Genome-wide identification of the SWEET gene family in Phaseolus vulgaris L. and their patterns of expression under abiotic stress

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In this study, 24 putative PvSWEET genes were identified. They can be categorized into four subgroups based on a phylogenetic analysis, exon–intron structure, cis-regulatory elements, and MEME motifs. A collinearity analysis showed that the transport and recycling of sugars in plants from the source to sink maintain the balance between source and sink, and this process is driven by sugar transporters. In addition, the SWEET genes in clade III encode proteins that control the efflux of sucrose, whereas the SWEET genes in clades I and II encode proteins that transport hexoses, such as glucose and fructose. Moreover, the SWEET genes in clade IV encode proteins that preferentially transport fructose. AtSWEET11 and AtSWEET12 are localized on the plasma membrane and function as sucrose transporters, while the SWEET double mutant is expressed in developing seeds and regulate the incretion of sucrose from the seed coat to the embryo through the endosperm. AtSWEET8/RPG1 and AtSWEET13/RPG2, which have been found to play key roles in pollen nutrition, are expressed in the tapetum and anthers, respectively.

Introduction

Sugars are the main product of photosynthesis, the main energy source for growth, development, and reproduction, and are involved in a variety of biological processes (Ruan 2012). The transport and recycling of sugars in plants from the source to sink maintain the balance between source and sink, and this process is driven by sugar transporters. Currently, many sugar transporter families, including monosaccharide transporters, sucrose transporters (SUT), and sugars Will Eventually be Exported Transporter (SWEET) proteins, have been found and identified in a variety of species of plants (Ayre 2011; Chen et al. 2012; Eom et al. 2012; Yadav et al. 2015). The SWEET proteins contain seven conserved α-helical transmembrane (TM) domains and two MtN3/saliva motifs (Xuan et al. 2013). It has been reported that the SWEET gene family in both prokaryotes and eukaryotes (Chen et al. 2010). In addition, the SWEET gene family is not only involved in sugar efflux and influx but is also extensively involved in intercellular sugar transport and photosynthetic carbon transport throughout the plant (Chen et al. 2012).

Recently, the identification and evolution of the SWEET gene family have been studied in Arabidopsis (Chen et al. 2012; Wei et al. 2021), rice (Oryza sativa L.) (Yuan and Wang 2013), poa pratensis (Poa annua L.) (Zhang et al. 2020), wheat (Triticum aestivum L.) (Gao et al. 2018), maize (Zea mays L.) (Sosso et al. 2015), alfalfa (Medicago truncatula) (Hu et al. 2019), and soybean (Glycine max L.) (Patil et al. 2015). An analysis for sequence homology and phylogeny revealed that this gene family is usually categorized into four subgroups. It had been shown that the SWEET genes that belong to different subgroups preferentially transport different sugar substrates in Arabidopsis and rice (Yuan and Wang 2013). In addition, it has been shown that the SWEET genes in clade III encode proteins that control the efflux of sucrose, whereas the SWEET genes in clades I and II encode proteins that transport hexoses, such as glucose and fructose (Eom et al. 2015). Moreover, the SWEET genes in clade IV encode proteins that preferentially transport fructose (Chen et al. 2012; Klemens et al. 2013; Guo et al. 2014).

The SWEET genes are involved in many physiological processes. For example, AtSWEET11 and AtSWEET12 are localized on the plasma membrane and function as sucrose transporters, while the atsweett11/12 double mutant has been found to accumulate higher contents of starch and sugar in leaves, indicating a defect of phloem loading in atsweett11/12 (Chen et al. 2012). AtSWEET11, AtSWEET12, and AtSWEET15 are expressed in developing seeds and regulate the incretion of sucrose from the seed coat to the embryo through the endosperm (Eom et al. 2015). AtSWEET8/RPG1 and AtSWEET13/RPG2, which have been found to play key roles in pollen nutrition, are expressed in the tapetum and anthers, respectively.
participate in drought stress responses in GmSWEET21

diminuus plant that is rich in protein and contains a variety

eic stresses. Recently, several studies have been conducted
family plays an important role in plant growth and devel-

