Research Note

RAPD-fingerprinting to Identify *Eucalyptus grandis* Clones

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SYNOPSIS

DNA was isolated from 12 clones of *Eucalyptus grandis*, and subsequently amplified with arbitrary primers (RAPD-PCR). It was possible to distinguish between the different clones on the basis of the resultant fingerprints. We conclude that the RAPD technique is suited for the identification of *E. grandis* clones, and should also be useful in genetic studies.

INTRODUCTION

Clonal propagation of *Eucalyptus grandis* has become a major resource on which the South African forestry industry depends. The broad range of clonal variation found in *E. grandis* necessitates accurate identification of individual clones. Clones can be identified with morphological techniques, which can be regarded as slow, as certain characters take years to develop. In contrast, molecular techniques promise quick and accurate identifications. In this study we consider the potential use of Random Amplified Polymorphic DNA (RAPDs) in the identification of *E. grandis* clones.

Williams *et al.* (1990) introduced the RAPD technique as a tool for the construction of molecular genetic maps. This technique is based on the Polymerase Chain Reaction (PCR), but is unique in that arbitrarily chosen primers are used to randomly amplify DNA (Williams *et al.*, 1990; Waugh and Powell, 1992). DNA isolated from different individuals can be amplified in this manner. This results in DNA fragments of different sizes, each individual potentially having a unique set of different-sized DNA fragments (Waugh *et al.*, 1992).

The RAPD technique, while relatively new, has been used in population studies of a wide range of organisms including plants (Waugh *et al.*, 1992; Baird *et al.*, 1992; Klein-Lankhorst *et al.*, 1991; Martin *et al.*, 1991; Hu and Quiros, 1991; Devos and Gale, 1992; He *et al.*, 1992; Tulisieram *et al.*, 1992; Echt *et al.*, 1992; Prince and Tanksley, 1992; Struss *et al.*, 1992; Van Heusden and Bachmann, 1992), insects (Black *et al.*, 1992; Caswellchen *et al.*, 1992; Chapco *et al.*, 1992), and microbes (Guthrie *et al.*, 1992; Khush *et al.*, 1992; Mazurier *et al.*, 1992; Mazurier and Wernars, 1992; McMillin and Muldrow, 1992; Paran *et al.*, 1991; Reiter *et al.*, 1992; Schraft and Untermann, 1991; Sellstedt *et al.*, 1992). RAPD assay has also been used for the detection and mapping of polymorphic DNA markers in tomato (Klein-Lankhorst *et al.*, 1991), segregation analysis in diploid cultivated alfalfa (Echt *et al.*, 1992), and also for genetic linkage mapping of a single spruce tree using haploid DNA from individual seeds (Tulsieram *et al.*, 1992). These applications will pave the way for the use of the RAPD technique in genetic linkage, where DNA banding patterns could be linked, for example, to disease resistance (Klein-Lankhorst *et al.*, 1991).

The RAPD technique is currently being used to distinguish between individuals in populations of many different organisms. In this sense, it is already proving to be most valuable in many sectors of agriculture. The aim of this study was therefore to look at the usefulness of this technique for the identification of *E. grandis* clones of importance to the South African forestry industry.

MATERIALS AND METHODS

DNA isolation

Young leaves from 12 *E. grandis* clones were washed overnight in a 1:1 solution of Dimethylsulphoxide and Acetone. The leaves were then freeze-dried and crushed with a mortar and pestle in the presence of liquid nitrogen. Ten milligrams of fragmented leaf material was then incubated with Pronase (2 mg/ml) for four hours at 37°C. A further 1 mg/ml Pronase was added and allowed to incubate a further four hours. One millilitre Guanidinium thiocyanate buffer (4 M Guanidinium thiocyanate, 0,1 M TRIS-HCl and 1 % β-mercaptoethanol) was added. This mixture was maintained at 4 °C for 1 h. The cellular debris was pelleted by centrifugation for 20 min. at 8 000 revolutions/min. The supernatant was retained and

