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UniQuest Project No. **13955**

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Subject: **Proof of Polyploidy and Source Parent and Performance Profiling**

Date: **10 March 2006**

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Signed for and on behalf of UniQuest Pty Limited

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Dr Andrew Lowe

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1. INTRODUCTION

This is an interim report produced after 3 months of a 6 month project to examine the ploidy level, genomic stability and growth performance of a number of artificially synthesised plant (which are putative polyploids) for a range of tree species by Spontaneous Evolution Technologies. Sufficient growth material has been received and all protocols have now been optimised and we expect that a full set of data, as described in the contract will be produced in time for a final report for the end of the contract. Delivery of this final outcome depends on continued provision of material and cooperation from SET.

2. BACKGROUND

In order to determine how ploidy levels affect plant growth characteristics, plant physiology and morphology were investigated. We are currently examining plant growth and leaf properties of diploid (parent) and modified *Paulownia tomentosa* seedlings, as well as *Agathis robusta* seedlings. Seedlings were received from SET when they were approximately 10 cm tall, and have been grown since October and February, respectively, in a naturally lit glasshouse. The details on the methods will be provided in the final report.

3. REPORT BODY

3.1 Studies of plant physiology

We are in the process of completing measurements of plant morphology (height growth, stem diameter, number of branches, total biomass, leaf area) and plant physiology (net photosynthesis rate, transpiration rate, stomatal conductance, chlorophyll a fluorescence, and chlorophyll content).

Two sets of results for *Paulownia* are presented in the graphs below. The Fig. 1 compares height increments of parent plants (K1) and clones (K3) during growth for 8 weeks from October to December 2005. Bars represent means and standard errors of means of 3 plants for parent and clones. The data show that clones had on average a 39% greater height increment than parents. These data are statistically significant at the $P<0.05$ level. Note that height increment indicates only tallness of the plants, not plant biomass; biomass information will be provided after destructive harvest.

The Fig. 2 shows, for the same parent and clones as in the Fig. 1, total leaf chlorophyll content on a leaf area basis. Significantly ($P<0.05$) higher chlorophyll levels were found

in leaves of the clone compared with the parent plant. The significance of the findings shown below for overall plant growth and plant physiology will be examined and put into context in the final report.

Fig. 1

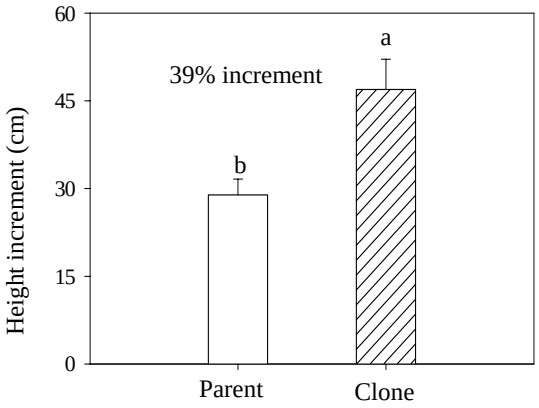
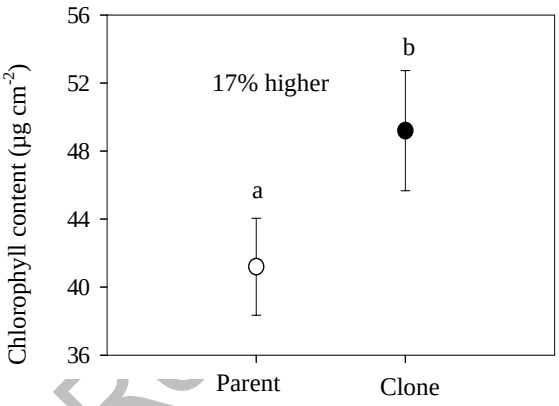
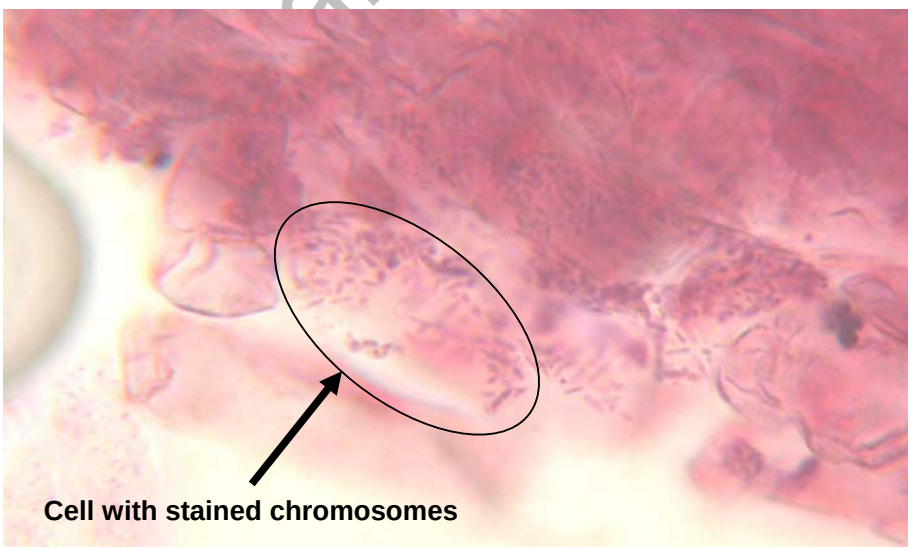


