An R2R3-MYB Transcription Factor PgFLP Directly Activates PgPIN10 to Regulate the GSA of Adventitious Root in Pomegranate

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Research article

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Abstract

**Background:** The self-rooted seedling is widely used in pomegranate planting industry currently; however, the root system of self-rooted seedling is shallow and poor cold resistance. Therefore, the study of the molecular mechanisms of pomegranate adventitious root gravitropism is very important for developing deep-rooted pomegranate cultivars.

**Results:** We report the pomegranate FOUR LIPS (PgFLP) that play a key role in regulating the gravitropic set-point angle of pomegranate adventitious root in response to gravity signal. In our study, PgFLP directly regulates the transcriptional expression of *PgPIN10* by binding to its promoter, thus regulating the GSA of adventitious root in pomegranate. Additionally, the 35S::PgFLP show stronger gravitational response than wild-type, leading to a smaller GSA in *Arabidopsis* lateral roots, indicating that PgFLP participates in regulating the GSA of adventitious root via *PgPIN10* in pomegranate.

**Conclusion:** Our results confirm that the transcriptional regulation of *PgPIN10* by R2R3-MYB transcription factor PgFLP in setting the gravitropic set-point angle of pomegranate adventitious root in response to gravity signal.

Background

Pomegranate (*Punica granatum* L.) is believed to originated in Afghanistan, present-day Iran, India, and Turkmenistan and other central Asia countries [36, 23]. In China, many recognized pomegranate varieties with high genetic diversity have been cultivated [33]. The self-rooted seedling is widely used in pomegranate planting industry currently. However, the development of gravitropic set-point angle (GSA) between self-rooted seedling and pomegranate seedling is a great difference, leading to the adventitious root of pomegranate was poor in shallowness and solidity, resulted in poor cold resistance [28].

The plant hormone auxin is explicit for a common integrator to many environmental and endogenous signals regulating lateral root formation [12]. In the lateral root cap, the auxin stimulating prebranch site formation [32]. Subsequently, the dynamic auxin flows orchestrated lateral root patterning and morphogenesis [3]. In addition, it interacts with the surrounding tissue in a complex manner [10, 16, 19, 26]. The gravitropic set-point angle can be defined by orientation of plant growth with respect to the gravity vector [5]. In addition, auxin controls gravitropic set-point angle of lateral branches through auxin signaling pathway dependent on TIR1/AFB-Aux/IAA-ARF in the higher plant [22].

MYB TFs have been found to regulate plant development and metabolism [7]. In *Arabidopsis*, the two R2R3-MYB proteins, MYB88 and FOUR LIPS (FLP), can bind directly to the promoters of *PINs* genes that harbour an [A/T/G][A/T/G]C[C/G][C/G] motif [31]. FLP and MYB88 have been found to play redundantly role in limiting terminal divisions in *Arabidopsis*. Compared with *flp* single mutants, the stomatal defects of *flp-1myb88* double mutants are more severe [11]. In addition, FLP and MYB88 have been found to can regulate root gravitropism [27], the late stages of stomatal development [11], cold hardiness [30], female reproductive development [17], and guard mother cell proliferation [11, 31]. The *PINs* have been found to
conducive to nearly every step of auxin fluxes [3, 18, 35, 20]. Following the primary roots are stimulated by gravity, the PINs have been repolarized [21, 29], the auxin flux redirection to the lower side of roots, leading to root tip bending [8, 9, 22, 25]. In addition, the polar auxin transport is beneficial to the root bending under obstacle avoidance [13]. The auxin distribution patterns have been predicted and explained through auxin transport [2, 6, 1]. The AtPIN3 shows remarkably dynamic expression during the root gravitropism and patterning of stomatal complexes in Arabidopsis [14, 27]. However, how regulators regulate PIN expression combining with auxin signalling pathway during root development is nothing known in pomegranate. We investigated the mechanisms regulating PgPIN10 expression during pomegranate adventitious root gravitropism.

