Papers

INTERNATIONAL SYMPOSIUM-CUM-WORKSHOP

Resource Management

Public-Private Partnership and Knowledge Sharing

Consortium of :
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August 27-31, 2001 at SEARCA, Los Baños, The Philippines

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IDENTIFICATION OF *Musa textilis* BY USING RAPD MARKERS*

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ABSTRACT

Musa textilis (abaca or Manila hemp) is a fiber producing plant, long time known being native to the Philippines. Its fiber has been used for many purposes i.e. for marine cordage, clothes, filters, document paper, meat casings, curtain stiffness. As a rich country of biodiversity, it is believed that the Indonesian archipelago is also a potential habitat of this plant, scattered out among many kinds of Musaceae. The plant identification and characterization for further development were carried out through assessing the patterns of DNA polymorphisms. The value of this RAPD approach is supported by the chromosome number and the morphological characteristics.

Five decamer primers (5'-3') were used to reveal the banding patterns of 35 numbers of plants collected from different places in Java and Sulawesi. A dendogram generated from a matrix of genetic distances demonstrated four distinct clusters (A, B, C, D) which were separated each other by more than 50% of the products differing. Evidences from the chromosome number, the plant morphology, and this genetic characteristic indicated that 13 plants confined in the cluster A are *Musa textilis*.

Keywords: *Musa textilis*, genetic characterization, RAPD markers

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Introduction

Wood is the principal material for pulp and paper. The increasing world demands for pulp and paper has driven to uncontrollable destruction of tropical forests, including those in Indonesia. Alternatives to reduce or to replace wood consumption in pulp and paper industries are indispensable.

The use of abaca (*Musa textilis* Nee) for paper was started as early as 1906 (Tabora, 1978). Thereafter many pulp and paper manufacturers in the USA conducted separate research studies on the dockyard wastes that bore the "Manila paper" which earned reputation as a paper of high strength. By 1975, more than 80% of the total abaca fiber produced in the Philippines went more to paper products, half of it from pulp, which completely turned a new episode in the industry.

The Indonesian archipelago is abundant in Musaceae, and it is believed that many varieties of abaca are scattered over the islands. This research aims ultimately to reveal the potentiality of abaca plants discovered in the Indonesian islands in view of developing some of them as material for pulp and paper in the future. This first step of study has an objective to identify the collected plants through random amplified polymorphic DNA (RAPD) patterns, which was supported by cytological and morphological observations. Plants grouping were resulted through a dendogram generated from the RAPD results.

Materials and Method

Plant material. Thirty-five *Musa* plants had been collected from Palu (Central Sulawesi), Malang and Banyuwangi (East Java) and Bogor (West Java). They were then planted in the university research field in Bogor to be used as the materials of observation.

DNA extraction. DNA was extracted by using modified method of Orozco-Castillo *et al.* (1994). About 0.2 g of young leaf pieces were ground with liquid nitrogen in a sterile mortar and pestle. While grinding, 0.02 g of PVPP and β-mercaptoethanol were added as antioxidant. The powder was transferred to an Eppendorf tube and resuspended in 1 mL extraction buffer (10% CTAB, 0.5 M EDTA (pH 8.0), 1 M *Tris*-HCl, (pH 8.0), 5 M NaCl). Samples were left for 15 minutes in room temperature, then incubated at 65° C for 30 minutes. To extract the DNA, the tube was added with equal volume of chloroform:isoamylalcohol (24:1) and subsequently shaked on vortex, centrifuged at 11 000 rpm for ten minutes. The supernatant was transferred to a new Eppendorf tube. This extraction procedure was repeated twice by adding once again one volume of

chloroform:isoamylalcohol. The DNA solution was precipitated with equal volume of isopropanol for 30 min. at 4° C and repeatedly centrifuged. The crude pellet was washed with 70% ethanol, dried at room temperature by carefully inverting the tubes on tissue papers and resuspended in 200 µL TE buffer.

DNA was quantified in agarose electrophoresis gels after ethidium bromide staining.

DNA amplification. Five primers were selected to generate DNA polymorphisms in *Musa* spp. All primers were 10-mer random oligonucleotide sequences (from Operon Technologies, USA and BRESATEC, Australia). The sequences (5' - 3') used were as follows: OPB 18 (5'-CCACAGCAGT-3'), OPC 15 (5'-GACGGATCAG-3'), OPD 08 (5'-GTGTGCCCCA-3'), ABI 117.17 (5'-GCTCGTCAAC-3'), and ABI 117.18 (5'-ACTCGTAGCC-3').

