

The Functional Characterization of the *SLC* Gene in *Populus trichocarpa*, and its Potential Role in Lignin Biosynthesis

Tyler Irwin, Lan Tran, Jürgen Ehling

Department of Biology, Centre for Forest Biology, University of Victoria

Introduction

- Wood is composed largely of cellulose, hemicellulose, and lignin, a non-soluble phenolic polymer making up approximately 25% of its biomass.
- Lignin is an essential component for plant growth and defense; it impacts anthropogenic uses of wood such as paper, construction materials, and biofuels.
- Genome-wide association studies identified markers that were linked to lignin content in Poplars, among them the relatively unknown *SoLute Carrier (SLC)* gene (Porth *et al.*, 2012).
- SLC* belongs to the nitrate transporter superfamily (von Wittgenstein *et al.*, 2014), but its actual substrate is unknown.
- Members of this family may transport nitrate, peptides, hormones, small secondary metabolites, or they may serve other purposes such as transporting soluble phenolic compounds (including lignin precursors).
- Poplars are a model organism for studying tree biology, having a rapid growth rate, easy ability to transform, and a sequenced genome.
- Working hypothesis: *SLC* is a transporter gene involved with the movement of soluble phenolic compounds.**

Methods

- β -glucuronidase (*GUS*) staining performed on poplar and Arabidopsis that had *GUS* fused to the *SLC* promoter.
- Transgenic hairy roots were made with both an empty vector and *SLC* overexpressing construct by *Agrobacterium rhizogenes* transformations. GFP expression used as a reporter gene and reverse transcriptase PCR performed to confirm overexpression.
- 16 wild-type and *SLC* overexpressing poplars were generated from cuttings and grown in a greenhouse, then being treated with high (10 mM) or low (0.1 mM) NH_4NO_3 . Physiological differences were observed, with this experiment being repeated with a larger sample size of 97 poplars.
- HPLC used to analyze phenolic profiles from roots and leaves of *SLC* overexpressing or RNAi knock down trees, hairy root cultures, and poplars subjected to nitrogen depletion.

Results

SLC- Expression (Promoter::*GUS* Plants)

