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Research paper

Transgenically altered lignin biosynthesis affects photosynthesis and water relations of field-grown *Populus trichocarpa*

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ABSTRACT

Concerns over energy security and environmental sustainability have stimulated interest in development of high-yield, low-lignin trees for bioenergy. Black cottonwood (*Populus trichocarpa*) has been targeted as a potential bioenergy species due to its high productivity, but it is unclear how transgenically altered lignin biosynthesis will affect plant function. We investigated the physiology of two transgenic *P. trichocarpa* genotypes grown in short rotation woody cropping systems at two sites in southeastern USA: (1) mesic mountain site and (2) warmer, drier Piedmont site. Our results suggest that lignin is fundamental for tree growth and survival in field environments. Lignin deficiency can decrease biochemical photosynthetic processes and interfere with the temperature-response of photosynthesis. Significantly, hydraulic conductivity of transgenic genotypes was 15–25% that of wildtype trees, resulting in decreased leaf-specific whole-plant hydraulic conductance. In the Piedmont, decreased hydraulic efficiency drastically reduced productivity of low-lignin genotypes by 50–70% relative to wildtype. Transgenic trees at the mountain site recovered stem lignin concentrations to levels observed in wildtype trees, but still had severely impaired hydraulic traits, highlighting the major consequences of genetic transformation on whole-plant function. Surprisingly, substantial loss of hydraulic conductivity had only minor effects on productivity at the mesic site and resulted in an alternative advantage for bioenergy systems – lower water consumption. In the hottest month (July), higher intrinsic water use efficiency resulted in total water savings of roughly 1 kg d^{-1} per transgenic tree without sacrificing productivity. Decreased hydraulic conductivity could therefore be a promising trait for selection of water-efficient genotypes in *Populus*.

1. Introduction

Biofuel production from cellulosic perennial crops, such as grasses and poplars, reduces greenhouse gas emissions relative to gasoline [1] and provides numerous advantages over non-cellulosic crops such as corn [2]. Poplars (i.e. *Populus* species) are among the fastest growing trees in temperate regions and thus are ideal candidates for short rotation woody cropping systems (SRWC) for bioethanol production in the United States [3,4]. The high productivity of poplars requires a large water supply [5], so they are restricted to regions with adequate water availability. The genus *Populus* contains species that are highly susceptible to drought stress due to low stomatal sensitivity to water potential [6] and high vulnerability to xylem cavitation [7–10]. Higher temperatures in the future are expected to increase the probability of drought and temperature-induced drought stress [11], so selecting genotypes that minimize water use and maximize carbon assimilation and growth will be of critical importance [12]. Explicit quantification of stand-level water use efficiency (WUE), and its improvement, will be important components of engineering climatically robust and sustainable bioenergy production systems [12].

Before commercial-scale production of cellulosic biofuels can occur, however, the cost of converting cellulose to ethanol or other biofuels must be decreased to a level competitive with gasoline and corn-starch ethanol (i.e. first generation biofuels). Reduction of lignin in cellulosic plant biomass, in particular, holds promise to increase ethanol yield while decreasing processing inputs [13].

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Abbreviations

R_d	daytime respiration rate
$K_{L,p}$	leaf-specific whole-plant hydraulic conductance
V_{cmax}	maximum carboxylation velocity of Rubisco
g_m	mesophyll conductance
Ψ_{leaf}	midday leaf water potential
A_n	net CO_2 assimilation rate
J	photosynthetic electron transport rate
g_s	stomatal conductance
E	transpiration rate
TPU	triose phosphate use
k_s	xylem-specific hydraulic conductivity

Lignin in plant secondary cell walls hinders the enzymatic degradation of cell wall polysaccharides to simple sugars destined for fermentation to ethanol [13]. Another strategy for increasing the biochemical efficiency of cellulose conversion to biofuels is to increase the ratio of syringyl (S) to guaiacyl (G) monolignol units in angiosperm lignin [14]. Syringyl-rich lignin is substantially easier to separate from cellulose than guaiacyl-rich lignin during pulping for paper production [15], which should theoretically decrease pretreatment costs during biofuel production. Genetic transformations of genes that encode for enzymes along the lignin biosynthetic pathway have been developed that successfully decrease lignin quantity and increase the S/G ratio of stem tissue in *Populus* [16–19]. Although transgenic plants grown in controlled conditions have dramatic decreases in stem lignin content [18,20], evidence is mounting that transgenic alteration of lignin biosynthesis may not remain stable when grown under natural field conditions over long time periods [21–23]. There are 23 genes that likely encode monolignol biosynthesis enzymes during wood formation in *P. trichocarpa* (Torr. & Gray), and there is likely functional redundancy in many of these enzymes [24]. In the 4-coumarate:coenzyme A ligase (*4CL*) gene family, three genes are expressed in wood-forming tissue of *P. trichocarpa* and were suggested for biochemical roles in lignin synthesis [24]. Increased lignin biosynthesis during wood formation can be induced by biotic or abiotic environmental stressors, including low temperature [25], pathogen attack [26], and drought [27]. Lignin composition has also been observed to change in response to natural abiotic factors such as wind [28]. Better understanding of the role of lignin in whole-plant functional responses to environmental variation is needed in order to improve purpose-grown trees.

