Isolation of the Histone Lysine Methyltransferase Gene *PhSDG8* and Characterization of Function in Shoot Branching

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ABSTRACT. *Petunia* (*Petunia ×hybrida*) is an important ornamental plant, and its branch development has become a hot research topic. In this study, *PhSDG8*, an ortholog of *SET domain group 8 (SDG8)*, was cloned from the petunia cultivar Mitchell Diploid. It had an open reading frame (ORF) of 5070 bp and encoded 1689 amino acids, with Suppressor variegation 3–9, Enhancer of zeste, Trithorax (SET), Zinc finger-cysteine and tryptophan conserved (Zf-CW), associated with SET (AWS) and Post SET domains. The predicted amino acid sequence of *PhSDG8* was most closely related to *Nicotiana sylvestris* ASHH2 (NsASHH2). Expression analysis revealed that *PhSDG8* expressed highest in the stems and lowest in the axil. Subcellular localization analysis showed that *PhSDG8* was localized in the nucleus. Overexpression of *PhSDG8* reduced the branch number of *Arabidopsis thaliana sdg8-2*. The silencing of *PhSDG8* resulted in an increase in the number of branches of petunia and significant upregulation of *PhUGT74E2*. These results suggested that *PhSDG8* may be a candidate gene for regulating the branching of petunia.

Petunia (*Petunia ×hybrida*) is one of the most important ornamental plants. In its cultivation, artificial topping is required to promote branch development to achieve the ornamental effects, but this task greatly increases the production cost. Therefore, breeding new cultivars with different branching characteristics through genetic engineering is an important way to solve the efficient production of petunia. At the same time, petunia has the characteristics of easy regeneration and short life cycle, and its genome has been successfully sequenced (Bombarely et al., 2016). Therefore, petunia has become a model plant for branch development and other research.

In recent years, great progress has been made in the branch development of petunia, such as identification of the strigolactone synthesis gene *decreased apical dominance 1 (DAD1)*, *DAD3*, *P. hybrida* more axillary buds 1 (*PhMAX1*), signal transduction gene *PhMAX2*, *DAD2*, and transporter gene *pleiotropic drug resistance (PDR1)* (Drummond et al., 2009, 2011; Hamiaux et al., 2012; Kretschmar et al., 2012; Snowden et al., 2005). Other studies mainly focus on the start time of the axillary meristem and environmental factors affecting petunia branching (Dal Santo et al., 2011; Drummond et al., 2015; Revel et al., 2015; Zenoni et al., 2011). These studies lay a good foundation for revealing the mechanism of petunia branch development.

However, the regulatory network of branching is extremely complicated. The formation of branches depends on the initiation of axillary meristems and the activity of axillary buds

(Shimizu-Sato et al., 2009). The initiation of axillary meristems is regulated by many genes (Dun et al., 2012). *Branched1 (BRC1)*, known as the class II Teosinte Branched1/Cycloidea/Pcf (TCP) family of transcription factors, has been shown to be an integrator of multiple internal and external signals to suppress branching (Shen et al., 2019). *Regulations of axillary meristems 1 (RAX1)*, *RAX2*, *RAX3* redundantly regulate axillary bud formation at different stages of shoot development in a daylength-dependent manner (Müller et al., 2006). *Lateral suppressor (LAS)* is mainly expressed in a band-shaped domain at the adaxial side of young leaf primordia, and loss of *LAS* function causes strong defects in axillary meristem (AM) formation (Fambrini et al., 2017). *Regulation of axillary meristem formation (ROX)* mRNA accumulates at the adaxial boundary of leaf and flower primordia, and it acts in concert with *RAX1* and *LAS* to modulate AM formation (Yang et al., 2012). *WRKY71* affects AM initiation and bud activity by positively regulating the transcription of *RAX1*, *RAX2*, and *RAX3* (Guo et al., 2015).

