Biochemical and Biophysical Research Communications 424 (2012) 239–244

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Overexpression of *PeRHD3* alters the root architecture in *Populus*

Meng Xu [*,1], Wenfan Xie [1], Minren Huang [*]

Jiangsu Key Laboratory for Poplar Germplasm Enhancement and Variety Improvement, Nanjing Forestry University, Nanjing 210037, China
Key Laboratory of Genetics and Biotechnology, Ministry of Education, Nanjing Forestry University, Nanjing 201137, China

ARTICLE INFO

Article history:
Received 30 May 2012
Available online 22 June 2012

Keywords:
Root hair defective 3 (*RHD3*)
Adventitious root
Hardwood cutting
Poplar transformation

ABSTRACT

Adventitious rooting is essential for the vegetative propagation of economically important woody species. A better understanding of the genetic and physiological mechanisms that promote or hinder rooting will enhance the potential for successful commercial deployment of trees. ROOT HAIR DEFECTIVE 3 (RHD3), a large GTP-binding protein, is ubiquitously expressed in plants. Our previous microarray study identified differential expression patterns of genes belonging to the *RHD3* family during adventitious root development from hardwood cuttings, and indicated that the *RHD3* genes were involved in adventitious rooting in *Populus*. In this study, we cloned and characterized cDNAs of the two *Populus RHD3* genes, designated as *PeRHD3a* and *PeRHD3b*. Transcripts encoded by the two genes were detected in roots, stems, leaves and petioles. To characterize the cellular functions of the genes, *Agrobacterium tumifaciens* was used to transform poplar with a vector that places expression of the target gene under the control of the strong constitutive promoter, Cauliflower Mosaic Virus 35S (Pro35S) promoter. Both *PeRHD3a* transgenic lines and *PeRHD3b* transgenic lines showed very similar phenotypic characteristics. Overexpression of *PeRHD3a* or *PeRHD3b* in poplar plants resulted in the formation of only a single prominent adventitious root with well-developed lateral roots, characteristic abnormalities in the root tip, and longer and more plentiful root hairs. These results imply that *RHD3* may control adventitious and lateral root formation, as well as root hair development by regulating anisotropic cell expansion.

1. Introduction

Adventitious rooting ability is an ecologically and economically important trait in programs focused on the genetic improvement of forest trees, and is a major determinant of the economic and biological viability of tree plantations. *Populus* species and other forest trees are often planted as dormant hardwood cuttings. The success of plantations established using such systems depends largely upon the effectiveness of the initiation and development of adventitious roots. Successful and extensive adventitious rooting of hardwood cuttings, not only favors rapid fixation of elite genotypes to ensure a stable supply of large amounts of genetically uniform plant material. It also decreases costs associated with both the establishment and management of tree plantations by reducing the time needed for crown closure and increasing the flexibility of planting schedules needed to accommodate fluctuations in environmental conditions [1]. Over the past several decades, adventitious rooting of *Populus* hardwood cuttings has been extensively studied at the physiological and morphological levels because of its commercial importance for the forestry industry. Previous studies have suggested that latent or preformed root initials in *Populus* cuttings are established during stem development and that, under the proper environmental conditions, they quickly develop into adventitious roots the following spring [2–4]. Moreover, adventitious rooting of *Populus* hardwood cuttings, which involves complex postembryonic developmental processes, is under strong genetic control [1,5]. However, despite extensive physiological and anatomical studies of adventitious rooting of stem cuttings, the molecular mechanisms involved in determining the competence of cells to generate adventitious roots are not well defined in long-lived woody species.

The molecular mechanism of adventitious root formation in herbaceous model plants have been analyzed extensively [6]. Physiological and genetic studies in *Arabidopsis thaliana*, *Oryza sativa* and *Zea mays*, have shown that plant hormones play important roles in adventitious root formation, and that hormone signaling interacts at multiple levels in this developmental process [7–9]. In woody species, far less is known about the genes involved in adventitious root formation. The *5NG4* gene from *Pinus taeda* [10], the *SCARECROW-LIKE* genes from *Pinus radiata* and *Castanea sativa* [11], and the *SHORT-ROOT* (*PrSHR*) gene from *P. radiata* are active during the earliest stages of adventitious root formation in hypocotyls cuttings [12]. The *Adventitious Rooting Related Oxygenase* (*ARRO-1*) gene from *Malus domestica* regulates hormone

* Corresponding author. Fax: +86 25 85427891.
E-mail addresses: xum@njfu.edu.cn (M. Xu), njmrhuang@njfu.edu.cn (M. Huang).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.06.083

homeostasis during adventitious root formation [13]. A recent study revealed that the cytokinin type-B response regulator PtRR13 negatively regulates adventitious root development in *Populus* softwood cuttings [14].

