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RESEARCH ARTICLE

Molecular cloning and functional characterization of *MdPIN1* in apple

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Abstract

Auxin has been identified to play critical roles in regulating plant growth and development. The polar transport of auxin is regulated by auxin transporters. In the present study, an auxin efflux carrier gene *MdPIN1* was cloned from *Malus*×*domestic*, Royal Gala, and introduced into wild-type *Arabidopsis thaliana* (Col-0). The transgenic plants exhibited the phenotype of inhibition of primary root (PR) elongation and increased lateral root (LR) number in compared with Col-0. Overexpression of *MdPIN1* affected auxin transport, and enhanced phototropic responses and geotropism reaction, whereas had no significant difference in the auxin biosynthesis. These findings suggest that the *MdPIN1* gene plays a vital role in auxin transport and root development.

Keywords: *MdPIN1*, auxin transport, root development, apple

1. Introduction

Auxin is an important phytohormone which plays critical roles in almost all process in plant growth and development, including the plants organogenesis and morphogenesis (Benková *et al*. 2003; Bohn-Courseau 2010), tropic response and apical dominance (Kepinski and Leyser 2005) and tissue differentiation (Reinhardt 2003). The unidirectional cell-to-cell polar transport of auxin is an important feature that is different from other hormones, by which way auxin is transported from the top to the bottom of the plant morphology, and which is not relevant to gravity (Liu *et al*. 1993; Lupini *et al*. 2014). Auxin polar transport exists in plant stems, roots and leaves, which is essential for the auxin asymmetrical distribution and influences the plants morphology (Friml 2003). The directionality of auxin movement is controlled by the transportation carriers, including a series of membrane proteins which are directly involved in auxin flow (Kepinski and Leyser 2005; Mravec *et al*. 2009).

Auxin transportation carriers, including auxin influx carrier AUXIN RESISTANT 1 (AUX1) and auxin efflux carriers PIN-FORMEDs (PINs), are involved in various aspects of plants growth and development by regulating auxin movement and distribution (Petrášek and Friml 2009; Haga and Sakai 2012). In *Arabidopsis*, there are eight *PIN* genes (*AtPIN1*–*AtPIN8*), and the *AtPIN1* is the first identified gene that is proved to be associated with auxin transport (Kramer *et al*. 2004). The AtPIN1 protein encodes 622 amino acid residues, containing 8–12 transmembrane regions that

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are necessary for the activity of auxin transport. Previous studies have shown that *AtPIN1* is involved in the lateral root (LR) organogenesis (Marhavý *et al*. 2014), phototropic responses (Blakeslee *et al*. 2004), morphogenesis and auxin transport (Heisler *et al*. 2010). The functions of *AtPIN1* could be blocked by the auxin transport inhibitors (Geldner *et al*. 2001), which further reveals the importance of AtPIN1 in auxin transport. Recently, some studies have shown that auxin and strigolactones work together to regulate shoot branching and *AtPIN1* plays a key role in this process (Shinohara *et al*. 2013). The *PIN1* gene in rice, named *OsPIN1*, is cloned and involved in auxin-dependent adventitious root emergence and tillering (Xu *et al*. 2005). Adventitious root emergence and development are significantly inhibited in the *OsPIN1* RNA interference (RNAi) transgenic plants (Xu *et al*. 2005). In addition to *PIN1*, the other *PINs* genes also play relative functions in plant development. *PIN2* protects LR formation during the early stages of iron stress (Li *et al*. 2015); *PIN3* and *PIN7* are involved in phototropism (Haga and Sakai 2012); *PIN4* functions in the establishment of an auxin sink below the quiescent center of the root apical meristem (Blakeslee *et al*. 2005); and *PIN5* and *PIN8* are involved in the instracellular auxin transport in pollen (Dal Bosco *et al*. 2012). *PIN6* is required for nectary auxin response and short stamen development (Bender *et al*. 2013).

A limiting basipetal auxin transport in shoots contributes to the dwarfing of apple rootstocks (Soumelidou *et al*. 1994). In modern fruit production, dwarfing and closely spaced planting has become a general tendency of apple development because of maturation early fruit and high yield. However, this cultivation mode results in the shallow and weak root system and then reduces absorption ability of both water and fertilizer. It's an effective pathway to regulate root development and increase apple production through the genetic improvement of root system. It is also found that auxin is one of the limiting factors for apple fruit size (Devoghalaere *et al*. 2012). Auxin plays an important role in regulating root development, and it is transferred by multiple carrier transporters in apple. MdPIN1 is considered to be a key carrier transporter that affects auxin transport and root development just like the functions in *Arabidopsis*. And explaining the functions of *MdPIN1* contributes to realize the target of regulating morphological structure, root development and finally increasing apple production.

In this study, we identified an apple PIN1 protein and named it MdPIN1, based on its homology with AtPIN1. Through the functional identification of MdPIN1-overexpressing *Arabidopsis*, we found that MdPIN1 played an important role in auxin transport and root development, which might contribute to the agricultural produce in the future.

2. Materials and methods

2.1. Plant materials and growth conditions

Samples of seed, root, leaf, shoot, bud, flower, fruit and fruit peel were collected from adult apple trees (Royal Gala) in Shandong Agricultural University, China, in August, 2016.

Wild-type *Arabidopsis thaliana* (Col-0) was used for genetic transformation. Seeds of Col-0 and transgenic lines were sown on Murashige and Skoog (MS) medium, vernalization for 3 days at 4°C, then transferred to normal environment under 16-h-light/8-h-dark conditions at 22°C.

2.2. Sequence alignment and phylogenetic analysis

To obtain the homologs of *MdPIN1*, BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) was performed. Phylogenetic analysis was conducted in MEGA software ver. 5.0. The protein secondary structure of MdPIN1 was predicted using Simple Modular Architeture Research Tool (SMART) Software (http://smart.embl-heidelberg.de/).

2.3. Polymerase chain reaction (PCR) analysis

The transcript levels of *MdPIN1* were examined using specific primers *MdPIN1*(RT)-F and *MdPIN1*(RT)-R. *ACTIN* was used as the control. All the primers were shown in Appendix A.

2.4. Plasmid construction and generation of the *MdPIN1* transgenic *A. thaliana*

The overexpression vector *MdPIN1-Flag* was constructed by inserting the DNA fragment of MdPIN1 open reading frame (ORF) into the transformed vector pCAMBIA1300. The *Agrobacterium tumefaciens* LBA4404 strain was grown in lysogeny broth (LB) media supplemented with 50 mg mL^{-1} of hygromycin and 50 mg mL^{-1} of rifampicin. The *MdPIN1-Flag* consists of the coding sequence under the control of a 35S promoter. Transgenic *A. thaliana* was generated using the floral dip transformation method (Clough and Bent 1998).

2.5. Gravitropism assays and phototaxis assays

7-d-old seedlings were gravistimulated by a 135° rotation and monitored in a lightproof box equipped with a spectrum-enhanced camera (EOS035 Canon Rebel T3i, Japan) modified by Hutech Technologies (Japan) by a built-in clear wide band multicoated filter and operated by EOS Software. Embedded light-emitting diode (LED) infrared diodes (880 nm) dispense light for illuminating the samples. The angle of the