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The WUSCHELa (PtoWUSa) is Involved in Developmental Plasticity of Adventitious Root in Poplar

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Abstract: WUSCHEL-RELATED HOMEBOX (WOX) transcription factors play critical roles in cell fate determination during plant development. As the founding member of the WOX family, WUSCHEL (WUS) is characterized for its role in maintaining stem cell in meristem. In this study, we investigated the function of Populus tomentosa WUSCHELa (PtoWUSa) in adventitious roots (ARs) in poplar. Expression profile analysis showed that PtoWUSa was not only expressed in shoot apical meristem and stem, but also expressed in ARs. Ectopic expression of PtoWUSa in Arabidopsis resulted in shortened primary root, as well as agravitropism and multiple branches. Overexpression of PtoWUSa in poplar increased the number of ARs but decreased their length. Moreover, the AR tip and lateral root tip became larger and swollen. In addition, the expression of auxin transporter genes PIN-FORMED were downregulated in ARs of transgenic plant. Taken together, these results suggest that PtoWUSa could be involved in AR development in poplar through regulating the polar auxin transport in ARs.

Keywords: adventitious roots; auxin; poplar; PtoWUSa; transgenic plant

1. Introduction

Cutting is a main method of vegetative propagation for woody plants. An essential step of cutting depends on the formation and development of adventitious roots (ARs). Therefore, dissecting the molecular mechanisms of the development of ARs and exploring the function of genes regulating adventitious rooting will be beneficial for improving propagation efficiency. ARs are always generated from the stem-, hypocotyl- or leaf-derived cells. The development of ARs is regulated by multiple endogenous and environmental factors. Among them, phytohormone auxin plays a key role [1]. Auxin is mainly synthesized in young aerial parts and flows down to the root tip columella in which an auxin maximum is formed, and then transports to other parts of the root through root epidermal cells [2]. During this process, the formed auxin concentration gradients regulate the root growth and plasticity. The auxin transport from cell-to-cell is mediated by auxin transport carriers, such as AUX1-like influx carrier, PIN-FORMED efflux carrier (PIN) and P-glycoprotein auxin carrier (PGP) [3].

The PIN is characterized by its polar plasma membrane localization that regulates the direction of auxin flow [4]. PGP can improve the stability of PINs in plasma membrane and enhance cellular auxin transport activity [5]. PIN, PGP efflux transporters, and AUX1 influx transporters determine
the directionality of intercellular auxin flow [6]. One of the prominent growth responses regulated by auxin polar transport is root tropism, such as the mutants of PIN2, PIN3, PIN5, PIN7, and AUX1 with root gravitropism [7–11].

The WUSCHEL-related homebox (WOX) gene family is a plant-specific transcription factor and belongs to the homeodomain superfamily [12]. The function of WOX genes in Arabidopsis thaliana have been well characterized and show that WOXs play critical roles in stem cell maintenance in shoot and root apical meristems (SAM and RAM), root development, et al. [13–15]. AtWOX1/AtWOX3 regulates lateral axis-dependent development of lateral organs [16]. AtWOX2, AtWOX8 and AtWOX9 are required for apical-basal axis formation of pre-embryo, and AtWOX9 also plays a role in maintaining the cell division in early embryogenesis [17,18]. AtWOX4 is expressed in vascular and regulates the vascular stem cell proliferation, and it acts redundantly with AtWUS in the maintenance of vascular meristem organization during secondary growth [19,20]. AtWOX5 is specifically expressed in the cells of quiescent center and functions in organizing stem cells in RAM [21,22]. AtWOX7 regulates the lateral root (LR) development [23]. AtWOX11/12 are involved in AR de novo organogenesis by activating the expression of AtWOX5 and AtWOX7 [24,25].

As the founding member of WOX family, WUSCHEL (WUS) is originally identified in Arabidopsis [26]. AtWUS serves as a central regulator in the maintenance of stem cell identity in the central zone of SAM and floral meristems [26,27]. AtWUS expression is restricted in organizing center of SAM, and AtWUS protein can move to the stem cell region to promote the expression of CLAVATA 3 (CLV3), then the activated CLV3 signaling pathway inhibits the expression of WUS, resulting in maintaining stem cell homeostasis in SAM [28–30]. At the reproductive phase, the WUS-AGAMOUS feedback loop controls the flower meristem (FM) identity [31]. In atwus mutant, plants develop in stop-and-go mode, and defective SAM and FM are initiated repetitively, leading to many disorganized bunches of leaves along the plant [26]. In Oryza sativa, OsWUS is expressed in young leaf primordia, axillary meristems (AMs) and transient expressed in rice SAM [32–34]. OsWUS is required for initiation of the AMs, but not for maintenance of the SAM [34]. The mutants of OsWUS, tab1-1 [24], moc3-1 [33], and srt [35] exhibit various degree defects in tillering and flower development. Overexpression of cotton (Gossypium hirsutum) GhWUS can induce somatic embryo and shoot formation [36]. Despite the function of WUS in developmental processes, which has been studied in herbaceous plant, little is known about its function in woody plants.