GTP-binding proteins are important for a wide variety of cellular processes, including signal transduction, intracellular trafficking, cytoskeleton organization, and protein synthesis. As a member of the atlastin GTPases [15], *Arabidopsis* ROOT HAIR DEFECTIVE 3 (RHD3), which contains the putative GTP-binding motifs GXXXXGKS and DXXG, was originally isolated during screens for mutants with defective root hair growth. Mutations affecting the *RHD3* gene altered cell size, but not cell number, in all organs. The *rhd3* mutants exhibited abnormal vacuole enlargement and vesicle distribution in root hairs, causing a short and wavy root hair phenotype [16,17]. Mutations of the *RHD3* gene in *Arabidopsis fragile fiber 4* (*fra4*) mutants caused dramatic reductions in the secondary wall thickness of fibers and vessels and the thickness of the primary walls of pith cells. This indicates an essential role for RHD3 in cell wall biosynthesis and actin organization, both of which are important for cell expansion [18]. A GFP-based assay suggested that RHD3 was required for normal rates of membrane traffic between the endoplasmic reticulum and Golgi apparatus [19]. In plant cells, the endoplasmic reticulum and Golgi apparatus form a unique system in which single Golgi stacks are motile and closely associated with the underlying endoplasmic reticulum tubules. *Arabidopsis* RHD3 mediates the generation of the tubular ER network and is required for normal distribution and motility of the Golgi apparatus [20].

With the completion of the *Populus trichocarpa* genome sequence [21,22], *Populus* now offers many possibilities for helping to unravel the molecular and genetic mechanisms of adventitious root development in stem cuttings. There are no reports regarding isolation and characterization of the *RHD3* gene in *Populus*, despite extensive and detailed analysis of the *RHD3* genes in herbaceous model organisms. Our previous study identified differential expression patterns of members of the *RHD3* gene family during adventitious root development from hardwood cuttings, and indicated that the *RHD3* genes were involved in adventitious rooting in *Populus*. In this study, we cloned and characterized cDNAs of the two *Populus RHD3* genes, and demonstrated accumulation of their transcripts in roots, stems, leaves and petioles. To characterize the cellular functions, *Agrobacterium tumifaciens* was used to transform poplar with a vector that placed expression of the target gene under the control of a strong, constitutive promoter, Cauliflower Mosaic Virus 35S (Pro35S). Unlike non-transgenic plants, the *PeRHD3a* or *PeRHD3b* transgenic lines all have only one prominent adventitious root with abundant lateral roots, exhibit abnormal enlargement and distortion of the root tip, and produce longer and more plentiful root hairs.

2. Materials and methods

2.1. Plant materials and growth conditions

Dormant hardwood cuttings from the hybrid poplar (*P. deltoides* × *P. euramericana* cv. 'Nanlin895') were collected from stool beds and water-cultured in plastic boxes. Aquarium pumps provided aeration for the plastic boxes, and the trees were kept in a greenhouse at 23–25 °C during the day and 16–18 °C at night. The process of adventitious root development in *Populus* hardwood cuttings has been described in several previous studies [5,23–25]. Tissues from three independent biological replicates were harvested at five macroscopic developmental time points (DB: dormant bark tissues, RC: root calli, ER: emerging roots, PR: primary adventitious roots, and LR: lateral roots) that define distinct phases during adventitious rooting from hardwood cuttings. In addition to these samples, roots, stems, leaves and petioles were harvested from 15-day-old seedling ('Nanlin895') grown in tissue culture. After harvesting, all samples were immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

2.2. Cloning of full-length ORFs

Total RNA was extracted from different organs or tissues as described by Xu et al. [28], and was further purified using the RNeasy Plant Mini Kit (Qiagen). One microgram of the DNase-treated RNA was then reverse transcribed into first strand cDNA using the SuperScript III First-Strand Synthesis System (Invitrogen). Our previous microarray analysis of adventitious root development in *Populus* identified differential expression for the probe sets PtpAffx.211104.1.S1_s_at and PtpAffx.212250.1.S1_at, which correspond to the predicted *P. trichocarpa* gene models *POPTR_0013s06310.1* and *POPTR_0019s05730.1*(unpublished data), respectively, both of which are highly homologous to *AtRHD3*. Based on the sequence information of *POPTR_0013s06310.1* and *POPTR_0019s05730.1*, the primer pairs (S-Table 1) were designed to amplify the open reading frames (ORFs) from emerging roots (ER). The PCR fragments with the expected lengths were cloned into the pMD19-T Vector (TaKaRa) and subsequently sequenced. The nucleotide sequences, ORFs, and deduced amino acid sequences were analyzed using the BioEdit software and FGENESH (http://mendel.cs.rhul.ac.uk/mendel.php?topic = fgen). The deduced amino acid sequences were used to predict the molecular weights and isoelectric points using the Compute pI/MW tool (http://www.expasy.ch/tools/protparam.html).

