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Transcription Factor MdPLT1 Involved Adventitious Root Initiation in Apple Rootstocks

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Abstract: The induction of adventitious roots is a key factor restricting the vegetative propagation of apple dwarf rootstocks. PLETHORA (PLT) transcription factors are involved in the regulation of plant stem cell niche and adventitious root development. In this study, we identified the *PLT1* gene in apples by bioinformatics and analyzed its evolutionary relationship. The *MdPLT1* gene was cloned from M9-T337 to verify its subcellular localization and analyze its function in transgenic tobacco. The MdPLT1 protein contained two conserved AP2 domains which may be similar to those of poplar the PtrPLT2a and PtrPLT2b with 85% support. The CDS sequence of the *MdPLT1* gene was 1638 bp, encoding 545 amino acids. The transcription factor MdPLT1 was localized in the nucleus. The number of adventitious roots of tobacco plants overexpressing *MdPLT1* significantly increased. In the adventitious roots of *MdPLT1*-overexpressed plants, the expression levels of genes related to the *NtPINs* family and the *NtYUCCAs* family were significantly increased. The results showed that *MdPLT1* positively regulated adventitious root formation. This study provided a theoretical basis for the establishment of the fast vegetative propagation of apple dwarf rootstocks.

Keywords: apple; MdPLT1; rootstock; adventitious root; gene function analysis

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1. Introduction

Apple (*Malus domestica* Borkh.) is a major commercial fruit tree and its fruits have high nutritional and economic value. Dwarfing and high-density planting can maintain high yield, quality, and efficiency in the long term, which is the main cultivation model for the current apple industry development. Therefore, orchard renewal requires a large number of dwarfing rootstock seedlings. Apple rootstock breeding mainly adopts asexual reproduction methods, including grafting, layering, and tissue culture [1]. The breeding technology system for dwarfing apple rootstocks has gradually been established. Still, in production, some dwarfing rootstocks with excellent ecological adaptability have poor rooting ability, which is an important bottleneck problem restricting the breeding of dwarfing rootstocks. Adventitious roots (ARs) induction from stem basal tissues is a major step in the vegetative propagation of apple rootstocks [2]. The AR primordia of apples arises from interfascicular cambium cells adjoining phloem cells [3]. The mechanisms of AR formation have been studied in different plants, including Arabidopsis [4,5], rice [6,7], tobacco [8], poplar [9,10], and larix [11,12]. However, studies on apple AR formation and development mainly focus on the identification, cloning, expression analysis, and transcriptional regulation mechanisms of differentially expressed genes using omics approaches [2,13–23]. AR initiation is a very complex process regulated by many plant hormones and gene networks. Therefore, the molecular regulatory mechanisms are still unclear.

The transcription factor PLETHORA (PLT) belongs to the AP2/ERF family and plays an important role in plant embryonic development, organ development, and the main-

tenance of meristematic tissues. In the model plant Arabidopsis, *PLT* was found to be involved in the morphogenesis and maintenance of the root apical meristem during root development, especially in determining the position of the quiescent center (QC) [24,25]. The expression of the *PLT1* and *PLT2* genes in the root meristematic region, along with the accumulation of their corresponding proteins and the radial expression of the GRAS family transcription factors SHORT-ROOT (SHR) and SCARECROW (SCR), have been found to play a crucial role in determining the position of the quiescent center (QC) [24]. These molecular mechanisms and gene expressions contribute to the precise regulation of root development and the maintenance of the root stem cell niche. Overexpression of the *PLT1* and *PLT2* genes can ectopically induce root meristem formation during embryogenesis and, in most extreme cases, lead to the conversion of shoot apical meristem to root meristem [24]. In addition, inducing the overexpression of the *PLT2* gene during the seedling stage in Arabidopsis can induce the formation of ARs from the shoot apex [25]. *PLT1* and *PLT2* genes are responsive to auxin induction, but exogenous auxin treatment cannot restore the phenotype of *plt1plt2* mutants, indicating that the auxin cannot replace the function of the *PLT1* and *PLT2* genes. The AN3 (ANGUSTIFOLIA3) complex directly binds to the promoter regions of the *PLT1* and the *SCARECROW* genes, and the enlargement of the meristematic tissue in *an3* mutants is largely due to an increase in the expression of the *PLT1* gene [26]. RGF1 mainly maintains the characteristics of root tip meristematic tissue and transient cell proliferation by regulating the expression levels and patterns of transcription factors PLT1 and PLT2 [27]. Salvi [28] found that after the germination of Arabidopsis seeds, cell division will lead to a decrease in distal *PLT2* levels, thereby ensuring the formation of the transition zone and the activation of *ARR12* (ARABIDOPSIS RESPONSE REGULATOR 12). The antagonistic control of *PLT2-ARR12* determines the division zone, and continuous *ARR1* activation antagonizes *PLT2* by inducing the cell cycle inhibitor gene *KRP2* (*CYCLIN-DEPENDENT KINASE INHIBITOR2*), thereby ultimately determining the size of the meristematic tissue. The *cystathionine beta-lyase* (*CBL*) gene is expressed in embryos and is enriched in the quiescent center of roots. *cbl* mutants have defects in embryo patterning, root stem cell niches, and poor root growth and development and also reduce the accumulation of *PLT1* and *PLT2*. Mutations in the *CBL* gene lead to a reduction in *H3K4me3* modifications on the *PLT1/2* genes and the downregulation of the expression of these two genes. The overexpression of the *PLT2* gene can partially rescue the root meristem defect phenotype of *cbl* mutants, indicating that the function of the *CBL* gene is partially mediated by *PLT1/2* [29]. It can be concluded that *PLT1* plays an important regulatory role in embryonic formation and root development, but its mechanism of action in AR formation remains unclear. Does the *MdPLT1* gene have the same function in apples? What is its mechanism of action?

