

# GIBBERELLIN INSENSITIVE DWARF1 Plays an Important Role in the Growth Regulation of Dwarf Apple Rootstocks

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**Abstract.** Dwarfing rootstocks can improve the plant architecture of apple trees and increase production. Gibberellins (GAs) are crucial for plant growth and dwarfing traits. The receptor, GIBBERELLIN INSENSITIVE DWARF1 (GID1), plays an important role in the regulation pathway. However, the growth regulatory mechanism of GID1 in dwarf apple rootstock seedlings is not clear. In this study, we selected dwarf apple rootstock ‘SH6’ and its cross parents as materials to clone the GA receptor gene *GID1c*. There were two different sites in the alpha/beta hydrolase domain. The expression of *GID1c* in ‘SH6’ was lower than that in *Malus domestica* cv. Ralls Janet, with the decrease of GA content. We further conducted GA<sub>3</sub> treatment and overexpression of *GID1c* in tissue culture seedlings of ‘SH6’, and the results showed that the expression of *GID1c* and biosynthesis genes increased and promoted the accumulation of hormone contents, which ultimately regulates the growth of ‘SH6’ dwarf apple rootstock seedlings. Our results suggest that *GID1c* may affect the plant architecture and dwarf traits of dwarfing rootstock and accelerate its application in orchards.

Gibberellins (GAs) are increasingly proving to be a crucial regulator of plant growth and a controlling factor of plant architecture. They regulate various physiological and biochemical processes of plant growth and de-

velopment, promote germination, control plant height and root growth, improve tillering, regulate flowering, and improve seed production (Andres et al., 2014; Qin et al., 2013; Resentini et al., 2015). Key enzymes involved in GA biosynthesis, such as ent-kaurene oxidase (KO), ent-kaurenoic acid oxidase (KAO), and ent-kaurene synthase (KS), catalyze the early step(s) of the cyclization and oxidation reactions and affect the GA signal pathway, regulating plant growth phenotype (Fambrini et al., 2011; Toyomasu et al., 2016; Yamamura et al., 2018). The GA 20-oxidases can regulate plant stature and disease development by affecting GA biosynthesis in rice (Qin et al., 2013). Mutants defective in GA biosynthesis, such as GA1, GA2, and GA3, present relatively low internal concentrations of GA, resulting in dwarf phenotypes for both monocot and dicot plants; in turn, GA applications promote growth elongation (Lohr et al., 2014). In the GA signal pathway, GIBBERELLIN

INSENSITIVE DWARF1 (GID1) acts as the GA receptor, can combine with GA to induce the degradation of DELLA repressor proteins, following the GA-dependent complex formation of GID1-GA-DELLA, and then regulate many physiological and biochemical processes in plants (Griffiths et al., 2006; Nemoto et al., 2017; Schwechheimer, 2012; Yan et al., 2014). *Arabidopsis* has three gibberellin receptors: GIBBERELLIN INSENSITIVE DWARF1A (GID1A), GID1B, and GID1C. GID1B and GID1C have specific roles in seed development and root elongation. A previous study suggested that GID1A can bind gibberellin in the cytosol to initiate gibberellin signaling and regulate plant growth and fruit set (Ferreira et al., 2018; Gallego-Giraldo et al., 2014; Livne and Weiss, 2014). In wheat, there are three TaGID1 genes, and overexpression of each TaGID1 gene in the *Arabidopsis* double-mutant *gid1a/1c* partially rescued the dwarf phenotype (Li et al., 2013). In woody plants, GID1 also plays an important role in growth and in the dwarf phenotype (Cantin et al., 2018); however, the regulation function of GID1 in the determination of the plant growth traits of dwarfing rootstocks is still unclear.

Plant growth is also controlled by the regulation of different hormone signals, including auxin, brassinolide, and abscisic acid (Ren et al., 2018). During the petal elongation growth process, auxin promotes cell elongation and increases the number of cells in the petal basal region, regulating flower growth and development (Wang et al., 2017). Auxin response factor8 interacts with BIGPETALp, which has been shown to limit petal growth by influencing cell expansion, to modulate petal expansion. In the BR signaling pathway, the BR molecule was shown to bind with receptor-like kinase BRI1 and then promote the activation of BES and BZR to regulate multiple aspects of growth and development (Clouse and Sasse, 1998; Kim et al., 2011; Tang et al., 2011; Wang and He, 2004; Yin et al., 2002; Zhu et al., 2013). During plant growth, environmental stress regulates the transcription of ABA-insensitive (ABI) and NCED genes, affecting plant growth and stress responses. In the ABA catabolism pathway, ABI promotes plant growth and fruit development during strawberry ripening; this promotion is regulated by microRNAs involved in posttranscriptional modification (Li et al., 2016). Therefore, crosstalk may occur between different hormones to regulate plant growth and development.

Plant architecture, including plant height, number of tillers, and root system characteristics, which are regulated by both genetic and environmental factors, is the focus of intense research aiming to improve crop performance (Hedden 2003). In modern apple breeding, the application of dwarf rootstocks can reduce vegetative growth and lead to dwarfing phenotype, which improves the fruit yield and quality (Andrea et al., 2006;

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Cohen et al., 2007; Foster et al., 2017; Greenwood et al., 2010; Jöst et al., 2015; Smolka et al., 2010); however, the biochemical and molecular mechanisms that regulate dwarfing traits in dwarf apple rootstocks have

not been elucidated. Specifically, the function of GA signaling in dwarf apple rootstocks as well as the crosstalk between GA and other hormones remain unclear. In this study, we selected the apple dwarfing inter-

stock 'SH6' as experimental material. We cloned the *GID1c* gene and compared both its sequence and expression specificity in 'SH6' and its parents. By using transient transformation, we also analyzed the transcript level

