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Full Length Research Paper

Genetic diversity analysis of *Musa* species using amplified fragment length polymorphism and multivariate statistical technique

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Little systematic work has been done on productivity, breeding and diversity studies in *Musa* species. It is highly cross-pollinated and is known for continued seedling propagation, and this study has described the reality of wide genetic variability offering significant scope for selecting superior genotypes and provides a sound scientific basis. Few attempts have been directed to improve *Musa* species due to its complex genetic structure and characterize it at molecular level. Understanding the genetic relationship and variation is important for efficient parental selection for the development of improved cultivar. Restriction site variation in 3 selective primer combination sequences amplified using the AFLP polymerase chain reaction (PCR) method, were examined by digesting each fragment with restriction endonucleases. The analysis of the three primers combined genetic data generated 285 polymorphic bands, and average number of bands per assay was 95. The three primer pairs clearly define two lines between Borneo (maternal parent) and SF 247 (paternal parent). The 285 polymorphic bands scored across the parents and their hybrids were used to generate Jaccard's similarity coefficients which were analysed phenetically. The UPGMA analysis defined 2, 3 and 2 clusters for the individual primers that diverged at different phenol levels, ranging from 0.56 to 0.98 with base pairs ranging from 50 to 800 bp size. Each primer combination varied at 21, 11 and 22% with 11, 14 and 26 polymorphic bands. Cluster analysis using the unweighted pairgroup method of mathematical averages (UPGMA) separated the two parents into distinct groupings based on the genome classification. Some of the hybrids formed loose clustering, indicating high genetic diversity in the group.

Key words: *Musa* species, Jaccard's coefficient, similarity, dendrogram, polymorphism, multivariate.

INTRODUCTION

Banana and plantain belong to Musaceae family and are among the tallest monocotyledons. They are basic food for millions of people in the inter-tropical area and represent a very important economic crop. *Musa spp*. AAB and AAA groups are important staple food for rural and urban consumers in the humid forest and mid-altitudes of sub-Saharan Africa (Vuylsteke *et al.,* 1993a). The cultivars are derived from natural hybridization between wild diploid *Musa* species. They are diploid, triploid or tetraploid, the current cultivated cultivars are mostly triploids*. Musa acuminata* (A genome, 2n=2x=22) is involved in all the cultivars*, M. balbisiana* (B genome, 2n=2x=22) in many of them (Simmmonds and Shepherd 1955) and *M.schizocarpa* (S genome, 2n=2x=22) and *Australimusa* species (T genome, 2n=2x=20) in a few of them Classification of cultivated genotypes has been based on their morphological similarities to wild diploid species and their ploidy level (Cheesman 1947, Simmonds and Shepherd 1955), the main groups being AA, AAA, AAB, and ABB. A better knowledge of the available genetic diversity and genome structure will be useful for cultivar improvement. Many crops are being investigated on the molecular level using more improved

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Table 1: Selective amplification of Genomic DNA of *Musa acuminata* and their hybrids

Component	Concentration (µl)			
	1rxn	5rxn	30rxn	35rxn
Mix 1				
EcoR1	0.18	0.9	5.4	6.3
Distilled water	0.32	1.6	9.6	11.20
Mse1	4.5	22.5	135	157.5
Mix 2				
Distilled water	5.4	27	162	189
$MgCl^{2+}$	2.5	12.5	75	87.5
10X buffer	2.0	10	60	70
Taq enzyme	0.10	0.5	3	35

Note: only 1rxn in this table was used for this work. Rxn = reaction

marker systems. Tropical crops such as *Musa* are seldom, if ever, included in international genome analysis initiatives to study their sometimes complex genetic structures. Molecular markers that provided useful information and new insight into the classification have been available for several years (Lebot *et al.* 1993; Baurens *et al*. 1997; Grapin *et al*. 1998). Genomic *in situ* hybridization (GISH) often enables visualization of the genome contributed by each parental species in interspecific hybrids and thus constitutes a powerful complimentary tool for estimating genetic diversity. A thorough evaluation with a better understanding of the diversity and evolution of species and subspecies within this genus is important. Molecular markers represent one of the most effective and successful application of molecular biology to plant genetics and breeding. This study was conducted to determine the extent of genetic diversity and relationship in diploid *Musa acuminate* species and their hybrids. Detailed morphological and molecular characterization of such a relationship will certainly enhance the understanding of genetic basis of offering, and hence facilitate the utilization of the inter-specific heterosis. Similar diversity analysis has been conducted on *Jatropha curcas* L. (Sun et.al, 2008; Suyathan et.al, 2008), and in triploid *Musa* species (Ude et al, 2002). The objective of the study was to reveal relationship among the 2 accessions of the female and male parents.