level in leaves (Seo et al. 2011; Eom et al. 2015). The overexpression of AtS-
WEET15 can accelerate senescence and caused hypersen-
sitivity to salt stress in Arabidopsis. In rice, the overexpression of OsSWEET5 retards growth and induces premature senescence (Zhou et al. 2014). The AtS-
WEET16 and AtSWEET17 genes are vacuolar transporters that play a key role in promoting the bidirectional transfer of fructose across the tonoplast, as well as in modifying germination, growth, and stress tolerance (Chardon et al. 2013; Klemens et al. 2013). The expression of AtS-
WEET4 and AtSWEET16 enhanced cold tolerance and nitrogen use efficiency in Arabidopsis (Klemens et al. 2013; Liu et al. 2016). The levels of expression of the AtSWEET11, AtSWEET12, and AtSUC2 genes in Arabi-
dopsis leaves were upregulated by drought stress to promote sucrose transport from the leaves to roots and enhance drought tolerance (Durand et al. 2016). Similar results were identified in our previous studies on soybean. GmSUC2, GmSWEET6, and GmSWEET15 were upregulated in soybean seedling leaves and roots in response to drought stress (Du et al. 2020b). Previous studies also indicated that GmSUC2, GmSWEET12, and GmSWEET21 participate in drought stress responses in different development stages of soybean (Du et al. 2020a).

Common bean (Phaseolus vulgaris L.) is an edible leguminous plant that is rich in protein and contains a variety of micronutrients (Wu et al. 2020). The SWEET gene family plays an important role in plant growth and development, as well as in the response to biological and abiotic stresses. Recently, several studies have been conducted on the effects of soil problems, such as salt stress and heavy metal pollution, on crop plants (Sasaki et al. 2012). Although a large number of SWEET gene families have been identified in different plants, studies on the function of PvSWEET genes in common bean and its response to metal stress are still limited. Therefore, 24 PvSWEET genes in common bean were analyzed in terms of their gene structure, chromosome distribution, evolutionary relationship, cis-regulatory elements, gene replication, collinearity, and their spatiotemporal patterns of expression. The results of this study provide crucial information to elucidate the evolving functions of PvSWEETs in common bean.

Materials and methods

Identification of the PvSWEET genes in common bean

In this study, the reference P. vulgaris L (PhaVulg1_0) genome information was obtained from Ensembl Plants (http://jul2018-plants.ensembl.org/index.html). HMM software (http://hmmer.janelia.org/) and the Pfam database (http://pfam.xfam.org) were used to identify candidate SWEET proteins that contained the MtN3/saliva domain (PF03083). Ensembl Plants (http://plants.ensembl.org/index) was used to obtain the protein annotation file. The analysis conducted using InterPro (http://www.ebi.ac.uk/interpro/) (Finn et al. 2017) and SMART (http://smart.embl-heidelberg.de/) (Letunic et al. 2015; Han et al. 2019) software further confirmed the reliability of the SWEET domain prediction. Subsequently, InterPro (http://prosite.expasy.org/) and WoLF PSORT (http://wolfpsort.hgc.jp/) were used to verify the integrity of SWEET domains in the candidate genes. The SWEET genes were named according to their precise locations on the chromosome.

Phylogenetic analysis

The sequences of SWEET proteins in Arabidopsis, rice, and soybean were also obtained from Ensembl Plants by searching for SWEET domains. The JTT + G model was found to be the best model through MEGA X prediction. A phylogenetic analysis was also performed using the maximum likelihood method with 1,000 bootstrap replicates. Therefore, a phylogenetic tree was modified and constructed by MEGA X.

Gene structure and conserved motifs, and promoter predictions of the PvSWEET genes

The GSDS platform (http://gsds.cbi.pku.edu.cn/) (Guo et al. 2007) was used to analyze the gene structure of the PvSWEET gene family. The correspondence between DNA and protein sequences was detected using Gene-Wise (Birney et al. 2004). The SWEET domain coordinates in the protein sequence were then converted to the coordinates in the nucleotide sequence using in-house Perl scripts. The motifs outside the SWEET domain were detected using the MEME tool (http://meme.nbcr.net/meme/) (Bailey et al. 2009). The length of characteristic motifs ranged from 10–50 amino acids, and the E value < 1e–20. The motifs of PvSWEET were compared with identify group-conserved signatures and numbered based on their order.

Collinearity analysis

The chromosome localization provided by Ensembl Plants was used to map the PvSWEETs to chromosomes. Gene duplication events were examined using a Multiple Collinearity Scan toolkit (MCScanX) (Wang et al. 2012). Circos (version 0.6912), a visualization tool, was used to generate the visualization in this study (Krzywinski et al. 2009).