As a model woody species, Populus is characteristic with rapid growth and high production of plant biomass. In our previous study, 18 WOX genes were identified in Populus tomentosa, and 2 P. tomentosa WOXs (PtoWUSa and PtoWUSb) were identified as the orthologous genes of Arabidopsis AtWUS [37]. In this study, we characterized the function of PtoWUSa in AR development. Expression profile analysis showed that PtoWUSa was not only expressed in SAM and stem, but also expressed in ARs. Transgenic results displayed that overexpression of PtoWUSa affected the root morphogenesis by down-regulation of PIN expressions.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

A. thaliana (ecotype Columbia-0) was grown at 20–22 °C under 16 h/8 h light/dark photoperiod in 1/2 Murashige-Skoog (MS) medium or soil. Hybrid poplar (P. alba × P. glandulosa) 84K was performed tissue culture to obtain poplar clonal plants for transgenic acceptors and was grown at 23–25 °C under 16 h/8 h light/dark photoperiod.

2.2. Domain, Conserved Motifs and Phylogenetic Sequence Analysis

The WUS amino acid sequences of different species were obtained from website (http://www.phytozome.com, https://www.ncbi.nlm.nih.gov/genbank). The multiple sequences alignment of WUS was generated using DNAMAN 7.0 (Lynnon Corporation, San Ramon, USA). The GenBank accession numbers of the WUS from different species were: AtWUS (A. thaliana, AT2G17950), OsWUS (O. sativa,
BAE48303.1), PtoWUSa (P. tomentosa, AHC29308.1), PtoWUSb (P. tomentosa, ACO55494.1), PtoWUSa (P. trichocarpa, Potri.005G114700), PtoWUSb (P. trichocarpa, Potri.007G01210), SsuWUS (Salix suchowensis, SapatV1A.0487s0060.1), SLWUS (Solanum lycopersicum, NP_001234015.2), VvWUS (Vitis vinifera, XP_002266323.1). Clustal X2 was used for sequence alignment, and MEGA v6.0 was used to construct phylogenetic tree with neighbor-joining (NJ) method with 1,000 bootstrap replicates.

2.3. RNA Extraction and qRT-PCR Assays

Total RNAs were extracted from plant samples using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The quality and concentration of RNAs were detected by agarose gel electrophoresis and NanoDrop 8000 (Thermo, Wilmington, USA). The first strand cDNA was acquired using SuperScript III first-strand synthesis system (Life technologies, Carlsbad, USA) with 1 µg mRNA. Quantitative real-time PCR (qRT-PCR) was performed on the Roche LightCycler 480 (Roche Applied Science, Penzberg, Germany) following manufacturer’s instructions. Actin7 and PtActin were chose as reference genes. The 2^−ΔΔCT method was used to calculate the relative expression level [38]. Three biological replicates and four technical replicates of each sample were performed for qRT-PCR analysis. The primers used in this study were listed in Table S1.

2.4. Plasmid Construction and Transformation

The coding sequence and the promoter of PtoWUSa were amplified and cloned into pDNOR222.1 for sequencing. The right coding sequence was sub-cloned respectively into pMDC32 and pMDC7, represented as p35S::PtoWUSa and pXVE::PtoWUSa. The right promoter sequence was conducted into pMDC164, represented as PtoWUSa::GUS. The resulting constructs were then transformed into Agrobacterium GV3101 by electroporation. Agrobacterium carrying p35S::PtoWUSa::GUS, p35S::PtoWUSa, or pXVE::PtoWUSa were used for poplar transformation. The RNAs were extracted from roots of two-week-old p35S::PtoWUSa transgenic A. thaliana plants and three-week-old p35S::PtoWUSa transgenic poplar plants, and the transcription levels of PtoWUSa were detected using qRT-PCR. Two independent transgenic A. thaliana lines (#31 and #43) and two independent transgenic poplar lines (#16 and #18) with high PtoWUSa abundance were used for further study. The p35S::PtoWUSa::GUS was used for GUS staining according to the previous studies [39].