In recent years, it has been found that epigenetics is also an important factor regulating shoot branching. The histone lysine methyltransferase *SDG8*, as the homologous protein of the histone H3K36 methyltransferase *SET2* in fungi (Sui et al., 2013), is also called Absent, Small, or Homeotic Disc 1 (*ASH1*) homolog 2s (*ASHH2*). *SDG8* was first identified as *early flowering in short days (EFS)* in *Arabidopsis* (*Arabidopsis thaliana*), the mutation of which leads to a phenotype of early flowering (Kim et al., 2005). Later, it was found that *SDG8* was able to regulate shoot branching (Cazzonelli et al., 2009; Dong et al., 2008; Liu et al., 2016; Zhao et al., 2005). The current study suggests that *SDG8* reduces the trimethyl level of histone 3 lysine 4 (H3K4) near the transcriptional start position of carotenoid isomerase (*CRTISO*) and increases the level of dimethylation, which lead to an increase of *CRTISO* mRNA levels, thereby regulating the content of carotenoids and the synthesis of strigolactone (Cazzonelli et al., 2009). On the other hand, *SDG8* can affect shoot branching by regulating the

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Table 1. Primer names and sequences used for polymerase chain reaction amplification.

Primer name	Primer sequences (5'-3')
<i>BRC1-RT-F</i>	ACTTAAAGAAGGTACAACTGG
<i>BRC1-RT-R</i>	AAAATAACTGGACTGAAGGGGT
<i>RAX1-RT-F</i>	TGACAAGAACAAAGTGAAAAG
<i>RAX1-RT-R</i>	GAGAGAGAGAAGAGAACCAGC
<i>RAX2-RT-F</i>	AAAGAAAATGTAAAAAGGGGT
<i>RAX2-RT-R</i>	GAGATGATAGGTCAAGAGCCA
<i>RAX3-RT-F</i>	AGATGTGGAAAGAGTTGTAGG
<i>RAX3-RT-R</i>	AAGATATCAAATTGATGGGAT
<i>LAS-RT-F</i>	GTTATCCTCCTCTACTCTTCG
<i>LAS-RT-R</i>	CACACTTGTTCCACTGTCATTC
<i>ROX-RT-F</i>	ATTCTTCTTGTTTATCTTCTTC
<i>ROX-RT-R</i>	CACTTATTCTATGCCTTCTTTC
<i>WRKY71-RT-F</i>	TTTCTACTATCACCAGCCATT
<i>WRKY71-RT-R</i>	TTCTTCTCTCCTTTCTTTTTT
<i>UGT74E2-RT-F</i>	GCAGTTGGCTCAGTTGTCTATG
<i>UGT74E2-RT-R</i>	GCGTTACTCCTTTCTCTTCCTC
<i>SDG8-RT-F</i>	AAATGTGTCTGTGGCTCG
<i>SDG8-RT-R</i>	AACCAAAAGCAGCAACAGAGT
<i>Beta-Actin-F</i>	GTTGGACTCTGGTGATGGTGTG
<i>Beta-Actin-R</i>	CCGTTTCAGCAGTGGTGGTG
<i>Tublin-F</i>	GAGCCTTACAACGCTACTCTGTCT
<i>Tublin-R</i>	ACACCAGACATAGTAGCAGAAATC
<i>SDG8-vigs-F</i>	CGGAATTCCTCTATTCTACAGGTCCCATATC
<i>SDG8-vigs-R</i>	CGGGATCCTTGGGTATTGCTCATACTATCTGGA
<i>SDG8-F-SL</i>	GAGAACACGGGGGACTCTAGAATGGTGCAAGCGGTTGAACT
<i>SDG8-R-SL</i>	GCCCTTGCTCACCATGGATCCCATTCTTGGGTTTTGCGCA
<i>PhSDG8-R-OE</i>	GCCCTTGCTCACCATGGATCCCCTACATTCTTGGGTTTTGCGCA

expression of *SPS/BUS* (*supershoot/bushy*) and *UDP-glycosyltransferase 74E2* (*UGT74E2*) (Dong et al., 2008). As we know, *UGT74E2* is an UDP-glucosyltransferase that affects auxin homeostasis (Tognetti et al., 2010). Therefore, it is speculated that *SDG8* may regulate the branch development mainly by affecting the dynamic balance of strigolactone and auxin.