2.3. Phylogenetic analysis

The Basic Local Alignment Search Tool (BLASTp; http://blast.ncbi.nlm.nih.gov/) was used to search for full-length protein sequences (RHD3) from different species in the non-redundant protein sequence repository of the public GenBank database (National Center for Biotechnology Information). The following sequences from GenBank were used in multiple sequence alignment analysis: AlRHD3 (XP_002865275.1) from *Arabidopsis lyrata* subsp. *lyrata*, AtRHD3 (NP_188003.1, NP_974308.1, NP_177439.2 and NP_199329.1) from *A. thaliana*, BdRHD3 (XP_003569246.1) from *Brachypodium distachyon*, CmRHD3 (ADN34108.1) from *Cucumis melo subsp. melo*, GmRHD3 (XP_003529864.1, XP_003546242.1) from *Glycine max*, OsRHD3 (NP_001068156.1, NP_001067218.1, NP_001043388.1) from *Oryza sativa*, OtRHD3 (XP_003078580.1) from *Ostreococcus tauri*, PpRHD3(XP_001783917.1) from *Physcomitrella patens subsp. patens*, PtRHD3(XP_002332571.1, XP_002327435.1) from *Populus trichocarpa*, RcRHD3 (XP_002527405.1) from *Ricinus communis*. SmRHD3 (XP_002967197.1, XP_002960525.1) from *Selaginella moellendorffii*, TaRHD3 (AAS67855.2) from *Triticum aestivum*, and VvRHD3 (XP_002270213.2, XP_002279673.2, CBI16767.3) from *Vitis vinifera*. Sequences were aligned using ClustalW with default settings, and a phylogenetic tree was constructed using the Maximum Parsimony method from MEGA5 [26]. The different clusters in the tree indicate their relationships with one another. Sequences that lie in the same cluster are more closely related with those lying in distant clusters.

2.4. Plant expression vector construction and poplar transformation

The ORFs of *PeRHD3a* and *PeRHD3b* were amplified and subsequently cloned into the pH35GS binary vector using the Gateway System (Invitrogen) to replace the *ccdB* gene, which was downstream of the CaMV35S promoter. The binary vector harboring the Pro35S::*PeRHD3a* or Pro35S::*PeRHD3b* was transformed into *Agrobacterium tumefaciens* strain EHA105, respectively. Poplar ((*P.*

tremula × *P. tremuloides*. clone T89) was transformed using *A. tumefaciens* strain EHA105 with the vector Pro35S::*PeRHD3a* or Pro35S::*PeRHD3b*, respectively, according to a previously described method [27].

2.5. Molecular detection of transgenic poplars

The RNA isolation and cDNA synthesis from the leaves of transgenic poplar and non-transformed (NT) T89 plants were performed as described in Section 2.2. Semi-quantitative RT-PCR was calibrated by a non-saturating PCR reaction (25 cycles) involving primers designed to amplify 18S cDNAs (Table 1). Quantitative real-time PCR (qRT-PCR) was performed using the ABI 7500 Real time PCR system (Applied Biosystems) with the SYBR Green Real-Time Master Mix (Toyobo) according to the manufacturer's protocol. All reactions were performed in triplicate. The specificity of the PCR reactions was confirmed by melting curve analysis of the amplicons. The delta–delta-Ct method was used to calculate the relative quantities of each transcript in the sample, using *EF1a* as the reference gene [28].

3. Results

3.1. Identification and characterization of the expression of PeRHD3a and PeRHD3b from poplar

Our previous study, which involved a genome-wide transcription survey conducted using fifteen GeneChip Poplar Genome Arrarys (Affymetrix) to identify and isolate genes associated with adventitious rooting in *Populus* hardwood cuttings, revealed the involvement of poplar *RHD3* in the adventitious rooting process. We thus cloned full-length ORFs of the two poplar *RHD3* genes, named *PeRHD3a* and *PeRHD3b* using previously identified probe sequences from GeneChip and reverse transcription (RT)-PCR. The *PeRHD3a* gene contained a 2094 bp open reading frame (ORF), encoding a putative protein of 698 amino acids with a predicted molecular weight (MW) of 79.15 kDa and an isoelectric point (pI) of 5.26. The o*PeRHD3b* gene contained a 2235 bp ORF, encoding putative protein of 745 amino acids with a predicted MW of 82.89 kDa and a pI of 5.74.

Tissue-specific expression levels of *PeRHD3a* and *PeRHD3b* in different tissues of 15-day-old seedlings ('Nanlin895') were evaluated using quantitative real-time PCR (qRT-PCR). Transcripts of both *PeRHD3a* and *PeRHD3b* were found in roots, stems, leaves and petioles of the seedling. However, their expression patterns differed between certain tissues (S-Fig. 1). Whereas *PeRHD3a* transcript levels were lower in roots, than higher in leaves. *PeRHD3b* transcripts were detected in roots but not in leaves.

3.2. Conserved motif analysis of PeRHD3a and PeRHD3b

A search of the Conserved Domain Database (CDD) for conserved protein domains indicated that the predicted amino acid sequences of *PeRHD3a* and *PeRHD3b*, contained multiple domains characteristic of the GBP, P-loop_NTPase superfamily and RHD3, respectively.