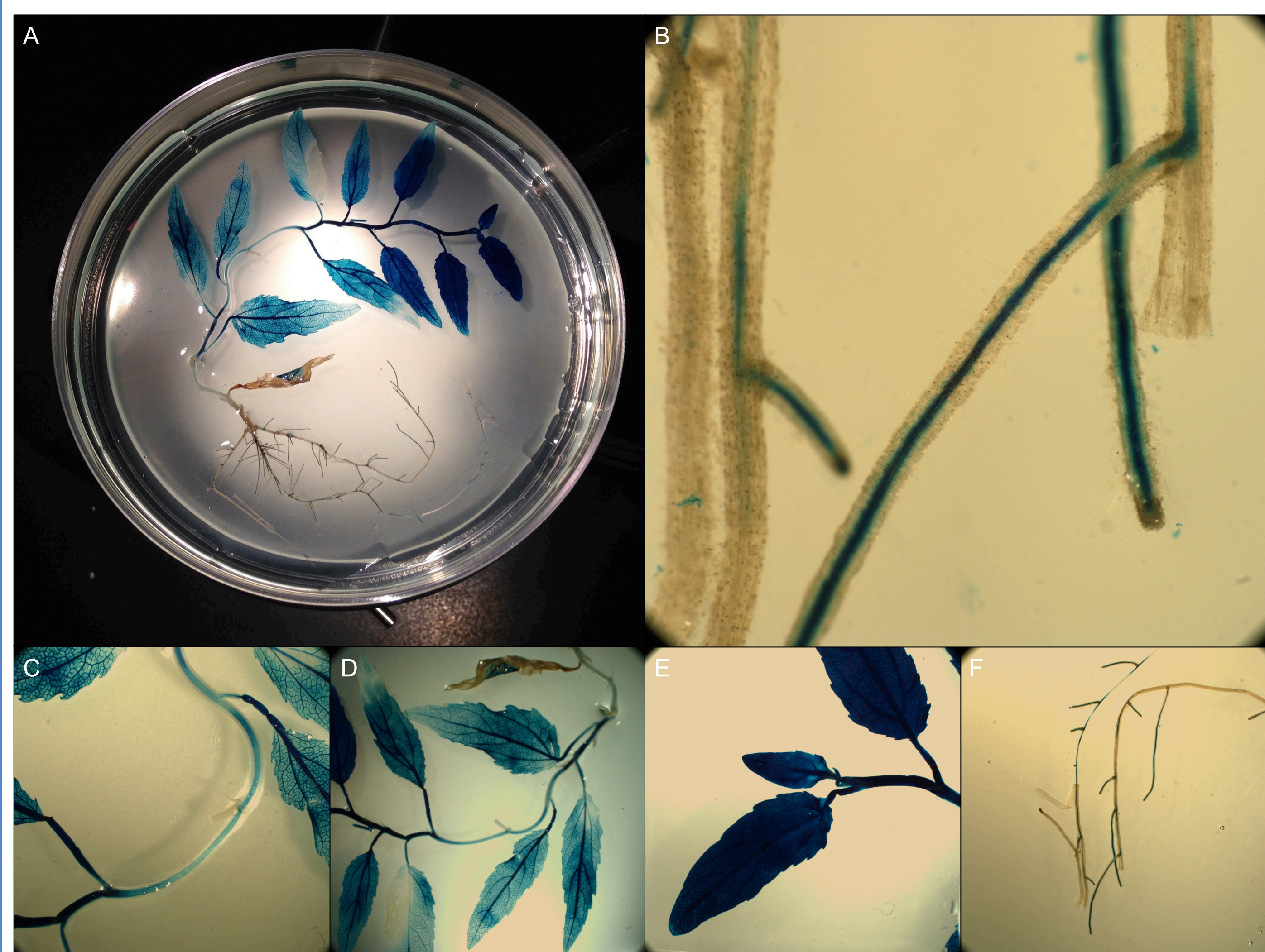


Figure 1. *GUS* staining results from transgenic three month old poplars, grown *in vitro* with the *GUS* gene fused to the *SLC* promoter, and stained with x-gluc. Shown in: a) whole mount of line # 5; b) close up of root tips; c) view of stem section; d) older leaves; e) younger leaves and apical meristem; f) larger area of roots.

- Expression of *SLC* is strongest in the vascular tissue.
- SLC* is more prevalent in younger, actively growing tissues.

Hairy Root Transgenics: *SLC*-Overexpression

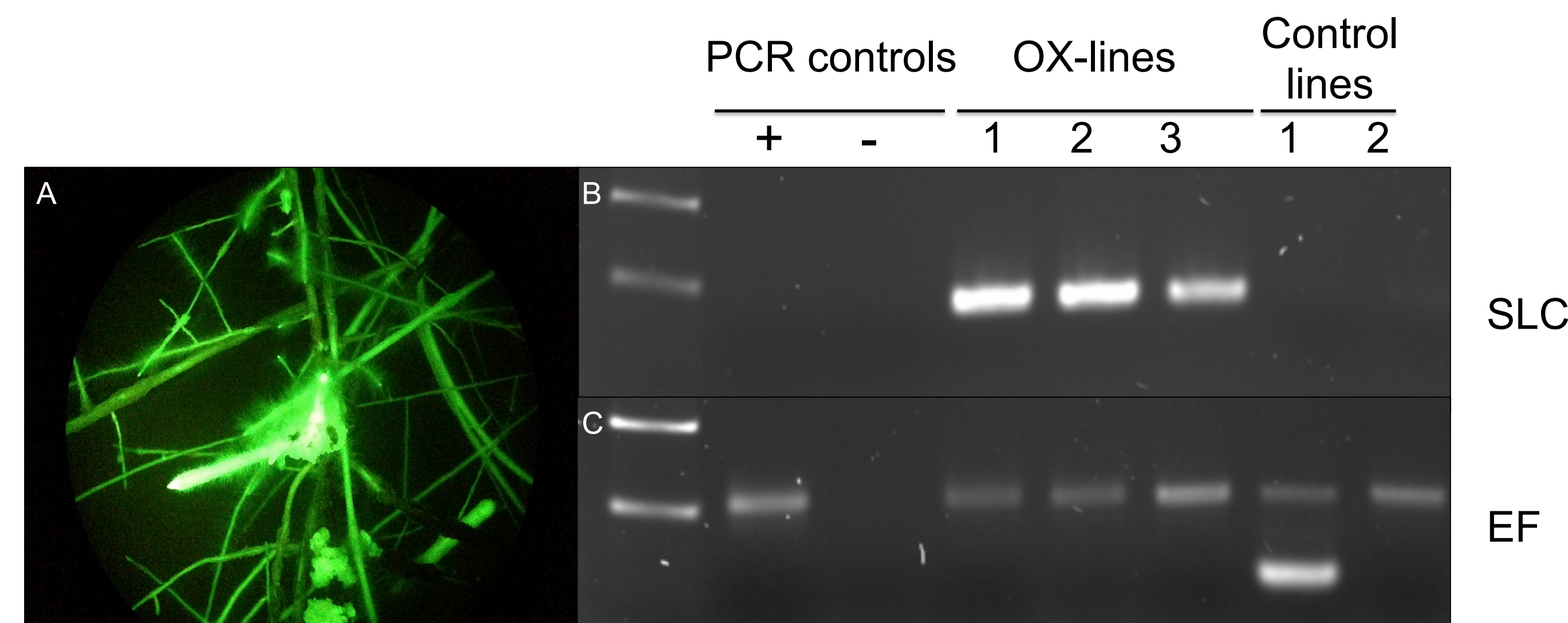


Figure 2. a) GFP expression in hairy roots with the 35S::*SLC* construct; b) RT-PCR using an *SLC* specific primer; c) RT-PCR using primers for the housekeeping gene elongation factor (*EF*). OX: overexpressing *SLC*; control lines: vector only transformed.