Lignins are functionally important in mechanical support [29,30], wood strength [31], water conductance [32], carbon storage, and disease resistance [26]. Lignified secondary cell walls provide significant compressive strength against stresses up to 40 MPa, relative to non-lignified walls [29]. Low-lignin *Populus* trees have been found to have decreased hydraulic conductivity and a greater susceptibility to cavitation [33,34] as a result of collapsed cells and blockage of vessels by tyloses and phenolics [35]. Such reductions in water transport capacity are likely to have negative effects on the productivity of transgenic trees, especially *Populus* because of its large water requirement [5]. It is unknown how universal these physiological responses are among *Populus* genotypes and different genetic transformations, especially for plants grown under field conditions for multiple years.

The main objective of this study was to quantify physiological water relations of three genotypes of *P. trichocarpa* (wildtype and two low-lignin transgenics) under natural environmental conditions in the southeastern USA. A previous study by Stout et al. [22] established that biomass production of these two low-lignin transgenic genotypes (PT-1, PT-3) was high, but the low-lignin and high S/G phenotypes were not fully maintained after three years of growth in North Carolina (Table 1). Therefore, we compared physiological responses at two different study sites: (1) a warmer 'Piedmont' site more prone to drought stress where the two transgenics maintained lower wood lignin concentrations (but not higher S/G ratio) than the wildtype, and (2) a mesic 'mountain' site where low-lignin phenotypes and high S/G ratio were not stable (i.e. not significantly different from wildtype after three years) but total yields were competitive with other proposed perennial cellulosic biofuel crops [22]. We measured seasonal changes in growth and hydraulic traits (leaf water potential, gas exchange, leaf-specific whole-plant hydraulic conductance, intrinsic WUE) of each genotype and compared responses of transgenic lines to the wildtype control at each site. We also determined if photosynthesis, foliar nitrogen concentration, and wood density differed among genotypes. We hypothesized that transgenic trees will have decreased rates of biophysical and biochemical photosynthetic processes and decreased hydraulic efficiency relative to the wildtype control.

2. Materials and methods

2.1. Study species

Populus trichocarpa (black cottonwood) is the largest hardwood tree in western North America and can grow to over 60 m in height [36]. It grows primarily on moist sites west of the Rocky Mountains and is most productive in bottomlands of major streams and rivers [36]. The female *P. trichocarpa* (genotype Nisqually-1) was found near the Nisqually River in central Washington, USA, and was the first tree to have its complete genome sequenced [37]. Stout et al. [22] performed an environmental screening of twelve transgenic *P. trichocarpa* lines, and in the current study, we performed a detailed analysis of the physiology and water relations of the two most promising genotypes. These two transgenic lines (PT-1, PT-3) were produced by antisense suppression of the gene encoding *4CL* in the Nisqually-1 genotype (V. Chiang, North Carolina State University, Raleigh, NC, USA; see Ref. [38]). The transformations used the *4CL* promoter from *P. tremuloides* and were mediated by *Agrobacterium tumefaciens*. The transgenic trees were produced from tissue culture under greenhouse conditions during fall 2008. After several months of growth, the pre-transplants were transferred to small pots and placed in a covered greenhouse in January 2009 for cold acclimation before planting in April 2009. Under greenhouse conditions, the two low-lignin lines had mean lignin mass fractions of 13.9% and 17.4%, while wildtype contained 22% lignin ([22], Table 1). The S/G ratios of the low-lignin lines were approximately 40% greater than the control at the time of planting (Table 1). After three years of growth in North Carolina, wood lignin concentration of all genotypes increased by 1.4–9.2% and S/G ratios decreased by 0.5–2.2, with the largest changes occurring in the transgenic lines (Table 1). As a result, lignin concentration or S/G ratios did not vary between transgenic and wildtype trees at the mountain site in the study year 2012. At the Piedmont site in 2012, the PT-1 and PT-3 transgenic lines had lower wood lignin concentrations than wildtype by 3.4% and 4.8%, respectively.

2.2. Experimental study sites

Trees were planted in the mountain, Piedmont, and coastal regions of North Carolina, USA, although only mountain and Piedmont sites were measured in this study as physiological responses