DNA amplification was performed in an Amplitron II Thermal Cycler in 25 µL reaction mixture containing 10 mM *Tris*-HCl (pH 8.0), 50 mM KCl. 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 10 pmol primer, 10 units *Taq*-polymerase (Promega) and 50 ng genomic DNA overlaid with paraffin oil. Following a preduell step at 94° C for 1 min., the amplification program was 45 cycles that consisted of denaturizing (1 min. at 49° C), annealing (1 min. at 37° C) and extension (2 min. at 72° C), followed by a final extension at 72° C for 4 min. Amplification products were separated by electrophoresis on 1.4% agarose gel in 1xTAE, stained with ethidium bromide and visualized under a UV transilluminator (312 nm). The images were documented with polaroid camera. One kb ladder DNA was taken as molecular size marker.

The data were analyzed with Numerical Taxonomy and Multivariate Analysis System (NTSSys) 1.8 version. A dendogram was generated by Unweight Pair-Group Method Average (UPGMA).

Chromosome counting was prepared by the method developed by Sharma and Sharma (1980) with slight modification. Carnoy's solution was utilized for fixing the root-tip and one to two drops of 2% aceto-orcein were added to the plant material for staining.

Results and Discussion

The five primers have revealed banding patterns that consisted of 1 to 11 products with molecular weight ranging from 0,25 to 3.0 kb. The 35 numbers of plants were scored for the presence or absence of the polymorphic bands; each of the five primers produced an average of eight polymorphic loci. A dendogram

displayed in Figure 1 was generated from a matrix of genetic distances resulted from NTSSys analysis.

Plants in the group A present similarities among them, ranging from 51 to 100%. Plant no. 48 joins the group A at 46.6% similarity and it stands in the group B. Plants no. 8 and 29 are separated from the groups A and B by only 32.4% similarities or 67.6% differences, while the rest are confined in a large group D with 25.3% of genetic distances from the other groups.

In order to obtain additional confirmation on the genetic level, chromosome counting was performed. Among the 34 numbers of plants (plant no. 42 died in the course of study), plants no. 4, 7, 9, 10, 34, 35, 36, 37, 38, 39, 40, 41, 44, 45, 46, 47, 49 are diploid with 2n = 20, while the rest possesses 16 to 44 chromosomes (see Table 1). The plant no. 41 has 40 chromosomes and was identified to be tetraploid. According to Purseglove (1983), *Musa textilis* is diploid plant with 2n = 20. In the study carried out by Chikmawati *et al.* (1998), *Musa balbisiana* 'Klutuk Wulung" gave a count of 22 chromosomes.

Table 1: Chromosome counts in the 35 no. of Musaceae plants

Chromosome no.	Plants no.
16	28
18	11, 18, 24, 29, 31
20	4, 7, 9, 10, 34, 35, 36, 37, 38, 39, 40, 44, 45, 46, 47, 49
22	1, 8, 13, 15, 16, 22, 26, 30, 33, 48
40	41
44	43

Morphological observations showed that plants belonged to the group A can easily be distinguished through the following characteristics: glossy pseudo stem, leaf sheaths are closely overlapping to form a compact tall pseudo stem; half of the leaf blades is shorter than the other at the base; flower bracts lay one after another to form scale-like heart shape. These common characteristics were also

described for abaca by Batugal and Tabora (1978). The most marked characteristics of the plants from the groups B, C, and D are among others waxy pseudo stem, petiole and leaf blades; both laminas are symmetrical, rounded or acute at the base; flower bracts are arranged completely cover one to another.

By synergizing the genetic and morphological characters, it was suggested that plants belonged to group A are abaca or *Musa textilis*. The plants no. 37 and 41 demonstrated exactly the same banding pattern so that they have 100% similarities. They perform similar morphology as well although the chromosome number of the plant no. 41 is twice (tetraploid, 2n = 40) as many as those in the plant no. 37 (diploid, 2n = 20). These evidences indicate that the plant no. 41 may be derived from the plant no. 37.

The three kinds of data support the fact that the plants no. 34, 38, and 40 are identical each other. This is true as well to the plants no. 46, 47 and 49. The plant no. 48 (group B) has 2n = 22 chromosomes with the most striking characters of morphology is the grey-purple-green colored and large-tall pseudo stem. It is likely to be classified in *Musa balbisiana*.