To understand the role of *MdPLT1* in the initiation of the apple rootstock AR, the expression level of *MdPLT1* was detected by real-time fluorescence quantitative PCR during apple AR formation. The *MdPLT1* gene was cloned and overexpressed in tobacco to analyze its function in AR initiation. This study provides a theoretical basis for further research on the molecular mechanism of apple AR formation and development.

2. Materials and Methods

2.1. Plant Materials

The stem base of apple rootstock T337 and *Malus prunifolia* (MP) were used as material for tissue culture, and tissue culture plantlets were collected from the tissue culture room of the College of Horticulture, Northwest Agriculture and Forestry University. Tissue culture plantlets of MP and M9-T337 apple rootstock were grown in tissue culture in Tianshui, China, and were used as plantlets for AR formation. The tissue culture plantlets of MP and M9-T337 plants were cultured on a root-inducing medium containing half-strength MS [22] supplemented with 0.1 mg/L indole3-butyric acid (IBA) to promote root formation. The cultures were grown at a consistent temperature of 22 ± 2 °C under a 16-h photoperiod at 50 μmol m^{-2} s^{-1} provided by cool-white, fluorescent tubes [2,30]. When

T337 plantlets were taken root and cultured for 1 month, different tissue materials were collected. *Nicotiana benthamiana* seeds were placed in 2.0 mL centrifuge tubes and soaked in 1 mL of 70% ethanol and 1 mL of 10% H_2O_2 solution for 30 s and 15 min, respectively, for surface disinfection. After that, the seeds were rinsed 5–6 times with sterile distilled water and transferred to sterile Petri dishes containing 4 layers of moist sterile filter paper. Then, 2 mL of sterile water was added, and the dishes were incubated at 28 °C in the dark for 5 days. After germination, the seeds were inoculated onto MS solid culture medium for photoperiod cultivation and used for tobacco genetic transformation. Tissue culture conditions: temperature of 21–23 °C, light/dark cycle of 16 h/8 h.

2.2. Construction of Phylogenetic Tree and Multiple Sequence Alignment Analysis

The PLT homologous protein sequences were obtained from the Plant Transcription Factor Database v5.0 (http://planttfdb.gao-lab.org/index.php), (accessed on 5 June 2023) and the NCBI Genbank (https://www.ncbi.nlm.nih.gov/genbank/) (accessed on 5 June 2023) and accession numbers were listed in Supplementary Table S1. Multiple sequence alignment was performed using the Muscle program, and a phylogenetic tree was constructed using the Neighbor-Joining (NJ) method in MEGA7 software [31], with a bootstrap value set at 1000. Multiple sequence alignment analysis of the homologous sequences of PLT1 was performed using DNAman 6.0 software.