2.5. Estradiol Treatments

The 17 β-estradiol was dissolved in dimethyl sulfoxide (DMSO) as 10 mM stock solution. The apicals (2–3 cm) from 84 K and two independent pXVE::PtoWUSa transgenic lines (#26 and #27) with two leaves were induced by adding 17 β-estradiol into the nutrient solution, while the untreated condition was corresponded to plants treated with DMSO. Phenotypic observation was carried out on 20-day-old poplars. The concentration of 17 β-estradiol was determined based on treatment in poplar [40].

2.6. Statistical Analysis

The statistical analysis of all the experimental data was performed with SPSS 16.0 software (SPSS Inc, Chicago, USA). Significant differences were detected using t test and the differences were considered significant if p < 0.05.

3. Results

3.1. Phylogenetic Analysis and Protein Sequence Comparisons

Two P. tomentosa WOXs (PtoWUSa and PtoWUSb) were identified as the orthologous genes of AtWUS in our previous study, but only PtoWUSa was expressed in AR [37]. Therefore, PtoWUSa was chosen for function investigation in AR development in the present study. PtoWUSa contained a helix-loop-helix-turn domain in the N-terminal which was known as the homeodomain (HD),
an EAR-like domain in the C-terminal, and a conserved WUS-box domain (TLxLFP) between them (Figure 1). The result of phylogenetic relationships between PtoWUSa and AtWOXs showed that PtoWUSa belongs to the WUS/modern clade and had a close genetic relationship with AtWUS (Figure S1). Compared with WUS protein sequences from different species, PtoWUSa shared 29.83–98.86% identity with WUS sequences from other species (Figure 1). PtoWUSa and PtrWUSa shared 98.86% identity and had only three residues difference over the entire amino acid sequence. These results indicate that WUS had conserved domain, although the sequences were variable in different plant species.

![Figure 1.](image)

**Figure 1.** PtoWUSa was highly conserved in a broad range of plant species. (A) Amino acid sequence alignment of PtoWUSa with AtWUS (*Arabidopsis thaliana*), OsWUS (*Oryza sativa*), PtrWUS (*Populus trichocarpa*), SiWUS (*Solanum lycopersicum*), SsuWUS (*Salix suchowensis*), and VvWUS (*Vitis vinifera*) orthologous genes in other plant species. The conserved regions (homeodomain, WUS-box motif, and ethylene-responsive element binding factor associated amphiphilic repression (EAR)-like domain) were underlined. (B) WUS amino acid sequence identity.

### 3.2. Expression Pattern of PtoWUSa

The GUS reporter of *PtoWUSa* was conducted to confirm the expression pattern of *PtoWUSa*. GUS staining, showing that the GUS signals were detected in the SAM (Figure 2A) and stem (Figure 2B) and roots (Figure 2C–F). The roots from the poplar stem were named ARs, and the roots located in the ARs were named LRs. In roots, the GUS signal was specially detected in the AR tip (ART) and the LR tip (LRT) (Figure 2C–F). These results indicated that the *PtoWUSa* accumulated in LRT and ART in the roots, suggesting that *PtoWUSa* might play roles in root development.
Figure 2. Spatial expression patterns of *PtoWUSa* detected by GUS staining. (A,B) Spatial expression patterns of *PtoWUSa* in the longitudinal section of shoot apex (A) and cross-section of stem (B). (C–F) Expression patterns of *PtoWUSa* in root (C); LRT (D and E) and ART (F) were close-ups of the boxed regions in (C). Arrows highlighted the GUS signal. Scale bars: (A) = 20 µm; (B) = 10 µm; (C) = 5 mm; (D,E) = 500 µm.