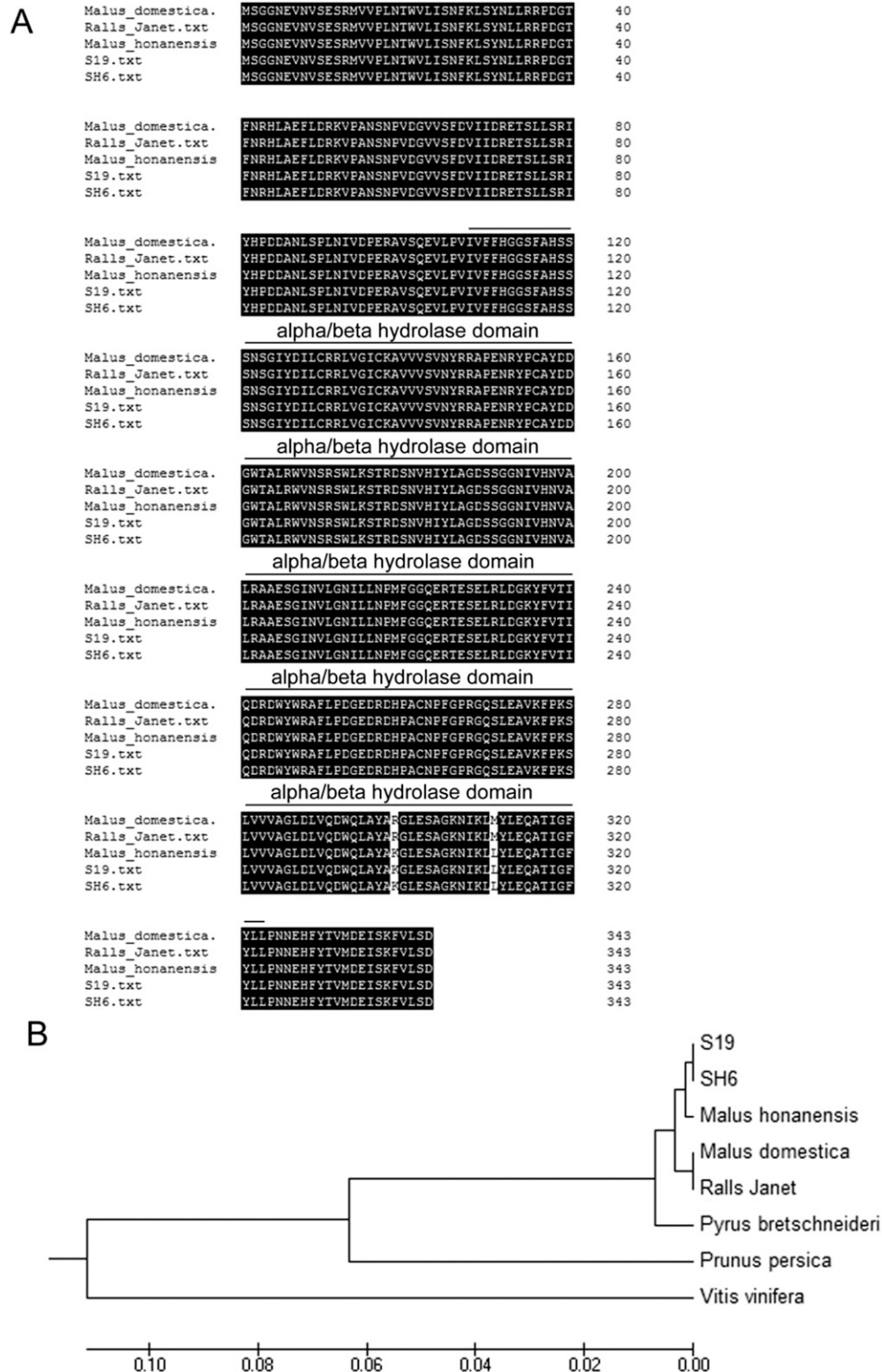


Fig. 1. Bioinformatic analysis of *GID1c* in *Malus domestica*, *M. domestica* cv. Ralls Janet, *Malus honanensis*, *M. honanensis* cv. S19, and 'SH6'. (A) Amino acid sequence alignment analysis of *GID1c* in *Malus domestica*, *M. domestica* cv. Ralls Janet, *M. honanensis*, *M. honanensis* cv. S19, and 'SH6'. Two specific sites in the alpha/beta hydrolase domain are indicated with under lining. (B) Phylogenetic analysis of *GID1c* in 'SH6', *M. honanensis* cv. S19, *M. honanensis* (MH), *M. domestica* cv. Ralls Janet (RJ), *M. domestica* (NM\_001294351.1), *Pyrus bretschneideri* (XM\_009337376.2), *Prunus persica* (XM\_007207866.2), and *Vitis vinifera* (KY765598.1). The genetic distance is closest between 'SH6' and *M. honanensis* cv. S19.

of related genes and hormone contents in the tissue of 'SH6' seedlings, and the results showed that *GID1c* may promote the biosynthesis and metabolism of GA and other hormones, which further regulate plant growth characteristics. We aim to analyze the regulation mechanism of *GID1c* on plant architecture and dwarfism characteristics in 'SH6' dwarf apple rootstocks and promote its application value in orchards.