The regulation of root gravitropism in pomegranate is very little known currently. In our study, PgFLP participates in regulating the gravitropic set-point angle of pomegranate adventitious root. PgFLP directly regulates the transcriptional expression of PgPIN10 by binding to its promoter, thus regulating the GSA of adventitious root in pomegranate. Our results confirm that the transcriptional regulation of PgPIN10 by PgFLP in setting the gravitropic set-point angle of pomegranate adventitious root in response to gravity signal.

**Results**

**The expression pattern of PgPINs in different rootedness pomegranate self-rooted seedling adventitious root**

Two different rootedness pomegranate self-rooted seedling are screened in this study, compared with each other, the two self-rooted seedling show different rootedness adventitious roots branch angle (Table 1). The two self-rooted seedling provide appropriate material development for the study of GSA. We had screened the shallow-rooted pomegranate breed ‘Taishanhong’(TSH) and the deep-rooted pomegranate breed: ‘Lanbaoshi’(LBS) (Fig. 1a). The PINs have been found to conducive to nearly every step of auxin fluxes [3, 18, 35, 20] and the auxin distribution patterns have been predicted and explained through auxin transport [2, 6, 1]. In this study, the expression of PgPIN1 and PgPIN10 showed higher in LBS than TSH (Fig. 1b). We also investigated the expression of other PgPINs, PgPIN3 and PgPIN7 showed lower in LBS than TSH (Additional file 1: Figure S1). These results indicated that PgPINs may participate in the regulation of GSA in pomegranate adventitious root.

**Molecular cloning of PgFLP**

To further characterize the function of PgPINs in pomegranate, we performed the Y1H screening via pomegranate cDNA library. Eventually, we used the promoter sequences of PgPIN10 as baits to obtained a cDNA fragment of PgFLP. To identify the pomegranate PgFLP that is involved in adventitious root gravitropism, a genome-wide analysis was performed. Comparison of amino acid sequences of PgFLP with their close homologs, the PgFLP protein is highly conserved identity to R2R3-MYB protein (Fig. 2a). To understand the evolutionary relationship of PgFLP, using the neighbor-joining (NJ) method [24], we constructed a phylogenetic tree. As a result, We found that PgFLP had been clustered within the R2R3-
MYB clade including AtFLP, MdFLP and others for which root gravitropism have been identified. Within the R2R3-MYB clade, PgFLP is closely associated with AtFLP, which regulate lateral root gravitropism, indicating that PgFLP may participate in the regulation of GSA in pomegranate adventitious root (Fig. 2b).

Subcellular localization analysis of PgFLP

To further characterize the function of PgFLP in pomegranate, we used the coding sequence of PgFLP and GFP reporter to constructed a 35S::PgFLP-GFP translational fusion. We found that in contrast to 35S::GFP where a fluorescent signal was observed throughout the whole epidermal cell, the 35S::PgFLP-GFP showed a signal in nucleus (Fig. 3a-b), suggesting that as a transcription factor, PgFLP may be involved in pomegranate adventitious root gravitropism.

PgFLP expression pattern in pomegranate

To further determine the function of PgFLP, we performed the qRT-PCR to determine the expression patterns of PgFLP in pomegranate. We found that the expressing PgFLP were detected in various organs of pomegranate, especially higher expressing in the leaves and the fruit peels (Fig. 4a). The auxin participate in triggering lateral root development [4], we investigated whether PgFLP expression is induced by auxin. The expression of PgFLP was rapidly up-regulated in auxin-treated roots (Fig. 4b). We also investigated the expression of PgMYB88 was not significantly induced by auxin (Additional file 1: Figure S2b). Subsequently, we found that the transcripts of PgFLP in LBS was increased (~2-fold) upon the TSH (Fig. 4c). We also investigated the IAA content in different rootedness pomegranate self-rooted seedling and found that the level of IAA was slightly increased in LBS compared to that in TSH on shoot cutting at 30 d and 40 d (Fig. 4d). These results indicated that PgFLP plays an key role in pomegranate adventitious root gravitropism.