In this study, we cloned the petunia homolog gene of *SDG8*, carried out bioinformatics analysis on its sequence, and detected the expression level of *PhSDG8* in different tissues using quantitative real-time PCR (qRT-PCR). Also its subcellular localization and function were analyzed. This study lays a foundation for further research on the mechanism of regulation of branch development in petunia.

Materials and Methods

PLANT MATERIALS AND GROWTH CONDITIONS. The petunia cultivar Mitchell Diploid was used in this study. Petunia seedlings, the *Arabidopsis* Columbia wild type (WT), *sdg8-2* (Zhao et al., 2005), transgenic plants were all planted in the pots and placed in tissue culture rooms with light-emitting diode lamps (Green Power TLED V5; Philip, Shanghai, China), with the temperature of 23 ± 2 °C, the illumination of $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the photoperiod of 16/8 h (light/dark).

ISOLATION OF THE *PhSDG8* GENE. Total RNA was extracted from stems of petunia plants using EZ-10 Total RNA Mini-Preps Kit (Sangon Biotech, Shanghai, China). DNA removal and First-strand cDNA synthesis was performed using Easy-Script One-Step gDNA Removal and cDNA Synthesis Super-

Mix (Transgene, Beijing, China). Primers were designed according to the petunia genome sequence (Bombarely et al., 2016) (Table 1). The products amplified by using KOD-Plus-Neo DNA Polymerase (Toyobo, Shanghai, China) were cloned into pEASY®-Blunt Simple Cloning Vector (Transgene) to determine the sequence of *PhSDG8*.

BIOINFORMATIC ANALYSIS. Based on the published *Arabidopsis* ASH1s, other ASH1 proteins were obtained from the National Center for Biotechnology Information (NCBI) database by blasting with known amino acid sequences of ASH1s. Multiple sequence alignment was calculated with DNAMAN software (version 5.2.2; Lynnon Biosoft, Quebec, QC, Canada). Phylogenetic analysis was conducted using the MEGA 5.05 program (Tamura et al., 2011) by the neighbor-joining method. A ProParam online tool in ExPASy (Gasteiger et al., 2005) was used to analyze the basic physical and chemical properties of the protein sequences.

EXPRESSION ANALYSIS. For the tissue expression experiments, roots, stems, leaves, axil, and apex of 30-day-old petunia seedlings were collected. We performed qRT-PCR and analysis as described by Dong et al. (2013). *SDG8-RT-F/SDG8-RT-R* was used to detect the expression level of *PhSDG8*. β -actin was used as a normalization control with primers Beta-Actin-F/Beta-Actin-R. For analysis of gene expression of plants inoculated with pTRV1/pTRV2-0 or pTRV1/pTRV2-*PhSDG8*, after 60 d of infiltration, axillaries were taken to extract RNA for reverse transcription, and the expression levels of *PhSDG8*, *PhBRC1*, *PhRAX1*, *PhRAX2*, *PhRAX3*, *PhLAS*, *PhROX*, *PhWRKY71*, and *PhUGT74E2* were detected (Table 1).

SUBCELLULAR LOCALIZATION ANALYSIS. To construct the *PhSDG8*-GFP fusion protein, *PhSDG8* cDNA, not containing

Fig. 1. Protein sequence alignment of PhSDG8, AtASHH2, MtASHH2, PaASHH2, and SlSDG8. The accession numbers were listed as follows: *Arabidopsis thaliana* AtASHH2 (AT1G77300.1), *Medicago truncatula* MtASHH2 (XP_024634357.1), *Populus alba* PaASHH2 (TKS00672.1), *Solanum lycopersicum* SlSDG8 (XP_010322942). Four functional domains, Zf-CW, AWS, SET, and Post SET, are marked by red frames.

