

UniQuest Project No. 13955

Report Prepared for: Mr Malcolm Lamont and Mr Hamish Macdonald
Bio Adapt International Pty Ltd
981 Tamborine Oxenford Rd
Wongawallan QLD 4210

Subject: Proof of Polyploidy and Source Parent and Performance Profiling

Date: 6 June 2006

Report Prepared By: Dr Susanne Schmidt
Dr Harshi Gamage
Dr Andrew Lowe
Ms Judith Lowe
Mr Peter Prentis

Executive Summary

This report and associated research was commissioned by Bio Adapt International Pty Ltd (formerly Spontaneous Evolution Technology), to examine nuclear DNA content and growth characteristics of forestry plants modified by Arbortechnologies, using the intellectual property licensed by Star Biotechnology. Arbortechnologies have putatively developed a laboratory procedure which produced stable polyploid lines, and can be applied to a wide range of plant and tree species. The potential advantage of polyploid compared to diploid lines is higher growth rate, increased physiological performance and environmental resilience. To examine these claims, modified (putative polyploidy) and unmodified (diploid) lines of a range of tree species were subjected to three sets of scientific investigations:

1. Verification of polyploidy – a combination of flow cytometry and chromosome counts was used to verify the ploidy of clone compared to diploid lines.
2. Verification of genome stability – a total genomic marker, AFLP, was used to examine the genome stability of cloned individuals and genome differences between different cloned lines.
3. Quantification of growth and physiology – a range of techniques were used to compare the growth and physiology of polyploid clone lines with diploid parents, including: leaf morphology, leaf physiology, chlorophyll fluorescence, biochemical properties and plant growth.

Based on these data it appears that the polyploidisation process used by Arbortechnologies can increase the amount of nuclear DNA in cells of *Elaeocarpus* and *Paulownia* clones (the two species successfully tested), and is consistent with individuals experiencing a genome duplication or polyploidisation event.

However the process was found to produce variable results in some *Paulownia* lines, which experience a reduction in genomic content (for example P7 and P3).

Morphological changes in *Paulownia tomentosa* and *Agathis robusta* polyploid clone lines, such as gigantism of stomatal cells (known as a gigas affect), are consistent with changes expected following genome duplication.

All clone lines of the ten species tested, had extremely high genomic stability demonstrating that mass clonal production programs should result in phenotypically stable clone lines. However, most different clone lines tested had slightly divergent genotypes, indicating that all of the clone lines have resulted from an independent polyploidisation process. The different genotypes also signify that the polyploidisation process may create novel genetic variation.

Although most clone lines possess different genotypes, all closely resemble those of the parental lines from which they were synthesised.

Selected *Paulownia* and *Agathis* polyploid clone lines were morphologically different, exhibited better growth, had an increased photosynthetic rate and different biochemical properties than diploid parental lines. Specifically, clones had larger leaves and total leaf area per plant, thicker leaf blades, palisade mesophyll and epidermis, and larger stomata than their parents. Selected polyploid clone lines had increased growth rates compared to parental lines, and had higher, carboxylation, electron transport rate, chlorophyll content and nitrogen use efficiency than parental lines. In addition, growth measures of plant height increment and shoot biomass were higher for selected polyploid clones compared to diploid parental lines. Similarly, there was a trend for greater stem and leaf growth in polyploidy compared to diploid clone lines.

Taken together data from the genetic and physiological studies strongly suggest that the polyploidy clone lines have, as claimed by Arbortechnologies and Bio Adapt, undergone a successful polyploidisation process. Many of the physiological and genetic differences observed between parent and clone lines are consistent with those observed in previous studies comparing polyploid and diploid plants.

Introduction

1.1 Advanced breeding techniques in forestry

One of the fundamental problems associated with the development of many tree species for forestry is the time taken to produce a marketable crop. For instance, many commercial tree species have a rotation length in excess of 15-50 years and will often not produce a marketable crop for greater than 25 years. Although improvements in the rate of plant growth have been achieved through conventional tree breeding, this process is also extremely time consuming as most forest trees do not reach reproductive maturity before 8-20 years. In practical terms for tree improvement this means that it will take between 8-20 years for single breeding cycle and much longer before significant increases in growth are achieved. Consequently, there is a need to examine non-conventional breeding and genetic improvement practise which may speed up the rotation length and breeding cycle of important forest tree species.

Recently, accelerated breeding techniques have been developed which can substantially reduce the breeding cycle of some promising timber trees. These methods include flower induction techniques and genetic engineering. In the case of flower induction techniques a 20-year breeding cycle can be reduced to 16 years in loblolly pine by accelerating flowering in the greenhouse. While this holds promise to reduce the breeding cycle of some forest trees it will not speed up the rotation length of these species. Genetic engineering has received a considerable amount of attention but few examples of forest tree application are available. In fact it is unlikely that genetic engineering will result in a significantly improved growth rate for many forestry species, but it holds promise in transferring pest resistance into susceptible species. One overlooked technique that holds great promise to accelerate the growth rate of forest tree species is the induction of polyploidy or genome doubling.

The induction of polyploidy has been a very important step in improving the growth rate of many crop species and holds great promise for improving growth rates for forest tree species. Almost all currently cultivated crop species are either newly synthesized or ancient polyploids, and in the case of some newly synthesized polyploids there has been an increase in vigor and growth rate associated with genome doubling. Therefore, the induction of polyploidy in conjunction with flower induction has the potential to decrease both the breeding cycle and rotation length of forest trees.

1.2 Background to polyploidy

Polyploidy, the process of genome doubling, has been and continues to be a pervasive force in plant evolution (Abbott and Lowe 2004, Adams and Wendel 2005). Most modern plant genomes harbor evidence of multiple rounds of past polyploidisation events. Although we don't fully understand why polyploids are so successful (Osborn in press), the prominence of polyploidy in flowering plants is possibly due to extensive modifications of the genome and/or

transcriptome, creating cascades of novel expression patterns, regulatory interactions and new phenotypic variation. Consequently, polyploid plants tend to be more successful than their diploid relatives often having larger distributions, habitat amplitude, and colonizing ability (Rothera and Davy 1986). Furthermore, novel changes to the genome or transcriptome allow polyploids flexibility to grow in a greater range of environments, have increased tolerance to drought and resistance to pests than their diploid progenitors (Stebbins, 1971, Levin 1983, Ramsey and Schemske 2002). The advantages of polyploids have seen many selected for use in agriculture and horticulture over their diploid progenitors (Ezura *et.al* 1993, Ortiz and Vuylsteke 1995, Osborn in press).

1.3 Aims and Objectives

Verify ploidy of clones lines – a combination of flow cytometry and chromosome counts was used to verify ploidy of clone compared to diploid lines.

Verify genome stability – a total genomic marker, AFLP, was used to examine genome stability within cloned individuals and between different cloned lines.

Quantity growth and physiology – a range of techniques were used to compare the growth and physiology of polyploid clone lines with diploid parents, including: leaf morphology, leaf physiology, chlorophyll fluorescence, biochemical properties and plant growth.