PgFLP directly regulate PgPIN10 transcription

To further characterize the mechanism of PgFLP to regulate GSA of adventitious root in pomegranate, we performed the Y1H assays. Y1H assays showed that PgFLP could interact with the promoter of PgPIN10 to activate its expression (Fig. 5a). To further investigate whether PgFLP could activate the transcriptional expression of PgPIN10 in planta, we conducted a transient expression assay. As a result, the proPgPIN10::GUS plus PgFLP showed signicantly higher GUS activity in tobacco leaves than the control (Fig. 5b-d). These results showed that PgFLP could activate the expression of PgPIN10 in pomegranate.

It is well established that FLP can bind directly to the promoters of PINs genes that harbour an [A/T/G][A/T/G][C/G] motif [31]. We found that there was two potential binding sites including AGCGG and TACCC in the promoter region of PgPIN10 (Fig. 5e and Additional file 1: Figure S3). To further determine if PgFLP could directly bind to these binding sites in plants, we performed Y1H assays. We found that PgFLP could interact with FBS (AGCGG). On the other hand, PgFLP could not interact with the mutated
FBS motif (mFBS) (Fig. 5f). These data demonstrate that PgFLP directly regulate *PgPIN10* transcription by binding to the AGCGG motif.

**Ectopic overexpression of *PgFLP* in *Arabidopsis* seedlings results in a smaller GSA of lateral root**

As it is difficult to obtain transgenic pomegranate plants overexpressing *PgFLP*, *PgFLP* was ectopically expressed in *Arabidopsis* to further characterize if PgFLP functions in the regulation GSA of pomegranate self-rooted seedling adventitious root in planta. Three independent transgenic lines, OE3, OE4 and OE7, were chosen for further analysis (Fig. 6a). To investigate phenotypes of the WT and *PgFLP* overexpression lines grown for 21 days on MS media, we found that *PgFLP* (OX-3, OX-4, OX-7) (Fig. 6b-e), exhibited a smaller GSA and grew downwards faster than WT. For example, over 35% of *PgFLP*-OX lateral root increased within a 30-50° range, meanwhile, within a 50-70° range, over 30% of lateral root was decreased (Fig. 6f). These results indicated that PgFLP functions in the regulation GSA of pomegranate self-rooted seedling adventitious root in planta.

**Discussion**

**PgFLP is involved in the pomegranate self-rooted seedling adventitious root gravitropism**

The self-rooted seedling is widely used in pomegranate planting industry currently. However, the development of gravitropic set-point angle (GSA) between self-rooted seedling and pomegranate seedling is a great difference, leading to the adventitious root of pomegranate was poor in shallowness and solidity, resulted in poor cold resistance [28]. FLP is angle-inducible gene in response to gravity in *Arabidopsis* and apple [27, 28]. We found that PgFLP had been clustered within the R2R3-MYB clade including AtFLP, MdFLP and others for which root gravitropism have been identified. Within the R2R3-MYB clade, PgFLP is closely associated with AtFLP, which regulate lateral root gravitropism, indicating that PgFLP may participate in the regulation of GSA in pomegranate adventitious root (Fig. 2b).

The *PINs* have been found to conducive to nearly every step of auxin fluxes [3, 18, 35, 20] and the auxin distribution patterns have been predicted and explained through auxin transport [2, 6, 1]. FLP and MYB88 have been found to can regulate root gravitropism [27], the late stages of stomatal development [11], cold hardiness [30], female reproductive development [17], and guard mother cell proliferation [11, 31]. To further understand the function of *PgFLP*, the expression of *PgFLP* was rapidly up-regulated in auxin-treated roots (Fig. 4b) and the transcripts of *PgFLP* in LBS was increased (~2-fold) upon the TSH (Fig. 4c). We also investigated the IAA content in different rootedness pomegranate self-rooted seedling and found that the level of IAA was slightly increased in LBS compared to that in TSH on shoot cutting at 30 d and 40 d (Fig. 4d). *PgFLP* (OX-3, OX-4, OX-7) (Fig. 6b-e), exhibited a smaller GSA and grew downwards faster than WT. In addition, *PgMYB88* transcripts were detected in various pomegranate organs, but the expression of *PgMYB88* was not significantly induced by auxin (Additional file 1: Figure S2a-b). We also found that the transcripts of *PgMYB88* in LBS was increased (~3-fold) upon the TSH (Additional file 1: Figure S2c). Whether *PgMYB88* is also involved in transcriptional regulation of adventitious root gravitropism in pomegranate requires further investigation. These results suggested that PgFLP
participates in regulating the GSA of adventitious root via the basal expression of *PgPIN10* in pomegranate.

