Targeted analysis of differentially expressed transcripts in *EgrMYB103-SRDX* transgenic poplar

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INTRODUCTION

Poplar growth trial layout a month before harvesting.
Picture by Dr S.G Hussey.

- Plant secondary cell walls (SCWs) constitute the bulk of wood biomass. The biosynthesis of these walls is tightly regulated at the transcriptional level by two main transcription factor families; NAC and MYB[1,2].
- *MYB103* is required for normal secondary wall biosynthesis in *Arabidopsis* and its activation is regulated by *SND1*, a master switch regulating SCW biosynthesis[3].
- *MYB103* is involved in cellulose biosynthesis[3]. In another study mutant lines showed a decrease in *FERULATE-5-HYDROXYLASE* (*F5H*), a key gene in the synthesis of syringyl (S) lignin[4].
- SCW biosynthesis has been extensively studied in *Arabidopsis,* but little is known about the functions of *MYB103* in SCW formation in *Eucalyptus*, an important tree species for paper and pulp industry.

AIM

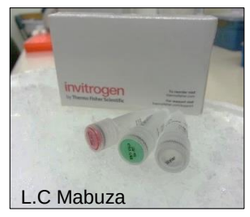
- Phenotype and identify differentially expressed SCW-associated genes affected by dominant repression of *Eucalyptus grandis MYB103* (*EgrMYB103-SRDX)* in hybrid poplar.

STRATEGY

Poplar growth trial (one year and four months).

Stem basal width measurements and harvesting poplar developing secondary xylem.

Optimisation of RNA extraction with Promega RNA isolation kit, CTAB protocol and Trizol reagent (Invitrogen)



cDNA synthesis and amplification of *EgrMYB103* and the hygromycin resistance gene (*HRG*).

Gene expression analysis of 168 poplar SCW gene targets with the Quantstudio open array plate (RT-qPCR)

RESULTS AND DISCUSSION

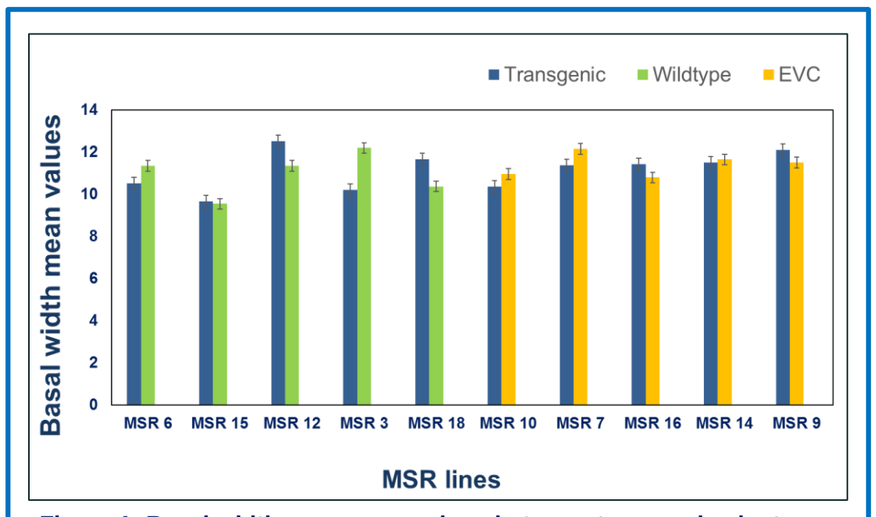


Figure 1: Basal width mean comparison between transgenic plants, wildtype and empty vector control (EVC).

- *EgrMYB103* dominant repression did not result in any visible change in phenotype.

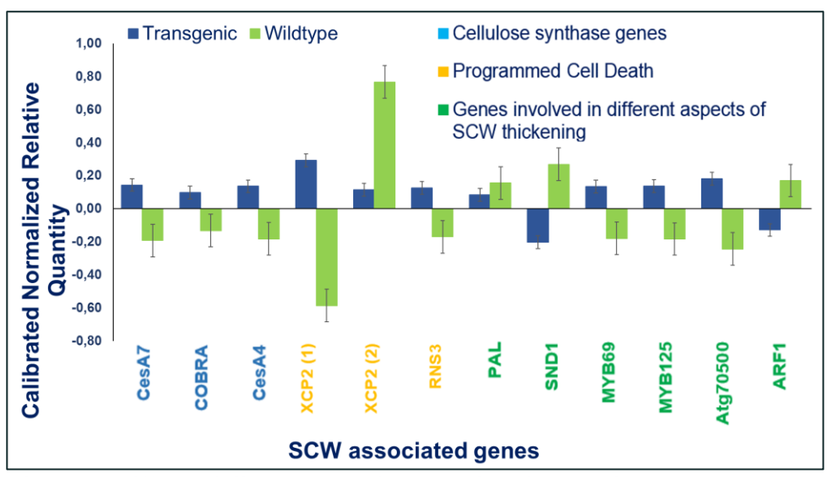


Figure 4: Differentially expressed SCW-related genes between MSR18 and the wildtype. Four biological replicates of MSR 18 line and three wildtypes were used for the open array plate. The Calibrated Normalized Relative Quantity was used to calculate the student t-test and genes with a p value <0.05 were plotted in this graph.