GO enrichment

Blast2GO and WEGO software were used to predict gene ontology (GO) annotation (Conesa et al. 2005) and for gene function classification, respectively (Ye et al. 2018).
**Growth conditions and treatments**

The common bean seeds were soaked in 5% sodium hypochlorite for 5–10 min and then cleaned three times with distilled water. Subsequently, 15 common bean seeds were incubated on two layers of medium-speed qualitative filter papers for each Petri dish with a diameter of 9 cm. Next, 11 mL of distilled water was added to each Petri dish and then incubated at 28°C in the dark in an incubator. Seed germination was defined as the emergence of a radicle from the seed coat. After 5 days of germination, 10 uniformly germinated soybean seeds in the same Petri dish were selected and subjected to four treatments: CK, 70 mM NaCl (Zhang et al. 2021), 0.5 mg/L CdCl$_2$ (Zhao et al. 2020), and 60 mg/L HgCl$_2$ (Mohammadi et al. 2021) for 24 h. The radicles were then collected to conduct a qRT-PCR assay for the PvSWEET genes. On day 5 after germination, CK cotyledons, hypocotyls, and radicles of common beans were collected for qRT-PCR genes. The soybean seeds in the same Petri dish were considered as one experimental unit. All the treatments were conducted in triplicate and considered as biological replicates.

**RNA extraction, cDNA synthesis and gene expression by qRT-PCR**

Total RNA was extracted from samples using an RNA isolation Total RNA Extraction Reagent (Vazyme Biotech Co., Ltd., Nanjing, China). The quantity and integrity of extracted RNA were determined based on their absorbance at 260 nm and through 1.0% agar gel electrophoresis, respectively. Single-stranded cDNA was synthesized using a PrimeScript™ RT Reagent Kit (Perfect Real Time; Takara Bio, Inc., Tokyo, Japan). The qRT-PCR reactions were performed on an ABI StepOne Plus Real-Time PCR System with 2 × ChamQ Universal SYBR qPCR Master Mix Kit (Vazyme Biotech Co., Ltd., Nanjing, China). The quantity and integrity of extracted Total RNA was extracted from samples using an RNA Iso-

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**Table 1. Detailed information of the SWEET genes in common bean.**

| Gene Name | Gene ID | Gene Bank | Chr | Location | Protein Length (aa) | Molecular Weight (Da) | pI     |
|-----------|---------|-----------|-----|----------|---------------------|----------------------|--------|
| PvSWEET1  | Phv001G061900 | ESW33346  | 1   | 7604890–7607275 | 245                 | 27244.47             | 9.37   |
| PvSWEET2  | Phv001G064300 | ESW33377  | 1   | 8039774–8042757 | 251                 | 27297.14             | 9.11   |
| PvSWEET3  | Phv002G023600 | ESW31034  | 2   | 9364810–9366784 | 273                 | 30413.24             | 8.76   |
| PvSWEET4  | Phv002G028380 | ESW31995  | 2   | 44735395–44737728 | 304              | 34505.55             | 9.16   |
| PvSWEET5  | Phv002G028390 | ESW31987  | 2   | 44733241–44734881 | 258              | 28878.71             | 9.73   |
| PvSWEET6  | Phv002G000900 | ESW23188  | 2   | 46311623–46312510 | 249              | 27582.77             | 9.47   |
| PvSWEET7  | Phv003G199300 | ESW27407  | 3   | 41224642–41226388 | 229              | 26223.18             | 9.32   |
| PvSWEET8  | Phv004G017100 | ESW23084  | 4   | 1747562–1749299 | 244               | 27297.54             | 8.75   |
| PvSWEET9  | Phv004G017200 | ESW23085  | 4   | 1754335–1756093 | 244               | 27239.29             | 8.62   |
| PvSWEET10 | Phv004G017300 | ESW23086  | 4   | 1761790–1763040 | 228              | 25772.54             | 8.94   |
| PvSWEET11 | Phv004G017400 | ESW23087  | 4   | 1789159–1796874 | 245              | 27169.23             | 8.93   |
| PvSWEET12 | Phv005G076300 | ESW21504  | 5   | 15075256–15075375 | 235            | 26292.46             | 8.86   |
| PvSWEET13 | Phv006G006000 | ESW17947  | 6   | 128976–130139 | 259              | 28547.73             | 9.08   |
| PvSWEET14 | Phv006G021800 | ESW20456  | 6   | 31236755–31239016 | 273            | 29926.38             | 6.58   |
| PvSWEET15 | Phv008G001100 | ESW11093  | 8   | 201637–204350 | 270               | 30479.37             | 8.93   |
| PvSWEET16 | Phv008G001200 | ESW11094  | 8   | 208201–209779 | 272               | 30735.62             | 8.64   |
| PvSWEET17 | Phv008G007600 | ESW11168  | 8   | 779573–781567 | 259              | 20503.69             | 8.63   |
| PvSWEET18 | Phv009G134300 | ESW09518  | 9   | 1979172–19794523 | 247            | 27256.49             | 9.68   |
| PvSWEET19 | Phv009G137700 | ESW09565  | 9   | 2023066–20232439 | 254            | 28230.67             | 9.07   |
| PvSWEET20 | Phv009G162700 | ESW09864  | 9   | 23670710–23672989 | 264        | 29784.77             | 9.31   |
| PvSWEET21 | Phv009G162800 | ESW09865  | 9   | 23692658–23700111 | 195          | 2142.9               | 9.8    |
| PvSWEET22 | Phv009G162900 | ESW09866  | 9   | 2371836–23720821 | 291          | 32547.72             | 8.75   |
| PvSWEET23 | Phv009G249700 | ESW10922  | 9   | 36274394–36277639 | 245          | 27087.97             | 8.38   |
| PvSWEET24 | Phv011G168100 | ESW03295  | 11  | 43841568–43843435 | 235        | 21867.37             | 8.98   |

