Observed number of alleles
* ne = Effective number of alleles
* h = Nei's (1973) gene diversity
* I = Shannon's Information index
* Co-efficient of gene differentiation
* N_m = estimate of gene flow from G_{ST}, $N_m = 0.5(1 - G_{ST})/G_S$

Table 4. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values among studied aromatic Genotypes

Genotypes	Y-281(P)	Kalizira (P)	40-1-2-1	10-14-5-1-3	43-28-5-3-1	43-28-5-2-2	10-14-5-1-1	43-28-5-2-1	48-1-2-3	37-4-1-2	41-12-3-1-2	33-144-1-1	37-1-3-1	48-8-1-2-6
Y-1281(P)	****													
Kalizira (P)	0.2876	****												
40-1-2-1	0.2374	0.3502	****											
10-14-5-1-3	0.3788	0.6343	0.4527	****										
43-28-5-3-1	0.4817	0.7620	0.2748	0.2360	****									
43-28-5-2-2	0.3588	0.4044	0.1691	0.4506	0.2718	****								
10-14-5-1-1	0.4671	0.4194	0.3145	0.5233	0.5161	0.2553	****							
43-28-5-2-1	0.2635	0.3646	0.4779	0.5954	0.7634	0.4406	0.2573	****						
48-1-2-3	0.2298	0.4266	0.3494	0.3427	0.4278	0.2831	0.4132	0.4247	****					
37-4-1-2	0.3182	0.4741	0.1986	0.4012	0.3483	0.3187	0.4208	0.5307	0.1851	****				
41-12-3-1-2	0.2613	0.6210	0.3489	0.3059	0.2841	0.3463	0.5460	0.4919	0.3820	0.2507	****			
33-144-1-1	0.1812	0.4494	0.2959	0.3173	0.3152	0.2930	0.4807	0.4613	0.2021	0.3152	0.3059	****		
37-1-3-1	0.3693	0.4150	0.5022	0.3683	0.3088	0.4494	0.5313	0.5571	0.2402	0.4472	0.3568	0.3314	****	
48-8-1-2-6	0.1634	0.2798	0.2995	0.5083	0.4597	0.3097	0.3247	0.3125	0.2492	0.4597	0.3762	0.2514	0.2312	****