MATERIALS AND METHODS

10 g of leaf tissue per sample, collected from the field and immersed in a liquid nitrogen, and the sample were ground with a mortar and pestle. DNA and extraction procedure were as previously described by Ude et al (2002).

DNAs from the parental species and the F_1 progenies were digested with restriction endonucleases, EcoR1, and Mse1 (Table 1), according to the suppliers' instructions (Gaithersburg Life technologies, USA). Genomic DNA was isolated and digested with two restriction endonulceases simultaneously. 1:50 DNA sample (5 µl), Mix 1, (5 µl) and Mix 2, (10 µl) (total volume of 20 µl) per sample were loaded on Perkin Elmer Geneamp PCR System 9600 and was left for 2 h. 20 µl of bromophenol blue dye was added for 1 h 30 min for stop reaction (final volume = 40 µl) and 5 µl of DNA samples were loaded on the sequencing system powered by electrophoretic power supply at a voltage of 50 W to run for two hours. This step generates the required substrate for ligation and subsequent amplification. The restriction fragments for amplification were generated using two endonucleases: EcoR1 and Mse1. EcoR1 has 6-bp recognition site, and Mse1 has a 4-bp recognition site. Samples were incubated for 37^{o}C and 70^{o}C for two hours and 10 minutes and the temperature held at 4^{o}C, enzyme reaction was stopped and samples were transferred for adapter ligation and amplification. These enzymes were used and they generated small DNA fragments that amplified in optimal size range (<1 kb) i.e. less than 1000 bp for separation on denaturing polyacrylamide gels which generate fingerprints. Bands were scored as zero (0) where there is no band and one (1) where a band is present**.**

Data analysis

The genetic data generated using modified AFLP technique was analysed using the NTSYS-pc software package version [2.02f] (Rohlf 1998). The polymorphic data was used to calculate Jacard's similarity coefficients and the coefficients were also subjected to an

Table 2; Number and size range of bands amplified in the AA *Musa* acuminata and their hybrids.

S/no	Primer combination	no. of fragments	Basepairs	no. of polymorphism
1	**E-$_{AGC}$/M-**$_{CTC}$	68	50-800	14
2	**E-$_{AGC}$/M-**$_{CTA}$	101	160-410	11
3	**E-$_{AGG}$/M-**$_{CAT}$	116	160-970	26
Total		**285**	**50-1000**	**51**
Average no. of bands per assay		**95**	-	-

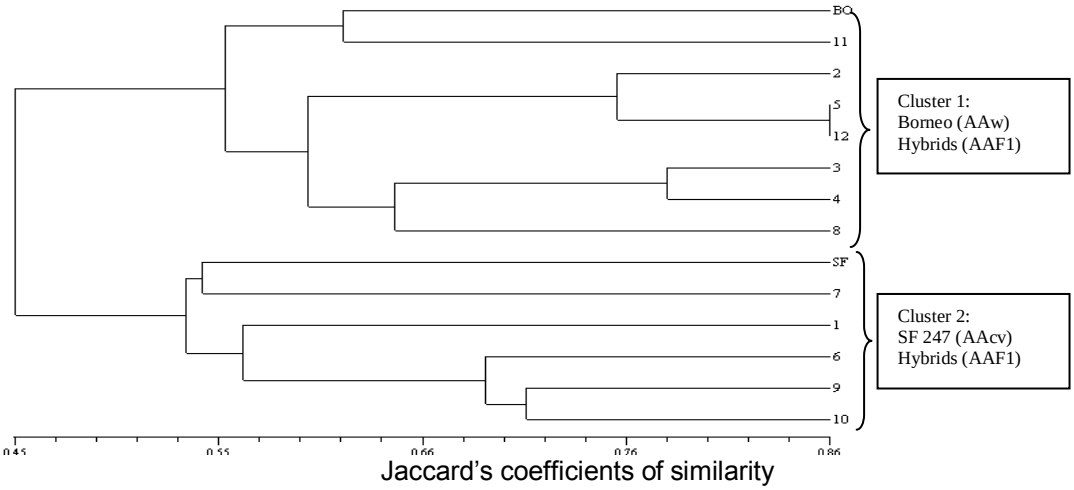

Figure 1: Phenetic dendrogram of Borneo, SF 247 and their hybrids obtained by unweigheted pair-group method of mathematical averages (UPGMA) clustering of amplified fragment length polymorphism (AFLP) data with a selective primer combination E-$_{AGC}$/M-$_{CTC}$. Scale on bottom is Jaccard's coefficient of similarity.