**Statistical analysis**

The data were analyzed using Microsoft Office 2013 (Redlands, CA, USA) and SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) (Li et al. 2018a). The data were presented as the means ± standard deviation of three independent experiments.

**Results**

**Identification and characterization of the SWEET genes in common bean**

Based on the results of an HMM profile analysis, 52 putative SWEET protein sequences were identified in the genome of P. vulgaris. Moreover, 24 PvSWEET genes were also confirmed through InterPro and SMART analyses and renamed PvSWEET1–PvSWEET24 based on their chromosomal position (Table 1 and Figure 1). The 24 predicted PvSWEET proteins ranged from 195 (PvSWEET21) to 304 (PvSWEET4) amino acid (aa) residues in length, with an average length of 252 aa. The relative molecular weight varied from 22,142.9 Da (PvSWEET21) to 34,505.55 Da (PvSWEET04). The pl values ranged from 6.58 (PvSWEET14) to 9.73 (PvSWEET05), with 23 members exhibiting pl values >7 (Table 1).

This study found that the 24 PvSWEET genes were distributed on 9 of 11 chromosomes (Figure 1). In addition, chromosome 9 contained the largest number of PvSWEETs (6) among the others, whereas no PvSWEET genes were found on chromosomes 7 and 10. In addition, chromosomal localization showed that the distribution and density of the PvSWEET genes on common bean chromosomes were uneven and basically clustered. Moreover, there were more PvSWEET genes distributed at two ends of the chromosomes, whereas fewer PvSWEET genes were distributed in the middle regions of the chromosomes.
Phylogenetic analysis of the PvSWEET proteins

To gain insight into the evolutionary relationships among the SWEETs in common bean, Arabidopsis, rice, and soybean, a phylogenetic tree was constructed by aligning 24 PvSWEET, 17 AtSWEET, 21 OsSWEET, and 52 GmSWEET protein sequences using MEGA X software (Figure 2). The phylogenetic analysis indicated that the different SWEETs could be clearly classified into four major clades (I, II, III, and IV). Among the PvSWEET proteins, clade III contained 10 members, and clade II contained six members, followed by clade I with six members, and clade III with only one member.

Gene structure and conserved motif analysis of PvSWEETs

The exon–intron structures of the 24 PvSWEETs were analyzed to reveal the gene structural features and evolutionary trajectory (Figure 3). The gene structure was generally characterized by a highly conserved distribution of exons and introns in the same clade or subclade (Figure 3(B)). In addition, it was noted that most PvSWEETs contained four exons, but several of the PvSWEETs members contained five exons.

In addition, the conservative motifs of 24 PvSWEETs were predicted using the MEME program. Ten motifs were identified based on the PvSWEET sequence characteristics and designated motifs 1–10 (Figure 3(C)). The SWEET proteins showed similar conserved motif compositions. In particular, motifs 1–6 were detected in all the PvSWEET proteins. This suggested that these six motifs are important components for the PvSWEET protein sequences. Moreover, specific motifs also existed in separate PvSWEET gene family subgroups, implying that the PvSWEET gene family diversified during evolution.