- Confirmed overexpression of *SLC* in hairy roots using RT-PCR

SLC-Overexpression: Nitrogen Response



Figure 3. Morphological differences from wild-type and *SLC* overexpressing poplars either treated with a high or low nitrogen treatment for 8 weeks. As seen in a) the two trees on the left and three on the right were administered high and low nitrogen, respectively; b) clockwise from top left: leaves from lines A8.5, A6.4, IF9, and wild-type, high nitrogen leaves on the left and low nitrogen leaves on the right; c) root architecture of trees treated with high and d) low nitrogen.

- Chlorosis in response to low nitrogen, but no difference in severity between *SLC* overexpressors and control plants.
- Higher density of roots in low nitrogen treated plants.

SLC-Overexpression: Nitrogen Response

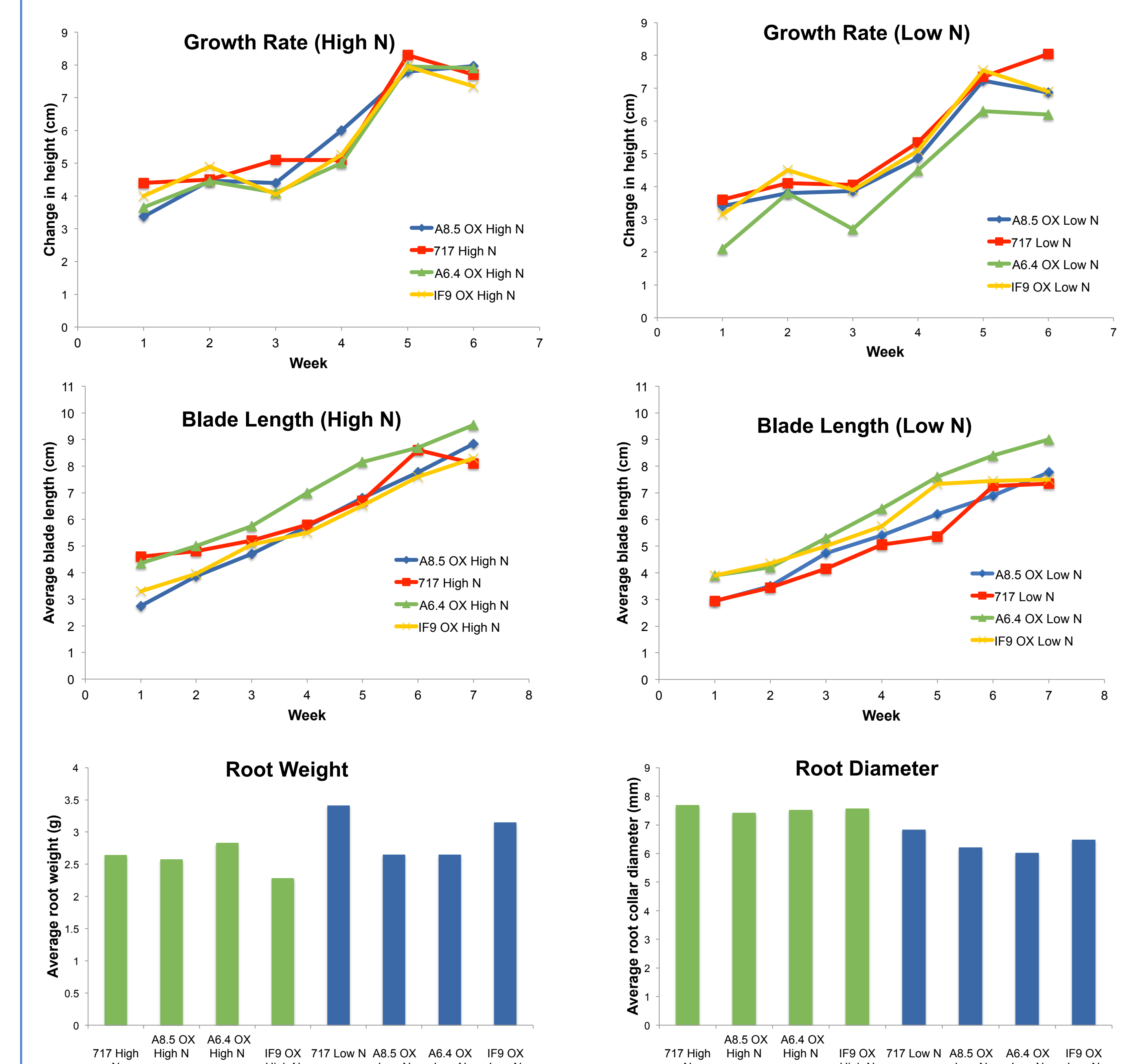


Figure 4. Results from nitrogen treatments on *SLC* overexpressing and wild type poplars grown in greenhouse (low nitrogen irrigation starting on week two). Clockwise from top left: average change in height (cm) when treated with high nitrogen, change in height (cm) when treated with low nitrogen, average blade lengths (cm) with low nitrogen, the average root collar diameter (mm) of trees under high or low nitrogen, the average dry root weight (g) of poplars receiving high or low nitrogen, and the average blade lengths (cm) of poplars with high nitrogen.

