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Assessment of Genetic Diversity in 13 Local Banana (*Musa* Spp.) Cultivars Using Simple Sequence Repeat (SSR) Markers

[1]M H Kabir, [2]A N K Mamun, [3]H Fatema, [4]R Amin

[1,2]Institute of Food and Radiation Biology, Atomic Energy Research Establishment G. P. O. Box 3787, Dhaka Bangladesh
[3]Department of Biotechnology and Genetic Engineering, Islamic University, Kustia, Bangladesh
[4]National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract: **A Study was conducted to investigate the genetic variability among 13 local banana cultivars using 3 SSR primers of Mb1-69, Mb1-113 and Mb1-134. All the primer pairs amplified a total of 29 different marker bands with an average of 9.6 bands per primer. Among the 29 bands only 4 bands were monomorphic and the rest 25 bands were polymorphic. The sizes of the amplified DNA bands in 13 local banana cultivars varied from 200 bp to 600 bp. The primer Mb1-113 amplified the highest (14) number of DNA bands and the primer Mb1-69 amplified the lowest (7) number of DNA bands whilst primer Mb1-134 amplified 8 DNA bands. The values of pair-wise genetic distances ranged from 1.00 to 9.00 indicating the presence of wide genetic diversity. The dendogram constructed based on phylogenetic relationship analysis revealed that the highest genetic diversity (9.00) found between the cultivars champa and jawayta and also the cultivars champa and jahazy whilst the lowest (1.00) between the cultivars doubled haploid and kathaly, doubled haploid and sorishafruity, doubled haploid and amritsagor and doubled haploid and ganasundory. The UPGMA dendogram has segregated the 13 local banana cultivars into two major clusters. Agnishwar and champa formed in cluster 1 and the rest of the cultivars like sobri jesore, sobri, anazy, kathaly, jawayta, sorishafruity, amritsagor, jahazy, bangle, ganasundory and doubled haploid have constituted the cluster 2.**

Keywords: **Genetic diversity, SSR markers, banana.**

1. INTRODUCTION

Banana is one of the most important cash crop in Bangladesh and grown round the year in the country for commercial purposes and homestead area for local consumption. It stood first position among the fruit producing in the country and supplies 42% of the total fruit requirement and also financial return is higher compared to other fruits and field crops (Haque, 1988b). It is a nutritious furit crop in the world and grown in many tropical areas where they are used both as a staple food and dietary supplements (Assani *et al*., 2001). There are 32 landraces including dessert and cooking banana cultivars in the country (Haque, 1988a). The average yield of banana in the country is about 15 t/ha that is far below the average world yield of 30.63 t/ha in India (FAO, 2006-2007). Edible bananas are mostly sterile polyploids and must be propagated vegetatively. Genetic variation is the starting point of any breeding program. Therefore, to set up an efficient strategy for breeding to improve banana cultivars and support the choice of crossing parents, a solid understanding of genetic diversity of available resources is needed. The crop is mostly damaged by *fusarium* wilt. It also faces banana bunchy top virus, banana leaf and fruit beetle, pseudo stem borer and post-harvest diseases such as anthracnose, crown rot, fruit rot and neck rot (Nik Masdek *et al.*, 1998). Recently, genetic identification has received special attention due to cultivar protection by the plant breeders and demanding cultivar identity for world trade as well as cultivar registration, efficient collection and preservation. The enormous increase in the availability of various molecular techniques over the past decades which has facilitated the classification of new banana cultivars, reassessment of traditional taxonomy,

diversity study and molecular characterization. Simple sequence repeat (SSR) have been successfully applied in the molecular genotyping of many important crops such as rice (Pessoa-Filho *et al*., 2007), cereals (Hayden *et al*., 2007), grapevine (This *et al*., 2004), Cacao (Zhang *et al*., 2006) and banana (Oriero *et al*., 2006) due to its co-dominant in nature. Thus, the present study was undertaken to investigate the potentiality of SSR markers for distinguishing individual accessions to rule out duplications among cultivars and to develop a standardized procedure for *Musa* genotyping which could serve as a basis for molecular characterization of newly introduced global *Musa* gene bank as well as *Musa* research and breeding community.