2.3. Extraction of Total RNA and Analysis of MdPLT1 Expression Patterns

MP (easy-to-root) and T337 (difficult-to-root) are two rootstock varieties with significant difference in rooting. When it comes to root, they have different degrees of difficulty. Samples were collected from different tissues of apple rootstock T337, MP (easy-to-root), and T337 (difficult-to-root) after 0, 2, 3, 5, 8, and 15 days of stem basal samples inoculation. Total RNA was extracted using the MiniBEST Universal RNA Extraction Kit (TaKaRa, Shanghai, China). First-strand cDNA was synthesized according to an instruction manual provided by PrimeScript™ RT reagent Kit (TaKaRa, China). Differential gene Primers were designed manually using Primer 6.0 software and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The expression level of the *MdPLT1* gene in different tissues and during adventitious root development was detected using the SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) quantitative reagent. The relative expression was quantified using the $2^{-\Delta\Delta CT}$ method from three independent biological replicates, with EF-1A (Actin) as the reference gene.

2.4. Cloning of the MdPLT1 Gene

Using M9-T337 apple rootstock as the material, the total RNA was extracted and reverse transcribed into cDNA using a reverse transcription kit (PrimeScript™ 1st Strand cDNA Synthesis Kit). Specific primers were designed using Primer6.0, and the primer sequences are shown in Supplementary Table S2. A *MdPLT1* gene fragment was amplified using the high-fidelity enzyme PrimeSTAR® HS (Premix). The sequence data has been submitted to the GenBank and has obtained the accession number: MK907602. The amino acid sequence of MdPLT1 was obtained by translating using DNAman software.

2.5. Construction of MdPLT1-GFP Fusion Expression Vector and Subcellular Localization

To validate the subcellular localization prediction results, the *MdPLT1* plasmid was cloned into the subcellular localization fusion expression vector pEGOEP-GFP to generate the *MdPLT1-GFP* fusion expression plasmid. The pEGOEP-GFP empty vector was used as a positive control. It was validated by an agroinfiltration-mediated transient expression in 1-month-old tobacco (*Nicotiana benthamiana*) leaves. In simple terms, pick a single clone of Agrobacterium and shake it for 12 h. After collecting the fungal mycelium, suspend the mycelium in a suspension solution (10 mM MES, 10 mM MgCl2, and 200 μM AS) until the OD600 reaches 0.75. Let the mycelium settle at room temperature for 3 h, while simultaneously incubating tobacco plants in the dark for three hours. Using a 1 mL syringe,

inject the backside of tobacco leaves with the suspension. After injection, incubate the tobacco plants in the dark for 8–10 h before transferring them to normal light conditions. On the third day after transformation, the expression of the pEGOEP-GFP empty vector and MdPLT1-GFP fusion protein was observed under a confocal laser scanning microscope [30].

2.6. Construction of MdPLT1 Overexpression Vector and Genetic Transformation of Nicotiana benthamiana

The *MdPLT1* gene was cloned into the overexpression vector pEGOEP-GUS and sequenced. The correctly sequenced plasmid was transformed into Agrobacterium GV3101 using the freeze-thaw method, and then the Agrobacterium-mediated leaf disc transformation method was used to transform *Nicotiana benthamiana*. The vector of pEGOEP-GUS-*MdPLT1* that we obtained was used for the leaf transformation of *Nicotiana benthamiana* [32]. Finally, the transgenic plants were screened by kanamycins (100 mg L^{-1}) resistance and identified by PCR. The T2 generation transgenic plants were used as materials to carry out the following experiments.

2.7. Identification and Phenotypic Analysis of Transgenic Nicotiana benthamiana with the MdPLT1 Gene

Genomic DNA was extracted from transgenic and wild-type *Nicotiana benthamiana* seedling leaves, and PCR was used to detect positive plants. Three lines (#1, #6, and #8) with consistent phenotypes were selected for propagation. Tobacco seeds were subjected to sterilization treatment, and germination tests were conducted on MS culture medium. The seedlings at the four-leaf stage were transplanted into nutrient soil and allowed to continue growing under normal conditions [32]. Transgenic and wild-type plants with good growth at 3 weeks old were selected, and the number of ARs was counted. Three biological replicates were set, with at least 12 seedlings measured each time, and the results were subjected to a *t*-test.

2.8. Relative Expression Analysis of NtPINs and NtYUCCA Gene Families

ARs were collected from both *MdPLT1*-overexpressing transgenic tobacco plants and wild-type tobacco plants. The Total RNA was extracted using the MiniBEST Universal RNA Extraction Kit (TaKaRa, China). First-strand cDNA was synthesized according to an instruction manual provided by PrimeScript™ RT reagent Kit (TaKaRa, China). Real-time qRT-PCR was performed to detect the expression levels using the SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) quantitative reagent on a Roche lightCycler 480 (Roche Applied Science, Penzberg, Upper Bavaria, Germany) according to the manufacturer's instructions. Relative expression was quantified using the $2^{-\Delta\Delta CT}$ method from three independent biological replicates. with EF-1A (Actin) as the reference gene. Specific primers of *NtPIN1*, *NtPIN3*, *NtPIN4*, *NtPIN9*, *NtYUCCA3*, *NtYUCCA4*, *NtYUCCA6*, *NtYUCCA8*, *NtYUCCA9*, and *NtYUCCA10* were designed using Primer6.0, and the primer sequences are shown in Supplementary Table S2.