Homology searches were performed using BLASTp algorithms to determine similarities between the query sequence and those found in the NCBI non-redundant protein sequences. The results indicated that the deduced amino acid sequence of *PeRHD3* shared a high sequence identity with the RHD3 proteins of various plant species, including *P. trichocarpa* (predicted proteins: XP_002332571.1 with 96% identity, XP_002332317.1 with 93% identity, and XP_002332572.1 with 88% identity), *Vitis vinifera* (unnamed protein: CBI16767.3 with 67% identity), *Cucumis melo subsp. melo* (proteins SEY1: ADN34108.1 with 60% identity). Similarly, the deduced amino acid sequence of PeRHD3b shared a high sequence identity with the RHD3 proteins of diverse plant species, including *P. trichocarpa* (predicted protein: XP_002327435.1 with 99% identity), and *C. melo subsp. melo* (proteins SEY1: ADN34108.1 with 91% identity), *Selaginella moellendorffii* (hypothetical proteins SELMODRAFT_439743: XP_002967197.1 with 87% identity), *Ricinus communis* (Proteins SEY1: XP_002527405.1 with 85% identity).

Multiple alignment of PeRHD3a and PeRHD3b with the known RHD3 proteins (NP_188003.1,NP_974308.1,NP_177439.2 and NP_199329.1) of *A. thaliana*, revealed that the RHD3 proteins have four typical motifs, including two conservative GTP-binding motifs (GXXXXGKS and DXXG) in eukaryotes, and the other two conserved amino acid motifs, FVIRD and NKDLDLP, which are unique to the RHD3 family. Although PeRHD3b lacks the GXXXXGKS motif, which is involved in coordination of phosphate binding, it contains the three other motifs (Fig. 1).

3.3. Phylogenetic analysis of RHD3 proteins

In order to understand the evolutionary relationship of RHD3 proteins from different species, the deduced amino acid sequences of 25 RHD3 proteins were aligned, and an un-rooted Maximum Parsimony (MP) phylogenetic tree was constructed using MEGA5 software (Supplementary Fig. 2). The phylogenetic analysis based on multiple sequence alignments was divided into three distinct groups. Whereas PeRHD3a fell into the group that included PtRHD3 (XP_002332571.1), SmRHD3 (XP_002967197.1 and XP_002960525.1), and VvRHD3 (XP_002270213.2 and CBI16767.3), PeRHD3b fell into the group that included AtRHD3 (NP_188003.1 and NP_974308.1), PtRHD3 (XP_002327435.1), and RcRHD3 (XP_002527405.1). This result indicates that PeRHD3a and PeRHD3b may have different functions.

3.4. Molecular characterization of transgenic poplar plants

To better understand the functions of the *PeRHD3a* and *PeRHD3b* genes in *Populus*, we constructed the plant expression vectors *Pro35S::PeRHD3a* and *Pro35S*::*PeRHD3b*, which were transformed separately into the poplar clone T89. The kanamycin resistant poplar transformants were screened and confirmed by PCR using specific primers. The expression levels of *PeRHD3a* and *PeRHD3b* in these transgenic lines were quantified using semi-quantitative RT-PCR and quantitative real-time PCR (qRT-PCR). The *PeRHD3a* or *PeRHD3b* transcripts of these transgenic lines were significantly higher than those of NT T89 plants (Fig. 2). These results indicated that the *Pro35S::PeRHD3a* or *Pro35S::PeRHD3b* expression cassettes were integrated into the genomes of these plants and that the appropriate transcript was expressed from these transgenes.

3.5. Microscopic analysis of transgenic poplar roots

Transgenic poplar plants expressing *PeRHD3a* or *PeRHD3b* under the control the Pro35S promoter were successfully generated using *Agrobacterium*-mediated transformation (Fig. 2). All independent transgenic lines were established and grown in a growth chamber. Light microscopy was used to investigate possible structural and developmental differences between NT T89 plants and the transgenic lines. There were no significant differences of aerial parts between 20-day-old NT plants and either class of transgenic lines. Interestingly, there were significant differences of the root systems between NT plants and the transgenic lines, with both *PeRHD3a* and *PeRHD3b* transgenic lines showing very similar phenotypic characteristics. *PeRHD3a*- or *PeRHD3b*- overexpression were both

