Molecular Plant • Volume 3 • Number 5 • Pages 818–833 • September 2010 RESEARCH ARTICLE

Virus-Induced Gene Silencing Offers a Functional Genomics Platform for Studying Plant Cell Wall Formation

Xiaohong Zhu[a], Sivakumar Pattathil[b], Koushik Mazumder[b], Amanda Brehm[b], Michael G. Hahn[b,c], S.P. Dinesh-Kumar[d] and **Chandrashekhar P. Joshi[a,e,1]**

a Biotechnology Research Center, School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA
b BioEnergy Science Center, Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, GA 30602, USA
c Department of Plant Biology, University of Georgia, Athens, GA 30602, USA
d Section of Plant Biology and Genome Center, University of California, Davis, CA 95616, USA
e Department of Bioenergy Science and Technology, Chonnam National University, Gwangju 500–757, Korea

ABSTRACT **Virus-induced gene silencing (VIGS) is a powerful genetic tool for rapid assessment of plant gene functions in the post-genomic era. Here, we successfully implemented a Tobacco Rattle Virus (TRV)-based VIGS system to study functions of genes involved in either primary or secondary cell wall formation in *Nicotiana benthamiana* plants. A 3-week post-VIGS time frame is sufficient to observe phenotypic alterations in the anatomical structure of stems and chemical composition of the primary and secondary cell walls. We used cell wall glycan-directed monoclonal antibodies to demonstrate that alteration of cell wall polymer synthesis during the secondary growth phase of VIGS plants has profound effects on the extractability of components from woody stem cell walls. Therefore, TRV-based VIGS together with cell wall component profiling methods provide a high-throughput gene discovery platform for studying plant cell wall formation from a bioenergy perspective.**

Key words: **Plant cell wall; VIGS; cellulose; xylan; lignin; Nicotiana.**

INTRODUCTION

Plant cells develop two types of walls: primary and secondary cell walls (Cosgrove, 2005). The formation of primary walls accompanies cell division and expansion where deposition of polysaccharides and structural proteins occurs outside the plasma membrane. The dynamic structure and composition of the primary wall determine cell shape while controlling the rate and direction of cell growth. The secondary wall consists of multiple layers of polysaccharides and polyphenolics deposited between the primary cell wall and plasma membrane after the cell ceases to expand. The relatively rigid structure of the secondary wall provides the plant with mechanical strength and serves as a physical barrier against pathogen attack (Lagaert et al., 2009). Moreover, primary and secondary cell walls are the major carbon-containing components in biomass (Pauly and Keegstra, 2008; Sandhu et al., 2009). Despite our knowledge of their crucial roles, we still lack mechanistic insights into the processes of cell wall formation. The structural complexity of the cell wall implies that a large number of genes may be involved in its biogenesis (Mohnen et al., 2008) and that their expression is orchestrated specifically in different cell types, tissues or organs, and species in response to developmental and environmental cues (Farrokhi et al., 2006).

Based on analyses of the available genome sequences of *Arabidopsis*, rice, and poplar, a number of genes have been annotated as having either known or unknown functions in cell wall formation (Aspeborg et al., 2005; Yong et al., 2005). Large collections of ESTs relevant to cell wall synthesis and wood formation have also been generated in poplar and other economically valuable tree species (Sterky et al., 1998, 2004). Furthermore, microarray profiling and co-expression analysis have led to the identification of numerous candidate genes potentially involved in cell wall synthesis (Brown et al., 2005; Persson et al., 2005). A system that enables rapid determination of the biological functions of genes involved in cell wall formation is necessitated by recent advances in genomic data collections.

[1] To whom correspondence should be addressed. E-mail cpjoshi@mtu.edu.

© The Author 2010. Published by the Molecular Plant Shanghai Editorial Office in association with Oxford University Press on behalf of CSPP and IPPE, SIBS, CAS.

doi: 10.1093/mp/ssq023, Advance Access publication 3 June 2010
Received 5 February 2010; accepted 24 April 2010

Considerable progress in understanding of cell wall formation has been made using *Arabidopsis* (Somerville, 2006). However, this system has limitations for increasing our understanding of wood formation, which involves massive secondary growth that does not typically occur in *Arabidopsis* (Sterky et al., 1998). *Nicotiana benthamiana*, a close relative of common tobacco (*N. tabacum*) that possesses the ability to undergo secondary growth, provides a potential model species for cell wall studies. In addition, availability of the tobacco genome sequence (www.pngg.org/tgi/) will facilitate novel gene discovery in cell wall formation in this plant model system. Most importantly, *N. benthamiana* is an excellent well proven platform for Virus Induced Gene Silencing (VIGS). VIGS, a transient RNAi-mediated gene silencing approach, facilitates rapid gene function assessment without the requirement of generating stable transgenics (Burch-Smith et al., 2004). VIGS has been successfully used to address biological questions related to plant defense, development, and metabolism in many plant species (Lu et al., 2003; Burch-Smith et al., 2004; Robertson, 2004).