the stop codon, was amplified using the primers SDG8-F-SL/SDG8-R-SL (Table 1), and then cloned into pCambia1300 vector using ClonExpress®II One Step Cloning Kit (Vazyme, Nanjing, China), producing the PhSDG8-GFP construct under the control of the 35S promoter. The *Agrobacterium tumefaciens* containing 35S::PhSDG8-GFP was injected into the abaxial side of *Nicotiana benthamiana* leaves (2 weeks old). After culturing at 25 °C for 48 h, the fluorescence was observed by confocal laser scanning microscopy (FV1200; Olympus, Tokyo, Japan).

ARABIDOPSIS TRANSFORMATION AND PHENOTYPE ANALYSIS. The full-length of *PhSDG8* sequence was amplified with SDG8-F-SL/PhSDG8-R-OE using KOD-Plus-Neo DNA Polymerase (Toyobo) (Table 1). The obtained sequence was ligated to pCambia1300 vector using ClonExpress®II One Step Cloning Kit (Vazyme), producing the 35S::PhSDG8 construct. The recombinant plasmid was transformed into *A. tumefaciens* by heat shock. Finally, the construct was introduced into homo-

zygous *sdg8-2* mutants through the *A. tumefaciens*-mediated flower-dipping method (Clough and Bent, 1998). Seeds were collected from the infiltrated plants and selected on a Murashige and Skoog (MS) medium supplemented with 50 mg·L⁻¹ kanamycin. Kanamycin-resistant plants were transferred to soil 15 d after germination and were grown in a growth chamber. The number of rosette branches (bud length ≥ 10 mm) and the height of the main stems were scored for phenotype analysis.

CONSTRUCTION AND TRANSFORMATION OF VIRUS-INDUCED GENE SILENCING (VIGS) VECTOR. Tobacco rattle virus (TRV)-based vectors, pTRV1 (pYL192) and pTRV2 (pYL156) were used in this study (Cao et al., 2015). The *PhSDG8* (300 bp) fragment was amplified with primers SDG8-vigs-F/SDG8-vigs-R. Then the *PhSDG8* fragment was digested using BamHI and EcoRI restriction enzymes and cloned into pTRV2 to generate pTRV2-PhSDG8. The recombinant plasmid-carrying target gene fragment and empty vector as

Fig. 2. Phylogenetic analysis of ASHs. The phylogenetic tree was constructed using the neighbor-joining method. Numbers above branches indicate bootstrap support percentages from 1000 replicates. The accession numbers of selected ASHs were listed as follows: *Morella rubra* MrASHH2 (KAB1203139.1), *Quercus lobata* QlASHH2 (XP_030933677.1), *Citrus sinensis* CsASHH2 (XP_024952142.1), *Manihot esculenta* MeASHH2 (XP_021598387.1), *Morus notabilis* MnASHH2 (XP_024019152.1), *Lactuca sativa* LsASHH2 (XP_023731848.1), *Nicotiana glauca* NsASHH2 (XP_009803898.1), *Capsicum annuum* CaASHH2 (XP_016575229.1), *Solanum tuberosum* StASHH2 (XP_006365937.1), *Arachis hypogaea* AhASHH2

control (pTRV2-0) were transformed into the *A. tumefaciens* strain GV3101 by a freeze-thaw method. According to Broderick's method, a single colony for each construct was selected for shaking culture to an optical density at 600 nm (OD_{600}) of 0.8–1.0. Cells were collected and resuspended in media (10 mM 4-morpholineethanesulfonic acid, 10 mM $MgCl_2$, and 200 μM acetosyringone) to an OD_{600} of 1.8–2.0. Then, pTRV1 cultures were mixed with pTRV2-PhSDG8 (or TRV2-0) cultures in a 1:1 ratio (Broderick and Jones, 2014). For leaf agroinfiltration, agrobacterium cultures were introduced into leaves of petunia seedlings selected randomly at the four-to-six leaf stage using syringes. After infiltration, seedlings were grown in a culturing room. Sixty days later, the number of branches (bud length ≥ 10 mm) was counted; also the height of the plants was measured.