2. MATERIALS AND METHODS

Thirteen local banana cultivars namely sobri jessore (large size), sobri (small size), anazy (cooking, large), kathaly (cooking, small), jawayta (seeded, large), sorishafruity (seeded, small), amritsagor, jahazy, agnishwar, champa, ganasundory, bangle and doubled haploid (anther derived plant) were used for molecular characterization through SSR analysis. The cultivars were collected from the experimental garden of Plant Biotechnology and Genetic Engineering Division, Atomic Energy Research Establishment, Savar, Dhaka. Young and fresh leaf were used for DNA extraction. The harvested leaf of 0.25g was cut down into small pieces and isolated DNA using modified CTAB (Abdullah *et al*., 2012). The concentration of DNA sample was determined using UV spectrophotometer (PG Instruments Ltd. Korea) at 260/280 nm readings were 1.6 to 1.8. The PCR reaction mixture for 10 µl containing template DNA (50 ng) 1 µl, de-ionized distilled water 1.8 µl, Taq buffer A 10X (15mM Tris-Hcl with $Mgcl_2$) 1 µl, primer (2 µM) 5 µl, dNTPs (2.5 mM) 1 µl and Taq DNA polymerase (5u/ µl) 0.2 µl. The reaction with each primer was replicated thrice to check the reproducibility of DNA. DNA amplification was carried out in an oil-free DNA thermal cycler (Finnzymes Instruments, EU) at the following thermal profile: initial denaturation for 5 minutes at 94ºC followed by denaturation for 60 seconds at 94ºC, annealing at 55ºC for 60 seconds and extension at 72ºC for 120 seconds. A final extension at 72ºC for 7 minutes was allowed for complete extension of all amplified fragments. Amplified fragments were separated on a 1.4% agarose gel containing 0.5 µg/ml ethidium bromide in 1X TBE buffer for 1 hour at 100 volts. The gel was visualized by UV-transilluminator and photographed by gel documentation system (Major Science, USA). The amplified bands were visually scored as present (1) and absent (0) of bands, size of bands and overall polymorphism of bands. The scores obtained using all primers in the SSR analysis were then pooled for constructing a single data matrix. This was used to estimate polymorphic loci, genetic diversity, genetic distance (Nei, 1972) and a UPGMA (Unweighted Pair Group Method with Arithmatic Means) dendrogram using computer program 'Statistica'.

3. RESULTS AND DISCUSSION

The three (3) primers generated 29 distinct bands of which 21 were considered as polymorphic. The percentage of polymorphic loci was 86.20% indicating a higher level of polymorphism. The three primers generated 9.66 score able bands per primer and 7.0 polymorphic per primer. Oriero *et al*. (2006) selected 44 SSR primers to assess 40 *Musa* accessions and found only 9 primers produced amplified product which is very low number of amplified product comparing to the present study. Thus, the primers are used in this study is useful for *Musa* genetic analysis. Creste *et al*. (2004) used 33 primers to investigate genetic diversity in *Musa* spp. and 15 primers gave amplified products. Thus, selection of primers played an important role in *Musa* genotyping. A diverse level of polymorphism in different crops has been reported in tomato (Moonmoon, 2006), eggplants (Biswas *et al*., 2009) and chili (Para *et al*., 1998). The values of pair-wise (Nei's, 1972) genetic distance ranged from 1.00 to 9.00. The highest genetic distances (9.00) were found between cultivars champa and jawayta and also champa and jahazy. The lowest (1.00) value were observed between the cultivars doubled haploid and kathaly, doubled haploid and sorishafruity, Doubled haploid and amrit sagor and doubled haploid and ganasundory. The difference between the highest and the lowest value of genetic distance revealed that wide range of variability persisting among the 13 banana cultivars. Moonmoon (2006) reported that assessment of genetic diversity, molecular markers were superior to morphological, biochemical and other method like heterosis.

Dendrogram based on Nei's (1972) genetic distance using UPGMA indicated segregation of 13 banana cultivars into two main clusters. Cultivar agnishwar and champa formed cluster-1 and the remaining 11 cultivars grouped in cluster-2. Cluster-2 was divided into 4 sub-clusters. Jahazy, jawayta, anazy formed sub-cluster-1, sub-cluster-2 and sub-cluster-3 respectively. Sub-cluster-4 includes sobri jesore, kathaly, sorishafruity, amrit sagor, ganasundory, doubled haploid, bangla and sobri. Sub-cluster-4 was again divided into more sub-clusters forming sub-sub-clusters. From this investigation, it

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was found that the highest genetic identity (9.00) remains between the cultivar champa and jawayta and champa and jahazy. On the other hand, the lowest (1.00) genetic identity was observed between the cultivar doubled haploid and kathaly, doubled haploid and sorishafruity, doubled haploid and amrit sagor, doubled haploid and ganasundory indicating these cultivars might be beneficial for banana improvement program. In this study, it is also revealed that each of the 13 banana cultivars possessed specific marker which could be used for their authentic identification. From the table, it was observed that most of the cultivars is linked with each other as they have equal genetic distance. Some of the cultivars also maintained close relationship with each other due to nearest genetic distance among the cultivars. Similar observation was reported by Alam *et al*. (2012) in tomato. Many authers namely Uma *et al*., 2006, Jain *et al*., 2007, Noyer *et al*., 2005, Hautea *et al*., 2004, Pillay *et al*., 2001 and 2000, Crouch *et al*., 2000, Newbury *et al*., 2000, Sunchez *et al*., 2000, Howell *et al*., 1994 studied relationships among banana populations.