3.3. Ectopic Expression *PtoWUSa* Affected Root Development in *Arabidopsis*

As the herb model plant, *Arabidopsis* has a rapid lifecycle and easy transformation system. Therefore, we firstly investigated the function of *PtoWUSa* by ectopic expression of *PtoWUSa* in *Arabidopsis*. Two independent transgenic lines (#31 and #43) with high abundance of *PtoWUSa* (Figure 3G) were chosen for functional study. After cultured on 1/2 MS medium for two weeks, the length of primary roots of *PtoWUSa*-overexpressing *Arabidopsis* seedlings was severely inhibited and was only 50% of WT seedlings (Figure 3A–C,H). Interestingly, the roots of transgenic plants appeared to curve and showed agravitropism (Figure 3A–C), and their root tip was enlarged and swollen (Figure 3D–F). After three weeks of growth, the abnormal root morphology of transgenic plants was intensified, such as the decreased primary root length, agravitropism, and curved root (Figure 4). The root hair and LRs were located closer to the root tip in transgenic plants, being different with WT plants (Figure 4F,H). It is noteworthy that the LR length of transgenic plants was increased compared with WT plants when the transgenic LRs were stretched out (Figure 4B).
Figure 3. Reduced root length and changed morphology by overexpression of PtoWUSa in Arabidopsis. (A–C) two-week-old of WT (A) and PtoWUSa-overexpressing Arabidopsis plants including two independent transgenic lines of #31 (B) and #43 (C). (D–F) The primary root of WT (D) and two transgenic lines (E,F). Arrows pointed to primary root tip. (G) Transcription levels of PtoWUSa in transgenic plants. AtACTIN was used as a reference gene. The expression level in WT was set to 1. (H) The length of root of WT and transgenic plants. * and ** indicated the difference between WT and each transgenic line at $p < 0.05$ and $p < 0.01$ levels, respectively. Scale bars: (A–C) = 0.2 cm; (D–F) = 200 µm.
Moreover, the *PtoWUSa*-overexpressing *Arabidopsis* plants exhibited more branches than WT plants (Figure 5A–D), and the stem number of transgenic plants reached to four to six (Figure 5F), but the height of primary stem of transgenic plants was reduced to 1/3–1/2 of WT plants after growing on soil for four weeks (Figure 5E).
Figure 5. Overexpression of *PtoWUSa* led to compromise of shoot branching in *Arabidopsis*. (A) Aboveground phenotypes of WT and *PtoWUSa*-overexpressing *Arabidopsis* plants. (B–D) Close-up views of shoot branches in the squares of the corresponding plants in (A). Arrows pointed to shoot branches. (E–F) Comparison of length of primary stem (E) and number of primary stem (F) between WT and transgenic plants. * indicated the difference between Ctrl and each transgenic line at $p < 0.05$ level, respectively. Scale bars: (A) = 2 cm; (B–D) = 1 cm.

3.4. Overexpression of *PtoWUSa* also Alters the Morphology of ARs in Poplar

In order to investigate whether *PtoWUSa* performed similar functions in poplar, we generated transgenic poplar plants overexpressing *PtoWUSa*. Two independent transgenic lines (#16 and #18) with high abundance of *PtoWUSa* were used for further study. After cultured for three weeks, the number of ARs increased to 6 to 8 in transgenic seedlings, which was about twice in control (Ctrl) (Figure 6A–C). Additionally, the AR length of *PtoWUSa*-overexpressing poplar plants (~2 cm) was significantly less than that of Ctrl plants (~6 cm) (Figure 6A–C). Moreover, the morphology of ARTs and LRTs in Ctrl plants was always sharp and small, whereas ARTs and LRTs became enlarged and swollen in transgenic plants. The location of LRs was closer to the ARTs in transgenic plants than in Ctrl plants (Figure 7).
Figure 6. Overexpression of *PtoWUSa* promoted AR formation and inhibited its elongation in poplar. (A,B) Comparison of three-week-old Ctrl and *PtoWUSa*-overexpressing poplar plants including two independent transgenic lines #16 and #18. (C,D) Comparison of AR number (C) and AR length (D) between Ctrl and transgenic plants. * and ** indicated the difference between Ctrl and each transgenic line at \( p < 0.05 \) and \( p < 0.01 \) levels, respectively. Scale bar: 1 cm.
Figure 7. Root morphology of Ctrl and PtoWUSa-overexpressing poplar plants. (A–C) ARs of Ctrl and transgenic plants. (D–F) Close-up views of ARTs of Ctrl and transgenic plants from (A–C). (G–I) Close-up views of LRTs of Ctrl and transgenic plants from (A–C). Scale bars: (A–C) = 1 mm; (D–F–I) = 1 μm; (E) = 3 μm.