3. Results

3.1. Identification and Evolutionary Analysis of the Apple PLT1 Gene

The phylogenetic tree was constructed using the full-length sequences of the MdAP2 and AtAP2 proteins (Figure 1A). Phylogenetic analysis showed that the transcription factor MdPLT1 clustered with AtPLT1 (AT3G20840.1) and AtPLT2 (AT1G51190.1), indicating a close evolutionary relationship among them (Figure 1A). MdPLT1 and MdPLT2 formed a clade with poplar PtrPLT2a and PtrPLT2b, with a support rate of 85% (Figure 1B). In addition, multiple sequence alignment revealed that MdPLT1 contains two conserved AP2 domains and shows higher similarity to the homologous proteins PtrPLT2a and PtrPLT2b in poplar (Figure 1C).

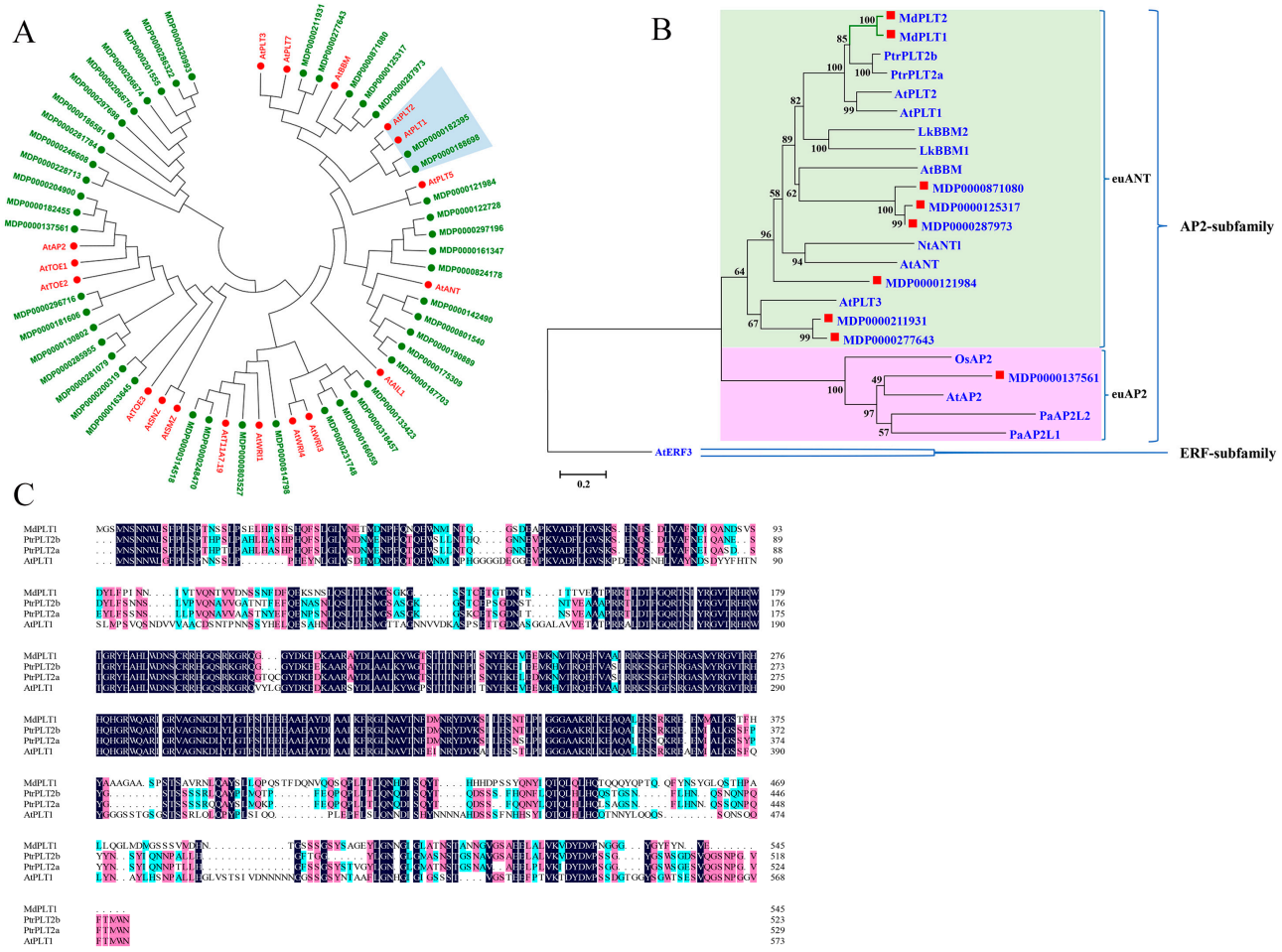

Figure 1. (**A**) Phylogenetic tree of MdAP2 subfamily; (**B**) Phylogenetic tree of 24 AP2 subfamily proteins from 5 land plant species. Numbers at each node in the phylogenetic tree are bootstrap values. Integration of MdPLT1 in the euANT group within the AP2/ERF family; (**C**) Multiple sequence alignment analysis of MdPLT1, AtPLT1, PtrPLT2a and PtrPLT2b.

3.2. Expression Pattern Analysis of MdPLT1 during Adventitious Root Formation

qRT-PCR experiments showed that *MdPLT1* is specifically expressed in ARs and stems (Figure 2A). The expression level of *MdPLT1* in MP (easy-to-root) was consistently higher than T337 (difficult-to-root) in both the treatment and control groups. After IBA treatment, the expression level of *MdPLT1* was higher in both MP and T337 compared to the control. Furthermore, the expression level reached its maximum on the 5th day after inoculation (Figure 2B). The expression analysis indicated that *MdPLT1* is involved in AR formation.

