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Research article

Evaluation of a novel promoter from *Populus trichocarpa* for mature xylem tissue specific gene delivery

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ABSTRACT

Wood (i.e., secondary xylem) is an important raw material for many industrial applications. Mature xylem (MX) tissue-specific genetic modification offers an effective means to improve the chemical and physical properties of the wood. Here, we describe a promoter that drives strong gene expression in a MX tissue-specific manner. Using whole-transcriptome genechip analyses of different tissue types of poplar, we identified five candidate genes that had strong expression in the MX tissue. The putative promoter sequences of the five MX-specific genes were evaluated for their promoter activity in both transgenic *Arabidopsis* and poplar. Among them, we found the promoter of Potri.013G007900.1 (called the *PtrMX3* promoter) had the strongest activity in MX and thus was further characterized. In the stem and root tissues of transgenic *Arabidopsis* plants, the *PtrMX3* promoter activity was found exclusively in MX tissue. MX-specific activity of the promoter was reproduced in the stem tissue of transgenic poplar plants. The *PtrMX3* promoter activity was not influenced by abiotic stresses or exogenously applied growth regulators, indicating the *PtrMX3* promoter is *bona fide* MX tissue-specific. Our study provides a strong MX-specific promoter for MX-specific modifications of woody biomass.

1. Introduction

Due to the demands for woody biomass in many industrial applications (e.g., lumber for construction, pulp for the paper industry, and feedstocks for biofuel production), genetic modifications have been attempted to improve quality and quantity of woody biomass (Baucher et al., 2003; Chen and Dixon, 2007; Han et al., 2007; Ragauskas et al., 2006; Weng et al., 2008). One such example is to produce woody biomass with reduced lignin content by suppressing lignin biosynthetic genes in an effort to increase fermentation yield in biofuel production (Chen and Dixon, 2007; Grima-Pettenati and Goffner, 1999; Hu et al., 1999). To increase biomass production, trees can be engineered for delayed flowering and improved tolerance to environmental stresses (Sticklen, 2006). These efforts could reduce competition with food or feed crops for water and other resources. In addition, metabolic engineering produce higher amounts of various secondary metabolites in the woody tissues (e.g., mature xylem), thus increasing pest resistance of the trees or utilizing trees as a molecular factory (Harfouche et al., 2011).

To date, the CaMV (Cauliflower Mosaic Virus) 35S promoter has been used in many tree genetic engineering applications. However, constitutive overexpression of transgenes by the CaMV 35S promoter often results in undesirable pleiotropic phenotypic consequences and sometimes is detrimental to tree growth (Coleman et al., 2006, 2008; No et al., 2000). For instance, overexpression of *AtMYB46*, a transcriptional master switch of secondary wall formation in *Arabidopsis*, under the control of 35S promoter, resulted in a dwarf phenotype due to ectopic lignification in the photosynthetic (i.e., mesophyll cells) and parenchyma cells (Ko et al., 2009). Overexpression of *PdGA20ox1*, a key gene in the production of bioactive gibberellins in *Pinus densiflora*, produced undesirable phenotypes, such as poor root growth and leaf development, in transgenic poplars (Jeon et al., 2015; Park et al.,

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2015). On the other hand, the use of a developing xylem tissue-specific promoter DX15 to overexpress *PdGA20ox1* could avoid those undesirable phenotypes (Jeon et al., 2015; Ko et al., 2012). Therefore, use of proper promoters, which enable targeted expression of the transgene in particular cell/tissue types or developmental stages, is critical to obtain desired transgenic phenotypes without collateral pleotropic consequences (Lee et al., 2014; Nookaraju et al., 2014; Ratke et al., 2015; Yang et al., 2013).

Mature xylem (i.e., secondary xylem) is produced by differentiation of vascular cambium and consists of three major cell types, which are tracheary/vessel elements, xylary fibers, and xylem parenchyma cells (Ménard and Pesquet, 2015; Schuetz et al., 2013; Zhang et al., 2014). When fully matured, both tracheary/vessel elements and xylary fibers are dead with thick secondary cell walls and function for water/solute transportation and structural support, respectively, in plants. However, xylem parenchyma cells are alive with no secondary cell walls and may be involved in a variety of functions, including a conduit for the movement of photosynthate for secondary wall formation in the adjacent vessel elements and fibers that are undergoing maturation (i.e., prior to programmed cell death). Secondary walls in mature xylem (MX) tissues are utilized as a feedstock for biofuel, fibers and solid wood products (Mellerowicz and Sundberg, 2008). Thus, MX-specific genetic modification offers a means to improve the quality of the woody biomass.