Cis-regulatory element analysis of the PvSWEETs

The 1.5-kb upstream sequences of the SWEET genes in common bean were analyzed using Plant CARE software to detect putative cis-regulatory elements. A total of 17 types of cis-regulatory elements, including hormone-related, light response, and resistance-related elements, were identified (Figure 4, Table S2). In the hormone-related element groups, ABRE was found in most PvSWEET genes (18 PvSWEET genes) in the form of multiple copies. In addition, 21 light response elements groups were also found. Among them, multiple copies of G-boxes existed in most PvSWEET genes (17). Three resistance-related elements, including ARE, MBSI, and LTR, were identified. Among them, ARE existed in 18 PvSWEET genes, MBSI in 3 PvSWEET genes, and LTR in 4 PvSWEET genes. These results suggest that the PvSWEET gene family may be involved in hormone response processes, growth regulatory, and stress responses.

Collinearity analysis and the tandem replication of PvSWEETs

Gene replication is an indispensable mechanism in plant evolution. It can extend new genes with similar or different functions. Three PvSWEET gene pairs (PvSWEET2/18, PvSWEET3/14, and PvSWEET4/20) were identified as duplicate gene pairs in common bean, and all of them were segmentally duplicated (Figure 5(A)). In addition, the selection pressure analyses of coding sequences can be represented by the ratio of the non-synonymous mutation rate (Ka) to the synonymous mutation rate (Ks), i.e. Ka/Ks < 1 represents a purifying or negative selection, and Ka/Ks > 1 represents a Darwinian or positive selection. Table S3 summarizes the Ka/Ks for three PvSWEET duplicates gene pairs < 0.4, indicating that the genes were under purifying selection. To further clarify the divergence during common bean evolution, the orthologous PvSWEET genes in the common bean genome were analyzed with those in the soybean and Arabidopsis genomes. In addition, collinearity was observed between the SWEET family genes in common bean, 31 genes in soybean, and nine in Arabidopsis (Tables S4 and S5, Figure 5(B,C)). This indicated that the SWEET genes may have similar functions.

Gene ontology (GO) enrichment of the PvSWEET genes

The GO enrichment related to the PvSWEET genes was analyzed to better understand the function of PvSWEET genes (Figure 6). Based on a GO enrichment analysis, 24 PvSWEET
genes were subdivided into three ontological categories: biological processes, cellular components, and molecular functions. There were 12 functional terms for biological processes, and most of the terms were related to the biosynthetic and metabolic processes of lipids, lipoooligosaccharides, and oligosaccharides. Twenty genes were important components of the membrane and are considered cellular components. For molecular functions, six genes showed potential ion transmembrane transporter activity, substrate-specific transport activity, and substrate-specific transport activity. These results indicate that the \textit{PvSWEET} genes are involved in many aspects of plant growth regulation.

**Tissue-specific expression of the \textit{PvSWEET} genes**

The transcriptional profiles were downloaded from the Phytozome database to understand the tissue-specific \textit{PvSWEET} genes (Figure 7). The transcriptional data contains nine tissues, including roots, stems, young trifoliate leaves, leaves, flower buds, flowers, young pods, green mature pods, and nodules. The results showed that 24 \textit{PvSWEET} genes exhibited different patterns of expression among varying tissues. Furthermore, most \textit{PvSWEET} genes were highly expressed in reproductive organs. Among all the \textit{PvSWEET} genes, \textit{PvSWEET5} was expressed the most highly in all the tissues tested, whereas \textit{PvSWEET8/9/10/13} were undetectable in the datasets. Twelve genes (\textit{PvSWEET5/6/7/11/12/17/18/19/20/21/22/24}) were highly expressed in flower buds or flowers, four genes (\textit{PvSWEET1/2/3/4}) in pods, four genes (\textit{PvSWEET5/6/22/23}) in leaves, four genes (\textit{PvSWEET1/4/11/23}) in stems, and two genes (\textit{PvSWEET15/16}) in the roots. However, only \textit{PvSWEET16} was highly expressed in nodules.

