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A Preliminary Analysis of Asexual Genetic Variability in Mulberry as Revealed by ISSR Markers

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ABSTRACT

DNA markers have been proved to be important for the genetic study of mulberry germplasm characterization, crop improvement, and molecular systematics. Genetic variations of mulberry clones were analyzed by ISSR markers in this paper. A total of 40 fragments produced by 8 ISSR primers were obtained, of which only one variation was tested in the clones within field and tissue culture, suggesting that this result highlighted the genetic stability in the clonally propagated.

Key Words: Mulberry; Genetic variability; ISSR

INTRODUCTION

Mulberry belongs to the Moraceae family and to the genus *Morus*. It is a perennial tree or shrub as an economically important plant used for sericulture. To maintain desirable hereditary traits and prevent varieties retrogression, the plants are mainly propagated asexually in the field, for instance, grafting and cutting. Tissue culture (micropropagation) is also used as a subculture and *in vitro* conservation for mulberry germplasm. Clonal propagation may play an important role through the rapid and massive propagation of genetically superior individuals for sericultural production. So it is important for mulberry preservation and utilization to investigate genetic variation of clonal mulberry (Pan, 2000).

Many approaches have been reported for assessing genetic diversity in clonal plants, but only minor mutations occurred in the process of asexual reproduction, they are often morphologically indistinguishable. Molecular markers allow detection of genetic variation at the molecular level by the analysis of variation at the genomic level, as is the case for clonal variation. This analysis will give a better estimate of the genetic variability in a clonal population and will provide information that is highly valuable in the conservation and use of germplasm (Esselman *et al.*, 1999; Diógenes *et al.*, 2002; Gerardo *et al.*, 2003). Since 1994, a molecular marker technique called inter simple sequence repeat (ISSR) has been available (Zietkiewicz *et al.*, 1994). ISSR analysis involves PCR amplification of genomic DNA by a single primer 14 - 18 bp long composed of a repeated sequence anchored at the 3' or 5' end by 2 - 4 arbitrary nucleotides. ISSR markers have found wide applicability in a variety of plants and are quick and easy to handle (Zhao *et al.*, 2006). In this work, we studied preliminarily the genetic variability in a clonally propagated population of field and *in vitro* culture of mulberry using ISSR markers.

MATERIALS AND METHODS

Materials. Seven individuals of mulberry variety, Fengweiyizhilai, were sampled from mulberry field in the Zhejiang University. Twenty-seven of sibling lines of mulberry variety, Fengchisang, induced from the same callus during 15 generation subculture and *in vitro* conservation were also sampled and used for further analysis of genetic variation.

DNA extraction and ISSR analysis. Total DNA was extracted using the modified CTAB method (Doyle & Doyle, 1987). PCR reactions were carried out in a volume of 15 μL containing 10 ng total DNA, 1.0 × PCR buffer (200 mm Tris-HCL pH 8.4, 2.5 mm $MgCl_2$, 500 mm KCl), 0.25 mm of each dNTP, 6 pM of each primer and 1 unit of *Taq* DNA polymerase. PCR cycling conditions for all species (Flexigene thermal cycler) were: 2 min initial denaturation step (94°C), followed by 36 cycles of 40 s at 94°C, 45 s at 55°C and 90 s at 72°C, ending with a final extension step of 7 min at 72°C. DNA amplification fragments were separated in a 2.2% agarose gel at 90 V for 2 h in 1 × TBE buffer (100 mm Tris-borate, pH 8.0, 2 mm EDTA) and stained with ethidium bromide.

Data analysis. Only distinct, reproducible, well-resolved fragments were scored as present (1) or absent (0) for each of the ISSR markers. Dendrograms based on Nei's (1979) genetic distance, were constructed by cluster analysis based upon the un-weighed pair group method with arithmetical averages (UPGMA) using Popgene software (Nei and LI, 1979).

RESULTS

Genetic variability of clonal mulberry within field and tube seedling. From prescreening assays with 5 mulberry individuals using 15 ISSR primers, eight markers generated

bright amplification products and were selected for further analysis. Six primers failed to generate any amplification products and 1 generated weak or ambiguous amplification products, were discarded. Of the 8 ISSR primers tested, 7 [$(GTC)_6$, $(AG)_6TA$, $(CT)_6RC$, $(AC)_8T$, $(AC)_8YC$, $(GACA)_4$, $(GT)_6CC$] ISSR primers produced amplification products that were monomorphic within the population of mulberry field and tissue culture. Only using the primer $(GAG)_4$ GC, one polymorphic band was present within two populations (Fig. 1 2 & 3). Within the natural cultivated mulberry population, a total of 33 reliable fragments were obtained, one bands were polymorphic with a ploymorphismic ratio of 3.03%, while within the population of *in vitro* culture plant, 35 bands were amplified among 27 indivaduals with the polymorphic ratio of 2.89%, indicating the polymorphism detected by ISSR markers is very low in the populations of mulberry asexual reproduction.