Unweigthed Pair-Group Method Arithmetic average analysis (UPGMA) to generate dendrograms. The frequency of co-migrating PCR amplification products in pair-wise comparisons of genotype was used to calculate Jaccard's (1908) similarity coefficients among the progenies as follows: $S_{ij} = N_{ij}/(N_{ii} + N_{ij} + N_{jj})$ where S_{ij} is the DNA marker similarity index between the i^{th} and j^{th} progeny, N_{ij} is the number of bands present in both progenies, N_{ii} is the number of bands present in the i^{th} progeny but lacking in the j^{th} progeny, and N_{jj} is the number of bands lacking in the i^{th} progeny but present in the j^{th} progeny. A dendogram was constructed from the matrix of similarity coefficients, using the Unweighted Pair-Group Method of the Mathematical Average (UPGMA), and genetic similarity/distance (GD) were calculated as [(1-Sij) X 100].

RESULTS AND DISCUSSIONS

AFLP marker technique was used on *Mus*a species and their hybrids to identify genetic diversity that will allow breeders to create complimentary breeding methods in the development of a genetic diversity/linkage map for crop improvement. Polymorphisms present in the parents and their hybrids using the three selective primers were demonstrated using AFLP techniques. AFLP, a novel PCR-based technique was used to identify multiple polymorphic bands in a denaturing gel using 3 of 12 primers tested.Out of 68 bands scored with a primer combination E-$_{AGC}$/M-$_{CTC}$, 14 (21%) were polymorphic. One hundred and one with 11 polymorphic bands (11%) with primer E-$_{AGC}$/M-$_{CTA}$, 116 fragments 26 polymorphic bands (22%) with primer E-$_{AGG}$/M-$_{CAT.}$ The 3 primer combinations used for the progenies and their parents that were screened in this study generated 285 fragments of which 59 (18%) were polymorphic from all the group of primer combinations and the average number of bands per assay was 95 (Table 2). A phenetic dendrogram of association generated with UPGMA clustering procedure defined 2 and 3 distinct clusters on specific selective individual nucleotides, and dendrogram of genetic similarity was obtained Figure 1, 2 and 3). Primer **E-$_{AGC}$/M-**$_{CTC}$ clearly defined the genotypes and their hybrids into 2 cluster groups at 0.55 phenol level. Cluster 1 includes Borneo which clustered at 0.66 phenol level and

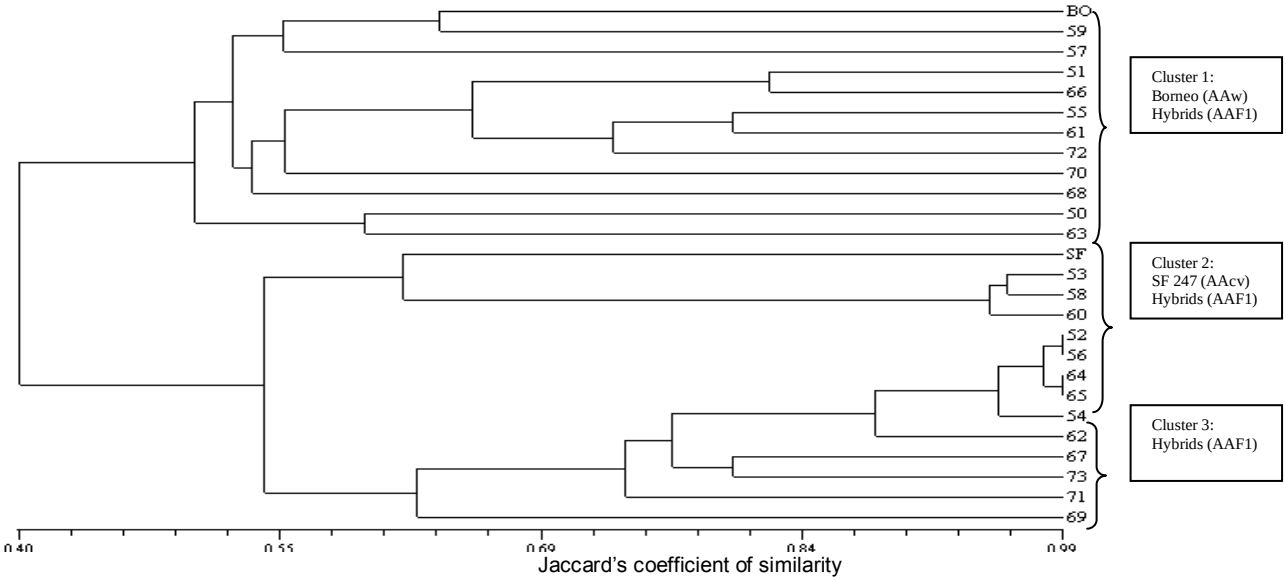

Figure 2: Phenetic dendrogram of Borneo, SF 247 and their hybrids obtained by unweighted pair-group method of mathematical averages (UPGMA) clustering of amplified fragment length polymorphism (AFLP) data with a selective primer combination **E**-$_{AGG}$/**M**-$_{CAT}$. Scale on bottom is Jaccard's coefficient of similarity.