STATISTICAL ANALYSIS. All the experiments in this study were repeated three times. Data were presented as mean value $\pm$ SD. Tukey's tests were performed for statistical analyses of phenotypic and expression data.

Results

CLONING OF *PhSDG8* AND PROTEIN STRUCTURE ANALYSIS. Primers were designed based on the published genome sequences of *Petunia inflata* to obtain the full length of *PhSDG8* sequence. The gene encoded 1689 amino acids with a relative molecular weight of 185.89 kDa and an isoelectric point of 6.92, while the relative molecular weight of *Arabidopsis* SDG8 was 191.22 kDa and the isoelectric point was 5.31. The instability index of PhSDG8 was computed to be 55.41. The relatively high concentration of amino acids in the protein was serine (11.6%), leucine (8.2%), glycine (7.7%), and lysine (6.9%). The total number of negatively charged residues (aspartic acid + glutamic acid) and positively charged resi-

Fig. 2. (Continued). (XP_025666034.1), *Cajanus cajan* CcASHH2 (XP_020212539.1), *Glycine soja* GsASHH2 (RZC18189.1), *Glycine max* GmASHH2 (XP_014631840.1), *Punica granatum* PgASHH2 (XP_031394924.1), *Syzygium oleosum* SoASHH2 (XP_030476498.1), *Dendrobium catenatum* DcASHH2 (PKU81992.1), *Arabidopsis thaliana* AtASHH1 (AT1G76710.1), *Brassica napus* BnASHH1 (XP_013717811.1), *Cicer arietinum* CaASHH1 (XP_004496445.1), *Vigna unguiculata* VuASHH1 (XP_027937757.1), *Arachis ipaensis* AiASHH1 (XP_016176356.1), *Ziziphus jujuba* ZjASHH1 (XP_015886513.1), MnASHH1 (XP_024023788.1), *Prunus avium* PaASHH1 (XP_021817357.1), *Pistacia vera* PvASHH1 (XP_031250764.1), *Citrus clementina* CcASHH1 (XP_024040947.1), *Durio zibethinus* DzASHH1 (XP_022773422.1), *Herrania umbratica* HuASHH1 (XP_021284799.1), *Carica papaya* CpASHH1 (XP_021898665.1), *Populus trichocarpa* PtASHH1 (XP_002306713.1), *Ricinus communis* RcASHH1 (XP_002528669.1), *Hevea brasiliensis* HbASHH1 (XP_021650631.1), MeASHH1 (XP_021630030.1), BnASHH4 (XP_013659981.1), *Raphanus sativus* RsASHH4 (XP_018488040.1), *Brassica rapa* BrASHH4 (XP_009104235.1), *Eutrema salsugineum* EsASHH4 (XP_024012774.1), AtASHH4 (NP_001326226.1), *Camelina sativa* CsASHH4 (XP_019082925.1), AtASHH3 (NP_566010.1), EsASHH3 (XP_006397594.1), BnASHH3 (XP_021814053.1), RsASHH3 (XP_018461825.1), *Tarenaya hassleriana* ThASHH3 (XP_010527044.1), SoASHH3 (XP_030456216.1), CcASHH3 (XP_024045341.1), CpASHH3 (XP_021907030.1), PtASHH3 (XP_024444632.1), *Malus domestica* MdASHH3 (XP_008394239.1), PaASHH3 (XP_021814053.1), *Rosa chinensis* RcASHH3 (XP_024185735.1), *Cucurbita maxima* CmASHH3 (XP_022969811.1), *Theobroma cacao* TcASHH3 (XP_007022844.1), *Jatropha curcas* JcASHH3 (XP_012081879.1), MeASHH3 (XP_021625182.1), and HbASHH3 (XP_021664231.1). PhSDG8 is marked with a red dot.