To further confirm the functions of the PtoWUSa, we constructed an inducible expression vector pXVE::PtoWUSa and transformed it into poplar. After being induced under 10 μM 17 β-estradiol for 15 days, the two transgenic lines (#26 and #27) with high expression of PtoWUSa were chose for subsequence study (Figure 8D). There was no different phenotype between the Ctrl and transgenic plants without 17 β-estradiol treatment (Figure 8A–C). After treatment by 17 β-estradiol, the AR primordia was observed, AR elongation was inhibited and the ARTs became enlarged and swollen in the pXVE::PtoWUSa transgenic plants (Figure 8E–I). All these results indicated that PtoWUSa was involved in AR development in poplar.
Figure 8. Growth phenotype of Ctrl and pXVE::PtoWUSa transgenic plants (including two independent transgenic lines #26 and #27) treated with 0 µM (A–C) and 10 µM (E–G) 17 β-estradiol for three weeks. (D) Expression levels of PtoWUSa in Ctrl and transgenic plants after treated 10 µM 17 β-estradiol. (H, I) Close-up views of ARs of transgenic line #26 from (F) and #27 from (G). PtoACTIN was used as reference gene. The expression level in Ctrl was set to 1. ** indicated the difference between Ctrl and each transgenic line at $p < 0.01$ level. Scale bar: (A–C, E–G) = 1 cm; (H, I) = 3 cm.

3.5. Expression Analysis of Auxin-Related Genes in PtoWUSa-Overexpressing Transgenic Poplar

The extensive researches have been shown that auxin acts as an important regulator in root development. The root morphology of transgenic plants overexpressing PtoWUSa was similar to the pin or aux mutants. This observation impelled us to clarify whether this phenotype in the present study was caused through altered distribution of auxin in transgenic plants. Thus, we examined the expression of auxin transport genes including AUX and PIN in the ARs of three-week-old PtoWUSa-overexpressing plants and Ctrl plants. The expression levels of eleven PINs and five AUXs were successfully detected. Among PIN genes, the expression levels of five PIN genes (PIN1c, PIN2, PIN3b, PIN5a, and PIN6a) for auxin polar transport were reduced, while other genes were slightly up-regulated (Figure 9). The expression levels of AUXs were no obvious change (Figure 9). These results suggested that overexpression of PtoWUSa might affect polar transport in the ARs by regulating the expression of PINs.
Figure 9. Expression analysis of auxin transport genes. Expression levels of PINs and AUX1-like genes in three-week-old Ctrl and PtoWUSa-overexpressing poplar plants were detected using qRT-PCR. The expression levels of each gene in Ctrl were set to 1. * indicated the difference between Ctrl and each transgenic line at $p < 0.05$ level.

4. Discussion

*WUS* is the founding member of the WOX family, which is the plant-specific transcription factor. It has been well documented that *WUS* is expressed in SAM and FM, and its main function is to maintain stem cell balance and to keep stem cells balance between division and differentiation in herbage plant. However, research on the homologues of *WUS* in woody plant is lacking and its function is unclear. In this study, the function of poplar *PtoWUSa* was characterized. The result of sequence analysis showed that *PtoWUSa* was a member of the WUS clade containing the conserved domains of HD, WUS-box, and EAR-like domain. Expression patterns exhibited that *PtoWUSa* was not only expressed in SAM, but was also expressed in root and stem, which was different from expression patterns of other *WUS* that have been reported. It has been confirmed that *WUS* also plays role in its expressed zone, such as flower and shoot meristem in *Arabidopsis* [26,27]. Therefore, its expression patterns indicated that *PtoWUSa* might participate in root and stem development.

In our study, multiple stems were present in *PtoWUSa*-overexpressing plants, implying that *PtoWUSa* affected the meristem growth. Moreover, the root development was disordered in *Arabidopsis* and poplar, indicating that *PtoWUSa* was involved in AR development. It is reported that *WUS* play key role in shoot regeneration. The *atwus* mutant is defective in shoot meristem development, and overexpression of *AtWUS* can promote the transition from vegetative stage to embryonic stage, and *GhWUS* is also involved in the formation of the embryogenic callus in cotton [36]. However, being different in *AtWUS* and *GhWUS* transgenic plants [36,41,42], embryo-like structures were not detected in the root tip of *PtoWUSa*-overexpressing *Arabidopsis* and poplar plants. These results implied that *PtoWUSa* might have a weaker function in somatic embryogenic than *AtWUS* and *GhWUS*.