- No obvious differences are seen between lines being treated with either high or low N.
- Low nitrogen plants might have smaller root collar diameters and higher root mass.

SLC-Overexpression and Knockdown: Phenolic Profile

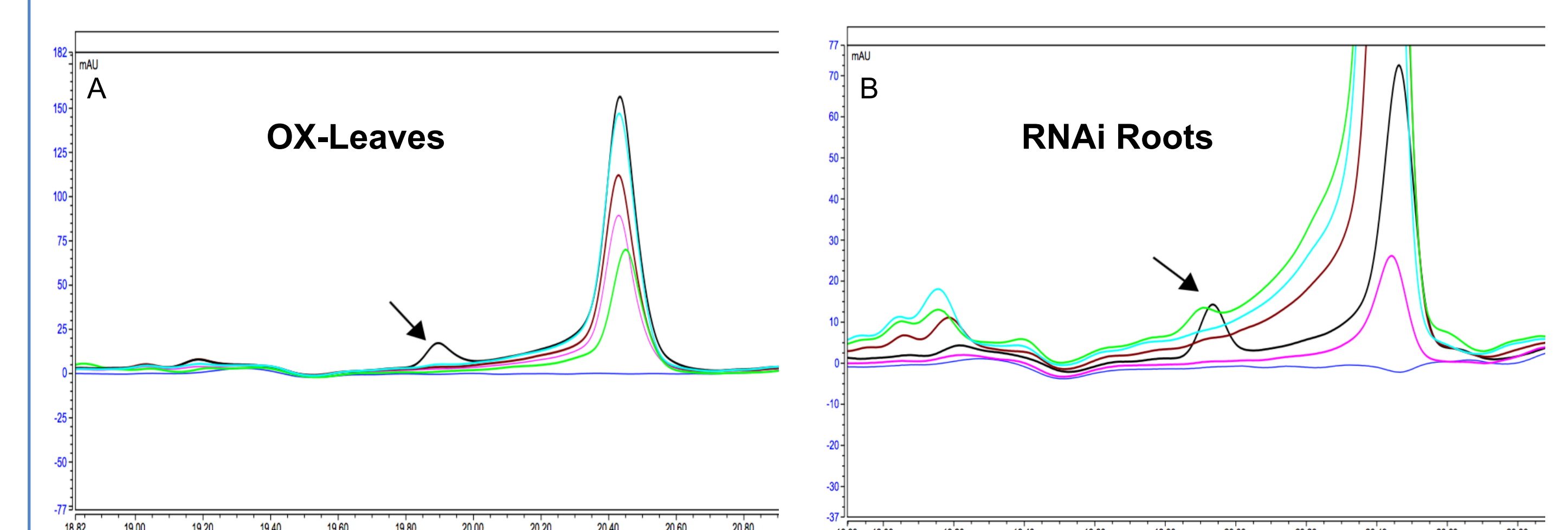


Figure 5. Chromatograms of HPLC data from soluble phenolic compounds of a) poplar leaves (overexpressing *SLC*) and b) poplar roots (*SLC* knockdown). Wild-type samples shown in black, all other colours representing transgenic samples.

- Possible differences in phenolic profiles apparent between wild-type and overexpressing lines.

Conclusions and Future Outlooks

- GUS* staining showed *SLC* localization in the vascular tissue of the plant, being seemingly stronger in younger tissues.
- Overexpression of *SLC* in hairy roots was shown through GFP expression and RT-PCR. Metabolic profiling will need to be done when there is sufficient tissue available.
- No obvious physiological differences can be seen in the nitrogen depletion experiment between transgenic and wild type lines, suggesting *SLC* is not a nitrate transporter, but this will be repeated with a larger sample size of 97 trees in order to have statistical significance.
- The *SLC* gene may be involved with soluble phenolic transport based on HPLC data, but further analysis is needed.