dues (arginine + lysine) were 222 and 218, respectively. Aliphatic index was 68.85, and the grand average of hydropathicity (GRAVY) was -0.663 . The amino acid sequence of PhSDG8 was analyzed by SMART software (Letunic et al., 2012). The results showed that PhSDG8 contained Zf-CW (aa 614–617 and 635–637), AWS (aa 715–767), SET (aa 773–892), and Post SET domain (aa 894–907) (Fig. 1), which are all the characteristic of SDG8 (He et al., 2010; Liu and Huang, 2018). The amino acid sequence of PhSDG8 shared 71% identity with those of SlSDG8 (Fig. 1). Alignment of the amino acid sequence encoded by PhSDG8 with those of other SDG8 proteins from *Arabidopsis*, *Medicago truncatula*, *Populus alba*, and *Solanum lycopersicum* showed that sequences were conserved mostly in the four domains (Fig. 1).

PHYLOGENETIC ANALYSIS OF *PhSDG8*. To determine the genetic relationship between PhSDG8 and other SDG8s, phylogenetic analyses were performed (Fig. 2). A total of 60 ASH1 SET protein sequences were collected from 43 species. The phylogenetic tree displayed four major clades that corresponded to the ASHH1, ASHH2, ASHH3, and ASHH4 subfamilies, while the PhSDG8 protein was clustered in ASHH2 clade, with the same clade with *Solanaceae* plants. PhSDG8 was most closely related to NsASHH2.

TISSUE-SPECIFIC EXPRESSION ANALYSIS OF *PhSDG8*. To analyze the expression of *PhSDG8* in different tissues, the relative expression level of *PhSDG8* in roots, stems, leaves, axils, and apices of petunia were detected (Fig. 3). The results showed that *PhSDG8* was expressed in all detected tissues, with the highest expression in stems and the lowest in axil. The expression level of *PhSDG8* in apex, axil, roots, and stems were 0.42, 0.21, 0.94, and 1.8 of leaves, respectively.

SUBCELLULAR LOCALIZATION OF *PhSDG8*. To clarify the function of the PhSDG8, we analyzed the subcellular localization of PhSDG8. A pCambia1300 vector containing PhSDG8 fused to Green Fluorescent Protein (GFP) at its N terminus was constructed in this research. We used pCambia1300 as a control. After transient transformation into *N. benthamiana*, GFP driven by 35S had green fluorescence on the cell membrane and nucleus, while PhSDG8 fused with GFP only produced green fluorescence in the nucleus, indicating that PhSDG8 was localized in the nucleus (Fig. 4).

FUNCTIONAL ANALYSIS OF *PhSDG8*. To analyze the function of *PhSDG8*, 35S::PhSDG8 vector was constructed and transformed into *Arabidopsis sdg8-2*. The obtained transgenic lines were used for branch phenotypic analysis, and the leaves of transgenic lines were used to detect expression level of *PhSDG8*. It was found that *PhSDG8* was detected in five transgenic lines, but not in the WT and *sdg8-2* mutants (Fig. 5D). Overexpression of *PhSDG8* restored the branch and height phenotype of *sdg8-2* to the wild type (Fig. 5A–C), suggesting that *PhSDG8* can regulate branch development of *Arabidopsis*.

SILENCING OF *PhSDG8* IN PETUNIA. To confirm *PhSDG8* suppression at the molecular level, the expression level of *PhSDG8* was determined by qRT-PCR. The average expression level of *PhSDG8* in pTRV2-0 plants was 4-fold higher than that in pTRV2-PhSDG8 seedlings (Fig. 6B), which indicated that *PhSDG8* was silenced. Silencing of *PhSDG8* led to an increase in the number of branches of petunia from 1.4 to 4.4 (Fig. 6C), and a decrease in plant height from 596 to 413 mm (Fig. 6D), indicating that the expression level of *PhSDG8* was correlated with the number of branches of petunia. The expression of *PhBRC1*, *PhRAX1*, *PhRAX2*, *PhRAX3*, *PhLAS*, *PhROX*,

Fig. 3. Expression analysis of *PhSDG8* in different tissues of petunia. All samples were taken from three pools of 12 plantlets. Detection of β -actin was used as a control. Error bars show the standard deviations (SD) of three biological replicates. Lowercase letters a and b indicate significant differences via Tukey's test at $P < 0.05$.