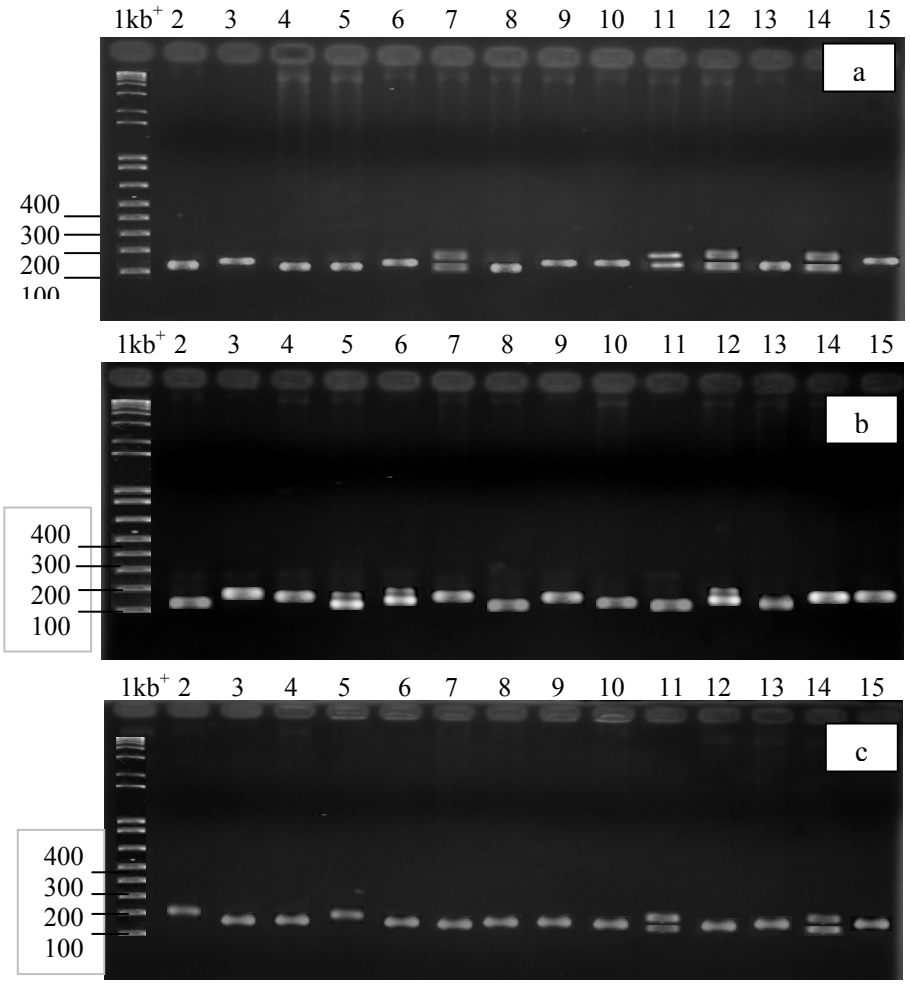

Figure 1. Banding pattern of some of the rice genotypes for a) RM223, b) RM342A, c) RM515 where Lane-1: 1 kb+ ladder; Lane-2: Y-1281 (parent); Lane-3: Kalizira (parent); Lane-4:40-1-2-1; Lane-5:10-14-5-1-3; Lane-6:43-28-5-3-1; Lane-7:43-28-5-2-2; Lane-8:10-14-5-1-1; Lane-9:43-28-5-2-1; Lane-10:48-1-2-3; Lane-11:37-4-1-2; Lane-12:41-12-3-1-2; Lane-13:33-144-1-1; Lane-14:17-1-3-1; Lane-15: 48-8-1-2-6

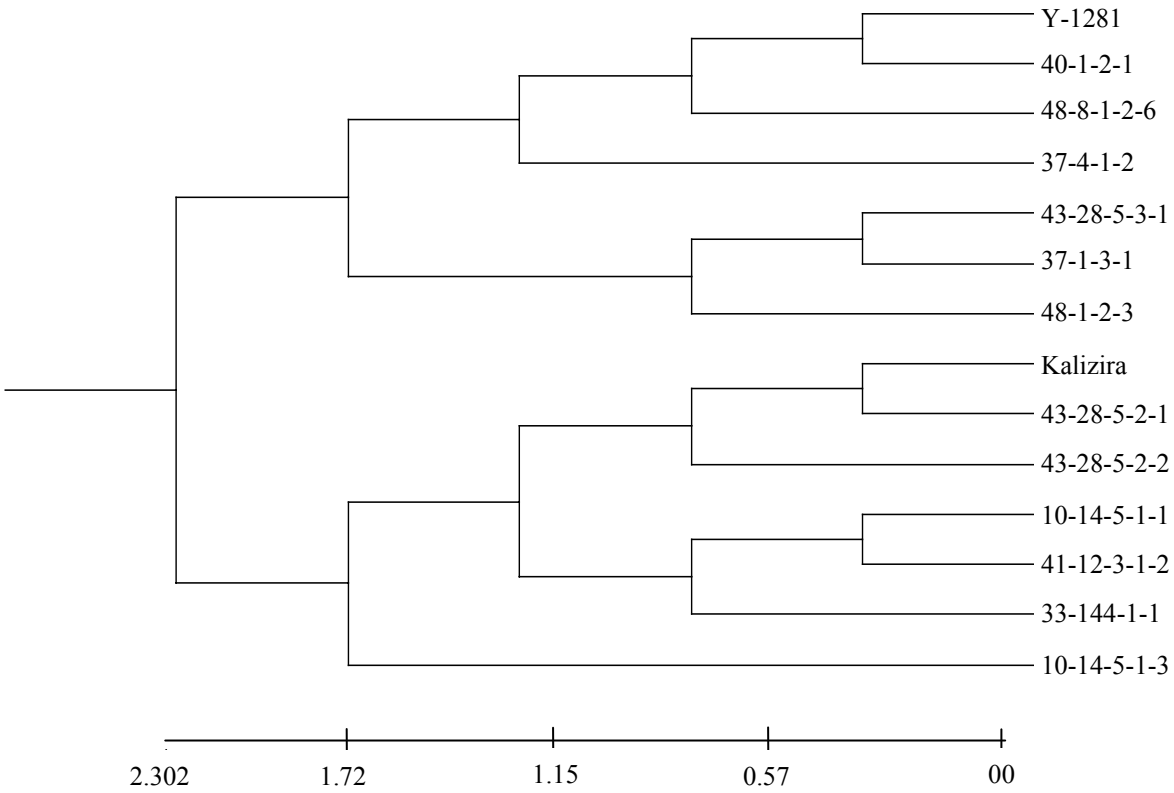

Figure 2. UPGMA dendrogram based on Nei's (1972) original measure of genetic distance summarizing the data on differentiation between aromatic rice genotypes according to SSR analysis