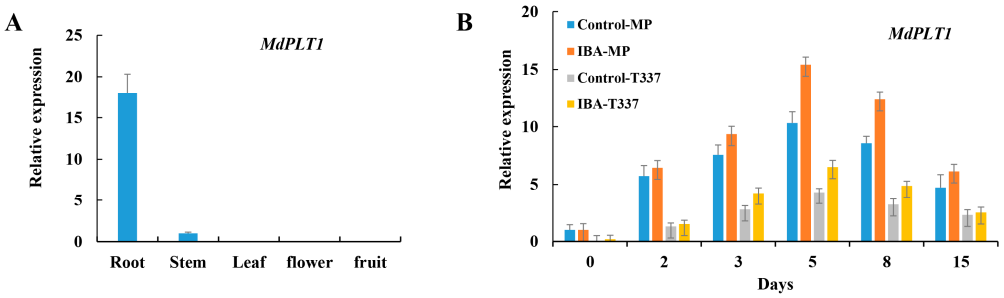

Figure 2. Expression pattern analysis of *MdPLT1* gene. (**A**) Tissue-specific expression analysis of *MdPLT1* (Set the expression level in the stem to 1); (**B**) Expression pattern analysis of *MdPLT1* during AR formation (Set the expression level of Control-MP 0 d to 1).

3.3. Cloning of MdPLT1 Gene

The *MdPLT1* gene was cloned using the specific primers *MdPLT1*-PCR-F and *MdPLT1*-PCR-R. The PCR product was then analyzed using 1.2% agarose gel electrophoresis, which revealed a distinct band between 1200 bp and 2000 bp. The length of the band is approximately 1600 bp, matching the expected size (Figure 3). Following the purification of the PCR product, sequencing was performed, revealing a coding sequence (CDS) of 1638 bp. This CDS encodes a protein consisting of 545 amino acids (Figure S1).

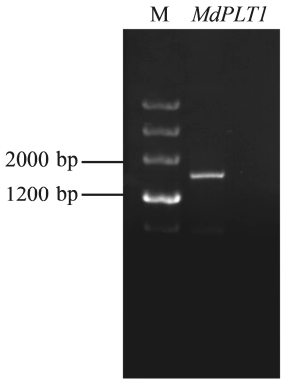

Figure 3. PCR products of *MdPLT1* gene.

3.4. Subcellular Localization Identification of MdPLT1 Protein

To verify the subcellular localization of MdPLT1, the pEGOEP35S-MdPLT1-GFP fusion expression vector was constructed using double enzyme digestion. The correctly sequenced recombinant plasmid was transformed into *Agrobacterium* GV3101 competent cells. Tobacco transient expression was performed and the fluorescence signal was observed using confocal laser scanning microscopy. As shown in Figure 4A, the MdPLT1 protein was localized in the nucleus (Figure 4A), while the positive control was expressed in all organelles (Figure 4B).