treated three times with Phenol-chloroform and twice with chloroform. After each addition of Phenol-chloroform, samples were heated to 65 °C and thoroughly mixed before centrifugation (5 min. at 8 000 revolutions/min.) The nucleic acid was then precipitated with 0,1 vol. 3 M Sodium-acetate and 0,6 vol. Isopropanol for 1 h at 4 °C. The nucleic acid was pelleted by centrifugation for 5 min. at 8 000 revolutions/min. The pellet was washed in 70 % Ethanol and resuspended in sterile water.

RAPD-PCR

Isolated DNA was then used in a RAPD-PCR reaction using two different oligonucleotides, 15 bases in size. The sequences of the primers were 5'-CGTCGTCCAGAGGTT-3', and 5'-CATGTGTGGCGGGCA-3' respectively.

Amplification reactions were performed in 50 µl volumes containing 5 µl 10x Buffer (Promega Corporation, Madison, USA); 3 mM $MgCl_2$ (Promega); 160 µM each of dATP, dCTP, dGTP, dTTP; 2 µM of primer; 7 µM DNA; 1,5 units of *Taq* Polymerase (Promega). The PCR reactions were performed in 0,5 ml Eppendorf tubes overlaid with mineral oil.

A Hybaid Thermal Reactor (Model No. HB-TR1) thermal cycler (Hybaid Ltd., Middlesex, UK) was used for the amplification reactions. The DNA was first denatured for 5 min. at 96 °C. The reaction was then cooled to 77 °C, at which point the Taq Polymerase was added. Then 35 cycles of; annealing at 34 °C for 1 min., elongation at 72 °C for 2 min. (ramp of 1,5 sec./°C), and denaturation at 92 °C for 15 sec. followed. A final elongation step at 72 °C for 10 min. ensured complete amplification of the fragments.

The DNA fragments that resulted from amplification were separated on a 1 % Agarose gel for 3 h with 80 V direct current. DNA bands were visualised, after Ethidium-Bromide staining, in the presence of UV light.

RESULTS AND DISCUSSION

DNA was successfully isolated from all 12 clones of *E. grandis* studied. Fingerprints resulting from electrophoresis of amplified DNA all revealed similarities and differences amongst 10 of the clones (*Figure 1*). Bands unique to each individual clone were present, and these could thus be used to characterise the clones. These results are consistent with those found by other researchers working with bacterial strains (Mazurier *et al.*, 1992; Mazurier and Wernars, 1992; McMillin and Muldrow, 1992; Schraft and Untermann, 1991; Sellstedt *et al.*, 1992), plant lines (Hu and Quiros, 1991; Baird *et al.*, 1992; He *et al.*, 1992), and even with nematodes (Caswellchen *et*