qRT-PCR was used to examine the levels of expression of 18 \textit{PvSWEET} genes in the cotyledons, hypocotyls and Figure 2. Phylogenetic tree of SWEET proteins in common bean (\textit{Phaseolus vulgaris}), \textit{Arabidopsis thaliana}, rice (\textit{Oryza sativa}) and soybean (\textit{Glycine max}). The phylogenetic tree was built using the Maximum-likelihood method with the JTT + G model implemented in MEGA X. Different colors represent the four various clades: red, clade I; blue, clade II; green, clade III; and yellow, clade IV. The red, green, blue, and yellow circles represent common bean, \textit{A. thaliana}, rice, and soybean SWEET gene family members, respectively.
radicles of common bean during the sprout stage (Figure 8). Most \( \text{PvSWEET} \) were found to be highly expressed in cotyledons, hypocotyls and radicles, while very few \( \text{PvSWEET7/18} \) transcripts were detected in hypocotyls, and few \( \text{PvSWEET11/15} \) transcripts were detected in hypocotyls and radicals.

**Stress-induced patterns of expression of the \( \text{PvSWEET} \) genes**

The patterns of expression of 18 \( \text{PvSWEET} \)s under three treatments were analyzed to obtain insights into the roles of \( \text{PvSWEET} \)s in response to plant stress (Figure 9). The levels of expression of the 18 \( \text{PvSWEET} \) genes differed among the three stress treatments. Eight genes (\( \text{PvSWEET1/3/4/10/12/13/18/24} \)) were upregulated by NaCl, CdCl\(_2\), and HgCl\(_2\) stresses; two genes (\( \text{PvSWEET19/21} \)) were downregulated by three stress treatments, and two genes (\( \text{PvSWEET5/20} \)) were downregulated by CdCl\(_2\) stress, although they were unaffected by NaCl and HgCl\(_2\) stresses. \( \text{PvSWEET6} \) was downregulated by CdCl\(_2\) and HgCl\(_2\) stresses, whereas it was unaffected by NaCl stress. \( \text{PvSWEET11} \) was upregulated by NaCl and CdCl\(_2\) stresses but downregulated by HgCl\(_2\) stress. \( \text{PvSWEET16} \) was upregulated by CdCl\(_2\) and HgCl\(_2\) stresses, whereas they were downregulated by NaCl stress. Three genes (\( \text{PvSWEET2/7/15} \)) were not affected by these three treatments.

**Discussion**

**Characteristics of the SWEET gene family in common bean**

As uniporters, the SWEET proteins can mediate both sugar uptake and efflux across tonoplast or plasma membranes and exhibit a low affinity for sugar (Chen et al. 2012). Diverse biological functions of the SWEET gene family have been widely studied in several plants (Chen et al. 2012; Yuan and Wang 2013; Sosso et al. 2015; Kryvoruchko et al. 2016; Gao et al. 2018). Common bean is one of the most common edible legumes consumed globally, and it contains abundant protein and vitamins. However, to our knowledge, a genome-wide characterization and functional analysis of the SWEET genes has not yet been reported in common bean. In this study, 24 full-length \( \text{PvSWEET} \) genes were identified and subjected to phylogeny, gene structure, domain architecture, and expression profile analyses.

It has been reported that the numbers of SWEET genes vary substantially across different species (Patil et al. 2015). To date, very few SWEET genes have been identified in animals or lower plant species. SWEET genes are much more ubiquitous in higher plants. One SWEET gene was found in humans (Chen et al. 2010), whereas 7, 17, 21 and 52 of the SWEET genes were found in the nematode Caenorhabditis elegans (Chen et al. 2010), Arabidopsis (Chen et al. 2012), rice (Yuan et al. 2014), and soybean...
respectively. These findings imply that the SWEET genes have multiple functions in higher plants.

In Arabidopsis, 17 AtSWEET genes were divided into four clades (Chen et al. 2012). This study showed that 24 PvSWEETs were classified into four clades based on their evolutionary relationships, with six genes in clade I, seven genes in clade II, 10 genes in clade III, and one gene in clade IV (Figure 2). Previous studies have shown that the proportions of SWEET genes in different clades varied across species indicating distinct expansion rates among the different species (Yuan and Wang 2013; Patil et al. 2015; Gao et al. 2018). In most plants, clades II and III are predominant in the SWEET gene family, although in different proportions. For example, clade II accounted for 19% of the genes in soybean, whereas they were 43% in rice, and clade III accounted for 44% in soybean and 26% in rice (Figure 2). In common bean, clade I accounted for 25%, clade II for 29%, clade III for 42%, and clade IV for 4%, suggesting that clades II and III are the predominant groups and may play important roles in the expansion of PvSWEET gene family (Figure 2). The results of a phylogenetic analysis of PvSWEETs were congruent with those of conserved motif and gene structural analyses. The PvSWEET proteins had similar conserved motif compositions, whereas the gene members in each clade had specific motifs (Figure 3). This suggests that the diversity and functions of PvSWEET genes may have diverged during evolution.