The banding patterns of different banana cultivars using 3 primers are shown in Figs. 1 to 3. Band size ranging from 200 to 600 bp in PCR amplification products scored for primers. Strong and weak bands were produced in the PCR reactions. Weak bands produced from low homology between the primer and the pairing site on the DNA strand (Thormann *et al*., 1994). This study shows the genetic diversity within *Musa* germplasm collection in Atomic Energy Research Establishment. There are several limitation in breeding and genetic improvement in *Musa* genotypes. Therefore, selection the most suitable parents that would result in higher diversity is crucial for significant genetic progress.

Table 1. Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values among 13 local banana cultivars.

	sobrijesore	sobri	anazy	kathaly	jawayta	sorishafruity	amritsagor	jahazy	agnishwar	champa	ganasundory	bangla	doubled haploid
sobrij	.00	6.00	7.00	3.00	7.00	3.00	3.00	7.00	6.00	8.00	3.00	5.00	4.00
sobri	6.00	.00	7.00	3.00	7.00	3.00	3.00	7.00	6.00	8.00	3.00	5.00	4.00
anazy	7.00	7.00	.00	4.00	8.00	4.00	4.00	8.00	7.00	9.00	4.00	6.00	5.00
kathaly	3.00	3.00	4.00	.00	4.00	3.00	3.00	4.00	3.00	5.00	3.00	2.00	1.00
jawayta	7.00	7.00	8.00	4.00	.00	4.00	4.00	8.00	7.00	9.00	4.00	6.00	5.00
sorishaf	3.00	3.00	4.00	3.00	4.00	.00	3 .00	4.00	3.00	5.00	3.00	2.00	1.00
amrits	3.00	3.00	4.00	3.00	4.00	3.00	.00	4.00	3.00	5.00	3.00	2.00	1.00
jahazy	7.00	7.00	8.00	4.00	8.00	4.00	4.00	.00	7.00	9.00	4.00	6.00	5.00
agnis	6.00	6.00	7.00	3.00	7.00	3.00	3.00	7.00	.00	4.00	3.00	5.00	4.00
champa	8.00	8.00	9.00	5.00	9.00	5.00	5.00	9.00	4.00	.00	5.00	7.00	6.00
ganas	3.00	3.00	4.00	3.00	4.00	3 .00	3.00	4.00	3.00	5.00	.00	2 .00	1.00
bangle	5.00	5.00	6.00	2.00	6.00	2.00	2.00	6.00	5.00	7.00	2.00	.00	3.00
doubleh	4.00	4.00	5.00	1.00	5.00	1.00	1.00	5.00	4.00	6.00	1.00	3.00	. 00

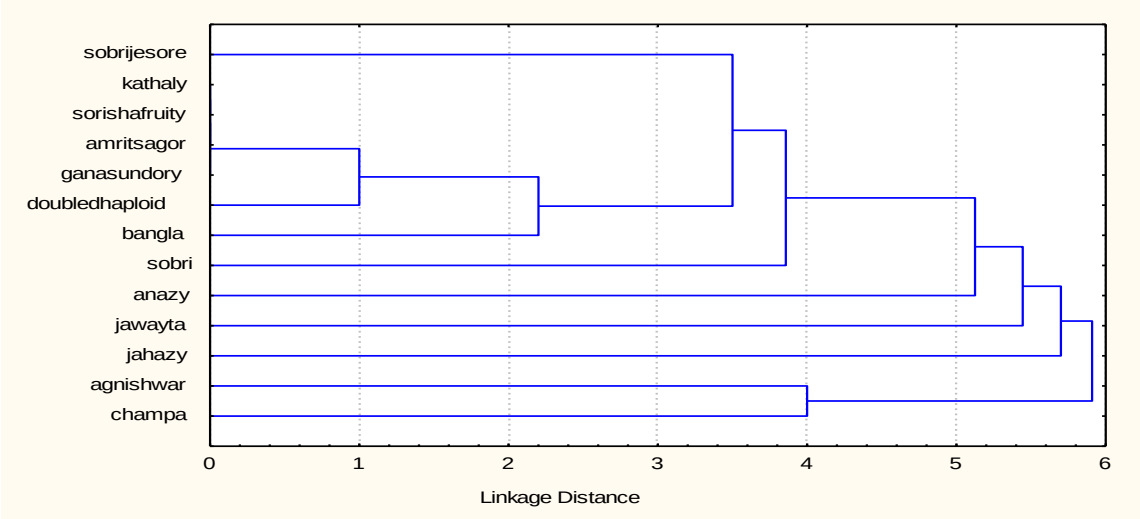

Figure1: UPGMA dendogram based on Nei's (1972) genetic distance, summarizing data on differentiation in 13 local banana cultivars according to SSR analysis.