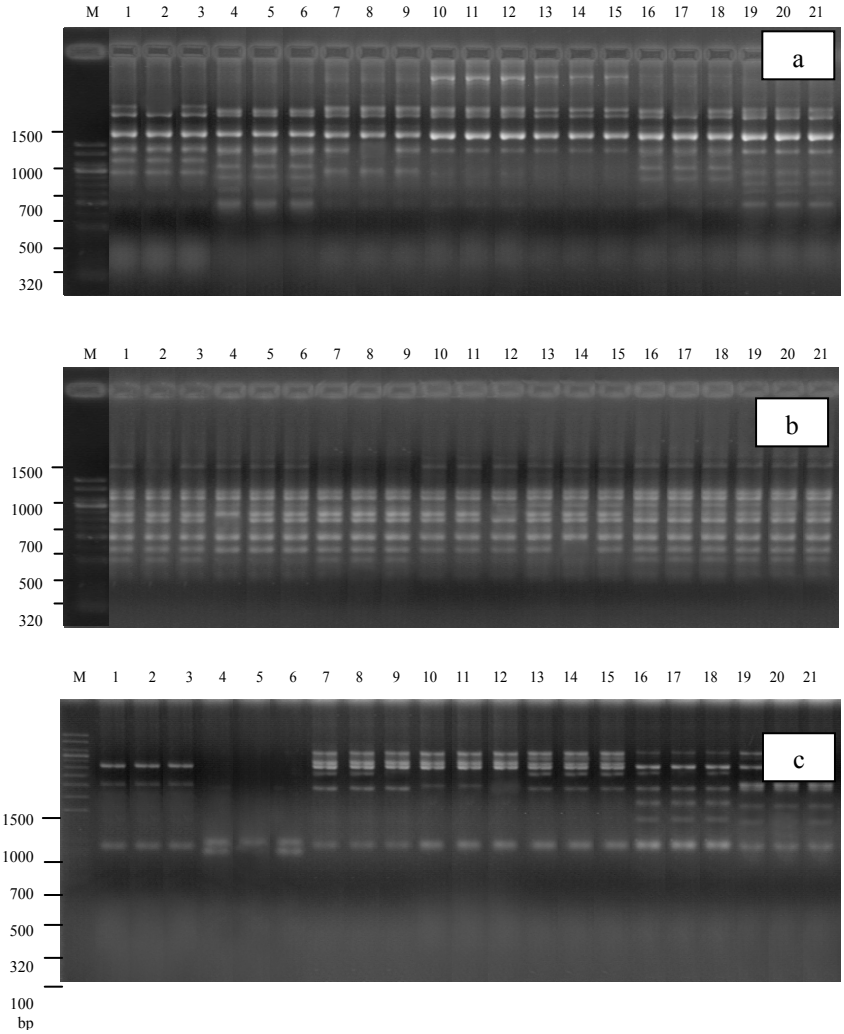

Figure 3. RAPD profiles of some aromatic rice genotypes a) OPA02, b) OPA10, c) 67AB10G7 where M: Molecular weight marker (20 bp DNA ladder). Lane 1-3: Y-1281 (P), Lane 4-6: Kalizira (P), Lane 7-9: 40-1-2-1, Lane 10-12: 10-14-5-1-3, Lane 13-15: 43-28-5-3-1, Lane 16-18: 43-28-5-2-2, Lane 19-21: 10-14-5-1-1.