The SWEET gene family in common bean is larger than that in Arabidopsis. This could be owing to the expansion of PvSWEET gene, which is consistent with the previous research on soybean and rice (Yuan and Wang 2013; Patil et al. 2015). Tandem duplication and fragment duplication are the main expansion methods for plant gene families (Cannon et al. 2004). It is evident that genes remain in the plant genome through tandem duplication and fragment replication and play an important role in adaptive responses to environmental stimuli (Hanada et al. 2008; Jiang et al. 2010). This study found that fragment repetition was the primary mode of expansion for the PvSWEET gene family. The gene duplication events in the PvSWEET gene family were analyzed, and three pairs of fragment duplication genes were identified in subgroups I and III, as well as being distributed on chromosomes 1, 2, 6 and 9. In addition, 31 PvSWEET genes homologous to soybean and nine PvSWEET genes homologous to Arabidopsis were detected in the phylogenetic tree. However, there were no serial replication events identified in this study. Therefore, it appears that fragment repetition may play a dominant role in the expansion
The tissue-specific expression of genes can typically preliminarily predict their corresponding functions (Xiao et al. 2019). In this study, the patterns of expression of 24 PvSWEET genes were examined, and 67% were highly expressed in flowers and pods. This suggests that the PvSWEET genes primarily function in the reproductive organs of common beans. In *Petunia axillaris*, the levels of transcription of *PaSWEET13c, PaSWEET9a, PaSWEET1d, PaSWEET5a*, and *PaSWEET14a* increase as the flowers mature and are the highest in fully open flowers (Iftikhar et al. 2020).

Lin et al. (2014) reported that *AtSWEET9* is critical for nectar production in Arabidopsis and can function as an efflux transporter. In other plants, Iftikhar et al. (2020) also reported that *PaSWEET9c* of *P. axillaris* was a homolog of nectar-specific SWEETs, whereas *AtSWEET9* is expressed in mature and fully open flowers. In addition, Chen et al. (2015b) reported that *AtSWEET11, AtSWEET12, and AtSWEET15* were highly expressed in seed development in Arabidopsis, whereas the triple mutant *atSWEET11/12/15* showed serious seed defects, including retarded embryonic development and reduced seed weight. The phylogenetic analysis conducted in this study showed that *AtSWEET9, AtSWEET11, AtSWEET12, and AtSWEET15* were highly homologous to the *PvSWEETs* in subgroup III, including *PvSWEET3, PvSWEET5, PvSWEET14, PvSWEET17, PvSWEET20, PvSWEET21,* and *PvSWEET22.* Therefore, it was hypothesized that these genes are involved in inflorescence and grain development because the homologous genes may have similar functions.

It is evident that the *AtSWEET11* and *AtSWEET12* genes are highly expressed in Arabidopsis leaves and mediate the efflux function of sucrose phloem (Chen et al. 2012). An evolutionary analysis also revealed that *AtSWEET11* and *AtSWEET12* are highly homologous to *PvSWEETs* in subgroup III, including *PvSWEET5* and *PvSWEET22.* Therefore, it was hypothesized that *PvSWEET5* and *PvSWEET22* also play an important role in sucrose efflux from leaf phloem. In Arabidopsis, *AtSWEET2, AtSWEET16,* and *AtSWEET17* were highly expressed in the roots (Guo et al. 2014; Chen et al. 2015a). In addition, the evolutionary analysis in this study showed that *AtSWEET2* was in subgroup I, whereas *AtSWEET16* and *AtSWEET17* were in subgroup II, and *PvSWEET11* and *PvSWEET15* in subgroup III were highly expressed in the roots of common bean. These findings could be owing to genetic evolution.