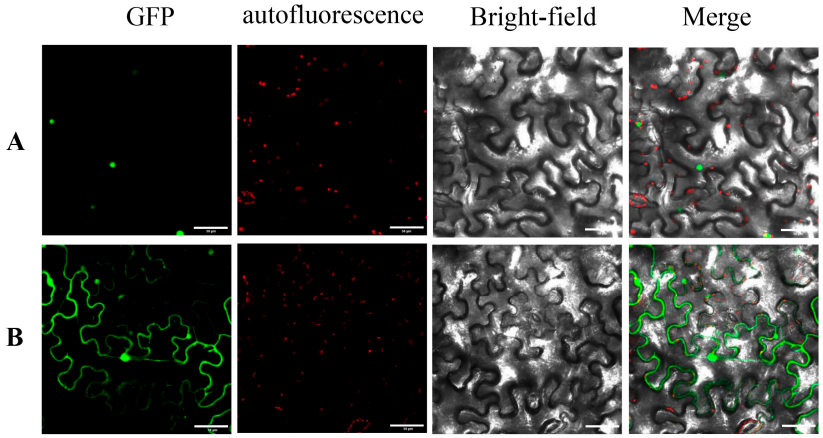

Figure 4. Subcellular localization of the MdPLT1 protein in tobacco leaf cells and onion epidermal cells transiently expressing the GFP-MdPLT1 fusion protein. (**A**) Images from tobacco leaf cells agroinfiltrated with the GFP-MdPLT1 fusion protein. (**B**) Images from tobacco leaf cells agroinfiltrated with GFP alone. Scale bar: 50 μm.

3.5. Tobacco Genetic Transformation and Phenotypic Analysis of MdPLT1

To further verify the function of *MdPLT1* in the regulation of AR development, the *pEGOEP-MdPLT1-GUS* plasmid was transferred into tobacco using the leaf disc method, and 9 transgenic lines were obtained. The DNA was extracted from the tobacco leaves for positive plant identification. The presence of the transgene in these transgenic lines was confirmed by PCR (Figure 5A). Once ARs developed from these transgenic tobacco lines,

we determined that the overexpression of *MdPLT1* affected AR development. The number of 2-week-old ARs from cultured transgenic stem tips was significantly higher than that from cultured stem tips derived from non-transgenic tobacco (Figure 5B,C). This implies that MdPLT1 might promote AR formation.

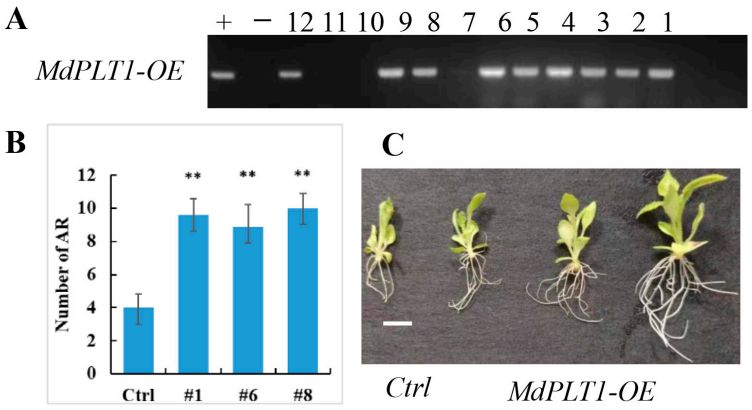

Figure 5. Identification and phenotypic analysis of overexpressed *MdPLT1* on AR development in stable transgenic tobacco plants. (**A**) Detection of *MdPLT1* overexpression in tobacco-positive seedlings by electrophoresis; (**B**) Numbers of ARs in 2-week-old transgenic tobacco plants; (**C**) Two-week-old transgenic tobacco plants. Bars indicate standard deviations (SDs), and asterisks indicate significant differences relative to non-transgenic plants at $p < 0.01$ (**). Values are reported as means ± SDs ($n = 12$). Scale bar: 2 cm.