Fig. 4. Subcellular localization of PhSDG8-GFP protein in tobacco epidermal cells. (A, C) Fluorescence images of PhSDG8-GFP fusion protein and control are shown. (B, D) The merged images of PhSDG8-GFP fusion protein and control are shown.

PhWRKY71, and *PhUGT74E2* were detected in three lines that infiltrated with VIGS vectors carrying *PhSDG8* inside (Fig. 6E). The results showed that only the *PhUGT74E2* expression level was significantly up-regulated, which suggested that *PhSDG8* could affect the development of branches by regulating the expression of *PhUGT74E2*.

Discussion

Proteins containing the conserved SET domain can be found in various species. SET domain proteins are categorized into seven classes: Enhancer of zeste [E (z)], ASH1, Trithorax (Trx), Suppressor variegation [Su (var)], and so on (Chen et al., 2020).

Fig. 5. Overexpression of *PhSDG8* restored the phenotype of *Arabidopsis thaliana* *sdg8-2*. (A) Comparison of phenotypes of the *A. thaliana* wild type (WT), *sdg8-2*, and *sdg8-2* transformed with 35S::PhSDG8. (B) The number of secondary rosette branches produced by WT, *sdg8-2*, and five independent homozygous lines carrying 35S::PhSDG8 are shown. L represents transgenic line, and n = 20. (C) The height of the main branches of WT, *sdg8-2*, and five independent transgenic lines are shown. (D) Transcripts of *PhSDG8* were assayed by reverse transcriptase polymerase chain reaction (RT-PCR) from total RNA of leaves. Detection of the *Tubulin* transcript was used as a control. Lowercase letters a and b indicate significant differences via Tukey's test at $P < 0.05$.

In *Arabidopsis*, there are five ASH1 SET domain proteins: SDG26/ASHH1, SDG8/ASHH2, SDG7/ASHH3, SDG24/ASHH4, and SDG4/ASHR3 (Zhu et al., 2013). Several recent studies found that *SDG8* was involved multiple biological processes, such as shoot branching (Dong et al., 2008), carotenoid composition (Cazzonelli et al., 2009), ovule and anther development (Grini et al., 2009), response to a mechanical stimulus (Cazzonelli et al., 2014), and embryogenesis (Cheng et al., 2018). Therefore, *SDG8* is an important gene for regulating plant growth and development. However, although there is some research on *SDG8* in *Arabidopsis*, no reports are found in petunia.

In this study, an ortholog of *SDG8* was cloned from petunia cultivar Mitchell Diploid. Sequence analysis revealed that, in addition to the presence of canonical domains (AWS, SET, post-SET), PhSDG8 contained a Zf-CW domain, which was unique to *SDG8* among all SET proteins. The conserved Cys-X-X-Trp and Trp-X-Cys sequence motifs in Zf-CW domain were considered to bind to DNA sequences or protein domains, and specifically target histone methylation (Christopher et al., 2009). In addition, studies have shown that the Zf-CW domain of SDG8 preferentially binds monomethylated H3K4 (Liu and Huang, 2018). A phylogenetic tree and sequence alignment showed that PhSDG8 had the same origin as ASHH2 protein of other species, and subsequently diverged at different phases of later evolution, indicating PhSDG8 indeed belonged to the ASHH2 class.

