The overall project objective was to probe the relationship between sucrose transporters and plant productivity in the biomass for biofuels woody perennial, Populus. At the time the proposal was written, sucrose transporters had already been investigated in many plant model systems, primarily with respect to the export of photosynthate sucrose from source leaves, and the uptake of sucrose in storage organs and seeds. Preliminary findings by the PI found that in Populus, sucrose transporter genes (SUTs) were well expressed in wood-forming tissues that comprise the feedstock for biofuels production. Because sucrose comprises by far the predominant form in which photosynthate is delivered from source organs to sink organs like roots and wood-forming tissues, SUTs control a gate that nominally at least could impact the allocation or partitioning of sucrose for potentially competing end uses like growth (stem biomass) and storage. In addition, water use might be conditioned by the way in which sucrose is distributed throughout the plant, and/or by the way in which sucrose is partitioned intracellularly. Several dozen transgenic lines were produced in year 1 of the project to perturb the expression ratio of multiple plasma membrane (PM) SUTs (intercellular trafficking), versus the single tonoplast membrane (TM) sucrose transporter that effectively regulates intracellular trafficking of sucrose. It was possible to obtain transgenic lines with dual SUT gene knockdown using the 35S promoter, but not the wood-specific TUA1 promoter. By the end of project year 2, a decision was made to work with the 35S plants while archiving the TUA1 plants. The PhD candidate charged with producing the transgenic lines abandoned the project during its second year, substantially contributing to the decision to operate with just the 35S lines. That student's interests ranged more toward evolutionary topics, and a report on SUT gene evolution was published (Peng et al 2014).

Water use:

The first of three post-docs to eventually transit in and out of the project conducted large scale greenhouse experiments to study the water use, growth, metabolite and gene profiles of the existing SUT4-RNAi lines (Frost et al, 2012). The Frost et al work found that shoot height growth and total biomass of SUT4-RNAi lines was especially sensitive to chronic mild drought. This was a surprise since photosynthesis increased in the SUT4-RNAi lines under chronic mild drought conditions. An RNAseq-metabolic profiling-water use study was eventually completed in which an abnormally high shoot-to-root sucrose concentration ratio was observed in RNAi plants. Our evidence supports the interpretation that this primed, and under increasing water deprivation, exacerbated osmotic imbalances within plant (Xue et al 2015, submitted). Constitutively increased transcript levels of abscisic acid (ABA) biosynthetic genes and bark/vegetative storage proteins suggested latent stress in root tips of well-watered RNAi plants. Stronger drought-stimulation of stress-inducible genes encoding late-embryogenesis-abundant proteins in transgenic roots was consistent with increased vulnerability to soil drying. Based on changes in aquaporin transcript levels during soil drying, there appeared to be an RNAi effect on intercellular water trafficking in stem xylem. In addition, drought caused aquaporin transcript reductions in transgenic roots that were sustained during recovery, but this was not observed in WT plants. Co-expression network analysis revealed altered integration of ABA sensing/signaling with ethylene and jasmonate sensing/signaling in RNAi compared to WT roots. Fatty acid and suberin biosynthesis were more transcriptionally integrated with ABA in stressed WT than RNAi roots. It was suggested that Intracellular trafficking of sucrose is linked both with whole-plant sugar gradients and with stress-sensing pathways that affect the uptake and intercellular distribution of water. Together, the chronic and acute drought stress studies point to the need for a broad dynamic range of SUT4 expression changes as poplar plants adjust carbon and biomass partitioning in response to soil moisture availability.

Acute drought RNA-Seq data accession number SRP041959.

An elaborate drought experiment was conducted with the 35S-multi-SUT-RNAi lines in which one or two well-expressed PM SUTs were down-regulated in relation to the tonoplast SUT4. Although gene and metabolite profiling data were collected, the results were equivocal, cast a certain degree of doubt on how the experiments were conducted, and will probably only be published with great difficulty. No biologically compelling effects on water use, metabolic profiles, growth or gas exchange were observed using the double 35S SUT transformants.

Sink-Source:

An experiment was completed in which the effect of SUT gene manipulation on shoot growth of partially defoliated plants was explored. The intention was to conduct such work with SUT4-RNAi as well as with the multi-SUT RNAi transgenics. Due in large part to post doc continuity and turnover problems, only the work with the SUT4-RNAi lines was completed. Two post-doctorals joined and abruptly left the project over a 15 month period after the first post doc left for a promising career opportunity. This is especially detrimental to greenhouse research with Populus plants because experiments overlap and plant maintenance, propagation and treatments are logistically challenging to coordinate under the best of circumstances. The last post doc left early during the third year of the project when finding a replacement was impossible. In short, the findings are that SUT4 expression is an important regulator of root biomass production in Populus. In turn, leaf/stem ratio of biomass partitioning in new shoot growth decreases as plant size and shoot/root ratio increases in SUT4-RNAi poplars. The ultimate cause of the progressive decline in transgenic root growth appears to be a premature onset of age-related reductions in sucrose exporting activity of lower leaves of SUT4-RNAi plants. RNAseq data found that flavonoid pathway transcript levels in bark, leaves and stem exhibited a larger defoliation treatment response in SUT4-RNAi than WT plants. Presently the conclusion is that the flavonoid gene changes reflect stress sensing, and were consistent with altered flavonoid metabolite levels in the transgenics. This along with supporting metabolic data are being analyzed, strong leads are being assessed, and a manuscript is in preparation.

RNA-Seq data accession number SRP059838.

One issue besides postdoc and student turnover throughout the project was the substantial residual expression of target genes in the RNAi lines. It was generally difficult to obtain more than 40-50% down-regulation in the multi-SUT-RNAi plants. This made phenotyping challenging, and tended to wear people down. It is likely that the advent of new gene KO technologies like CRISPR will facilitate multigene KO approaches in species like Populus where there are no natural knockouts, and where residual expression is a problem using RNAi technology.

Work is being completed on a SUT transgenic line that yielded a clear premature leaf senescence phenotype. This was the only SUT manipulation to yield a visibly obvious phenotype using INRA-717 poplar plants. The other SUT manipulations yielded altered physiological performance under stress conditions, but the anticipated phenotypes required significant effort to tease out under ambient growth conditions. An RNA seq experiment has been completed and when the manuscript is submitted, this grant proposal will be credited.