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Molecular Characterization of Banana (AA) Diploids with Contrasting Levels of Black and Yellow Sigatoka Resistance

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Abstract: Most banana cultivars are susceptible to many diseases, whereas Sigatoka leads to greatest yield losses. One of the strategies to overcome this disease is thorough banana genetic breeding which consists in the obtainment of improved (AA) diploids which are then crossed with triploids obtaining (AAAB) tetraploid disease resistant bananas also presenting other important agronomic characteristics. The prior knowledge of the genetic diversity of (AA) diploids, is therefore considered indispensable in order to direct the crosses being made. The objective of the present work was to analyze the genetic diversity of 20 (AA) banana diploids with contrasting levels of reaction to yellow and black Sigatoka caused by *Mycosphaerella musicola* and *M. fijensis*, respectively, using molecular markers. From the dendrogram data it is shown that a great number of experimental hybrids can be obtained from the combination of genetically different diploids, therefore making the banana genetic breeding program more efficient regarding its objectives.

Key words: *Musa acuminata*, *Mycosphaerella fijensis*, *Mycosphaerella musicola*, Molecular Markers, Sigatoka Resistance

INTRODUCTION

Most bananas that produce eatable fruits come from intra or interspecific crosses of the wild diploid species, *Musa acu*minata (A genome) and *Musa balbisiana* (B genome). Therefore, the cultivated varieties can present different genomic combinations: AA, AB, AAA, AAB, ABB, AAAA, AAAB, AABB and ABBB, diploids, tripods and tetraploids, depending on the basic number of chromosomes, two, three or four, respectively, being eleven, the basic number of chromosomes of the species[1].

The AA germplasm consists of many wild forms of the *Musa acuminata* species and in the cultivated varieties it is considered a source of genes of interest for banana genetic breeding. There are no cultivars of the group BB, BBB or BBBB, possibly due to the lack of parthenocarpy in the *M. balbisiana* species[2].

Banana crop is cultivated from northern to southern regions of the Country and practically all fruits produced are commercialized in the internal market, whereas the great majority of banana growers are made up of small farmers that use bananas as a source of income in their budget. The main available cultivars on the market are less productive; some are tall in height and the great majority susceptible to pests and diseases. Sigatoka disease has been considered one of the most devastating diseases in terms of yield loss of this crop[3].

The banana genetic breeding program, initiated at Embrapa Cassava and Fruit Crops in 1983, is based mainly on the improvement of (AA) diploids and subsequent crosses with AAB triploids Prata and Silk types generating AAAB tetraploids agronomically superior and resistant to diseases. The (AA) diploids are improved by crosses of selected parents for desired traits and that present fertile male and/or fertile female gametes, therefore obtaining improved diploid hybrids[4].

The use of molecular markers in the study of genetic diversity of banana diploids amply found in the literature[5-9]. The objective of the present work was to analyze the genetic diversity of 20 (AA) banana diploids to be used in the banana genetic breeding using RAPD (Random Amplified Polymorphic DNA) molecular markers.

MATERIALS AND METHODS

Genetic Material: Twenty (AA) banana diploids from the germplasm bank at Embrapa Cassava and Fruit Crops, presenting contrasting levels of yellow and black Sigatoka resistance were evaluated (Table 1).

DNA Extraction: The DNA was extracted from young banana leaves according to the methodology described by[10]. The samples were maintained in an ultra-freeze rat -80°C until the maceration with a mortar and pestle using liquid nitrogen until a fine powder was obtained. The DNA quality was checked in 0.8% agarose gel and the samples standardized to the concentration of 10 ng/ml to be used in the RAPD analysis.

DNA Amplification: The RAPD amplification reactions were carried out according to[11]. The reactions were completed to the final volume of 25mL, containing KCl 50 mm, Tris-HCl 10 mm (pH 8.3), $MgCl_2$ 2,4 mm, 100 mm of each dNTP´s (dATP, dTTP, dGTP, dCTP), 0,2 mm of primers (Operon Technologies, Alameda, CA-USA), 20 ng of DNA and one *Taq* polynerase Unit (Pharmacia Biotech, USA). The amplifications were carried out in the BioRad Nuclear Thermocycler with the following amplification conditions: initial denaturation step of the DNA strand at 95°C for 1 minute, followed by 45 cycles, each one consisting of denaturation at 94°C for 1 minute; primer pairing to the DNA strand at 35°C for one minute and fragment extension by *Taq* polymerase at 72°C for two minutes. After 45 cycles, the end of the extension was carried out for 7 minutes at 72°C. These fragments were amplified and separated by electrophoresis in 1.2% agarose gel, in TBE 1X (EDTA 2 mm and Tris-borate 90 mm), containing 0,5 ml/ml ethidium bromide. The samples were submitted to 90 v for approximately three hours and a half. The DNA bands were visualized under ultraviolet light and the images captured by the Kodak Digital Photo documentation System.

Molecular Data Evaluation: Only well visualized bands was used for the genetic divergence analyses. The molecular data evaluation followed the methodology of the Nei Li index, using the GENES program software[12] to obtain the distance matrix and the dendrogram was constructed by the statistics program[13] using the UPGMA (Unweighted Pair-Group Means Average) cluster analysis.