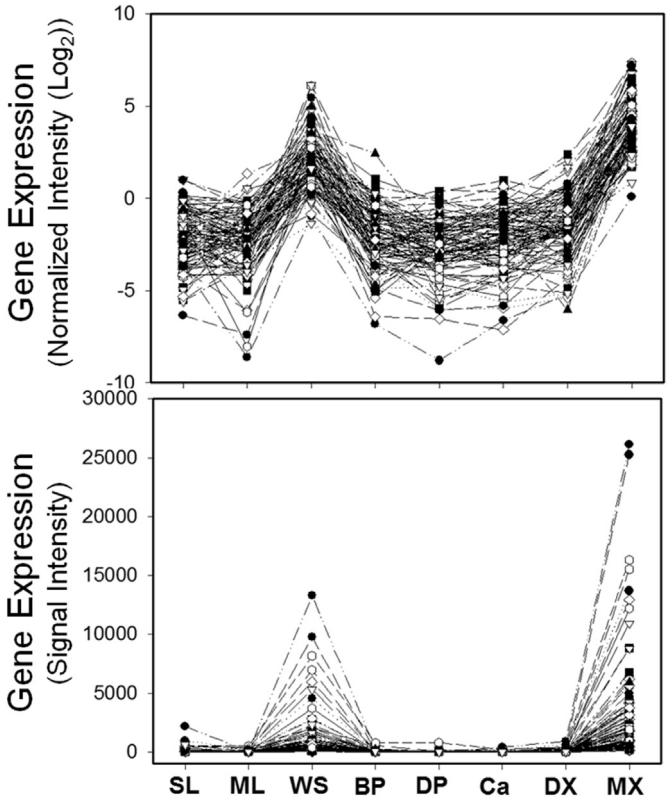

Fig. 1. Identification of MX-tissue abundantly expressed genes. A total of 116 genes were identified by applying the following sequential filters and plotted. First, genes that were up-regulated (>10 fold) in MX (mature xylem) compared to both SL (shoot apical meristem and leaf primordia) and ML (mature leaf without major veins) yielded 1699 genes; second, genes that were up-regulated (>10 fold) in MX compared to BP (bark and mature phloem), DP (developing phloem), Ca (cambial zone) and DX (developing xylem) narrowed the selection down to 123 genes; third, only genes with 'Presence' calling in MX were included in the final 116 genes. WS indicates 'whole stem' as an internal control. The lower panel shows the 'signal intensity' of each gene and the upper panel shows the log2 based 'normalized intensity'. A list of the genes is provided in Supplementary Table S1.

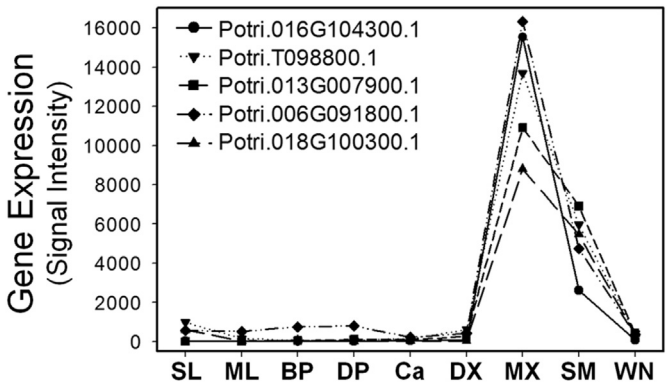

Fig. 2. MX-tissue specific and strong expression of the five selected candidate genes. Tissue specific expression (signal intensity) of candidate genes, Potri.016G104300.1 (*PtrMX1*), Potri.T098800.1 (*PtrMX2*), Potri.013G007900.1 (*PtrMX3*), Potri.006G091800.1 (*PtrMX4*), and Potri.018G100300.1 (*PtrMX5*), is plotted. SL, shoot apical meristem and leaf primordial; ML, mature leaf without major veins; BP, bark and mature phloem; DP, developing phloem; Ca, cambial zone; DX, developing xylem; MX, mature xylem; SM, WN; summer and winter stem, respectively (Ko et al., 2011).

Here, we report the identification and characterization of a MX-specific promoter sequence, called *PtrMX3*. This *PtrMX3* promoter activity was found almost exclusively in MX cells in both transgenic *Arabidopsis* and poplar plants. Furthermore, *PtrMX3* promoter activity was not altered by abiotic stresses or exogenously applied growth regulators, suggesting that it is genuinely MX tissue-specific promoter.

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis were grown on soil or on half-strength (1/2) Murashige and Skoog basal medium (MS; M0404, Sigma–Aldrich, St. Louis, MO) in agar medium containing 2% sucrose in a growth room (14 h light/10 h dark) at 25 °C. Hybrid poplar (*Populus alba* × *Populus tremula* var. *glandulosa*) was used for the production of transgenic poplar plants. Plants were propagated *in vitro* by cutting apical stems into segments (2–3 internodes, leaves removed) that were then cultivated on half-strength MS agar medium containing 0.2 mg/L IBA (Indole-3-butyric acid) in a growth chamber (16 h light/8 h dark) at 25 °C.

2.2. Generation of promoter::GUS transgenic plants

Based on our tissue-specific transcript data (Ko et al., 2012), we screened five MX-tissue specific genes using the strategy described in Fig. 1. Genetic information for these genes was retrieved from Phytozome v9.1 site (http://www.phytozome.net/). Approximately 1.5 kb of genomic DNA upstream of the start codon of putative MX-tissue specific genes (named PtrMX promoter) was isolated by PCR and inserted upstream of the *GUS* gene in the pBGWFS7 or pKGWFS7 vector using Gateway cloning (Invitrogen, Carlsbad, California) to produce PtrMX promoter::GUS constructs. The vector constructs were then introduced into *Agrobacterium tumefaciens* strain C58, which was used to transform *Arabidopsis* and poplar by the floral-dip method (Clough and Bent, 1998) or callus-mediated method (Choi et al., 2005), respectively. All constructs used in this study were verified by DNA sequencing. Promoters and primers used for their amplification are listed in supplementary material; Table S2.