- *CesA4*, *CesA7* and *COBRA*, were upregulated while *SND1* was downregulated.
- This is to be expected if *EgrMYB103* provides negative feedback loop to *SND1*.
- The *XCP2* homologs were inconsistently differentially expressed.
- The lack of *F5H* differential expression could be a result of differing roles of *MYB103* in *Arabidopsis* as opposed to tree species.
- Could also be due to heterologous expression of an *E. grandis* transcription factor in a non-native species.

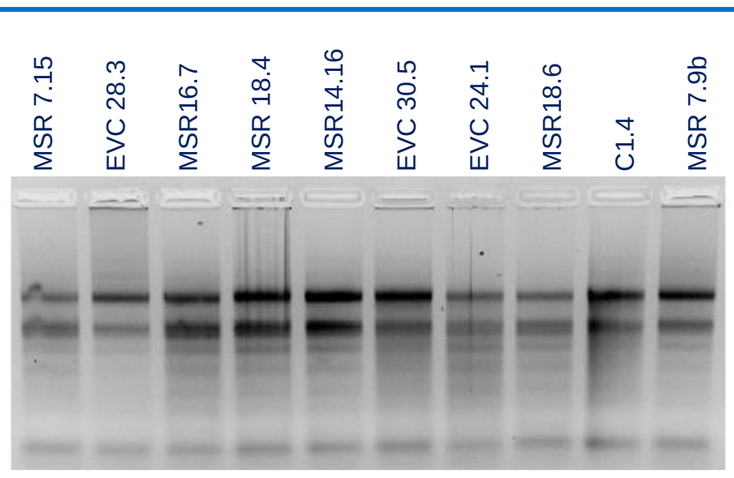


Figure 2: RNA Gel electrophoresis image of samples extracted with Trizol reagent (Invitrogen).

- RNA optimisation was achieved with Trizol reagent (Invitrogen) after attempting the SV total RNA isolation system kit (Promega) and CTAB protocol.
- Half of the Trizol reagent (Invitrogen) extracted samples sent for Bioanalyzer analysis (Inqaba) had an RNA Integrity Number >7. The gel images of these samples were used as reference for subsequent RNA extractions

CONCLUSION

- The amplification of the hygromycin resistance gene in the wildtypes suggests that contamination is more likely the case than ortholog cross amplification. We are further investigating this with sequencing
- SCW genes were differentially expressed in an unexpected way. However, only one transgenic line was analysed so this needs more data.

REFERENCES

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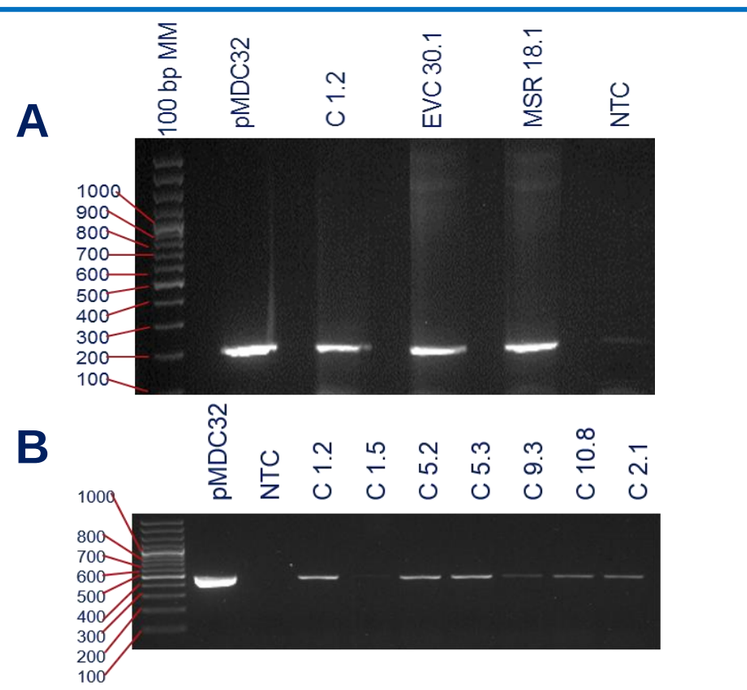

Figure 3: PCR gel electrophoresis images. (A) *EgrMYB103* transgene amplification in a transgenic plant (MSR), wildtype (C), empty vector control (EVC), pMDC32 (+ control) and NTC, no template - control. (B) Hygromycin resistance gene (HRG) amplification.

- The transgene amplified for the transgenic, the wildtype and EVC also showed amplification. It is possible the *MYB103* poplar ortholog was being amplified.
- To test this hypothesis, a gene that is part of the vector backbone, the hygromycin resistance gene, was amplified in the wildtype using cDNA as template. Amplification of this gene in the wildtype suggest possible contamination.

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