RESULTS AND DISCUSSION

A total of 150 polymorphic fragments were obtained by the amplification of 21 random primers. The genetic dissimilarity estimations were obtained by the Nei Li index and the groups observed by the dendrogram analysis (Fig. 1). The dendrogram analysis demonstrated that the smallest distance was observed between the 0323-03 and 4279-06 diploids, hybrids resistant to yellow and black Sigatoka. This distance between the hybrids is probably due to the fact that the Calcutta genotype was used as janitor in both hybrids.

Table 1: Contrasting Levels of Yellow and Black Sigatoka Resistance for Different (AA) Banana Diploids

(AA)-Banana Diploids	Black -Sigatoka Resistance	Black -Sigatoka Susceptibility	Yellow -Sigatoka Resistance	Yellow -Sigatoka Susceptibility
Calcutta	X		X	
Burmanica			X	
Microcarpa			X	
Raja Uter				X
Tjau Lagada				X
F2P2				X
Khai Nai On				X
Psang Berlin		X		X
Nyarma Yik		X		X
Sowmuk				X
Lidi	X		X	
Jari Buaya				X
0323-03	X		X	
SH32-63	X			X
1304-06	X		X	
1741-01			X	
9179-03			X	
0116-01	X		X	
1318-01			X	
4279-06			X	

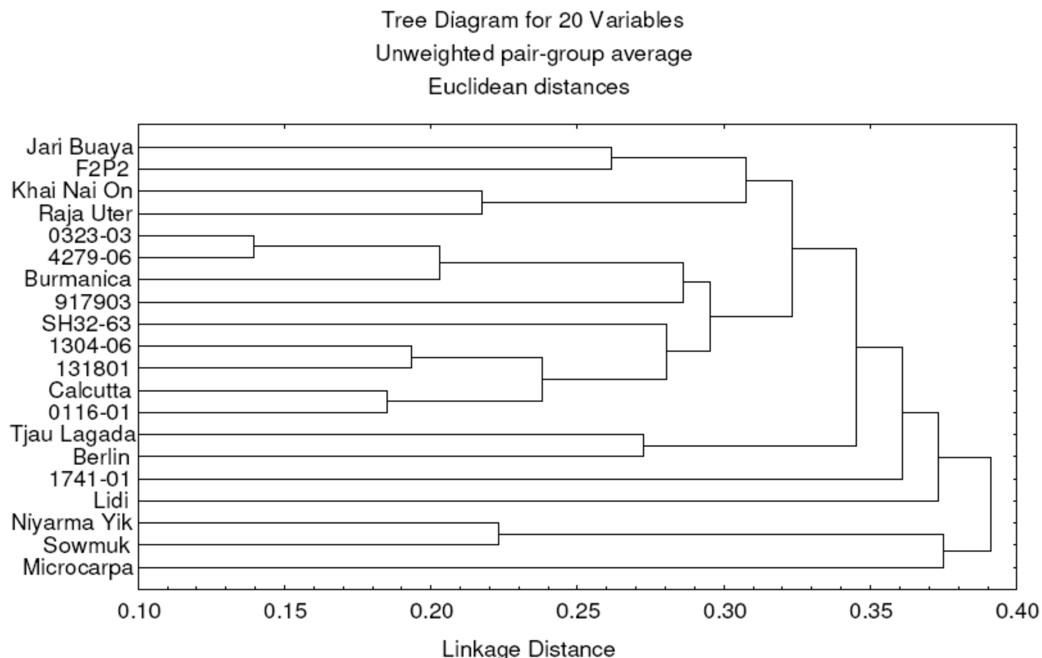

Fig. 1: Dendrogam Presenting the Genetic Distances of 20 (AA) Banana Diploids

In the hybrid 0323-03, Calcutta was used as the female janitor and in the 4279-06 hybrid, which corresponds to M 53 x (Tuu Gia x Calcutta), it participates as the male janitor. It was also observed that the individuals that presented the smallest genetic distances also presented similar reaction to Sigatoka; such as the hybrids 0323-03 and 4279-06, resistant to both sigatokas and Jari Buaya and F2P2, both susceptible to the disease.

The Microcarpa diploid, which presents greater genetic distance in relation to the Jari Buaya and F2P2 diploids, differs from these by presenting yellow Sigatoka resistance, whereas the other two are susceptible.

The literature regarding molecular markers in the characterization of natural banana hybrids is ample, demonstrating the importance of this study supporting the banana breeding program[8]. Studied the genetic diversity in *Musa acuminata*, *M. balbisiana* and other natural banana hybrids using AFLP (Amplified Fragment Length Polymorphism) technique, suggesting the existence of new relationships between subspecies inside *the M. acuminata* complex, different from those encountered only by the use of morphological data. Mitochondrial and Chloroplast DNA were also analyzed using RFLP (Restriction Fragment Length Polymorphism) in order to elucidate the center of domestication of parthenocarpic varieties. This kind of study, therefore complementing the morphological data, has been contributing for targeting.

This kind of study, therefore complementing the morphological data, has been contributing for targeting the main crosses being carried out in the banana breeding program aiming for the best possible combination of favorable alleles for important agronomical traits[5].

By the diversity data observed in the present work (Fig. 1), it is shown that a great number of experimental hybrids can be obtained from the combination of divergent diploids. An interesting cross would be between the Jari Buaya diploid, which presents nematode resistance with Microcarpa, for the banana breeding program aiming the obtainment of yellow Sigatoka and nematode resistance at the same time. Therefore, this analysis will contribute to a better understanding of the best cross combinations to be carried out in the banana genetic breeding program making it more efficient as to its main objectives.

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