Conclusion

The overall results of this study conclude that the 35 numbers of plants tested consist of 31 different varieties, and only 13 of them (in A group) are confirmed to be abaca or *Musa textilis*. RAPD analysis may be used to quantify the extent of genetic diversity among plants, but a reconfirmation is necessary through the support of other observations, genetical and/or morphological. The banding profiles of individual variety can be used for clonal identification.

Literatures Cited

Batugal, PA and PC Tabora, Jr. 1978. The abaca plant – its morphology and physiology. The Abaca. Intern. Doc. Center on Abaca. UPLB. Laguna. The Philippines. 5-17.

Chikmawati, T, R. Megia, U. Widyastuti, and IN Farikhati. 1998. Kariotipe Musa acuminata 'Mas Jambe' dan M. balbisiana 'Klutuk Wulung'. Hayati, 5(2): 54-57.

Orozco-Castillo, K; J Chalmera; R Waugh; and W Powell. 1994. Detection of genetic diversity and selective gene introgression in coffee using RAPD marker. Theor. Appl. Genet., 89: 934-938.

Purseglove, JW. 1983. Tropical crops. Monocotyledons. ELBS and Longman Group Ltd.: 377-384.

Sharma, K and A Sharma. 1980. Chromosome Technique, Vol. I. Third ed. Butterworth. London.

Tabora, PC Jr. 1978. Historical background. The Abaca. Intern. Doc. Center on Abaca. UPLB, Laguna. The Philippines: 1-4.

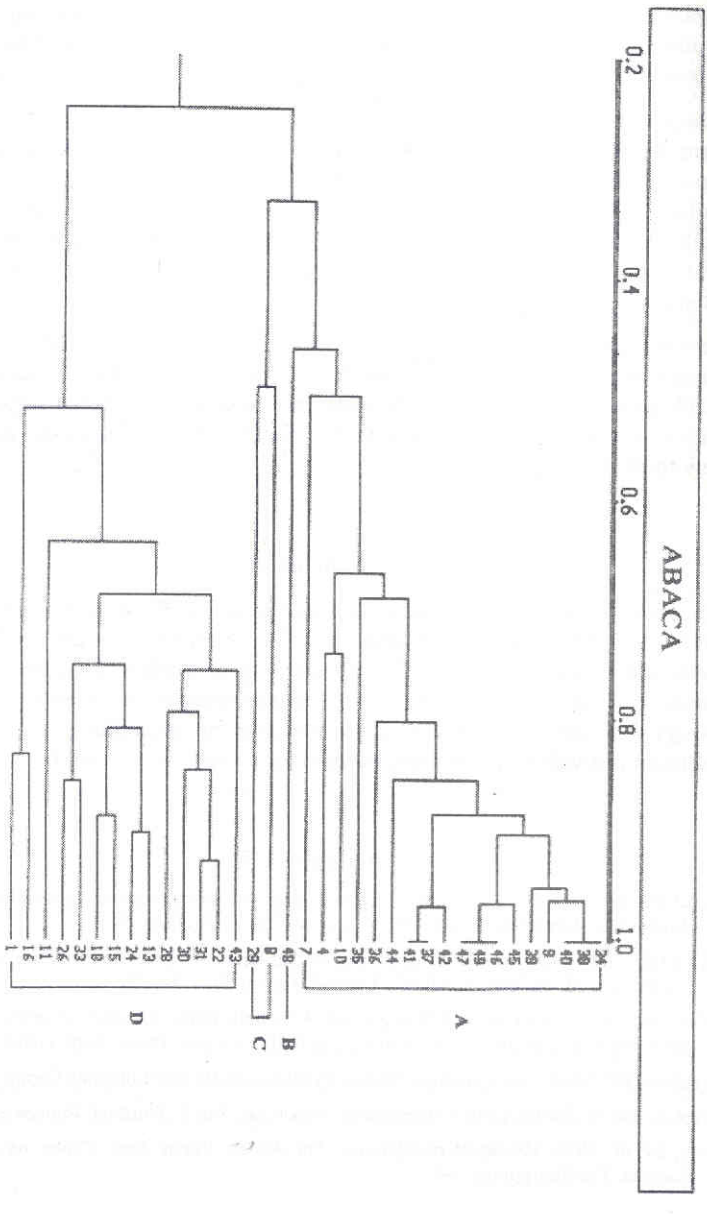

Figure 1. Dendogram of 35 plant no. of Musaceae generated by cluster analysis of RAPD data.