

CRISPR/CAS9-KNOCKOUT OF TRICHOME-REGULATING MYBS IN *POPULUS ALTER* LIGHT SENSITIVITY AND WAX COMPOSITION

William P. Bewg¹, Margot Chen¹, Scott Harding¹, Jingyin Yu¹, Ran Zhou¹, Timothy Tschaplinski², Nancy Engle², Braz Vaidya³, Nirmal Joshee³ and Chung-Jui Tsai¹

¹ School of Forestry and Natural Resources, Department of Genetics, and Department of Plant Biology, University of Georgia, Athens, Georgia, USA 30602 (bewg@uga.edu);

² Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA 37830;

³ Fort Valley State University, Fort Valley, Georgia, USA 31030.

In all habitats, plants face a variety of environmental stresses, and thus, have evolved an array of physiological and transcriptional coping mechanisms. Acting as a physical barrier between plant and its environment, hair-like trichomes act multifunctionally to provide pest defense and UV shielding whilst reducing transpiration rates. Additionally, they can also act as a locale for secondary metabolite synthesis and storage. The role and regulation of trichomes is well studied in herbaceous models, but less so in poplar, a woody perennial with bioenergy importance. Previous research identified the transcription factor PtaMYB186 as a positive regulator of trichome initiation during early stages of leaf development in *Populus tremula* x *P. alba* (IRNA 717-1B4). Here, the CRISPR/Cas9 system was utilized to target *PtaMYB186* and its close paralogs for knockout mutagenesis in poplar. The regeneration of trichomeless mutants confirmed the regulatory roles of the MYB transcription factors during trichome initiation. These trichomeless poplar had increased pest susceptibility, though unexpectedly, growth and leaf transpiration rates were not affected. Additionally, light-regulated genes were found to be differentially expressed and exposing the trichomeless mutants to a high-light environment significantly increased synthesis of anthocyanins, a class of known photoprotective metabolites. Notably, cuticle wax and whole leaf analyses found a complete absence of triterpenes in the mutants, suggesting biosynthesis and storage of triterpenes in poplar occurs in the trichomes. Together, these findings contribute further insights into the multifunctional role of trichomes in poplar as both a pest and light barrier, as well as a site of triterpene biosynthesis and storage.