## Materials and Methods

**Plant materials and growth conditions.** 'SH6', the hybrid offspring of *Malus domestica* cv. Ralls Janet and *M. honanensis* cv. S19 (a brachyplast bud mutation variety of *Malus honanensis*), is widely used as apple dwarfing interstock for its stable dwarfness and resistance. *Malus honanensis* and 'S19' also have the dwarfing and semidwarfing characteristics, but they have poor genetic stability and rootstock-scion affinity. 'Ralls Janet' has no dwarfing or semidwarfing characteristics. The leaves of field plants were collected from the Institute of Pomology in Taigu, Shanxi, China. The tissue culture seedlings of 'SH6' were cultivated in the Tissue Culture Center of Beijing University of Agriculture on solid medium [Murashige and Skoog (MS) medium] under a 16-h light cycle (1800–2000 Lx) at 23 °C. Rooting cultures were established on medium (1/2 MS medium) under a 16-h light cycle (1800–2000 Lx) at 23 °C.

**Gene cloning.** Leaves (with the same vegetative vigor) in annual shoots of 'SH6', *M. domestica* cv. Ralls Janet, *Malus honanensis*, and *M. honanensis* cv. S19 were collected from field plants for gene cloning at 180 d after full bloom (DAFB). The primers used are shown in Supplemental Table 1 and were designed based on the apple genome sequencing in the National Center for Biotechnology Information (NCBI). The total RNA was isolated using an RNA Pure Total RNA Extraction Kit (Aidlab, Beijing, China). After DNase I (Takara, Shiga, Japan) treatment to remove genomic DNA, first-strand complementary DNA (cDNA) was synthesized using a Promega Reverse Transcription System Kit in accordance with the manufacturer's instructions (Promega, Madison, WI).

**Bioinformatic analysis.** Amino acid sequence alignment analysis was conducted with DNAMAN, and the function domain analysis was performed at <http://smart.embl-heidelberg.de/>. A phylogenetic tree was constructed in accordance with the nearest-neighbor interchange method using the ClustalW program of MEGA5.2 software. Branches corresponding to partitions were produced from 1000 bootstrap replicates. The evolutionary distances were computed using the p-distance method.

**Dwarfing traits measurement.** We selected 10 plants of each cultivar for the measurement of dwarfing traits from field plants at 180 DAFB. The tape was used to measure the plant height and crown diameter

(m), a vernier calliper was used to measure the tapering (Kr) of annual shoots (diameter of stem apex: the diameter of stem base), and a resistivity detector was used for resistivity detection ( $\Omega\cdot m$ ) of annual shoots ( $p = RS/l$ ; R, resistance; S, cross-sectional area; l, length).

**GA3 and paclobutrazol treatments.** To analyze the growth phenotypes, each of 10 tissue culture seedlings of 'SH6' (with the same vegetative vigor) were cultivated on solid medium (4.74 g MS + 6 g agar powder + 30 g sucrose) and supplemented with both 50  $\mu M$  GA<sub>3</sub> (Sigma-Aldrich, St. Louis, MO) and 10  $\mu M$  paclobutrazol (PAC) under a 16-h light cycle (1800–2000 Lx) at 23 °C. The plant materials were collected after 20 d of treatment for the measurement of growth index and hormone contents.

**Quantitative real-time polymerase chain reaction.** Gene expression differences were analyzed via quantitative real-time polymerase chain reaction (qRT-PCR). The total RNA was extracted from the leaves in annual young shoots of different cultivars and 'SH6' tissue culture seedlings. First-strand cDNA was synthesized using a Tiangen Fast Quant RT Kit (Tiangen, Beijing, China) with 1  $\mu g$  total RNA. qRT-PCR was performed on an ABI7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) using GoTaq qPCR master mix (Promega) in accordance with the manufacturer's instructions. The conditions were as follows: 95 °C for 3 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 20 s. The relative gene expression was analyzed using  $2^{-\Delta\Delta Ct}$  values, which were normalized with the 18S gene. The primers used are shown in Supplemental Table 1 and were designed based on the conserved sequence in NCBI. At least three biological replicate samples were used. Differences were considered significant at  $P < 0.05$ .

**Vector construction and genetic transformation.** The coding sequence of *GID1c*

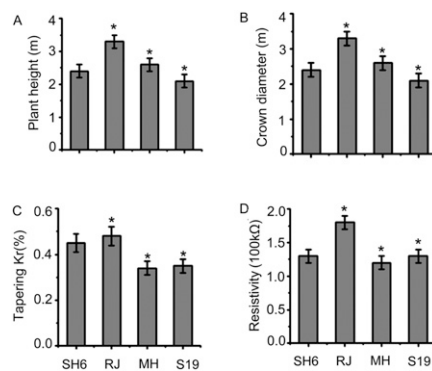


Fig. 2. The dwarfing traits of *Malus domestica* cv. Ralls Janet (RJ), *Malus honanensis* (MJ), *M. honanensis* cv. S19, and 'SH6'. (A) The plant height in different cultivars. (B) The crown diameter in different cultivars. (C) The tapering (Kr) in different cultivars. (D) The resistivity in different cultivars. The data are presented as the means  $\pm$  SD of three biological replicates with Duncan's statistical analysis. The asterisks represent the significant difference. \* $0.01 < P < 0.05$ , \*\* $P < 0.01$ .