WOX transcription factors have been found to be involved in AR development. In *Arabidopsis*, *AtWOX7* from WUS-clade is involved in LR development and it is activated by *AtWOX11/12* in the process of AR de novo organogenesis [25,37]. However, no *AtWOX7* homolog gene was identified in poplar. Based on the expression pattern and evolutionary relationship, we speculated that *PtoWUSa*, *PtoWOX5a* or other *PtoWOXs* might take place of the roles of *WOX7* in poplar AR development. In poplar, a member of WUS clade, *PtoWOX5a* also expressed in the ARs, and an overexpression
of PtoWOX5a affected the morphology of ARs and LRs [39]. WOX5 can rescue the deficiencies of wus mutants in Arabidopsis, indicating that WUS and WOX5 has similar function [43]. Furthermore, the similar phenotype of ARs in PtoWUSa- and PtoWOX5a-overexpressing plants indicated that PtoWUSa and PtoWOX5a also had similar function in AR development in poplar. The direct genetic evidences including CRISR-cas9 or RNAi need to further reveal its function in AR development in future.

In A. thaliana, WUS has been demonstrated to be involved in meristem growth and maintenance via acting its down-stream genes, such as CLV and type-A Arabidopsis Response Regulator 5/7/15 (ARR5/7/15). In SAM, WUS can directly activate CLV3 and in turn the CLV3 signal represses WUS expression. This WUS-CLV feedback loop controls SAM maintenance [28,29]. WUS is linked to cytokinin signaling through direct transcriptional repression of ARR5/7/15, which are negative regulators of cytokinin signaling and are necessary for proper meristem function [44]. In our study, we also found that overexpression of PtoWUSa repressed the expression of five PIN genes (PIN1c, PIN2, PIN3b, PIN5a and PIN6a) in ARs in poplar. PIN auxin efflux carriers and AUX1-like auxin influx carriers are two classes of proteins for polar auxin transport [10]. The PIN function has been confirmed to take part in polar auxin transport, which is important for root morphogenesis [2,45]. OsPIN1 is expressed in root primordial, and suppressing the expression of OsPIN1 significantly inhibits ARs development through affecting auxin transport [8,46]. AtPIN2 plays an important role in controlling gravitropism by regulating the redistribution of auxin from the stele towards the elongation zone of roots. In pin2 mutant, the root showed agravitropism and reduced elongation [7]. In our previous study, it has been confirmed that PtPIN1, PtPIN2, and PtPIN5a are specifically expressed in ARs, implying that they are involved in AR development by regulating the polar auxin transport in ARs [47]. In PtoWUSa-overexpressing plants, the expression levels of five PtPINs (PIN1c, PIN2, PIN3b, PIN5a and PIN6a) were down-regulated, and this might influence polar auxin concentration and result in agravitropism of transgenic plant (Figures 4 and 5). In the pin2 and pin5 mutants, the root elongation was inhibited [7,48]. The reduced expression of PtPIN5a and PtPIN2 might be the reason why the AR elongation was inhibited in PtoWUSa-overexpressing plant in our study (Figure 6). No obvious change of expression levels of AUX1-like suggested that PtoWUSa was mainly regulated the efflux transport of auxin, rather than auxin influx carrier. These studies indicate that PtoWUSa may have superimposed coordination of reprogramming towards developmental plasticity, and it processes multiple regulatory mechanisms and biological functions. Further investigations need to be carried out to reveal its complex regulatory network in future.

5. Conclusions

In this study, we explored the function of PtoWUSa and found it is involved in AR development by down-regulating the expression of auxin efflux carrier in PtoWUSa-overexpressing plants. Constitutive expression of PtoWUSa caused the defect of root development, including agravitropism, and enlarged and swollen root tip in transgenic Arabidopsis and poplar plants. Our results provide some new information about function of WOX in poplar, and this study will help us gain a better understanding the AR development in woody plant.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4425/11/2/176/s1. Figure S1. Phylogenetic analysis of PtoWUSa and AtWOXs. The 18 proteins were clustered into three distinct groups: the modern/WUS clade, an ancient clade, and an intermediate clade. Table S1. The primers sequences used in this study.

Author Contributions: M.L. and J.L. designed this research. J.L. and H.J. performed the experiments. J.L., H.J., PS., J.Z. and Y.X. performed the data collation and statistical analysis. J.L. wrote the manuscript. M.L., L.W. and J.H. contributed with suggestions. All authors have read and agreed to the published version of the manuscript.

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