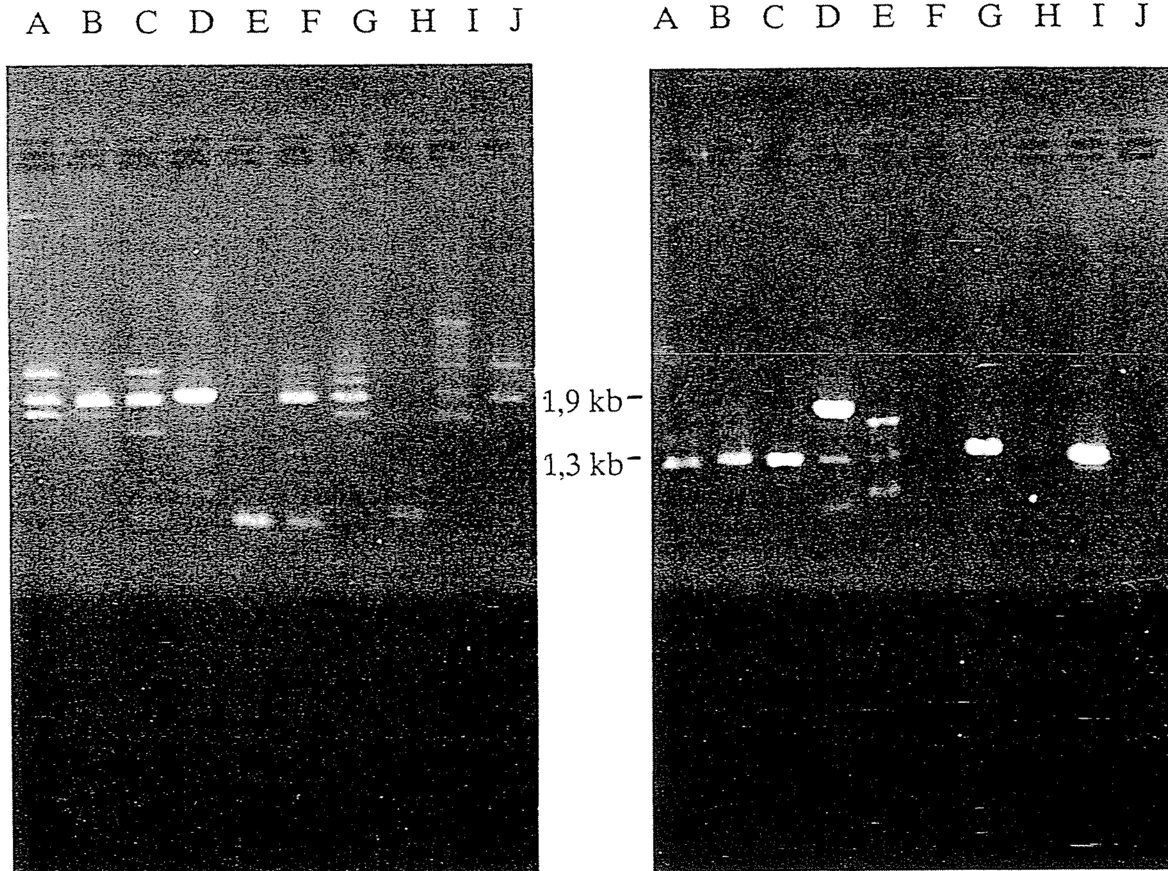

FIGURE 1. Two 1 % agarose gels. The RAPD-PCR products were loaded in lanes A to J of each gel. Each lane contains the PCR-RAPD product from amplification of DNA from 10 different clones of E. grandis. Each gel contains the results from RAPD reactions with a different primer. Molecular sizes are given in kilo-base pairs (kb).

al., 1992) and insects (Chapco *et al.*, 1992).

Two primers of 15 bases (15 mer) in length were used in this study. Being different in base composition, each primer was responsible for a unique fingerprint with each individual clone amplified. The unique banding patterns associated with each primer implies that with the correct primer, it would be possible to produce specific genetic markers. This would enable investigators to identify clones with certain traits, such as disease resistance or susceptibility, in a very short time.

Using arbitrary primers it should be possible to reveal genotype relationships. This was the case when Crawford *et al.* (1993) used RAPDs to indicate *Acaena argentea* and *Margyricarpus digynus* as the parents of *Margyracaena skottsbergii*. This and other similar studies (Hedrick, 1992; Van Heusden and Bachman, 1992; Quiros *et al.*, 1993) all indicate the usefulness of the RAPD technique in studies concerning parentage and inheritance data.

In this preliminary study, we were able to identify both differences and similarities between different *E. grandis* clones using only two primers. Although we have shown that these primer-sequences can be used, we recognise that a substantially greater number of primers will be required for the reliable identification of a large number of clones, and for the subsequent marker selection for desirable traits such as disease resistance. There is clearly tremendous potential for the application of this and similar techniques to the *Eucalyptus* breeding and clonal programme of the South African forestry industry.

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