**The *PgFLP* is participated in the regulatory network during the pomegranate adventitious root gravitropism**

It is well established that FLP can bind directly to the \([A/T/G][A/T/G]C[C/G][C/G]\) motif [31]. Because *PgFLP* is closely associated with AtFLP, which regulate lateral root gravitropism, and *PgFLP* functions in the regulation GSA of pomegranate self-rooted seedling adventitious root in planta (Fig. 6). Y1H experiments showed that *PgFLP* can interact with FBS (AGCGG). We also found the expression of *PgFLP* was rapidly up-regulated in auxin-treated roots (Fig. 4b), indicating that the function of *PgFLP* may be more diverse compared with *FLP* in *Arabidopsis*.

We found that FLP can activate the expression of *PIN3* and *PIN7* in *Arabidopsis* [27]. In our study, we found that *PgFLP* could activate the expression of *PgPIN10* in pomegranate (Fig. 5) and *PgFLP* could interact with FBS (AGCGG) (Fig. 5f). These data demonstrate that *PgFLP* directly regulate *PgPIN10* transcription by binding to the AGCGG motif. How *PgFLP* is participated in the feed-forward transcriptional regulation of *PgPIN10* during the pomegranate adventitious root gravitropism needs further investigation.

In our study, a model of *PgFLP* in response to gravity signal during pomegranate self-rooted seedling adventitious root gravitropism was proposed (Fig. 7). Under gravity, *PgFLP* directly regulates the expression of *PgPIN10* by binding to the AGCGG motif to regulate GSA. On the other hand, other *PgPINs* may also in response to IAA affecting auxin reflux to regulate GSA. Overall, our results confirm that the transcriptional regulation of *PgPIN10* by R2R3-MYB transcription factor *PgFLP* in setting the gravitropic set-point angle of pomegranate adventitious root in response to gravity signal.

**Conclusions**

An R2R3-MYB transcription factor *PgFLP* directly activates *PgPIN10* by binding to the AGCGG motif of its promoter region to regulate the GSA of adventitious root in pomegranate. Overall, our results confirm that the transcriptional regulation of *PgPIN10* by *PgFLP* in setting the gravitropic set-point angle of pomegranate adventitious root in response to gravity signal.

**Methods**

**Plant materials and growth conditions**

The deep-rooted pomegranate breed: ‘Lanbaoshi’(LBS) and shallow-rooted pomegranate breed: ‘Taishanhong’(TSH), provided by Professor Chuanduo Shi (Shandong Institute of Pomology), were for the study of GSA. The roots of ‘Taishanhong’(TSH) were used for gene cloning. *Arabidopsis thaliana* (ecotype ‘Columbia’), provided by Professor Xiang Shen (Shandong Agricultural University), was for
genetic transformation. The *Arabidopsis* seeds were grown on MS medium at 22°C for 16 hours of light / 8 hours of dark.

**RNA extraction and qRT-PCR analysis**

We used Total RNA Isolation System to extract total RNA from pomegranate tissues. Then, an appropriate amount of total RNA was taken to synthesize cDNA with the PrimeScript®1st Strand cDNA Synthesis Kit (Takara, Japan). The qRT-PCR was performed as previously described [15, 28]. The relative expression level of each target gene was normalized to that of the *ACTIN* gene. Three biological replicates were performed for each analysis. Details primers used in this study are shown in Additional file 2: Table S1.

