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PCR AMPLIFICATION AND DNA FINGER PRINTING IN *MORUS ALBA* PLANT BY USING RAPD PRIMERS

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Mulberry (Genus *Morus*) is an economically important plant, as it serves as the only food crop for the domesticated silkworm *Bombyx mori L* that produces silk. Besides few species of mulberry are also valuable for their edible fruits *i.e. M. Alba, M. indica* and *M. laevigata* and *M. serrota.* The isolation of DNA from *Morus alba* plants and PCR analysis with RAPD primers were carried out. The result of the PCR experiments was checked by running a portion of the amplified reaction mixture in an agarose gel. A total of two primers like OPC-11 to OPC-20 and OPA-11 to OPA-20 series were investigated for Mulberry species, each of the random primers produced distinct polymorphic banding patterns in the species examined and showed the visible bands in lane 7,8 and 17 were clear and distinct. In the present investigation the results of rapid gel profile of M*orus alba* showed Polymorphic banding with amplification molecular primers like OPC-11 to OPC-20 and OPA-11 to OPA-20 series had become fundamental tools for RAPD technique for plant biologists.

Keywords : DNA finger printing; *Morus alba;* PCR amplification; RAPD primers.

Introduction

Species identification in mulberry (*Morus*) continues to be a point of great debate among scientists despite the number of criteria such as floral characters, wood, and leaf anatomical and biochemical characters were used to identify the species within this genus[1]. The low genetic similarity between the group of varieties originating from the eastern regions with that of the southern region encourages formation of extensive breeding programs between these groups as to transfer the high yield potential of the southern varieties to the low yielding but highly adaptive eastern varieties[2]. The RAPD and ISSR markers are useful for mulberry genetic diversity analysis and germplasm characterization[3]. Random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers were used to study the DNA polymorphism in elite blackgram genotypes[4].The mulberry varieties could be distinguished by their RAPD and DAMD profiles. As many as five RAPD primers and one DAMD primer generated profiles that can together differentiate all the nine varieties in terms of unique bands[5]. Further analysis, using a more sensitive DNA amplification method with designed primers flanking the species-diagnostic ISSR and RAPD markers, revealed that such sequences are not generally species-specific because they are present in other spruce species[6].

The present investigation showed that the selection of the molecular primers OPC–11 to OPC-20 and OPA-11 to OPA-20 series have become fundamental tools for RAPD technique.

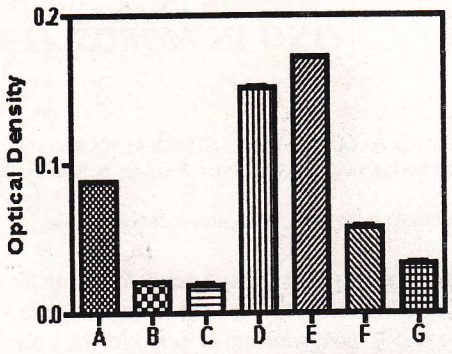
Materials and Methods

Plant material - The plant *Morus alba* was selected from the fields and orchards at Biotechnology Center, Hulimavu, Department of Horticulture, Bangalore, Karnataka, India.

Drying of plant material- The matured leaves of *Morus alba* were free from diseases and damages are available throughout the year was collected in brown paper covers for the isolation of DNA . Approximately 0.5-1.0 gm of fresh leaves were grinded to fine powder with a pestle and mortar after freezing in liquid nitrogen.

DNA extraction -The isolation of DNA from *Morus alba* plants was done according the method of Saghai-Maroof *et al.*[7]. And CTAB mini preparation of DNA extraction protocol was done according the method of Taylor and Powell[8].

PCR analysis with RAPD primers- Decamers from kits B, F, G and AP (Operon Technologies Inc., Alameda, California, USA) were used as primers. DNA was amplified essentially following Williams *et al.*[9]. Initially a pilot experiment was carried out varying primer, template DNA and Mg^{2+} ion concentrations. The final amplification reactions contained 10 mM TAPS (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.2 mM each dNTP, 10 picomoles primer, 0.6 U *Taq* DNA polymerase (Bangalore



A.Standard DNA B.Garuga C.Morus Alba D.Karuntaka E.Purple Convolvulus F.Wild Turmeric G.Coleus Forskohli

Fig. 1. Quantification of DNA yield in *Morus alba* with the standard comparison of other plants by using different primers of OPC-11 to OPC-20 and OPA-11 to OPA-20 series.

Easy 440 k UVT-20 S/W gel documentation system (Germany) after staining with ethidium bromide.

Results and Discussion

Randomly Amplified Polymorphic DNA (RAPD)- RAPD combines the polymerase reactions of PCR with non-specific DNA primers to amplify random DNA fragments. The randomly amplified polymorphic DNA (RAPD) technique uses two random primers of about 10 to 12 bases in length, each of which will hybridize at several locations in the genome. When the hybridization events occur close enough to one another to allow the polymerization reaction to proceed, the DNA segments lying between the primers are amplified.