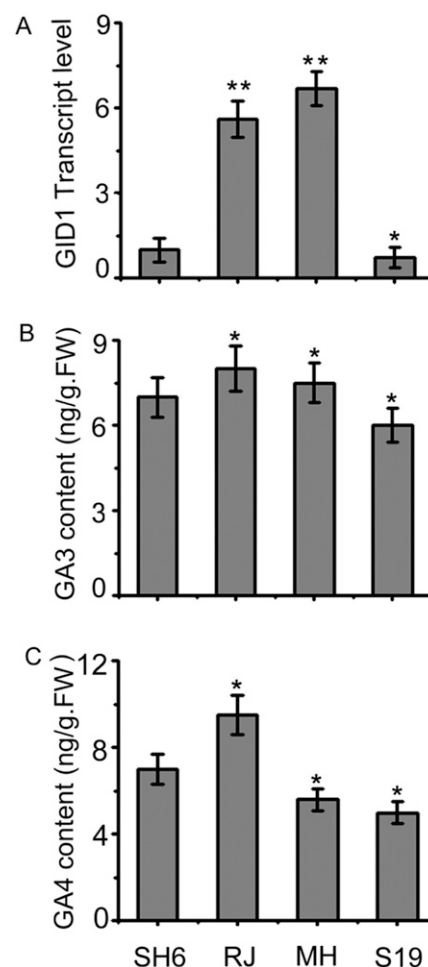


Fig. 3. (A) The *GID1c* transcript levels and gibberellin (GA) content analysis. The transcript level of *GID1c* in the different cultivars. (B) The GA<sub>3</sub> content in the different cultivars. (C) The GA<sub>4</sub> content in the different cultivars. The data are presented as the means  $\pm$  SD of three biological replicates with Duncan's statistical analysis. FW = fresh weight; MJ = *Malus honanensis*; RJ = 'Ralls Janet'. The asterisks represent the significant difference. \* $0.01 < P < 0.05$ , \*\* $P < 0.01$ .

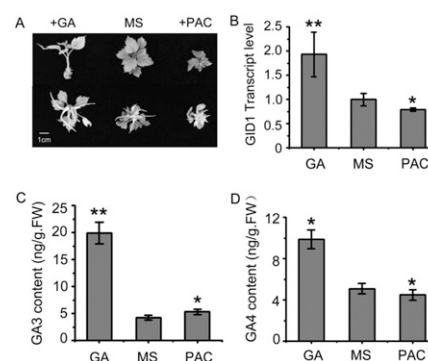


Fig. 4. (A) Growth phenotype of 'SH6' tissue culture seedlings under different treatments [GA<sub>3</sub> and paclobutrazol (PAC)]. (B) Analysis of *GID1c* transcript levels in response to different treatments. (C) The GA<sub>3</sub> content in the different cultivars. (D) The GA<sub>4</sub> content in the different cultivars. The data are presented as the means  $\pm$  SD of three biological replicates with Duncan's statistical analysis. The asterisks represent the significant difference. \* $0.01 < P < 0.05$ , \*\* $P < 0.01$ .

was inserted into ap35S-1300–green fluorescent protein (GFP) vector containing *Xba*I and *Kpn*I sites compared with the p35S-1300-GFP empty vector (WT), after which the vectors were transformed into *Agrobacterium tumefaciens* strain GV3101. To test the function of *GID1c* in the ‘SH6’ tissue culture seedlings, we performed a transient transformation using the vacuum draw method (Zhang et al., 2016). Then, the seedlings were cultured for 7 d under a 16-h light cycle (1800–2000 Lx) at 23 °C after 3-day shade treatment. The leaves of the seedlings were subsequently collected for PCR and qRT-PCR testing and related index measurement. The data of three biological replicates were used.

**Microscopic examination.** The GFP in transgenic ‘SH6’ tissue culture seedlings was examined using an upright fluorescent microscopic (Leica, Wetzlar, Germany) under blue excitation spectra and a Stereo Fluorescence Microscope (Leica) under green excitation spectra. The microscope images were then observed on a computer.

**Hormone content measurement.** The leaves in young annual shoots of different cultivars and the leaves of tissue culture seedlings ‘SH6’ with different treatments were collected and ground into powder. Then, 0.2 to 0.5 g powder was added to 4 mL phosphate-buffered saline extracting solution with polyvinylpyrrolidone to measure the hormone contents with an enzyme-linked immunosorbent assay.

**Statistical analysis.** Duncan’s statistical analysis for the measurement of growth index, expression level, and hormone content was performed using Origin Pro 8 (Origin Laboratory Corporation, Northampton, MA) and Photoshop (Adobe, San Jose, CA) software were used for image integration. Error bars for each symbol indicate the SD of the reactions of three biological replicates.

## Results

**Bioinformatic analysis of *GID1c* in different apple cultivars.** Given the homology with *M. domestica* sequences (NM\_001294351.1), we cloned the CDS of *GID1c* in ‘SH6’, *M. domestica* cv. Ralls Janet, *M. honanensis*, and *M. honanensis* cv. S19 leaves with the primers Md-GID1-F and Md-GID1-R. The open reading frame of the *GID1c* gene encodes a deduced amino acid sequence of 343 residues. There was little difference among the four cultivars except two amino acid sites in the alpha/beta hydrolase domain; the sequence in ‘SH6’ was more homologous with that in *M. honanensis* cv. S19 and *M. honanensis*, and the sequence in *M. domestica* cv. Ralls Janet was more homologous with that in *M. domestica* (Fig. 1). The *GID1c* sequence difference in different cultivars may have no effect on the GA signal pathway, but it was in accordance with the plant dwarfing traits. The plant height, crown diameter, tapering, and resistivity in dwarfing cultivars were lower than those in ‘Ralls Janet’ (Fig. 2). These findings revealed the closer genetic relationship between ‘SH6’ and *M. honanensis* cv. S19 and *M. honanensis*.

Table 1. Growth index of ‘SH6’ on MS solid medium and supplemented with 50  $\mu$ M GA<sub>3</sub> and 10  $\mu$ M PAC.

Treatment	MS	GA	PAC
Plant height (cm)	1.5 $\pm$ 0.06	2.8 $\pm$ 0.10	1.0 $\pm$ 0.15
Number of internodes	0	5.0 $\pm$ 0.10	0
Root length (cm)	2.0 $\pm$ 0.20	3.1 $\pm$ 0.15	0.8 $\pm$ 0.15
Leaf length (cm)	1.4 $\pm$ 0.10	2.0 $\pm$ 0.06	1.1 $\pm$ 0.10
Leaf width (cm)	1.2 $\pm$ 0.10	1.6 $\pm$ 0.10	1.1 $\pm$ 0.17
Petiole length (cm)	0.8 $\pm$ 0.06	1.1 $\pm$ 0.10	0.3 $\pm$ 0.10

GA = gibberellin; MS = Murashige and Skoog; PAC = paclobutrazol.

