

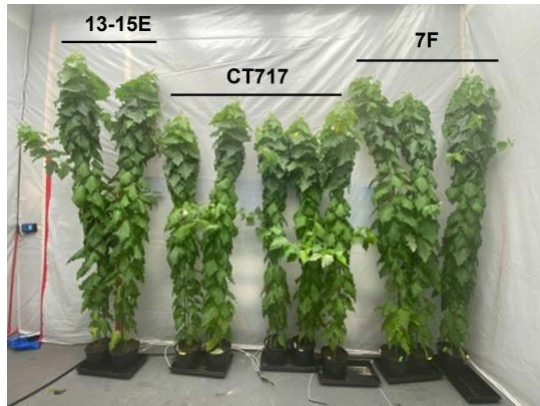
Harnessing the Power of Synthetic Biology to Enhance Tree's Natural Ability to Rebalance the Planet's Carbon Cycle

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Forest trees have played a pivotal role in maintaining the planet's carbon cycle, both in terms of carbon drawdown through photosynthesis and of carbon sequestration through wood production. The drastic increase in atmospheric CO₂ concentration since the industrial revolution presents a significant challenge for trees to quickly evolve an efficient mechanism to rebalance the carbon cycle. Recent advancement in synthetic biology opens the door to bioengineering plants to fix carbon more efficiently and to preserve carbon for longer. We strive to apply these learnings and tools in trees to enhance their natural ability in carbon cycle maintenance to increase net biome productivity. Using hybrid poplar as a model system, we have built a technology platform beyond poplar for tree bioengineering and provided proof-of-concept in several focus areas. An example of proof-of-concept using the technology platform was provided with testing in hybrid poplar INRA717-IB4 of a photorespiration bypass pathway design selected from literature reports showing efficacy in tobacco (South et al. 2019; Cavanagh et al. 2022). The design includes an RNAi strategy to reduce the transportation of the photorespiration byproduct, glycolate, out of the chloroplast and a shunt pathway to metabolize the retained glycolate back to CO₂ for fixation through the Calvin-Benson cycle. The shunt pathway includes a gene from algae encoding glycolate dehydrogenase and a gene from pumpkin encoding malate synthase. Working together, and alongside the endogenous enzymes in the malate metabolism cycle, these two enzymes located in the chloroplast convert glycolate to CO₂ for added carbon fixation by RuBisCO carboxylation. Molecular and physiological data collected from two separate growth experiments indicates that transgenic plants expressing genes in the photorespiration bypass pathway have reduced transportation of glycolate, increased photosynthetic efficiency, faster plant growth and elevated biomass production. One lead transgenic event accumulated 35-53% more above-ground dry biomass over four months of growth in a controlled environment (Fig. 1). Field testing of the performance of these genetically engineered poplar trees for photosynthesis enhancement is in progress. We have since made progress on providing proof-of-concept in several project areas directed towards increased carbon sequestration including wood decay resistance using our technology platform.

A



B

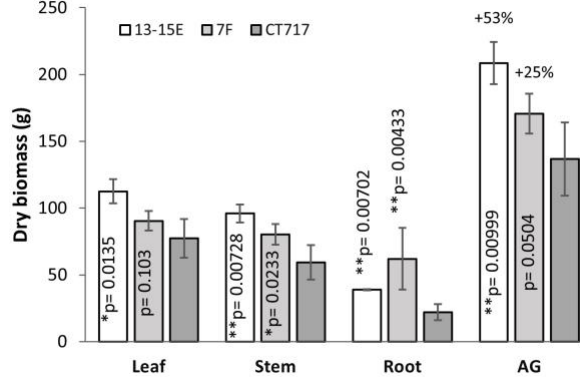


Figure 1. Plant growth and biomass analysis in selected transgenic events. A. Plant height difference between transgenic plants and CT717 non-transgenic control plants before harvesting for biomass measurement at week 21. Left: 13-15E, 2 ramets; Right: 7F, 3 ramets; Middle: CT717, 5 ramets. **B.** Dry biomass of harvested C1 experimental plants, including leaf, stem, root, and above-ground (AG; leaf + stem). Number of ramets: CT717 control plants, 5; Event 13-15E, 2 (one of the three initial ramets died earlier in the experiment); Event 7F, 3. All p -values were calculated using t-test and represent the difference compared to the non-transgenic CT717 control plants. * and ** represents a significant difference at $P < 0.05$ and $P < 0.01$, respectively when compared to non-transgenic CT717. For more data and information, see Tao et al. 2023.

References:

- South, P.F., A.P. Cavanagh, H.W. Liu, and D.R. Ort. 2019. Synthetic Glycolate Metabolism Pathways Stimulate Crop Growth and Productivity in the Field. *Science* 363: eaat9077, doi:10.1126/science.aat9077.
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