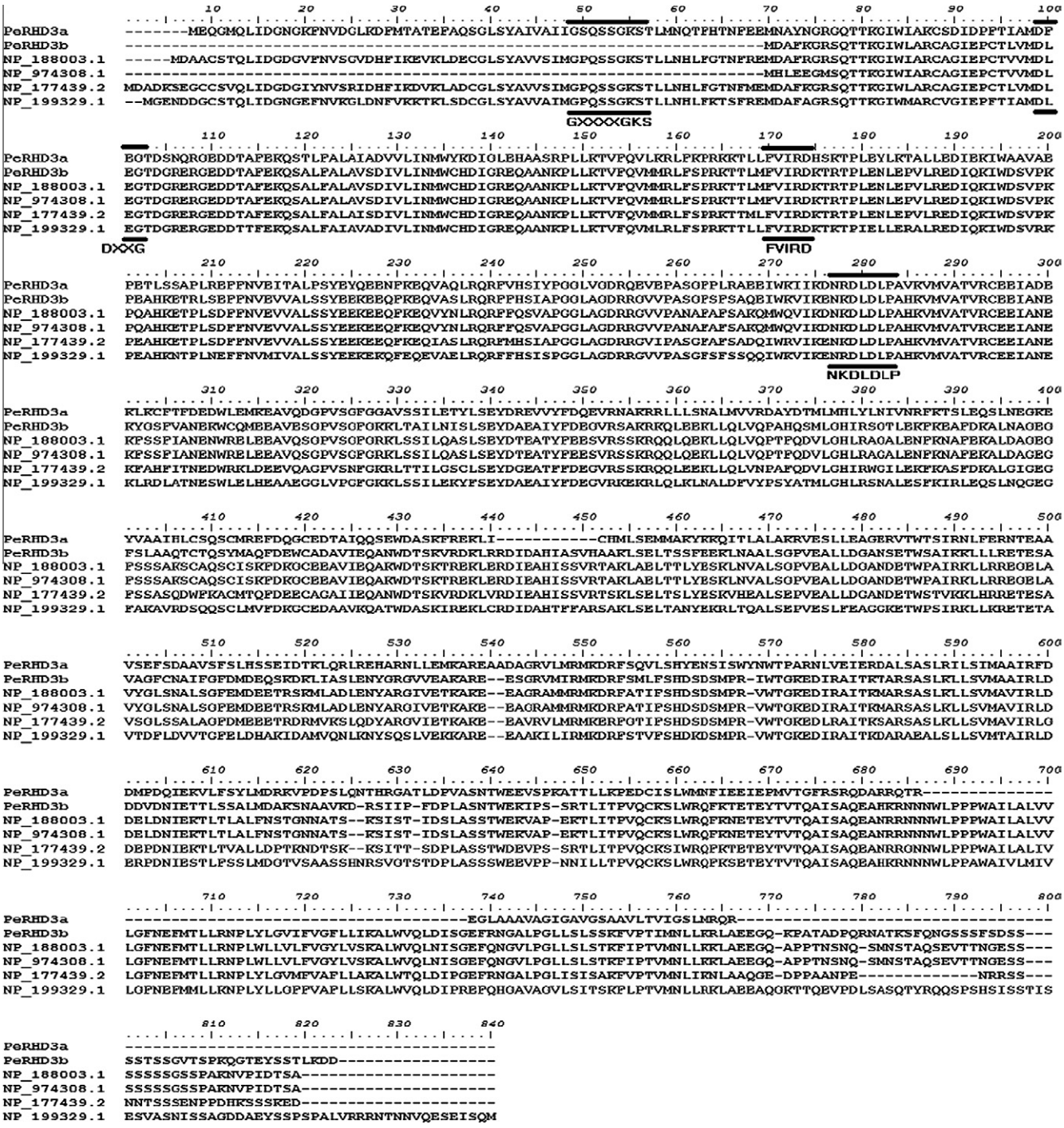

Fig. 1. Conserved protein domains of RHD3. Multiple alignment of PeRHD3a and PeRHD3b with the following *Arabidopsis* RHD3: NP_188003.1, NP_974308.1, NP_177439.2 and NP_199329.1. Four typical domains in RHD3 proteins are indicated with the black bar.

associated with adventitious root formation, and shown to reduce the number of adventitious roots in transgenic plants. All transgenic plants formed only one prominent adventitious root with more lateral roots that were well-developed, whereas there were more than two adventitious roots in NT T89 plants (Fig. 3A). Transgenic poplar plants that overexpressed either *PeRHD3a* or *PeRHD3b* exhibited characteristic abnormalities in the root tip (Fig. 3B). The nature of these abnormalities differed between *PeRHD3a* transgenic lines and *PeRHD3b* transgenic lines. Similar to previous studies, we concluded that *PeRHD3a* and *PeRHD3b* regulated root hair growth in *Populus*, with overexpression of either *PeRHD3a* or *PeRHD3b* in transgenic plants resulting in more root hair formation (Fig. 3C).

4. Discussion

In higher plants, morphogenesis of plant cells and organs is largely determined by the orientation and extent of cell enlargement during cell differentiation. The *RHD3* gene encodes an evolutionarily conserved protein with GTP-binding motifs and was required for regulating cell enlargement in *Arabidopsis* [16]. *Arabidopsis* has three isoforms of RHD3 that are analogous to the mammalian