Fig. 2



3.2 Proof of ploidy

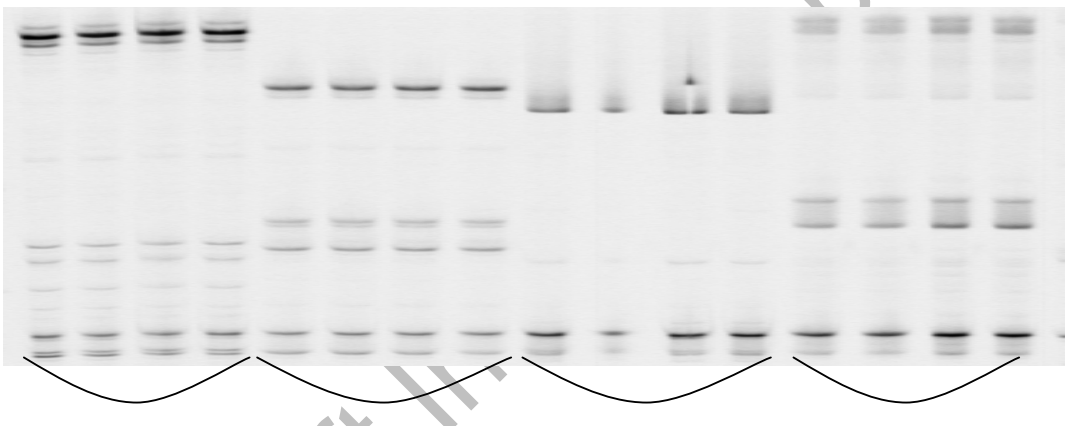
To verify multiplicated chromosome number, we are currently examining the ploidy of *Paulownia* clones using chromosome staining. Preliminary results depicting > 20 chromosomes (preliminary counts indicate n=40)/cell for clone line K2 are shown below and so appears to be a polyploid compared to the diploid chromosome number of n=20 for standard *Paulownia*. Further cell counts are being undertaken.



To verify multiplicated genome content, flow cytometry is currently being undertaken to determine the DNA content of cells in parents and clones of *Paulownia* and other species. This will allow high-throughput verification of polyploidy lines.

3.3 Source parent and genome stability

Genome stability and proof of parent in *Paulownia* clone lines are currently being verified using a total genome fingerprinting technique (Amplified Fragment Length Polymorphism). The image below depicts preliminary AFLP data showing high genome stability within clones lines (the four replicates of each line). However the results also indicate that there has been considerable genome reorganisation during the polyploidisation process, which has been seen in other studies. It appears therefore that each clone line is the product of a separate polyploidisation process.



4. CONCLUSION

Based on these preliminary data, the single *Paulownia* clone tested (K2) appears to be polyploid (probably tetraploid), and three clones screened (P3, P7 and P15), appear to have a stable genomic composition. However these different clones lines exhibit different genomic combinations, which is consistent with the fact they were separately synthesised. Also based on the preliminary, but unreplicated, growth experiments, the single putative polyploid *Paulownia* clone tested (K3) appears to have superior growth and greater chlorophyll content than the single unmodified, diploid parental line (K1).

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