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Research article

PzTAC and *PzLAZY* from a narrow-crown poplar contribute to regulation of branch angles

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ABSTRACT

Plant architecture, as a basic element influenced by genetic and environmental factors, has an important effect on grain yield via light transmission in agroforestry systems. The molecular mechanism underlying control of branch angle, an important aspect of tree architecture, is not well understood in poplars. Here, we cloned two genes from *Populus* × *zhaiguanheibaiyang* (a narrow-crown poplar), designated *PzTAC* and *PzLAZY*, which were predicted to be members of the *ITG* gene family through sequence homology. Transcript levels of the homologous genes were estimated by reverse transcriptase quantitative PCR (RT-qPCR) in different organs of *P.* × *zhaiguanheibaiyang* and *P. Deltoides* 'Zhonglin2025' (a broad-crown poplar). *TAC* expression was mainly confined to the leaves and annual shoots, whereas *LAZY* was mainly expressed in the annual shoots and axillary buds. Beside, we detected the promoter expression patterns derived from the *PzTAC* and *PzLAZY* genes using the *β-glucuronidase* (*GUS*) reporter gene in transgenic *Populus* × *euramericana* 'Neva'. *GUS* activity driven by the *PzTAC* and *PzLAZY* promoters was detected in mature leaves, leaf axils and vascular tissues of roots. The *PzTAC* promoter was mainly active in leaf veins, whereas the *PzLAZY* promoter was mainly active in mesophyll cells and root tips. The average branch angle in transgenic 35S::*PzTAC* plants was larger than that of transgenic 35S::*PzLAZY* plants. The results provide strong evidence that the two genes affect the vascular tissues of transgenic plants to modify branch angles.

1. Introduction

In agricultural production, plant architecture is an important factor for both herbaceous crops and forest trees. As a major constituent of agroforestry systems, trees form a network among agricultural land to enhance productivity, profitability and ecological sustainability (Wood and Burley, 1991). A well-designed tree architecture should minimize competition with adjacent crops for environmental resources such as light; thus, tree architecture may contribute to increasing the yield and yield stability of crops (Heath et al., 1994; Wang and Li, 2006; Yu et al., 2007). In recent years, researchers have paid much attention to plant architecture, from analysis of its structure to elucidation of regulatory molecular mechanisms, in herbaceous crops and fruit trees (Wang and Li, 2005; Schneider et al., 2012). Many factors, such as leaf dimensions, internodal elongation, and branch angle, influence plant architecture (Ward and Leyser, 2004; Dardick et al., 2013). Branch angle has been examined in diverse tree species (Wilson, 2000; Dardick et al., 2013). In agroforestry systems, the ideal branch angles of trees should be smaller than that optimal in other land-use systems (Dickmann and Keathley, 1996).

In addition to environmental and hormonal factors, branch angle, as an important determinant of plant architecture, is

Abbreviations: *ProLAZY*, *PzLAZY* promoter; *ProTAC*, *PzTAC* promoter; BC, broad-crown; *GUS*, *β-glucuronidase*; NC, narrow-crown; RT-qPCR, reverse transcriptase quantitative PCR; WT, wild type; MS, Murashige and Skoog; NAA, α-naphthaleneacetic acid; 6-BA, 6-benzylaminopurine; GA_3, gibberellic acid 3; Kan, kanamycin; Cef, cefotaxime; Leb, leader branch; Lab, lateral branch.

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regulated by specific genes which can be transformed into target plants to induce or improve plant architectural traits by transgenesis (Xueyong et al., 2003; Li et al., 2007; Yu et al., 2007; Yoshihara et al., 2013). Apical dominance, a well-characterized phenomenon, may influence branch angles. For example, the branch angle will be wider under strong apical dominance, whereas lateral branches will grow at a narrower angle when apical dominance is weak (Wilson, 2000). Branch angle can be manipulated by altering apical dominance through using transgenic methods (Booker et al., 2003; Takeda et al., 2003). Thus, identification of the molecular mechanisms for regulating apical dominance is of practical importance, especially the isolation of specific genes that affect the modification of branch angle.

In recent years, dozens of genes and regulatory factors associated with branch angle have been identified (Thomas et al., 2003; Takeda et al., 2003; Bai et al., 2012). Many factors are responsible for variation in branch angle including auxin distribution and the differences in auxin concentration (Wilson, 2000). Previous studies have shown that the directional transport of auxin results from the auxin asymmetric distribution (Friml and Palme, 2002; Michniewicz et al., 2007). Members of PIN protein family seem to play an unusual role in transportation of phytohormones (Friml et al., 2002), and some of them has been proved to be related with branch angle. For example, suppression of *OsPIN1* (*Oryza sativa L ssp*. Japonica, Nipponbare) expression by RNA interference (RNAi) in rice leads to an increase in till angles in rice (Xu et al., 2005). Thus, it is necessary to study the functional genes related to PIN protein family members. Over-expression of *OsPIN2* in rice exhibits a phenotype with a larger tiller angle through down-regulation of a gravitropism-related gene *OsLAZY1*, and enhances auxin transport from shoots to roots (Chen et al., 2012). In addition, OsPIN2 together with OsPIN1b and OsTAC1 (tiller angle controller 1) controls rice shoot architecture by a distinct auxin-dependent regulation pathway (Chen et al., 2012). *TAC*-like and *LAZY*-like genes are two different clades of the recently discovered *IGT* gene family which is responsible for gravitropic control of branches (Dardick et al., 2013). For instance, in *Arabidopsis thaliana*, *AtLAZY1* functions in gravitropic responses and mutants that impair its function markedly increase inflorescence branch angle (Takeshi and Moritoshi, 2007; Yoshihara et al., 2013). Moreover, as homologs of *LAZY*-like genes, *TAC*-like genes have been shown to change the branch angles in peach (Dardick et al., 2013). From this point of view, the characterization of members of the *IGT* family is of great importance.