Expression analysis showed that *PhSDG8* had higher expression in stems than in other detected tissues. This was consistent with the conclusion that *BnSDG8A* and *BnSDG8B*

were highly expressed in *Brassica napus* stems, compared with the expression in roots and leaves (Jiang et al., 2018). Study in *Arabidopsis* showed that the expression of *SDG8* in the shoot apical meristem was higher than that in leaves (Cazzonelli et al., 2010). Therefore, it appears that the expression patterns of *PhSDG8* are similar, and different compared with other species. Sub-cellular localization analysis showed that PhSDG8 was localized in the nucleus, indicating that PhSDG8 was a protein that acted in the nucleus, which is consistent with the conclusion of SDG8 in *Arabidopsis* (Xu et al., 2008). Similar to *BnSDG8* gene (Jiang et al., 2018), overexpression of *PhSDG8* in *Arabidopsis* completely restored the phenotype of *sdg8-2*, including branch number and plant height. This result not only demonstrates that *PhSDG8* is a functional gene for regulating shoot branching but also shows the function conservation of *SDG8s* among different species.

TRV has been successfully used as a VIGS vector in many species, although the experimental methods vary among species (Broderick and Jones, 2014). In this study, *PhSDG8* was effectively silenced in petunia. The decrease of *PhSDG8* expression level led to the increase of the number of branches in petunia, accompanied by the decrease of plant height and early flowering. These results suggest *PhSDG8* was an inhibitor of branch development and flowering regulation. *BRC1*, *RAX1*, *RAX2*, *RAX3*, *PhLAS*, *ROX*, *WRKY71*, and *UGT74E2* are important genes for regulating branching (Guo et al., 2015; Tognetti et al., 2010; Wang et al., 2018, 2019). Silencing of *PhSDG8* only caused significant up-regulation of *PhUGT74E2*; while this had no effect on other genes, especially without causing any changes in *PhBRC1* expression level, whose homologous genes have been confirmed to be involved in the response to multiple signals of regulating shoot branching (Barbier et al., 2019). The result indicated that *PhSDG8* acted independently of the above pathways. *UGT74E2* is a member of the *Arabidopsis* glycosylation transferase family 1 (Family-1) and encodes 453 amino acids, which can catalyze auxin for glycosylation (Rehman et al., 2018). Studies have shown that the overexpression of *UGT74E2* causes a significant increase in the number of branches (Tognetti et al., 2010). Therefore, it is speculated that *PhSDG8* can disrupt the dynamic balance of auxin by regulating the transcription of *PhUGT74E2* and cause abnormal development of plant branches, while the regulation mechanism of *PhSDG8* to *PhUGT74E2* still needs further study.

Our results presented here reveal the existence of *PhSDG8* and function of regulating shoot branching in petunia. It provides not only the experimental basis for revealing the branching mechanism of petunia, but also offers the gene

Fig. 6. Branch number phenotype and gene expression analysis of silencing of *PhSDG8* in petunia. (A) Comparison of phenotypes of control and plants infiltrated with pTRV2-0 or pTRV2-PhSDG8. L1, 2, 3 represents different lines infiltrated with pTRV2-PhSDG8. (B) Transcripts of *PhSDG8* were assayed by qRT-PCR from total RNA of leaves. Detection of the β -actin transcript was used as a control. Error bars show the sd of three biological replicates. Control, pTRV2-0, and pTRV2-PhSDG8 were all taken from three pools of 12 plantlets. (C) The branch numbers of control and plants infiltrated with pTRV2-0 or pTRV2-PhSDG8 are shown (n = 12). (D) The height of the main branches of control and plants infiltrated with pTRV2-0 or pTRV2-PhSDG8 are shown (n = 12). (E) Expression levels of *PhROX*, *PhRAX1*, *PhRAX2*, *PhRAX3*, *PhLAS*, *PhWRKY71*, *PhUGT74E2*, and *PhBRC1* in control and plants infiltrated with pTRV2-0 or pTRV2-PhSDG8 were detected. Detection of the β -actin transcript was used as a control. Error bars of control and pTRV2-0 show the sd of three biological replicates, while error bars of L1, L2, and L3 show the sd of three technical replicates. Lowercase letters indicate significant differences via Tukey's test ($P < 0.05$), while groups without letters indicate no significant difference.

resource for the use of *PhSDG8* to improve the plant architecture.

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