**Sequence and phylogenetic tree analysis**

The corresponding amino acid sequences were obtained from the NCBI nucleotide database and aligned using ClustalX 1.81 and DNAMAN software. The corresponding protein accession numbers: *PgFLP*, Punica granatum XP_031400371.1; *PgMYB88*, Punica granatum XP_031386277.1; *MdMYB88*, Malus domestica ASW25823.1; *MdFLP*, Malus domestica XP_017192102.1; *MdMYB124*, Malus domestica ASW25824.1; *AtMYB88*, Arabidopsis thaliana NP_001030957.1; *AtMYB124 (AtFLP)*, Arabidopsis thaliana NP_563948.1; *FvMYB a*, Fragaria vesca XP_004297324.1; *FvMYB b*, Fragaria vesca XP_011463081.1; *VvMYB a*, Vitis vinifera XP_010652303.1; *VvMYB b*, Vitis vinifera XP_010652298.1; *OsMYB a*, Oryza sativa EEC82494.1; *OsMYB b*, Oryza sativa NP_001060344.1. The phylogenetic tree was constructed according to Tamura et al. [24].

**Yeast one-hybrid assays**

Y187 (Clontech) was used for Y1H assays. The PgFLP was recombined into the pGADT7 vector to obtain the AD-PgFLP plasmid, and the *PgPIN10* promoter was cloned into the pHIS2 vector. Yeast Y187 cells containing different combinations of recombinant vectors were placed on medium SD/-Leu-Trp-His with different concentration of 3-AT for detection.

**GUS transient assays**

The tobacco leaves were used to conduct transient expression assays. The promoter of *PgPIN10* was cloned into pCAMBIA1300-GUS to obtain the *proPgPIN10::GUS* recombinant vector. The coding sequence of *PgFLP* were inserted into pRI101 plant transformation vector downstream of 35S promoter. The corresponding combinations were co-injected into tobacco leaves according to previously described [28]. The tobacco of the experimental group was cultured normally for 2 days. Then, we detected GUS activity as previously described [28].

**The construction of *PgFLP* overexpression vector and genetic transformation**

The complete *PgFLP* coding region was integrated into the pRI101 plant transformation vector downstream of 35S promoter. The 35S::*PgFLP* vector was transformed into *Arabidopsis* plants [27].
seeds of the transgenic Arabidopsis plants were individually harvested. Homozygous transgenic lines were used for further investigation.

Quantification of plant hormones

The pomegranate self-rooted seedling adventitious roots were immediately frozen with liquid nitrogen. Then, we used ELISA (Enzyme-Linked Immuno Sorbent Assay) to measure hormones IAA according to Zhang et al [34].

Measurement of gravity set-point angle

Transgenic Arabidopsis thaliana roots were used to measure GSA. The GSA angles were divided into 0-30°, 30-50°, 50-70°, 70-90° and 90-110°. The Image J software was used to measure GSA. Experiments were repeated independently three times. Statistical significance: **P < 0.01.

Abbreviations

FLP, FOUR LIPS; PIN, PIN-FORMED; IAA, indole-3-acetic acid; GSA, gravitropic set-point angle; TFs, transcription factors; TSH, ‘Taishanhong’; LBS ‘Lanbaoshi’; GFP, green fluorescent protein; MS, Murashige and Skoog; WT, wild-type; GUS, β-glucuronidase; qRT-PCR, quantitative reverse transcription-PCR; Y1H, yeast one-hybrid.

Declarations

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
YLY conceived the project. YLY, JHT and ZHW designed the experiments. ZHW performed most of the experiments, and ZHW wrote the paper. JLL helped with the transient expression assays; JLL, XMY, HXT, LJF and FW helped with the phenotypic observations and the paper revision. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Abas L, Benjamins R, Malenica N, Paciorek T, Wiśniewska J, Moulinier-Anzola JC, et al. Intracellular trafficking and proteolysis of the Arabidopsis auxin-efflux facilitator PIN2 are involved in root gravitropism. Nat Cell Biol. 2006;8:249-56.