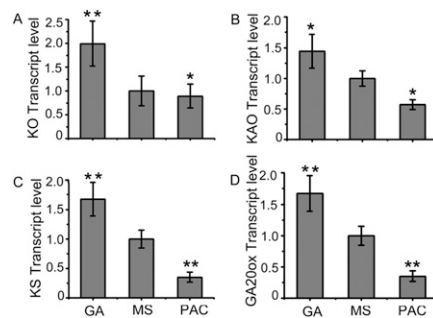


Fig. 5. Analysis of the transcript levels of genes related to gibberellin (GA) biosynthesis under different treatment [GA and paclobutrazol (PAC)] conditions. (A–D) The transcript levels of *KO*, *KAO*, *KS*, and *GA20ox*, respectively. The data are presented as the means  $\pm$  SD of three biological replicates with Duncan’s statistical analysis. The asterisks represent the significant difference. \*0.01 < *P* < 0.05, \*\**P* < 0.01.

**The transcript level of *GID1c* and *GA* contents in different apple cultivars.** In different cultivars, the transcript levels of *GID1c* in ‘SH6’ and *M. honanensis* cv. S19 were 5- to 7-fold lower than those in *M. domestica* cv. Ralls Janet (RJ) and *M. honanensis* (MH) (Fig. 3A). Moreover, the contents of GA<sub>3</sub> in ‘SH6’ and *M. honanensis* cv. S19 were also lower than those in *M. domestica* cv. Ralls Janet (RJ) and *M. honanensis* (MH) (Fig. 3B). In addition, the contents of GA<sub>4</sub> in ‘SH6’, *M. honanensis* (MH), and *M. honanensis* cv. S19 were lower than that in *M. domestica* cv. Ralls Janet (RJ) (Fig. 3C). These results suggest that *GID1c* may be an important factor in the regulation of plant growth by promoting GA content. The lower expression of *GID1c* in ‘SH6’ may result in the dwarfing phenotype of ‘SH6’ compared with ‘Ralls Janet’ (RJ).

***GID1c* transcript levels and *GA* contents in response to GA<sub>3</sub> and PAC treatments.** We treated ‘SH6’ tissue culture seedlings with GA<sub>3</sub> or PAC to explore the effects of GA<sub>3</sub> and their inhibitor PAC on ‘SH6’ growth phenotypes; normal MS medium served as a control treatment. The results showed that, compared with the control treatment, the GA<sub>3</sub> treatment significantly promoted plant height and root growth, whereas PAC clearly inhibited plant height and root growth (Fig. 4A). Moreover, the GA<sub>3</sub> treatment also promoted *GID1c* expression, with an increase of GA (GA<sub>3</sub>/GA<sub>4</sub>) contents, whereas PAC inhibited *GID1c* expression (Fig. 4B–D). These phenomena increased plant height and the number of internodes and promoted the growth of leaves and roots (Table 1). All these results

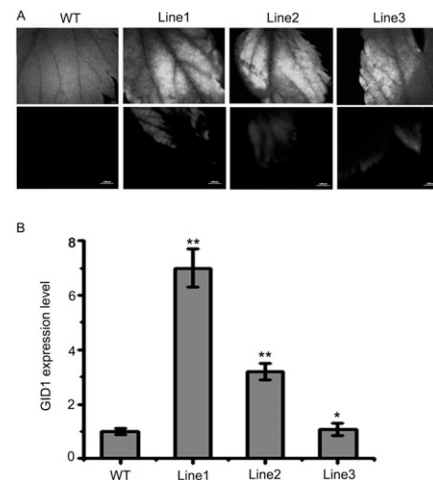


Fig. 6. Microscopic examination and *GID1c* transcript level analysis of transgenic ‘SH6’ tissue culture seedlings. (A) Examination of green fluorescent protein (GFP) in both transgenic lines (OE-GID1: Line1, Line2, Line3) and the p35S-1300-GFP empty vector (WT) line of ‘SH6’ tissue culture seedlings via an upright fluorescence microscopy and stereo fluorescence microscopy. (B) Analysis of the transcript levels of *GID1c* in transgenic line (OE-GID1: Line1, Line2, Line3) and WT line of ‘SH6’ tissue culture seedlings. The data are presented as the means  $\pm$  SD of three biological replicates with Duncan’s statistical analysis. The asterisks represent the significant difference. \*0.01 < *P* < 0.05, \*\**P* < 0.01.

demonstrated that, by increasing the transcript levels of the GA receptor *GID1c*, GA treatment promoted the growth of ‘SH6’ with the accumulation of GA content.

**The transcript levels of *GA* biosynthesis genes in response to different treatments.** We explored the expression patterns of genes related to GA biosynthesis in response to GA<sub>3</sub> and PAC treatments in ‘SH6’ tissue culture seedlings. GA<sub>3</sub> treatment increased the transcription level of *KO* 2-fold compared with the effects of MS treatment, and PAC treatment had little effect on the expression of *KO* (Fig. 5A). The transcription levels of *KAO*, *KS*, and *GA20ox* genes, all of which are also related to GA biosynthesis, were upregulated in the plants treated with GA<sub>3</sub> but were downregulated in the plants treated with PAC (Fig. 5B–D). The results showed that GA<sub>3</sub> treatment increased the expression of the GA receptor *GID1c* and upregulated the transcript levels of *KO*, *KAO*, *KS*, and *GA20ox*, promoting GA biosynthesis and the accumulation of GAs, which are crucial for the growth regulation of dwarf ‘SH6’ apple rootstock seedlings.