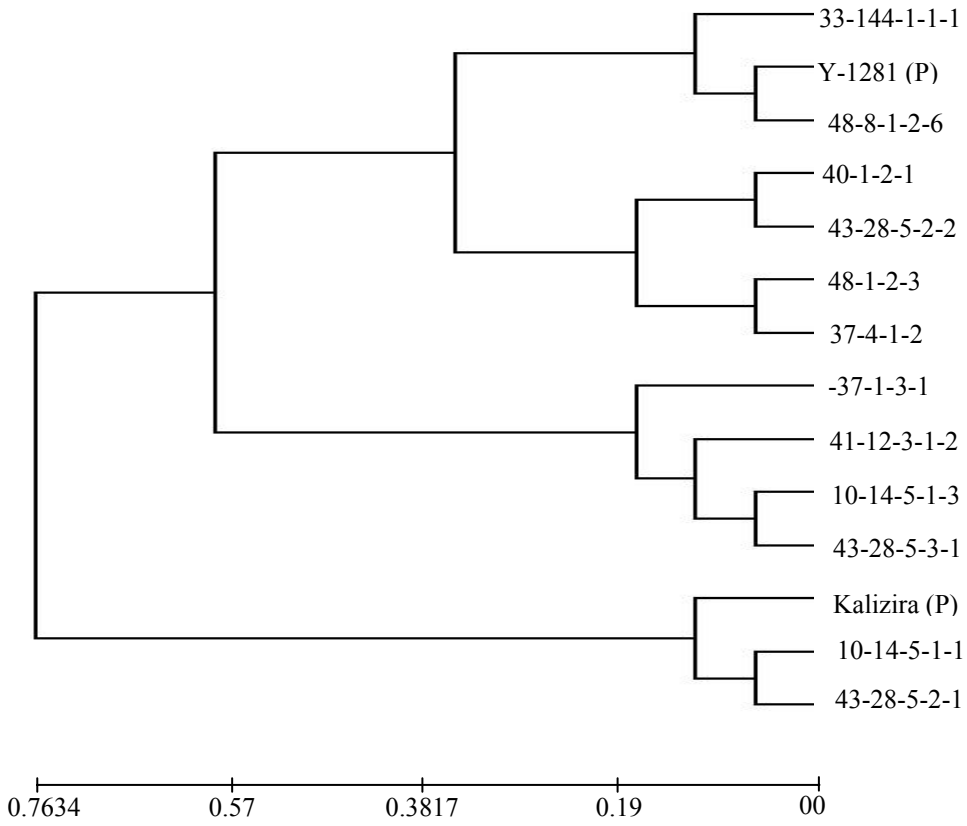

Figure 4. UPGMA dandogram based on Nei's (1972) original measure of genetic distance summarizing the data on differentiation between aromatic rice genotypes according to RAPD analysis

REFERENCES

Chakravarthi, B.K. and R. Naravaneni. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). African J. Biotech. 5 (9): 684-688.

Choudhury, P.R., Kohil, S., Srinivasan, K., Mohapatra, T. and Sharma, R.P. 2001. Identification and classification of aromatic rices based on DNA fingerprinting. Euphytica, 118: 243-251.

Cordeiro, G.M., Christopher, M.J., Henry, R.J. and Reinke, R.F. 2002. Identification of microsatellite markers for fragrance in rice by analysis of the rice genome sequence. Mol. Breed. 9(4): 245-250.

Fristsch, P. and Rieseberg, L.H. 1995. The use of random amplified polymorphic DNA (RAPD) in conservation genetics. In: Smither, T.B. and R.K. Wayne (eds.) Molecular Genetic Approaches in Conservation, Oxford University Press, Oxford. pp. 54-73.

Giovannoni, J.J., Wing, R.A., Ganal, M.W. and Tanksley, S. 1991. Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res. 19: 6553–6558.

Joshi, S.P., Gupta, V.S., Aggarwal, R.K., Ranjekar, P.K. and Brar, D.S. 2000. Genetic diversity and phylogenetic relationship as revealed by Inter simple sequence repeat polymorphism in the genus *Oryza*. Theor. Appl. Genet. 100: 1311-1320.

Kim, G., Kim, H., Lee, S., Chung, B., Song, B. and Cho, Y. 2005. Genetic diversity of glutinous rice collections based on agronomic traits and RAPDs. Korean J. Crop Sci. 50(3): 212-220.

McCouch, S.R., Chen, X., Panaud, O., Temnykh, S., Xu, Y., Chao, Y.G., Huang, N., Ishii, T. and Blair, M. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Mol. Biol. 35: 89-99.

Nagaraju, J., Kathirve, M., Ramesh, R. K., Siddiq, E. A. and Hasnain, E. 2002. Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Agric. Sci. 99(9): 5836-5841.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106: 283-292.

Qian. W., Song, G. and Yuan, H. D. 2006. Genetic diversity in accessions of wild rice (*Oryza granulate*) from South and Southeast Asia. China Genet. Res. Crop Evol. 53(1): 197-204.

Rahman, S. N., Islam, M. S., Alam, M. S. and Nasiruddin, K. M. 2007. Genetic polymorphism in rice (*Oryza sativa* L.) through RAPD analysis. Indian J. Biotech., 6(2): 224-229.

Saker, M. M., Sawsan, S. Y., Abdallah, N. A., Bashandy, H. S. and Sharkawy, A. M. E. 2005. Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. African J. of Biotech. 4 (9): 882-890.

Thanh N.D, Zheng F, Dong N, Tinh L. Ali M. and Nguyen H. 1999. Genetic variation in root morphology and microsatellite DNA loci in upland rice (*Oryza sativa* L.) from Vietnam. Euphytica (105): 43-51.

Tingey, S. V. and Deltufo, J.P.. 1993. Genetic analysis with Random Amplified Polymorphic DNA. Plant Physiol. 101: 349-352.

Yu, P., Li, Z., Zhang, H., Wang, M., Sun, J. and Wang, X. 2004. Genetic diversity of common wild rice (*Oryza rufipogon Griff*) by using SSR markers and phenotypic traits in Guangxi Province. Yi Chuan Xue Bao 31(9):934-40.