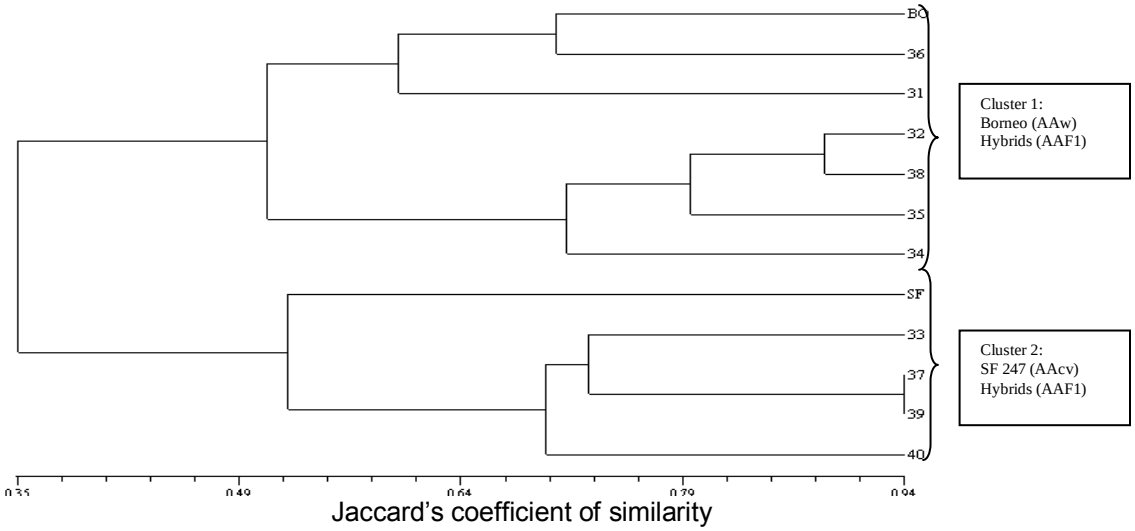

Figure 3: Phenetic dendrogram of Borneo, SF 247 and their hybrids obtained by unweighted pair-group method of mathematical averages (UPGMA) clustering of amplified fragment length polymorphism (AFLP) data with a selective primer combination **E**-$_{AGC}$/**M**-$_{CTA}$. Scale on bottom is Jaccard's coefficient of similarity.

the hybrids at 0.64, 0.76 and 0.78 phenol level (Figure 1). Based on Jaccard's similarity coefficients, SF 247 and Borneo at 0.53 phenol levels and their hybrids at 0.57, 0.69 and 0.70 phenol level. The dendrogram of similarity with primer **E**-$_{AGG}$/**M**-$_{CAT}$ from UPGMA analysis in (Figure 2) composed of 3 clusters at 0.55 phenol level. Cluster 1 consists of Borneo and most of the hybrids in that group. Cluster 2 compost of SF 247 and only 3 hybrids, cluster 3 includes only the hybrids diverged at 0.64, 0.73, 0.75 and up to 0.96 from SF 247 respectively. Consequently, the dendrogram of similarity with primer **E**-$_{AGC}$/**M**-$_{CTA}$ (Figure 3) gave 2 distinct clustering patterns at 0.51 phenol level. Cluster 1 includes Borneo (female parent) with some of the hybrids; cluster 2 includes the male parent (SF 247),

and their hybrids.

CONCLUSION

The major requirement for plant improvement is the availability of genetic variation in utilizable form. Two parent genotypes of *Musa* species Borneo (female parent) and SF 247 (male parent) AA group digested using
standard DNA extraction procedure and AFLP techniques, and was analysed pheneticlly using three primer combinations. Genetic data was subjected to unweighted pair group methods of arithmetic averages (UPGMA). The results explained the level of genetic diversity and association within the progenies and the three primer combinations separated the two parents, and this was consistent with genome classification with this group. One of the primary objectives of genetic diversity study in crop improvement is to unmask hidden traits and reveal relationship among cultivars for the basis of selection and further improvement. This results obtained from this study however gave a better understanding of relatedness and association among hybrids and their parental genotype and Jaccard's coefficient of similarity showed genetic variation among the hybrids and their parental genotypes (Borneo and SF 247). However, the use of AFLP and multivariate statistical techniques is a tool for determining parental contribution and relatedness among progenies. This indicates that the variables that are strongly associated in the same group may share some underlying biological relationship, and these relationship associations are often useful for generating hypothesis and understanding Behaviour of the association. The results from this study has confirmed based on the phonetic analysis that AFLP analysis is a powerful tool for determining the extent of morphological and genetic diversity and variation among or within crop species including characterization of breeding germplasm collections of*Musa* species and related field crops.

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