2. Barbosa ICR, Hammes UZ, Schwechheimer C. Activation and Polarity Control of PIN-FORMED Auxin Transporters by Phosphorylation. Trends Plant Sci. 2018;23:523-38.

3. Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, Jürgens G, et al. Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell. 2003;115:591-602.

4. Chen Q, Liu Y, Maere S, Lee E, Van Isterdael G, Xie Z, et al. A coherent transcriptional feed-forward motif model for mediating auxin-sensitive PIN3 expression during lateral root development. Nat Commun. 2015;6:8821.

5. Digby J, Firn RD. The gravitropic set-point angle (GSA): the identification of an important developmentally controlled variable governing plant architecture. Plant Cell Environ. 1995;18:1434-40.

6. Doyle SM, Rigal A, Grones P, Karady M, Barange DK, Majda M, et al. A role for the auxin precursor anthranilic acid in root gravitropism via regulation of PIN-FORMED protein polarity and relocalisation in Arabidopsis. New Phytol. 2019;223:1420-32.

7. Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L. MYB transcription factors in Arabidopsis. Trends Plant Sci. 2010;15:573-81.

8. Friml J, Wiśniewska J, Benková E, Mendgen K, Palme K. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis. Nature. 2002;415:806-9.

9. Kleine-Vehn J, Ding Z, Jones AR, Tasaka M, Morita MT, Friml J. Gravity-induced PIN transcytosis for polarization of auxin fluxes in gravity-sensing root cells. Proc Natl Acad Sci USA. 2010;107:22344-49.
10. Kumpf RP, Shi CL, Larrieu A, Sto IM, Butenko MA, Peret B, et al. Floral organ abscission peptide IDA and its HAE/HSL2 receptors control cell separation during lateral root emergence. Proc Natl Acad Sci USA. 2013;110:5235-40.

11. Lai LB, Nadeau JA, Lucas J, Lee EK, Nakagawa T, Zhao L, et al. The Arabidopsis R2R3 MYB proteins FOUR LIPS and MYB88 restrict divisions late in the stomatal cell lineage. Plant Cell. 2005;17:2754-67.

12. Lavenus J, Goh T, Roberts I, Guyomarc'h S, Lucas M, De Smet, et al. Lateral root development in Arabidopsis: fifty shades of auxin. Trends Plant Sci. 2013;18:450-58.

13. Lee HJ, Kim HS, Park JM, Cho HS, Jeon JH. PIN-mediated polar auxin transport facilitates root-obstacle avoidance. New Phytol. 2020;225:1285-96.

14. Le J, Liu XG, Yang KZ, Chen XL, Zou JJ, Wang HZ, et al. Auxin transport and activity regulate stomatal patterning and development. Nat Commun. 2014;5:3090.

15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-8.

16. Lucas M, Kenobi K, von Wangenheim D, Vobeta U, Swarup K, De Smet I, et al. Lateral root morphogenesis is dependent on the mechanical properties of the overlaying tissues. Proc Natl Acad Sci USA. 2013;110:5229-34.

17. Makkena S, Lee E, Sack FD, Lamb RS. The R2R3 MYB transcription factors FOUR LIPS and MYB88 regulate female reproductive development. J Exp Bot. 2012;63:5545-58.

18. Marhavý P, Vanstraelen M, De Rybel B, Zhaojun D, Bennett MJ, Beeckman T, et al. Auxin reflux between the endodermis and pericycle promotes lateral root initiation. EMBO J. 2013;32:149-58.

19. Péret B, Li G, Zhao J, Band LR, Voss U, Postaise O, et al. Auxin regulates aquaporin function to facilitate lateral root emergence. Nat Cell Biol. 2012;14:991-98.

20. Pernisova M, Prat T, Grones P, Harustiakova D, Matonohova M, Spichal L, et al. Cytokinins influence root gravitropism via differential regulation of auxin transporter expression and localization in Arabidopsis. New Phytol. 2016;212:497-509.

