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ASSESSMENT AND DEVELOPMENT OF MICROARRAY-BASED DNA FINGERPRINTING IN *EUCALYPTUS GRANDIS* AND RELATED SPECIES

BY

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DECLARATION

I, the undersigned hereby declare that the thesis submitted herewith for the degree *Philosophiae Doctor* to the University of Pretoria, contains my own independent work and hitherto has not been submitted for any degree at any university or faculty.

Sabine Lezar

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PREFACE

Eucalyptus species are an essential component of commercial forest plantations in tropical and subtropical regions of the world. Major efforts are now directed towards breeding and selection of elite genotypes with tolerance to diseases such as Chrysoporthe canker caused by *Chrysoporthe austroafricana*. Currently, DNA-based genetic markers are the most widely used techniques for fingerprinting and identification of superior genotypes. These techniques, however, are limited by the resolution of the detection system, which commonly involves electrophoresis to separate fragments based on size. This limitation has prompted the investigation of microarray-based DNA fingerprinting presented in this thesis.

The first chapter of this thesis presents a review of literature regarding the current opportunities and challenges of microarray technology for disease resistance breeding in forest trees. This review is arranged in four sections. The first of these deals with the impact of disease on forest trees, with particular reference to *Chrysoporthe austroafricana*. This is followed by an overview of the DNA microarray technique. The third section is concerned mainly with the uses of microarray markers for genetic analysis of forest trees. The last section deals with the challenges of microarray data analysis and management.

Chapter Two of this thesis reports on the development and assessment of microarray-based DNA fingerprinting in *Eucalyptus grandis*. *Eucalyptus grandis*

clones were previously identified using random amplified polymorphic DNA (RAPD) and microsatellite markers. While these techniques are constrained by their reliance on gel or capillary electrophoresis, recently developed microarray technology is not constrained by these requirements and holds promise for parallel analysis of thousands of markers in plant genomes. The aim of the study was to generate a small genotyping array for *Eucalyptus* and to determine the usefulness of microarrays for fingerprinting a full-sib progeny of *Eucalyptus grandis*. For this purpose, we implemented the recently developed Diversity Array Technology (DArT) for *E. grandis*.

Chapter Three concerns the identification of molecular markers associated with *Chrysoporthe austroafricana* tolerance in *Eucalyptus* using bulk segregant analysis (BSA) and DarT. Currently, the most effective way to reduce the impact of Chrysoporthe canker on *Eucalyptus* trees is the selection of elite genotypes with best disease tolerance. These trees were selected by artificial inoculations with a virulent isolate of *Chr. austroafricana* and monitoring disease progress. However, trials to test disease tolerance of clonal hybrids using artificial inoculations are extremely time consuming. The work presented in this chapter was, therefore, designed to develop PCR markers converted from microarray markers for the fast and unambiguous screening of breeding stock for disease tolerance.

Chapter Four reports on the genome-wide fingerprinting of commerciallygrown *Eucalyptus* species and hybrids using DArT. The most widely used tools for

molecular diagnostics and genetic diversity studies in *Eucalyptus* are amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and microsatellite markers. Large volumes of information about these fingerprinting techniques are currently available. However, information pertaining to the efficiency of genome-wide fingerprinting of commercial species of *Eucalyptus* and hybrids using an AFLP-like complexity reduction method is not available yet.

This thesis presents a collection of studies conducted over four years that deal with microarray-based DNA fingerprinting in *Eucalyptus*. All studies were conducted independently and have been written as separate publishable units. Thus, some repetition between parts of chapters, which contain a progression of knowledge accumulated over a relatively long period of time, has been unavoidable. It is my sincere hope that the work presented in this thesis has contributed to our knowledge regarding the exploitation of DNA microarrays as a marker analysis method in *Eucalyptus* tree breeding programs.

SUMMARY

DNA microarray technology is a new and powerful technology that could substantially increase the speed of forest tree breeding programmes. This thesis represents a compilation of investigations that focus on the exploitation of DNA microarray technology for genetic marker analysis of *Eucalyptus* trees. The major focus of the studies presented in this thesis was on the assessment and development of microarray-based DNA fingerprinting in *Eucalyptus*.

A DNA chip for *Eucalyptus* was not available at start of the study. As a result of this study a 384-prototype chip was developed to evaluate the potential of microarrays for fingerprinting closely related *Eucalyptus* clones, species and hybrids. These studies show that microarrays are an efficient DNA marker technology for genome-wide fingerprinting of complex organisms for which no sequence data exist. However, cross-hybridisation and the lack of dedicated software products remain a challenge.

The 384-probe array developed in this study was subsequently employed for the detection of putative markers associated with tolerance to *Chrysoporthe austroafricana* in *Eucalyptus grandis*. Putative tolerance-associated markers were identified by bulk segregant analysis (BSA) and converted to cleaved amplified polymorphic sequence markers for further characterization in segregating *Eucalyptus* populations. BSA revealed a total of 109 scorable, polymorphic loci, of

which nine appeared to be associated with tolerance or susceptibility. Two DArT markers were converted to cleaved amplified polymorphic sequence (CAPS) markers, which discriminate susceptible and tolerant individuals. These PCR markers can be used for the rapid screening for disease tolerance in *Eucalyptus* planting and breeding stock.

The collection of studies included in this thesis demonstrated that DArT is an efficient DNA marker technology for genome-wide genotyping, particularly for application in less-studied plant genomes. Whole-genome profiling using DArT raises significant opportunities for tree breeding programmes and for future genome analysis of *Eucalyptus*.