3.6. Relative Expression Analysis of NtPINs and NtYUCCAs Gene Families

Figure 6 illustrates the expression levels of the auxin polar transport proteins NtPINs and the NtYUCCAs gene family in ARs of both wild-type and transgenic tobacco plants. The overexpression of *MdPLT1* in ARs led to significant changes in gene expression. Compared to the wild type, the transgenic plants exhibited a 134% increase in *NtPIN1* expression, a 231% increase in *NtPIN3* expression, a 212% increase in *NtPIN4* expression, and a 178% increase in *NtPIN9* expression (Figure 6A). Moreover, the expression levels of *NtYUCCA3*, *NtYUCCA4*, *NtYUCCA6*, *NtYUCCA8*, *NtYUCCA9*, and *NtYUCCA10* increased by 285%, 356%, 441%, 567%, 335%, and 357%, respectively (Figure 6B). These findings suggest that the overexpression of the *MdPLT1* gene enhances auxin synthesis and polar transport, thereby facilitating the formation of ARs.

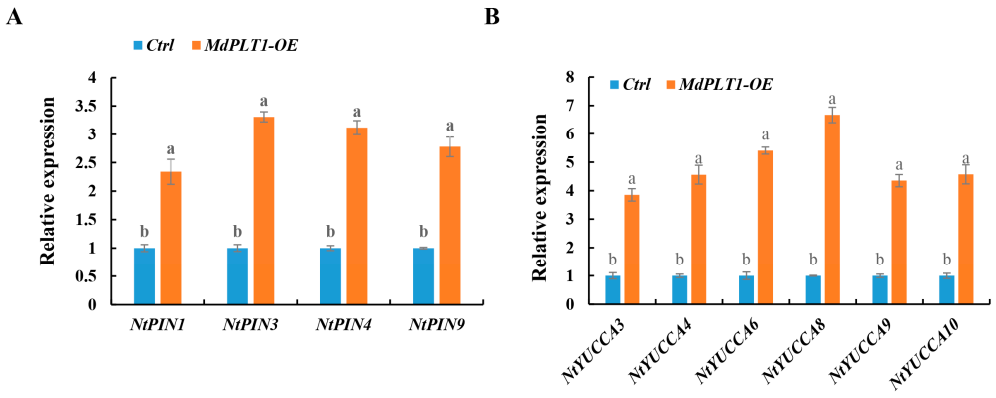

Figure 6. Relative expression analysis of *NtPINs* (**A**) and *NTYUCCAs* family genes (**B**) in *MdPLT1*-overexpressed ARs of tobacco. Biological replicates, tests, and *p* values are described above. The different letters mean significance, which was examined by the Duncan's range test ($p < 0.05$).

4. Discussion

AR is an important step in the vegetative reproduction of economically-important horticultural herbaceous and woody plants. Dwarfing rootstocks are widely used in modern apple production for high-density planting and efficient mechanical management of orchards. The apple rootstock breeding system has been established. However, in production, some dwarfing rootstocks with good ecological adaptability have poor rooting ability, which is a significant bottleneck problem in dwarfing rootstock breeding. Therefore, understanding the molecular mechanisms of AR formation and the regulatory gene network is consequently important for apple production.

At present, a lot of research work has been done on the mechanism of AR formation in apples. The research from Bai [17] showed that IBA was necessary for root primordium formation of apple rootstocks. IBA induced increased the number of ARs and upregulated the expression of the genes *MdWOX11*, *MdLBD16* and *MdLBD29* [30]. Another study suggested that the overexpression of *MdWOX11* promoted AR primordium formation in apples, while the interference of *MdWOX11* inhibited AR primordium production [2]. However, new research confirmed that CK content is related to AR formation and further revealed that a high auxin/CK ratio was beneficial to AR formation in apples. At the same time, it showed that cytokinin-responsive MdTCP17 interacts with MdWOX11 to repress AR primordium formation in apple rootstocks [30]. These studies suggest that IBA and *MdWOX11* play important roles in AR development in apples, which is consistent with the results that transcription factors *WOX11/12* directly activate *WOX5/7* to promote root primordia initiation and organogenesis [33]. Interestingly, *WOX5* and *PLT1* regulate the formation of AR primordia by responding to ARF10 and ARF16 [34]. Therefore, PLT1 plays an important role in root development, but whether it plays a role in AR formation in apples is not clear.