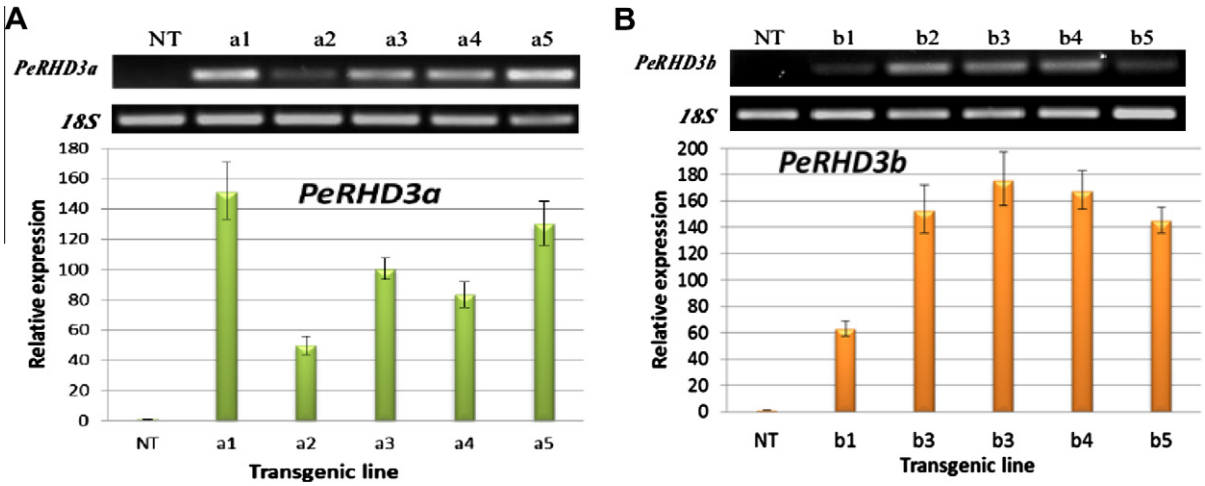

Fig. 2. Semi-quantitative RT-PCR and qRT-PCR of transgenic poplar plants. (A) Analysis of overexpression *PeRHD3a* in transgenic lines using semi-quantitative RT-PCR (25 cycles) and qRT-PCR. Lane NT, non-transformed T89 plants; lanes a1, a2, a3, a4, and a5, randomly selected *PeRHD3a*-overexpressing T89 lines. (B) Analysis of overexpression *PeRHD3b* in transgenic lines using semi-quantitative RT-PCR (25 cycles) and qRT-PCR. Lane NT, non-transformed T89 plants; lanes b1, b2, b3, b4, and b5, randomly selected *PeRHD3b*-overexpressing T89 lines.