**Transcript levels of relative genes and hormone content analysis in the *GID1c* overexpression lines.** To further investigate the function of *GID1c* in plant growth, we constructed a *GID1c* overexpression (OE) vector and obtained the transient transformation lines of 'SH6' tissue culture seedlings (OE-*GID1*: Line1, Line2, Line3) compared with the empty vector line (WT), which were examined microscopically. The leaves of the OE-*GID1* lines presented distinct green fluorescence (Fig. 6A). The transcript levels of *GID1c* in the OE-*GID1c* plants were higher than that in the WT plants (Fig. 6B). Furthermore, the expression of *KO*, *KAO*, *KS*, and *GA20ox* related to the GA biosynthesis pathway increased, which provided an advantage to GA biosynthesis (Fig. 7A–D). At the same time, the transcription levels of the *AUX* and auxin response factor (*ARF*) genes, which are related to the auxin signaling pathway, also increased in the OE-*GID1* lines (Fig. 7E and F). The expression of *BZR*, a negative regulator of the BR pathway, decreased in the OE-*GID1c* lines, whereas the expression of *BES*, a positive regulator, increased (Fig. 7G and H). Moreover, the expression of the *ABI* and *NCED* genes, which are related to ABA signaling, was significantly upregulated in

the OE-*GID1* plants compared with the WT plants (Fig. 7I and J). As a result, *GID1c* OE also increased the accumulation of hormones (*GA*<sub>3</sub>, *IPA*, *IAA*, *BR*, and *ABA*) in OE-*GID1* 'SH6' tissue culture seedlings, but there was no obvious change in *GA*<sub>4</sub> content (Fig. 8). These results indicated that the OE of *GID1c* may promote the relative gene expression and then increase the hormone contents to regulate plant growth. There may be a cross-talk between GA signals and other hormone (*BR*, auxin, and *ABA*) signals, resulting in synergistic effects that ultimately regulate the growth of 'SH6' dwarf apple rootstocks, including the control of plant architecture.

## Discussion

Height is a crucial biological characteristic and economic trait of plants. The selection of plant cultivars that present dwarf phenotypes, which allow higher planting densities, has been an important strategy in modern

crop production (Jöst et al., 2015). The largest individual increases in harvest index and yield occurred in semidwarf cultivation systems of wheat, maize, and rice; these increases involved GA-insensitive dwarfing alleles (Prassinos et al., 2009; Rebetzke et al., 2012). Apple dwarf plants include both dwarf and semidwarf types; these types can be mainly generated via combinations of dwarf rootstock-tall scion, dwarf interstock-tall scion, and dwarf rootstock-dwarf scion. 'SH6' is the hybrid offspring of *M. domestica* cv. Ralls Janet × *M. honanensis* cv. S19 (an *M. honanensis* bud mutation variety that presents short shoots). Owing to its dwarfing interstock properties, 'SH6' is widely applied to apple fruit production in China (Fig. 2). During the past few decades, researchers have proposed several hypotheses to explain plant dwarfing mechanisms. In endogenous hormone regulatory pathways, GA signals and the GA receptor *GID1* may perform key functions that regulate plant height and

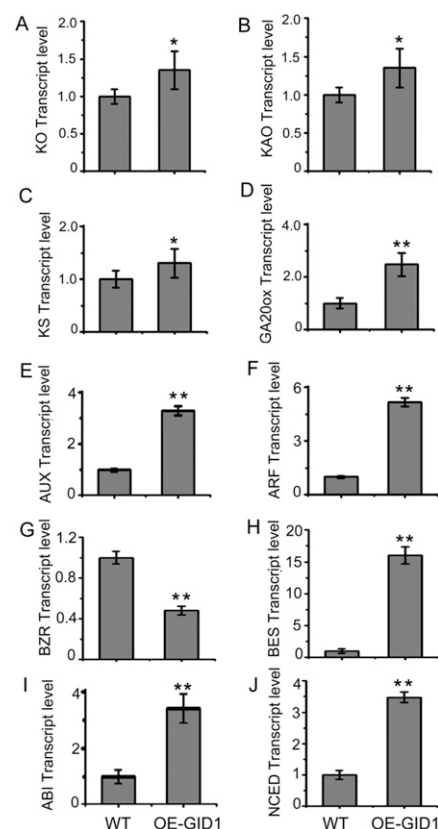


Fig. 7. Analysis of the transcript levels of genes related to hormone biosynthesis and metabolism in both transgenic line and WT line of 'SH6' tissue culture seedlings. (A–J) The transcript levels of *KO*, *KAO*, *KS*, *GA20ox*, *AUX*, *ARF*, *BZR*, *BES*, *ABI*, and *NCED*, respectively. The data are presented as the means  $\pm$  SD of three biological replicates with Duncan's statistical analysis. The asterisks represent the significant difference. \* $0.01 < P < 0.05$ , \*\* $P < 0.01$ .