A potato virus X (PVX)-based VIGS has been used to silence a cellulose synthase (CesA) gene in *N. benthamiana* plants to assess its function in primary cell wall formation (Burton et al., 2000). PVX-*NtCesA-1* infected plants showed a cellulose-deficient phenotype and alterations in leaf and whole-plant morphology. This study highlighted the specificity of VIGS for the functional analysis of cell wall synthesis genes because VIGS of two highly similar (80% homology) CesA genes produced completely different phenotypes. Here, we have optimized tobacco rattle virus (TRV)-based VIGS (Liu et al., 2002) in *N. benthamiana* plants as a rapid, reliable, and robust system to assess gene function in cell wall formation. We demonstrate that TRV-VIGS together with comprehensive microarray polymer profiling (CoMPP) assay (Moller et al., 2007) and glycome profiling using cell wall glycan-directed monoclonal antibody-ELISA (enzyme-linked immunosorbent assay) (Pattathil et al., 2010) serve as a genome-wide high-throughput gene discovery platform in lignocellulosic biomass synthesis.

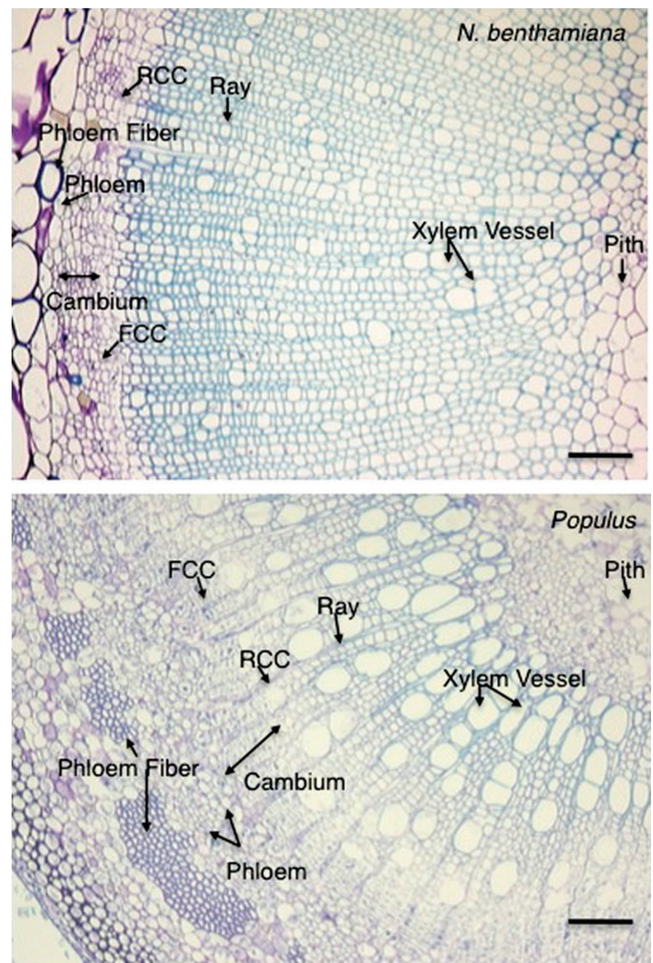

Figure 1. Comparison of Stem Structures of *N. benthamiana* and *Populus*.

Various structuralelements of the stem in TBO-stained transverse sections of 14th internodes of *N. benthamiana* (top panel) and *Populus* (bottom panel) are indicated by arrows. FCC, fusiform cambial cells (FCC); RCC, radial ray cambial cells. Scale bar represents 100 μm.

RESULTS

N. benthamiana Stems Are Similar to Poplar Woody Stems

To determine whether the mature stems of *N. benthamiana* possess anatomical characteristics similar to woody stem, we compared *N. benthamiana* and poplar stem structures. Transverse sections (TS) of the 14th internode of 6-week-old *N. benthamiana* and 8-week-old poplar show a similar secondary xylem structure (Figure 1). Phloem, cambium zone, xylem vessels, and rays are well distinguished in TS of *N. benthamiana* stems. Xylem vessels are small, solitary, and arranged in small groups in *N. benthamiana.* In poplar, large xylem vessels are seen in long radial multiples (Figure 1). In *N. benthamiana*, the vascular cambium is visible in TS as a layer five to six cells thick between the secondary xylem and phloem. Two types of initials were clearly distinguishable in the vascular cambium zone, the axially elongated fusiform cambial cells (FCC), and the radial ray cambial cells (RCC). Radial rays derived from RCC are an important determinant for horizontal secondary growth (Mellerowicz et al., 2001; Baucher et al., 2007). In both *N. benthamiana* and poplar stems, radial ray cells are arranged in a uniseriate band (Figure 1). However, in poplar, radial rays occur at high frequency and appear to be evenly distributed within secondary xylem. In both *N. benthamiana* and poplar, xylem parenchyma cells are organized in radial strands and are scattered. *N. benthamiana* and poplar phloem appear different in TS (Figure 1). Unlike poplar, in which phloem fiber cells form groups, they are relatively scattered as individual cells in *N. benthamiana.* Collectively, these data suggest that *N. benthamiana* plant stems possess most of the structural characteristics of poplar woody stems.

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