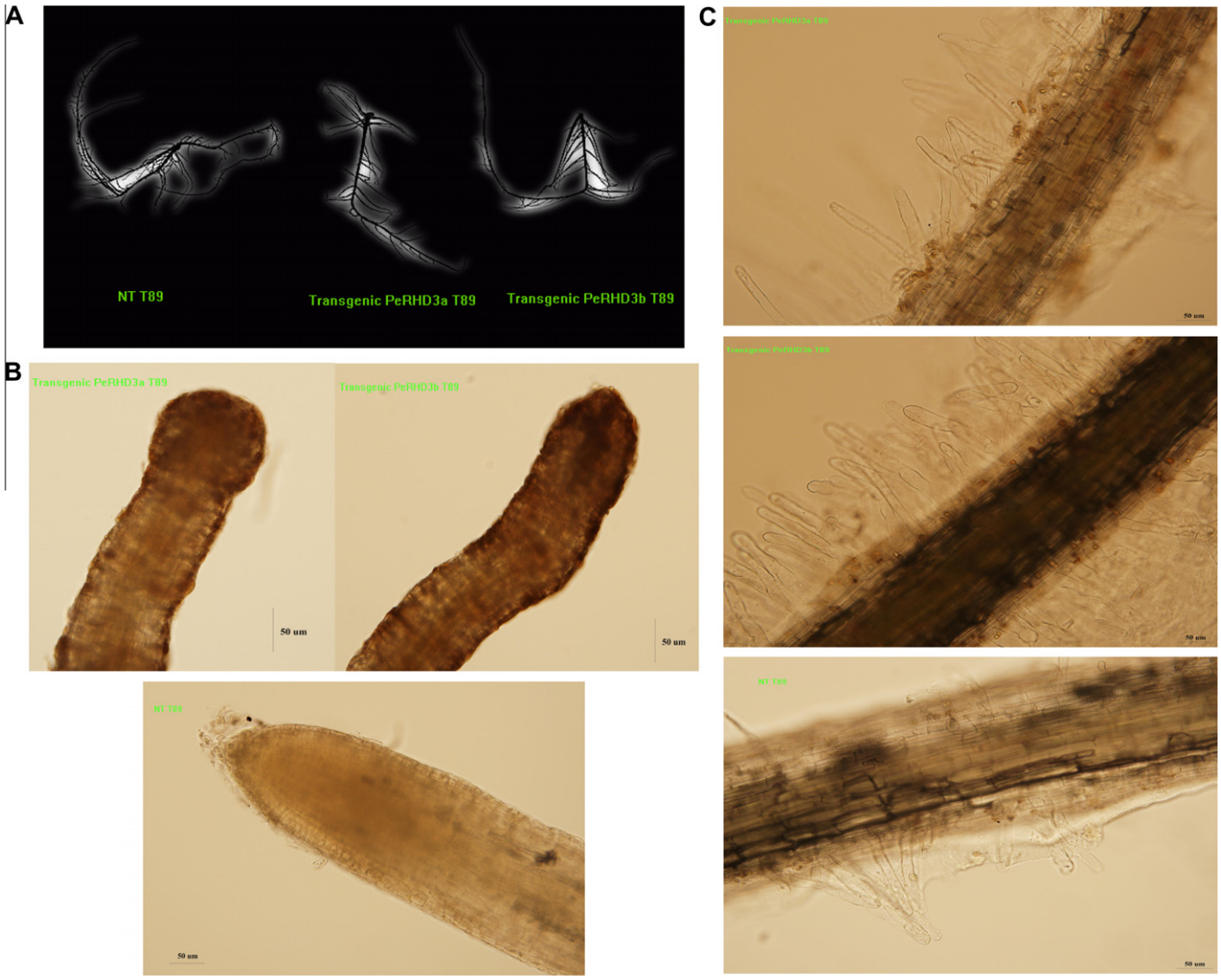

Fig. 3. Phenotypic analysis of transgenic poplar plants. (A) Poplar root systems of NT T89 plants and *PeRHD3a* or *PeRHD3b* transgenic lines. (B) Microscopic analysis of root tips of NT T89 plants and *PeRHD3a* or *PeRHD3b* transgenic lines. Bar = 50 μm. (C) Microscopic analysis of root hairs of NT T89 plants and *PeRHD3a* or *PeRHD3b* transgenic lines. Bar = 50 μm.

atlastin GTPases involved in shaping endoplasmic reticulum tubules. Whereas both *RHD3-like2* (*RL2*) and *RHD3* are ubiquitously expressed throughout plant tissues, *RHD3-like1* (*RL1*) is pollen specific [18], while knockout mutants of *RL1* and *RL2* produced no obvious developmental defect [20]. Recent work further revealed that members of the RHD3 family, at least when overexpressed, can substitute for other gene products normally responsible for maintaining the tubular morphology of the endoplasmic reticulum, indicating that they might act on similar targets in the cells [20].

In *Populous*, the members of the RHD3 family, their cellular functions, and their interactions with other genes and gene products remain to be determined. In this study, we cloned two RHD3 genes from poplar roots, *PeRHD3a* and *PeRHD3b*, which are ubiquitously expressed throughout poplar tissues (Supplementary Fig. 1). There is an obvious difference between these two genes in terms of their mRNA expression patterns. A search of the CDD database indicated that whereas the predicted amino acid sequences encoded by *PeRHD3a* contains four conversed domains, the putative PeRHD3b protein lacks a GXXXXGKS motif (Fig. 1). Further studies revealed that there were obvious developmental differences in the root systems between NT plants and both classes of transgenic lines, with similar phenotypes observed in both of the *PeRHD3a* and *PeRHD3b* transgenic plants (Fig. 3).

These results indicate that *PeRHD3a* and *PeRHD3b* are important for a wide variety of cellular processes in poplar, and that the prominent developmental abnormalities in the transgenic poplar plants we generated are not restricted to adventitious root development. New insights into members of the poplar *RHD3* family will improve comprehensive understanding of the molecular mechanisms controlling the extent and orientation of cell enlargement in plant development. Moreover, cloning and characterization of genes promoting or hindering rooting in poplar will enhance the potential for successful afforestation of difficult sites.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31100484).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.06.083.