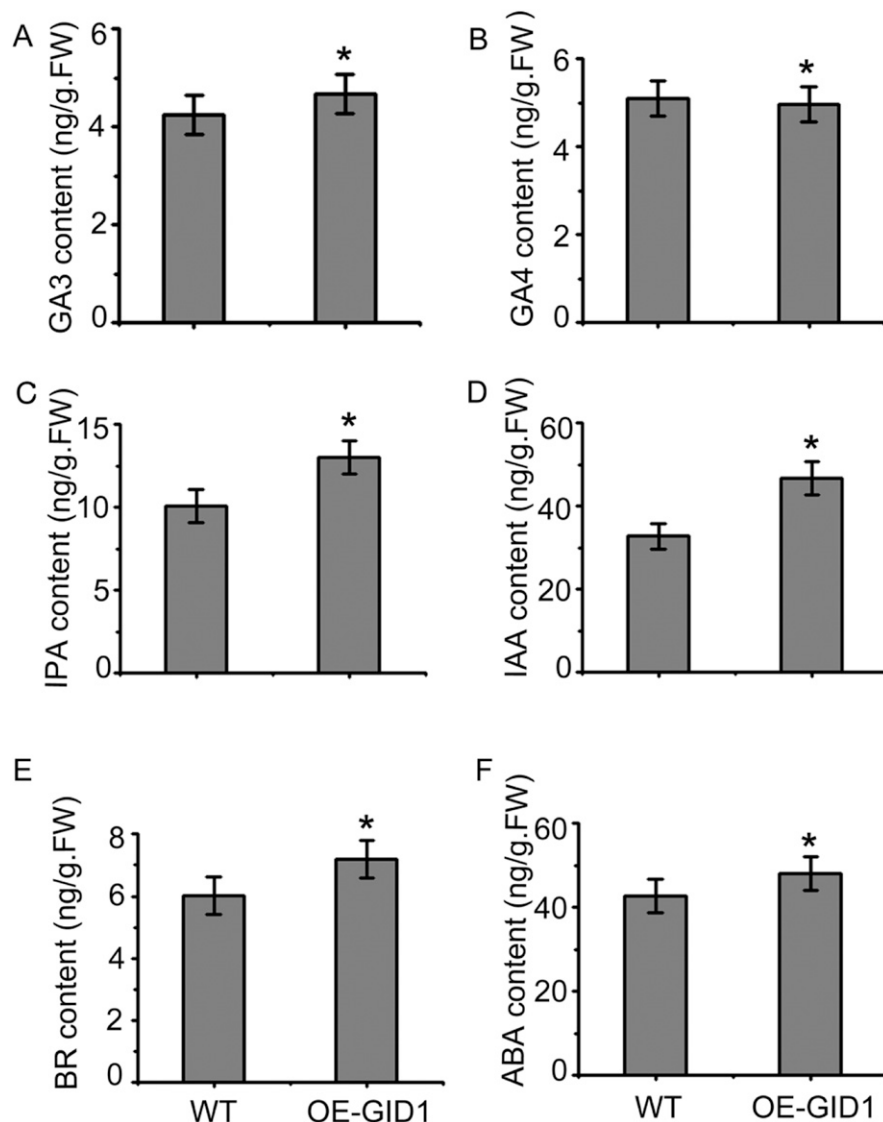


Fig. 8. Analysis of the hormone contents in transgenic line and WT line of 'SH6' tissue culture seedlings. (A–F) The content of *GA*<sub>3</sub>, *GA*<sub>4</sub>, *IPA*, *IAA*, *BR*, and *ABA*, respectively. The data are presented as the means  $\pm$  SD of three biological replicates with Duncan's statistical analysis. The asterisks represent the significant difference. \* $0.01 < P < 0.05$ , \*\* $P < 0.01$ .

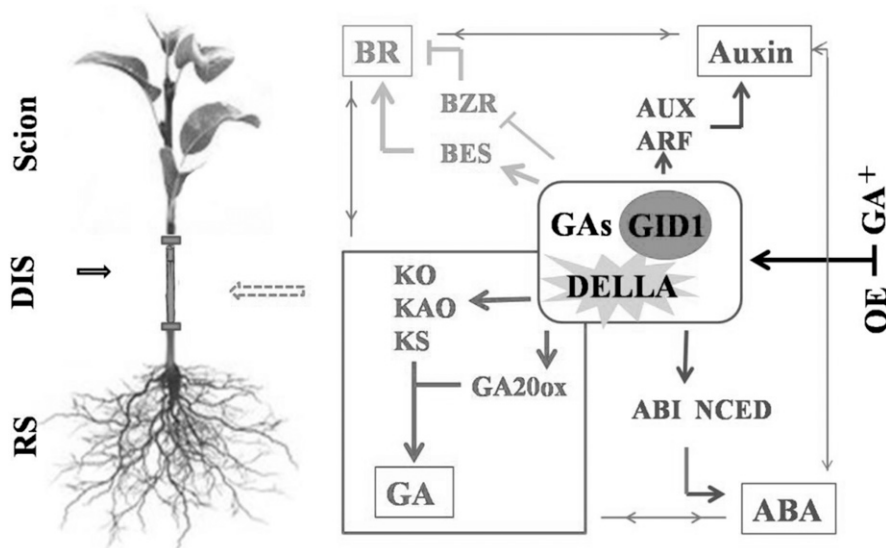


Fig. 9. Hypothesis model of the GID1c regulation mechanism in fruit dwarf rootstocks. RS = rootstock; DIS = dwarf interstock. The red mark represents gibberellin (GA) signal pathways [ent-kaurene oxidase (KO), ent-kaurenoic acid oxidase (KAO), ent-kaurene synthase (KS), and GA20-oxidases (GA20ox)], the green mark represents BR signal pathways (BZR and BES), the purple mark represents auxin signal pathways (AUX and ARF), and the blue mark represents ABA signal pathways (ABI and NCED). The arrow indicates a positive effect, and the T-shaped arrow indicates a negative effect.

architecture (Hollender et al., 2016). In our study, the *GID1c* expression in the leaves of 'SH6' and *M. honanensis* cv. 'S19' was lower than that in the leaves of both *M. domestica* cv. Ralls Janet and *M. honanensis*. Moreover, the GA contents in 'SH6' and *M. honanensis* cv. S19 were lower than in the leaves of *M. domestica* cv. Ralls Janet (Fig. 3). These results demonstrated that the expression of *GID1c* coordinated with the accumulation of GA, which affected the dwarf traits in 'SH6' apple rootstocks.