21. Petrásek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertová D, et al. PIN proteins perform a rate-limiting function in cellular auxin efflux. Science. 2006;312:914-18.

22. Roychoudhry S, Del Bianco M, Kieffer M, Kepinski S. Auxin controls gravitropic setpoint angle in higher plant lateral branches. Curr Biol. 2013;23:1497-1504.

23. Stover E, Mercure EW. The pomegranate: a new look at the fruit of paradise. HortScience. 2007;42:1088-92.

24. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28(10):2731-9.

25. Vanneste S, Friml J. Auxin: a trigger for change in plant development. Cell. 2009;136:1005-16.
Vermeer JE, von Wangenheim D, Barberon M, Lee Y, Stelzer EH, Maizel A, et al. A spatial accommodation by neighboring cells is required for organ initiation in Arabidopsis. Science. 2014;343:178-83.

Wang HZ, Yang KZ, Zou JJ, Zhu LL, Xie ZD, Morita MT, et al. Transcriptional regulation of PIN genes by FOUR LIPS and MYB88 during Arabidopsis root gravitropism. Nat Commun. 2015;6:8822.

Wang ZH, Li JL, Mao YF, Zhang MM, Wang R, Hu YL, et al. Transcriptional regulation of MdPIN3 and MdPIN10 by MdFLP during apple self-rooted stock adventitious root gravitropism. BMC Plant Biol. 2019;19:229.

Wisniewska J, Xu J, Seifertová D, Brewer PB, Ruzicka K, Blilou I, et al. Polar PIN localization directs auxin flow in plants. Science. 2006;312:883.

Xie Y, Chen P, Yan Y, Bao C, Li X, Wang L, et al. An atypical R2R3 MYB transcription factor increases cold hardiness by CBF-dependent and CBF-independent pathways in apple. New Phytol. 2017;218:201-18.

Xie Z, Lee E, Lucas JR, Morohashi K, Li D, Murray JA, et al. Regulation of cell proliferation in the stomatal lineage by the Arabidopsis MYB FOUR LIPS via direct targeting of core cell cycle genes. Plant Cell. 2010;22:2306-21.

Xuan W, Audenaert D, Parizot B, Moller BK, Njo MF, De Rybel B, et al. Root Cap-Derived Auxin Pre-patterns the Longitudinal Axis of the Arabidopsis Root. Curr Biol. 2015;25:1381-88.

Yuan Z, Yin Y, Qu J, Zhu L, Li Y. Population genetic diversity in Chinese pomegranate (Punica granatum) cultivars revealed by fluorescent-AFLP markers. J Genet Genomics. 2007;34:1061–71.

Zhang W, Cao Z, Zhou Q, Chen J, Xu G, Gu J, et al. Grain Filling Characteristics and Their Relations with Endogenous Hormones in Large- and Small-Grain Mutants of Rice. PLoS One. 2016;11:e0165321.

Zhang YZ, Friml J. Auxin guides roots to avoid obstacles during gravitropic growth. New Phytol. 2020;225:1049-52.

Zhao XQ, Yuan ZH, Feng LJ, Fang YM. Cloning and expression of anthocyanin biosynthetic genes in red and white pomegranate. J Plant Res. 2015;128:687–96.

Tables

Table 1 The angle between adventitious root and gravity (°)
| Root architecture | No. of Adventitious roots | Cuttings | $X \leq 30$ | $30 \leq X \leq 60$ | $X > 60$ |
|-------------------|---------------------------|----------|-------------|-------------------|---------|
|                   |                           |          | No. of roots | Percent of roots  | No. of roots | Percent of roots |
| Deep roots        | 47                        | LBS      | 11          | 23.40%            | 30        | 63.83%            | 6        | 12.77%            |
| Shallow roots     | 52                        | TSH      | 4           | 7.69%             | 28        | 53.85%            | 20       | 38.46%            |

$X$, represent as the angle between adventitious root and gravity; LBS, ‘Lanbaoshi’; TSH, ‘Taishanhong’.