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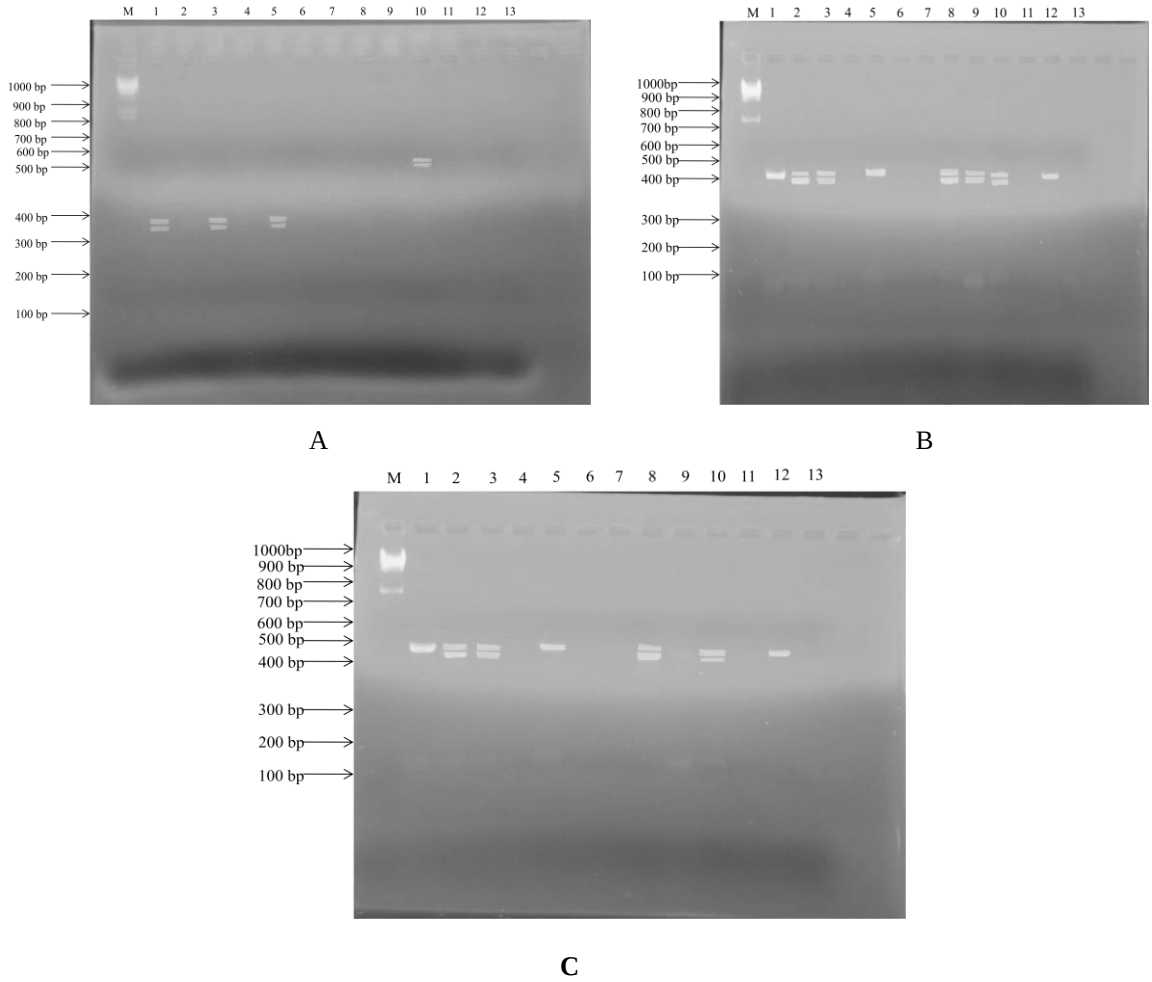

Figure2: SSR profiles of 13 banana cultivars DNA with different primers (a)Mb1-69, (b)Mb1-113 & (c)Mb1-134. LaneM-1.0 kb DNA ladder, Lane1-Sobri jessore (large size), Lane2-Sobri (small size), Lane3-Anazykola, Lane4-Kathaly, Lane5-Jawayta (large size seeded), Lane6-Sorishafruity (small size seeded), Lane7-Amrit sagor, Lane8-Jahazykola, Lane9-Agnishwar, Lane10-Champa, Lane11-Ganasundory, Lane12-Banglakola, Lane13-Doubled haploid.

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