Genetic variability between mulberry varieties. A total of 40 fragments produced by 8 ISSR primers were obtained between Fengweiyizhilai and Fengchisang. Of these, 13 bands were polymorphic (30.5%), indicating that between varieties had higher level polymorphism than within populations from the same mother plant. From the result of cluster, it is evident that individuals in a clonal population clustered together (Fig. 4).

DISCUSSION

DNA molecular markers can be used in assessing variation in natural and micropropagated populations as well as the variation existing among different species (Diógenes *et al.*, 2002). This analysis will give a better estimate of the genetic variability in a population and will provide information that is highly valuable in the conservation and use of germplasm. Genetic differences between clonally propagated plants have been found in other plants (Diógenes *et al.*, 2002; Gerardo *et al.*, 2003), which is usually propagated asexually. Also, no or little genetic variability was observed in the clonal population (Li *et al.*,2002; Li *et al.*,2004).

In this paper, the genetic variation of mulberry propagated clonally was analyzed for the first time.. The result showed that only one polymorphism was detected in two clonal populations, which affirms the genetic stability of the asexual propagation process and proven that the method of clonal reproduction can be used as resources preservation and seedling production. Despite its asexual reproductive mechanism, mulberry also presents minor genetic variability. This fact indicates the possibility of selection among individuals in a clonally propagated population and its use for an improvement program. In addition, whether genetic variability of clonal population is related to mulberry varieties, ecological environment conditions, population size and planting years or not, which will be investigated in a further study.

Fig. 1. All materials analyzed showed the same bands with primer (GTC) 6

Note: 1-27 represent tissue cultural clones; 28-34 represent mulberry clones from field. M is a DNA marker DL 2,000(2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp)

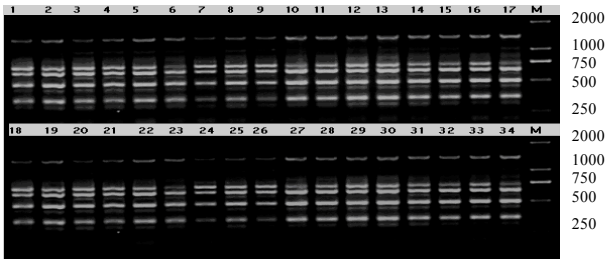


Fig. 2. Clones between field and tissue culture showed different bands with primer (CT)8RT

Note: 1-27 represent tissue cultural clones; 28-34 represent mulberry clones from field. M is a DNA marker DL 2,000

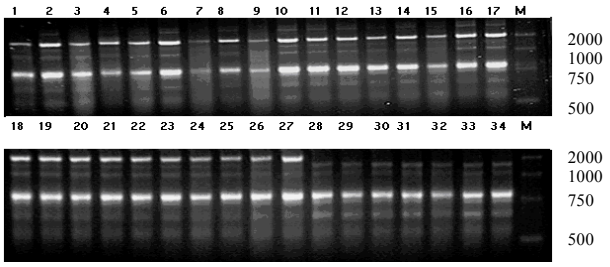


Fig.3 The special bands of clones of field and tissue culture were tested with primer $(GAG)_4$ GC

Note: A and B represent the polymorphic DNA fragments location as an arrow.

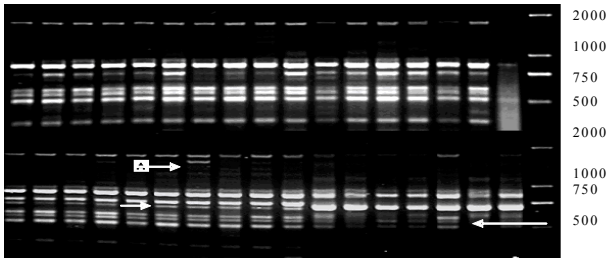
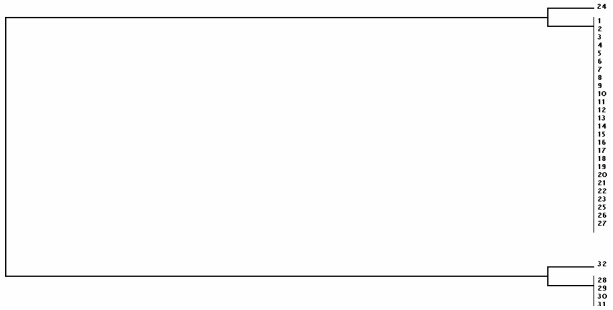


Fig. 4. A dendrogram obtained by UPGMA for 34 mulberry clones based on ISSR markers

Note: 1-27 represent tissue cultural clones; 28-34 represent mulberry clones from field.



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