References

[1] R.S. Zalesny, D.E. Riemenschneider, R.B. Hall, Early rooting of dormant hardwood cuttings of Populus: analysis of quantitative genetics and genotype × environment interactions, Can. J. For. Res. 35 (2005) 918–929.
[2] N.G. Smith, P.F. Wareing, The distribution of latent root primordial in stem of *Populus* × *rubusta*, and factors affecting the emergence of preformed roots from cuttings, Forestry 45 (1972) 197–209.
[3] M. Luxová, A. Lux, Latent root primordia in poplar stems, Biol. Plant 23 (1981) 285–290.
[4] S. Puri, F.B. Thompson, Effect of soil and plant water relations on rooting of *Populus* × *Euramericana* stem cuttings, New Forest 25 (2003) 109–124.
[5] B. Zhang, C.F. Tong, T.M. Yin, X.Y. Zhang, Q. Zhuge, M.R. Huang, M.X. Wang, R.L. Wu, Detection of quantitative trait loci influencing growth trajectories of adventitious roots in *Populus* using functional mapping, Tree Genet. Genomes 5 (2009) (2009) 539–552.
[6] F. Hochholdinger, R. Zimmermann, Conserved and diverse mechanisms in root development, Curr. Opin. Plant Biol. 11 (2008) 70–74.
[7] L. Gutierrez, J.D. Bussell, D.I. Pacurar, J. Schwambach, M. Pacurar, C. Bellini, Phenotypic plasticity of adventitious rooting in Arabidopsis is controlled by complex regulation of AUXIN RESPONSE FACTOR transcripts and microRNA abundance, Plant Cell 21 (2009) 3119–3132.
[8] Y. Coudert, C. Périn, B. Courtois, N.G. Khong, P. Gantet, Genetic control of root development in rice, the model cereal, Trends Plant Sci. 15 (2010) 219–226.
[9] P. Overvoorde, H. Fukaki, T. Beeckman, Auxin control of root development, Cold Spring Harb. Perspect. Biol. 2 (2010) a001537.
[10] V.B. Busov, E. Johannes, R.W. Whetten, R.R. Sederoff, S.L. Spiker, C. Lanz-Garcia, B. Goldfarb, An auxin-inducible gene from loblolly pine (*Pinus taeda* L.) is differentially expressed in mature and juvenile-phase shoots and encodes a putative transmembrane protein, Planta 218 (2004) 916–927.
[11] C. Sanchez, J.M. Vielba, E. Ferro, G. Covelo, A. Sole, D. Abarca, B.S. De Mier, C. Diaz-Sala, Two SCARECROW-LIKE genes are induced in response to exogenous auxin in rooting-competent cuttings of distantly related forest species, Tree Physiol. 27 (2007) 1459–1470.
[12] A. Sole, C. Sanchez, J.M. Vielba, S. Valladares, D. Abarca, C. Diaz-Sala, Characterization and expression of a Pinus radiate putative ortholog to the *Arabidopsis* SHORT-ROOT gene, Tree Physiol. 28 (2008) 1629–1639.
[13] A. Smolka, M. Welander, P. Olsson, A. Holefors, L. Zhu, Involvement of the *ARRO-1* gene in adventitious root formation in apple, Plant Sci. 177 (2009) 710–715.
[14] G.A. Ramírez-Carvajal, A.M. Morse, C. Dervinis, J.M. Davis, The cytokinin type-B response regulator PtRR13 is a negative regulator of adventitious root development in *Populus*, Plant Physiol. 150 (2009) 759–771.
[15] J. Hu, Y. Shibata, P.P. Zhu, C. Voss, N. Rismanchi, W.A. Prinz, T.A. Rapoport, C. Blackstone, A class of dynamin-like GTPases involved in the generation of the tubular ER network, Cell 138 (2009) 549–561.
[16] H. Wang, S.K. Lockwood, M.F. Hoeltzel, J.W. Schiefelbein, The *ROOT HAIR DEFFECTIVE3* gene encodes an evolutionarily conserved protein with GTP-binding motifs and is required for regulated cell enlargement in *Arabidopsis*, Genes Dev. 11 (1997) 799–811.
[17] M.E. Galway, J.W. Heckman Jr, J.W. Schiefelbein, Growth and ultrastructure of *Arabidopsis* root hairs: the *rhd3* mutation alters vacuole enlargement and tip growth, Planta 201 (1997) 209–218.
[18] Y. Hu, R. Zhong, W.H. Morrison III, Z.H. Ye, The Arabidopsis RHD3 gene is required for cell wall biosynthesis and actin organization, Planta 217 (2003) 912–921.
[19] H. Zheng, L. Kunst, C. Hawes, L. Moore, A GFP-based assay reveals a role for RHD3 in transport between the endoplasmic reticulum and Golgi apparatus, Plant J. 37 (2004) 398–414.
[20] J. Chen, G. Stefano, F. Brandizzi, H. Zheng, Arabidopsis RHD3 mediates the generation of the tubular ER network and is required for Golgi distribution and motility in plant cells, J. Cell Sci. 124 (2011) 2241–2252.
[21] G.A. Tuskan, S. Difazio, S. Jansson, J. Bohlmann, I. Grigoriev, U. Hellsten, N. Putnam, S. Ralph, S. Rombauts, A. Salamov, et al., The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray), Science 313 (2006) 1596–1604.
[22] S. Jansson, C.J. Douglas, *Populus*: a model system for plant biology, Annu. Rev. Plant Biol. 58 (2007) 435–458.
[23] G. Li, S. Lu, A study on rooting characteristics of cuttings of new poplar clones, For. Res. 7 (1994) 168–174.
[24] A. Kohler, C. Delaruelle, D. Martin, N. Encelot, F. Martin, The poplar root transcriptome: analysis of 7000 expressed sequence tags, FEBS Lett. 542 (2003) 37–41.
[25] A. Kohler, D. Blaudez, M. Chalot, F. Martin, Cloning and expression of multiple metallothioneins from hybrid poplar, New Phytol. 164 (2004) 83–93.
[26] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Mol. Biol. Evol. 28 (2011) 2731–2739.
[27] L. Shen, Y. Chen, X. Su, Sh. Zhang, H. Pan, M. Huang, Two FT orthologs from *Populus simonii* Carrière induce early flowering in *Arabidopsis* and poplar trees, Plant Cell, Tissue Organ Cult. 108 (2012) 371–379.
[28] M. Xu, B. Zhang, X. Su, Sh. Zhang, M. Huang, Reference gene selection for quantitative real-time polymerase chain reaction in *Populus*, Anal. Biochem. 408 (2011) 337–339.