GA participates in many aspects of plant growth and development, not only in seed germination, stem elongation, and floral development, but also in wood formation, and the differentiation of xylem fibers, in many plant species (Li et al., 2012). A previous study showed that GA biosynthetic and catabolic enzymes adversely influence plant height by regulating cell division and growth (Tyler et al., 2004). In our research, GA treatment also upregulated the expression levels of *GID1c*, which probably promoted the growth of 'SH6' tissue culture seedlings as well as the plant height, number of internodes, and leaf area by increasing the GA contents (Fig. 4 and Table 1). At high GA concentrations, the GID1 receptor binds with DELLA proteins, which leads to the formation of a GID1-GA-DELLA complex; this complex then targets the DELLA proteins for proteasomal degradation, which prevents dwarf phenotypes and impairs germination, flowering, and fertility (Fukazawa et al., 2015). In our study, the OE of *GID1c* in 'SH6' leaves may increase the GID1c protein, which promoted the increase of GID1-GA-DELLA complex, resulting in the upregulation of GA biosynthesis genes and GA accumulation, and ultimately regulated plant growth characteristics (Fig. 5). All these results indicated that GA treatment and OE

of *GID1c* may rescue the apple dwarfing traits by promoting the relative gene expression in GA biosynthesis and metabolism pathways and increasing the accumulation of GAs.

In the plant growth process, the crosstalk between different hormones is an efficient regulatory factor. By directly interacting between DELLAs and BZR1/BES1, BRs and GAs enhance one another's signals, thereby regulating cell elongation and plant growth (Allen and Ptashnyk, 2017; Jiang et al., 2015; Shahnejat-Bushehri et al., 2016). We performed *GID1c* transformations to further investigate the regulatory function of GID1c. The results showed that the OE of *GID1c* resulted in the upregulation of the expression of genes related to GA biosynthesis and the accumulation of GAs (Figs. 7 and 8). At the same time, the OE of *GID1c* downregulated the expression of *BZR1* transcription factors but upregulated the expression of *BES1* in the BR signaling pathway, which increased the content of BR. *GID1c* expression may promote the crosstalk between GA and BR to regulate the plant growth. Auxin is involved in root development, shoot growth, and fruit ripening. Plants can quickly sense and respond to changes in auxin levels; this sensing occurs via several major classes of auxin-responsive genes, including those of the auxin/indole-3-acetic acid (Aux/IAA) family and the ARF family (Luo et al., 2018). The OE of *GID1c* upregulated the transcript levels of *AUX* and *ARF* and also promoted the accumulation of IPA and IAA; therefore, we speculated that *GID1c* expression may affect the crosstalk between the GA and auxin signaling pathways to regulate the plant growth. ABA is involved in plant growth processes and abiotic stress responses (Hwang et al., 2018; Upadhyay et al., 2017; Wang et al., 2018).

Within the comprehensive core ABA signaling network, GID1c may upregulate the transcript levels of the related genes *ABI* and *NCED* to control ABA content and influence plant growth (Figs. 7 and 8). As such, GA treatment and the OE of *GID1c* in the leaves of the dwarf rootstock seedlings upregulated the expression of *GID1c*, and then increased the expression of genes related to the GA, auxin, BR, and ABA signal pathways and the hormone contents, which promoted the growth of the dwarf rootstock plants. Based on these data, we hypothesized that the upregulation of *GID1c* activated the primary genes involved in hormone biosynthesis and metabolism and thereby enhanced the coregulation of different hormones involved in plant growth and inhibited dwarfing traits (Fig. 9). In summary, our results indicated that GID1c is a key regulator in dwarfing plants, and it can be used to improve woody fruit rootstock breeding strategies for dwarf phenotypes and high-planting-density production. However, the rootstock-scion effect and the regulation mechanism in other dwarfing rootstock cultivars require further study.

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Supplemental Table 1. List of primers used in the study.

Primer name	Primer sequence	Objective
Md-GID1-F	CTCTAGAATGTCTGGGGCAATGAAGT	Gene Clone
Md-GID1-R	GGGTACCATTATCGGAGAGCACGA	
q18S-1	GTCACCTACCTCCCGTGTC	qRT-PCR
q18S-2	GAGCCTGAGAAACGGCTACC	
qGID1-F	AGCTAGCTTACAATCTCCTGCG	
qGID1-R	ACTTGGTGAAGTGCCAGCAT	
qKO-F	TGGCCTTCTTCTCCATGATC	
qKO-R	TACCACTGGAACAGAGGGGA	
qKAO-F	AGTGACTGAGCGCAGAAATC	
qKAO-R	GGTGTGGCCAGAGGATTCAT	
qKS-F	ACTGGCTAGTATCCTCGCGT	
qKS-R	TGAGCCCAATGGAATCGTCA	
q-GA20ox-F	GGCTGTTGAGTGCATGATCA	
q-GA20ox-R	CGGGAGTATTTTGAAGGC	
qAUX-F	TGGGGTTGAAGAGACAGAG	
qAUX-R	CCCACCAGTACTATTTGCCT	
qABI3-F	ATGGTCATCAGCAAGGATGG	
qABI3-R	AGGTGGCGGTGGATAGTTAT	
qARF-F	ATGGGGCTGTCATTACGAA	
qARF-R	TCCCACACAGTAAAGCTGAT	
qBES-F	ATCGCGGCGAAGATATTCAC	
qBES-R	ATGGGCTTATCTTGGTTCGAT	
qBZR-F	ATGAAGGAGAGGCAGAGGAA	
qBZR-R	TCCACAAGCAGGGCAGTAAT	
qNCED1-F	ACTACTACTACAAGACACCG	
qNCED1-R	GGGAGTTGAAGGTTGTTGAT	