Although *TAC*-like and *LAZY*-like genes have indicated to play a role in modifying branch angles in a variety of plant species, homologous genes have not been identified in poplars previously. Through breeding programs, many cultivars and hybrids of poplars have been raised. In the present study, *Populus* × *zhaiguanheibaiyang* (*P. Deltoides* 'Lux' ex I-69/55 × *P. leucopy-ramidalis* 4; a narrow-crown poplar; NC), a popular Chinese cultivar with small branch angles, was used as material to clone target *TAC* and *LAZY* genes. To determine the expression patterns of *TAC* and *LAZY* genes, we compared their relative expression in different organs between NC and *Populus deltoides* 'Zhonglin2025' (*P. deltoides* 'Lux' ex I-69/55 × *P. Deltoides*; a broad-crown poplar; broad-crown poplar; BC). As a kind of rough materials in paper-making industry, the growth and production of *Populus* × *euramericana* 'Neva' are limited by the stand-density (Tian et al., 2011; Zhou et al., 2012). Altering branch angles of *Populus* × *euramericana* 'Neva' may contribute to solve the problem. Thus we introduced the target genes into *Populus* × *euramericana* 'Neva' to detect the effect of *TAC* and *LAZY* genes on modifying the branch angles of poplars. It will provide new candidate genes to breed new varieties with an ideal plant architecture.

2. Materials and methods

2.1. Plant materials and bacterial strains

Five-year-old plants of *P.* × *zhaiguanheibaiyang* (*P. Deltoides* 'Lux' ex I-69/55 × *P. leucopy-ramidalis* 4; NC; Fig. 1A) and *P. deltoides* 'Zhonglin2025' (*P. deltoides* 'Lux' ex I-69/55 × *P. deltoides*; BC; Fig. 1B), which share the same female parent but have different male parents, were used to isolate *TAC* and *LAZY* genes and to compare differences in expression patterns in secondary roots, annual shoots, leaves and axillary buds. All samples were harvested from the arboretum of Shandong Agricultural University and stored at −80 °C. *Populus* × *euramericana* 'Neva', which is widely cultivated in northern China, was maintained by tissue culture for genetic transformation.

Escherichia coli DH5α (Tiangen Biotech, Beijing, China) was used for DNA manipulation, and *Agrobacterium tumefaciens* LBA 4404 was used to transform plants. The pMD18-T (Takara, Dalian, China) and pBI121 vectors were maintained in our laboratory.

2.2. cDNA synthesis and gene (TAC and LAZY) cloning from BC and NC poplars

Total RNA was isolated from leaves of the BC and NC poplars using the RN38 EASYspin Plus RNA Kit (Aidlab, Beijing, China) followed by RNase-free DNase treatment. The concentration of RNA in the extracts was determined with a spectrophotometer. First-strand cDNA was synthesized using a PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara, Dalian, China) as the PCR reaction template to clone the *TAC* and *LAZY* genes from the BC and NC poplar genotypes. According to the nucleotide sequences of western balsam poplar (*Populus trichocarpa*) in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov), gene-amplified primers were designed by Primer 5.0 (PREMIER Biosoft International, California, USA). The PCR protocol was 1 cycle of 98 °C for 3 min, then 30 cycles of 98 °C for 30 s, 55 °C

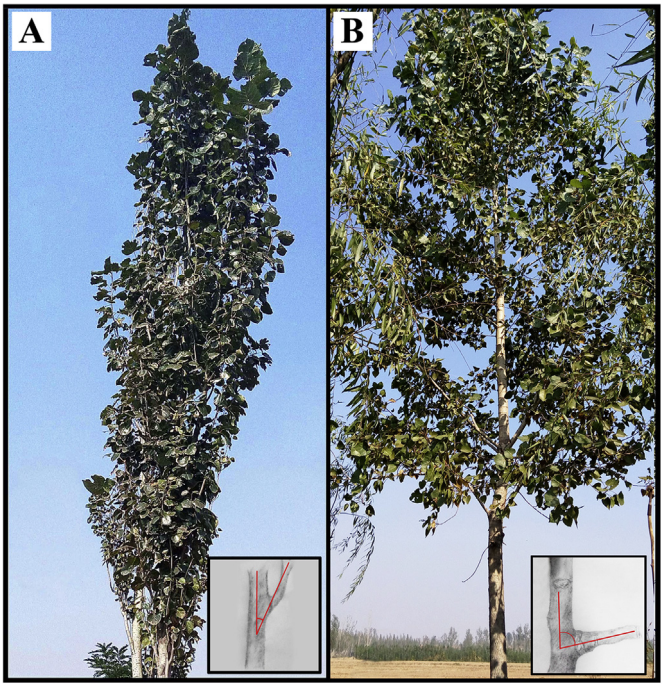

Fig. 1. Comparison of the crown structures of (A) narrow-crown and (B) broad-crown poplars.