Gel electrophoresis of PCR products- The result of the PCR experiments was checked by running a portion of the amplified reaction mixture in an agarose gel. The band representing the amplified DNA may be visible after ethidium bromide staining or if the DNA yield is low, the

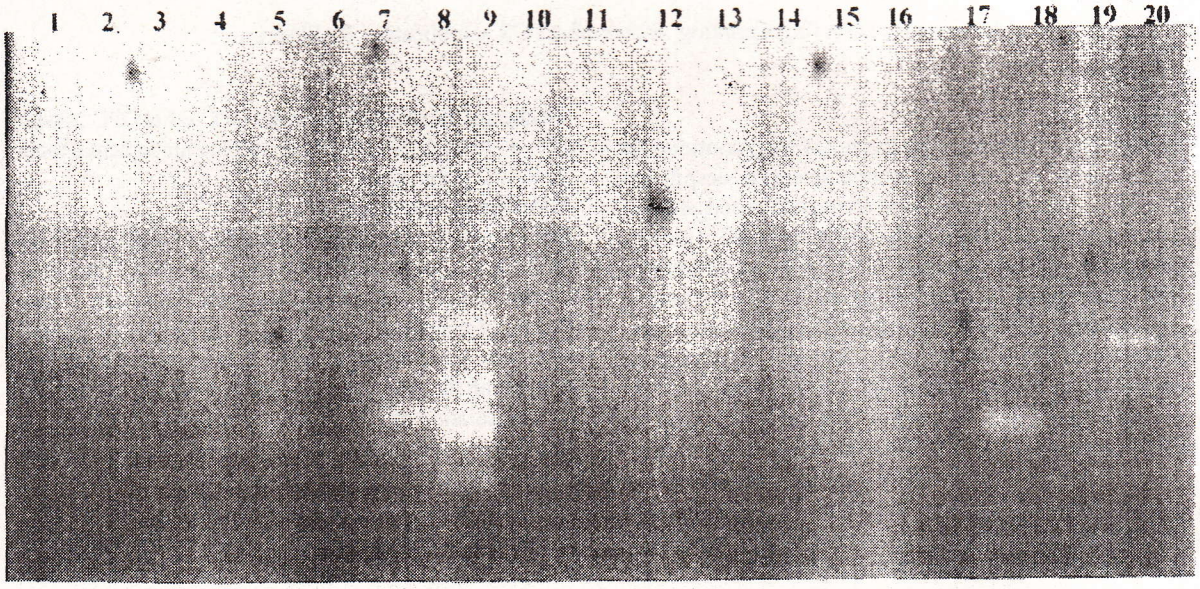


Fig.2 . Polymorphism generated by the primers OPC-11 to OPC-20 and OPA-11 to OPA-20 series

Genei, Bangalore,Karnataka, India) and 50 ng mulberry DNA template in a 25 µl reaction volume. The reaction was cycled 46 times at 94°C for 1 min, 35°C for 1.5 min and 72°C for 1.5 min in a thermal cycler (Eppendorf Master Cycler Gradient 96 wells, Germany). Additionally a final extension cycle allowed incubation for 5 min at 72°C. Amplification products were separated by electrophoresis (at constant current of 25 mA) through 1.0% agarose gels in 0.5X TBE buffer, visualized and imaged using Herolab

product can be detected by southern hybridization. Fig.1 showed the quantification of DNA yield was excellent in *Morus alba* with respect to the standard comparison of other plants by using different primers of OPC-11 to OPC-20 and OPA-11 to OPA-20 series.

A total of two primers were investigated for Mulberry species, each of the random primers produced distinct polymorphic banding patterns in the species examined. Typical results obtained with the primers OPC-

11 to OPC-20 and OPA-11 to OPA-20 series are shown in Fig.2 respectively.

The size of the amplified products ranged from 200-3000 bp with 3-4 bands per primer a total of 20 RAPD polymorphic markers were generated by the eprimers at a rate of 0.4 markers per primer.

The major strength of DNA markers is that they have a potential to reveal at almost unlimited number of polymorphism covering the whole genome. The DNA markers have very unique features like ubiquitous nature, detection at any developmental stage and independent of environmental effects and management practices and hence has direct applicability to breeding program. The most important application of DNA marker technology in the generation of saturated linkage maps, which have been extremely useful for mapping and tagging of genes of agronomically important traits.

A subset of the latter involves the use of a single arbitrary primer, which results in amplification of several discrete DNA products. The method is referred to as Random amplified polymorphic DNA (RAPD) analysis.

The present study reveals that PCR based fingerprinting techniques, RAPD, are informative for estimating the extent of genetic diversity as well as to determine the pattern of genetic relationships. The Genetic analysis with RAPD markers is more rapid, simpler and requires smaller amounts of DNA. Moreover, it does not require cloning of DNA or southern hybridization. RAPDs have been demonstrated to be well suited for use in plant and animal breeding program, population genetics and for estimating genetic diversity and relationship in Mulberry plant population or varieties[10-12].

The present investigation showed that the selection of molecular primers OPC –11 to OPC-20 and OPA-11 to OPA-20 series have become fundamental tools for RAPD technique for the plant biologists.

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