The AP2 subfamily genes *BABY BOOM* (*BBM*), *PLT1*, *PLT2*, and *PLT3* regulate root growth and development in Arabidopsis, with functional redundancy in the root apical meristem [25]. *PLT* genes play important roles in auxin induction during plant embryonic development and AR formation. Most studies on *PLT* genes have focused on Arabidopsis [24,25,35,36], rice [37], and poplar [38]. However, there have been no reports on the research of the *PLT* gene function in apples. In this study, through homology alignment and phylogenetic tree analysis, it was found that apple MD03G1023900 clusters with Arabidopsis *AtPLT1*, showing the highest sequence similarity, and was identified as the apple *MdPLT1* gene (Figure 1A). This discovery suggests that these genes may be involved in the regulation of root development because of their tight evolutionary relationship. Multiple sequence alignment revealed that MdPLT1 contains two conserved AP2 domains and clusters with poplar PtrPLT2a and PtrPLT2b with 85% bootstrap support, suggesting potential functional similarity (Figure 1B,C).

In previous studies, AR formation in tissue-cultured stem cuttings of the T337 apple rootstock was examined. Electron microscopy was used to observe AR formation at select stages (0, 3, 9, and 16 days) of AR formation. AR primordia originated from tissue near the vascular cambium and secondary phloem parenchymatous tissue [21]. The transcription factor PLT1 is induced by auxin and participates in regulating embryonic and AR development. In apples, *MdPLT1* is specifically expressed in roots and stems of the T337 apple rootstock (Figure 2A), potentially involved in regulating AR formation. In order to verify the induction of *MdPLT1* gene expression by Auxin, the MP and T337 apple rootstock plantlets were treated with IBA. The results showed that after IBA processing, the expression of *MdPLT1* significantly increased, indicating its induction by auxin. Previous studies have also shown that the *MdPLT1* promoter sequence contains IBA-influencing elements [39], further demonstrating that the *MdPLT1* gene is auxin-induced. Further research showed that *MdPLT1* expression in easy-to-root rootstock MP was significantly higher than in difficult-to-root rootstock M9-T337 (Figure 2B), suggesting a positive regulatory role in apple rootstock AR formation. This is consistent with previous studies in Arabidopsis and poplar [24,25,38].

Agrobacterium-mediated transient transformation in tobacco confirmed that MdPLT1 is localized in the nucleus and functions in the nucleus (Figure 4), consistent with the characteristics of a transcription factor. We overexpressed *MdPLT1* in the tobacco to investigate its function. The overexpression of *MdPLT1* in tobacco resulted in an increased number of ARs (Figure 5), it indicated that *MdPLT1* had a positive regulatory effect on Ars. Compared with wild-type tobacco, the expression of the auxin polar transport protein gene family *NtPINs* and the *NtYUCCA* gene family in *MdLT1*-overexpressing plants were significantly increased (Figure 6), indicating the involvement of apple *MdPLT1* in auxin signaling during plant AR formation. These results suggest that the *MdPLT1* gene may regulate the AR formation by regulating auxin synthesis and transportation. Xu [40] also showed that the auxin-affecting factor ARF was involved in the regulation of AR formation in apple. An found that RGF1 mainly maintains the characteristics of root apical meristem and transient cell proliferation by regulating the expression levels and patterns of transcription factors *PLT1* and *PLT2* [27]. Xiong [41] and Elena Salvi [28] found that Arabidopsis positively regulates the expression of *PLT1* in the root apical meristem by recruiting RNA polymerase II (Pol II) to *PLT1* and interacting with *PLT1*. These studies suggest that the transcription factor *PLT1* is involved in regulating AR formation and root development. This study preliminarily verified the function of the apple *MdPLT1* gene in tobacco, but further transformation experiments in apples are needed to confirm its impact on AR development in apples.

5. Conclusions

In this study, we identified the apple *PLT1* gene through bioinformatics methods and cloned it using T337 apple rootstock. The analysis of tissue-specific expression patterns and transcript levels in AR regeneration indicated that *MdPLT1* was specifically expressed in roots and stems, and its expression level was increased in MP (easy-to-root). We constructed the transgenic *MdPLT1* line and confirmed the nuclear localization and involvement in AR formation. Our study provides new insight for further understanding of the role of *MdPLT1* in AR development in apples.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10010064/s1, Figure S1: *MdPLT1* presumably encodes an amino acid sequence; Table S1: Protein and gene accession numbers used in this study; Table S2: Primers used in this study.

Author Contributions: H.W. conceived and designed the study, conducted all the experiments and data analysis and drafted the manuscript. G.B. and L.Y. helped perform phenotypic analysis and qRT-PCR assays. W.M. helped extract the sequence of *MdPLT1*. J.Y.U. helped edit the English text of a draft of this manuscript. H.W. coordinated the project design and helped write the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article and supplementary materials.

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Conflicts of Interest: The authors declare no conflict of interest.

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