Salt stress is one of the most important abiotic stresses that affects agricultural production. Unlike abiotic stresses, such as drought and cold stress, salt stress exist throughout the growth cycle of plants. However, seed germination is a critical period for plant growth and is very sensitive to salt...
stress. In addition, salt stress can destroy the composition of cells and their physiological activity, reduce the germination rate or delay germination (Thiam et al. 2013). Therefore, it is necessary to study the expression of \textit{PvSWEETs} genes in common bean during the germination stage. The expression of 18 \textit{PvSWEET} genes in the cotyledons, hypocotyls, and radicles of common beans showed that most of the genes were highly expressed, which indicates that the \textit{PvSWEETs} genes are involved in the development of common bean, particularly in the germination stage.

Abiotic stresses, such as salt, drought, and extreme temperature, can negatively affect plant growth and development (Rengasamy 2006; Cramer et al. 2011). Stress response \textit{cis}-acting elements, such as ARE, LTR, MBS, and MBSI, were found in the promoter region of \textit{PvSWEET} genes, which was associated with uniform abiotic stress. Except for \textit{PvSWEET4/6/9/10/13}, all 19 \textit{PvSWEET} genes contained at least one \textit{cis}-acting element involved in a stress response. Therefore, these results suggest that these genes may play an important role in stress responses. In addition, previous studies have shown that the SWEET gene helps to control plant responses to environmental stress (Li et al. 2018b; Zhang et al. 2020). In addition, it was evident that plants often suffer from various abiotic stresses during the germination stage, particularly owing to the adverse factors caused by the soil environment.

This study investigated the patterns of expression of 18 \textit{PvSWEET} genes in the radicles of common bean under different stresses, including NaCl, Cd, and Hg stress. Among the 18 \textit{PvSWEET} genes studied, more than 80% of them showed significant changes in expression under at least one stress. In addition, 40% of the \textit{PvSWEET} genes were upregulated, whereas 11% of them were downregulated under all three stresses. Therefore, this study indicates that the \textit{PvSWEET} genes play an important role in the response of common bean to abiotic stress.

The results of this evolutionary analysis showed that \textit{PvSWEET24} is highly homologous to \textit{AtSWEET2} in subgroup I. In Arabidopsis, \textit{AtSWEET2} has been found to be highly expressed in roots and is involved in resistance to \textit{Pythium} infection (Chen et al. 2015a). This study found that the expression of \textit{PvSWEET24} was significantly upregulated under all three stress treatments. This indicates that \textit{PvSWEET24} could help common bean roots resist environmental stress. \textit{PvSWEET5} and \textit{PvSWEET20} were only downregulated in CdCl\(_2\) but remained unchanged under NaCl and HgCl\(_2\) stress compared with the control. This suggests that \textit{PvSWEET5} and \textit{PvSWEET20} may be specifically expressed in common bean under Cd stress. In addition, \textit{PvSWEET11} and \textit{PvSWEET16} were differentially expressed under the three stress conditions, indicating that they have different roles in response to salt, Cd, and Hg stress. In conclusion, it is evident that the common bean may have developed specialized regulatory mechanisms for different abiotic stresses.

**Conclusions**

In this study, 24 \textit{PvSWEET} genes were identified and analyzed in terms of gene structure, chromosome distribution,
evolutionary relationship, cis-regulatory elements, gene replication, collinearity, and spatiotemporal patterns of expression. A phylogenetic tree was used to divide the *PvSWEET* genes into four subgroups based on their structure and motif composition. Tissue-specific patterns of expression were found for the genes indicating broad functional diversity. Most gene family members responded to NaCl, CdCl2, and HgCl2 stresses. This study lays a foundation for future studies on the role of *PvSWEET* genes in the growth, development, and response to metal stress in common bean.

Figure 7. Expression profiling of 24 *PvSWEETs* in nine tissues, including roots, stems, young trifoliate leaves, leaves, flower buds, flowers, young pods, green mature pods, and nodules based on the Phytozome database.

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Figure 8. A qRT-PCR analysis of the levels of expression of 18 *PvSWEET* genes in cotyledons (C), hypocotyls (H), and radicles (R) during the sprouting stage. The vertical bars represent the standard error of three replicates. Different letters on the bar represent significant differences between means at the $P < 0.05$ level. qRT-PCR, real-time quantitative reverse transcription PCR.
Figure 9. A qRT-PCR analysis of 18 *PsSWEET* genes under three different treatments (NaCl, CdCl$_2$, and HgCl$_2$). The vertical bars represent the standard error of three replicates. *Significant differences among the mean values compared with CK at the $P < 0.05$ level.
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