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Identification, characterization, and expression of the *SWEET* gene family in *Phalaenopsis equestris* and *Dendrobium officinale*

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Abstract

Sugars are important molecules that function not only as primary metabolites, but also as nutrients and signal molecules in plants. The sugar transport protein genes family *SWEET* has been recently identified. The availability of the *Dendrobium officinale* and *Phalaenopsis equestris* genome sequences offered the opportunity to study the *SWEET* gene family in this two orchid species. We identified 22 and 16 putative *SWEET* genes, respectively, in the genomes of *D. officinale* and *P. equestris* using comprehensive bioinformatics analysis. Based on phylogenetic comparisons with SWEET proteins from *Arabidopsis* and rice, the DoSWEET and PeSWEET proteins could be divided into four clades; among these, clade II specifically lacked PeSWEETs and clade IV specifically lacked DoSWEETs, and there were orthologs present between *D. officinale* and *P. equestris*. Protein sequence alignments suggest that there is a predicted serine phosphorylation site in each of the highly conserved MtN3/saliva domain regions. Gene expression analysis in four tissues showed that three *PeSWEET* genes were most highly expressed in the flower, leaf, stem, and root, suggesting that these genes might play important roles in growth and development in *P. equestris*. Analysis of gene expression in different floral organs showed that five *PeSWEET* genes were highly expressed in the column (gynostemium), implying their possible involvement in reproductive development in this species. The expression patterns of seven *PeSWEETs* in response to different abiotic stresses showed that three genes were upregulated significantly in response to high temperature and two genes were differently expressed at low temperature. The results of this study lay the foundation for further functional analysis of *SWEET* genes in orchids.

Additional key words: floral organs, high or low temperature, orchids, phylogenetic trees, sugar transporters.

Introduction

In higher plants, sugars are not only nutrients but also important signal molecules. Sugars are distributed through the plant *via* sugar transporters, which guarantee sugar allocation among source and sink cells (Afoufa-Bastien *et al.* 2010). The *SWEET* proteins, which contain MtN3/saliva transmembrane domains, are a recently identified family of sugar transporter-like proteins that are present in a diverse group of organisms ranging from protozoa to higher eukaryotes. The numbers of genes predicted to encode SWEET family proteins have been determined in the genomes of many plants, such as *Arabidopsis*, rice (Chen *et al.* 2010), grapevine (Chong *et al.* 2014), potato (Manck-Götzenberger and Requena 2016), and tomato (Feng *et al.* 2015). Most of the characterized or predicted SWEET proteins from different plant species consist of seven transmembrane helices (7-TM) that harbor two MtN3/saliva domains (Chen *et al.* 2012, Yuan *et al.* 2014a). SWEET proteins function in diverse physiological activities as sugar transporters or by facilitating ion transport *via* interaction with ion transporters (Han and Jiang 2015), in reproductive development (Yuan *et al.* 2014a, Han and Jiang 2015), senescence (Yuan *et al.* 2014a), and biotic and abiotic stress responses (Chong *et al.* 2014, Manck-Götzenberger

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Abbreviations: aa - amino acids; *BLASTP* - protein basic local alignment search tool; *GSDS* - gene structure displayer server; ME - minimum evolution method; ML - maximum likelihood method; NJ - neighbor-joining method; qPCR - quantitative polymerase chain reaction; RPKM - reads per kilobase per million reads; SWEET - sugars will eventually be exported transporters; TM - transmembrane; TMHMM - hidden Markov model for predicting transmembrane helices in proteins.
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and Requena 2016). Moreover, some non-typical SWEET proteins with more or less than 7-TM have also been predicted in rice (Yuan *et al.* 2014a), grapevine (Chong *et al.* 2014, Patil *et al.* 2015), and soybean (Patil *et al.* 2015), and a few contain only a single MtN3/saliva domain (Yuan *et al.* 2014a).

Phylogenetic analyses have shown that SWEET protein family members in plants can be divided in four clades (I - IV) in species such as *Arabidopsis* (Han and Jiang 2015), grapevine (Chong *et al.* 2014), rice (Yuan *et al.* 2014a), and tomato (Feng *et al.* 2015), suggesting evolutionary conservation of the *SWEET* gene family in plants. Some SWEET proteins in *Arabidopsis* have been shown to be involved in soluble sugar transport; for example, AtSWEET1-8 (clades I and II) function mainly as glucose transporters (Chen *et al.* 2010), AtSWEET9-15 (clade III) are sucrose and glucose transporters, and AtSWEET16 and 17 (clade IV) are mainly sucrose, glucose, and fructose transporters (Klemens *et al.* 2013, Yuan and Wang 2013, Han and Jiang 2015). The SWEET proteins in each clade may have similar sugar transport capabilities.

Phalaenopsis species are popular ornamental plants worldwide because of their elegant appearance and the extended longevity of their flowers (Cai *et al.* 2014). Another well known orchid species, *Dendrobium officinale*, is an important traditional Chinese medicinal herb (Yan *et al.* 2015). Among the various bioactive compounds produced in the stems of *D. officinale*, polysaccharide has been identified as one of the most important constituents (Yang *et al.* 2012, Hu *et al.* 2015b). In orchids, several previous studies have described the potential functions of SWEET sugar transporters. For example, the *SvNod9* gene, which encodes a predicted sugar transporter of the SWEET family, is expressed during orchid mycorrhizal symbiosis between protocorms of *Serapias vomeracea* and the fungus *Tulasnella calospora* (Perotto *et al.* 2014). However, detailed and comprehensive analyses of the *SWEET* gene family in orchids have yet to be explored.

Genome-wide analysis is a useful first step in identifying members of a gene family and in elucidating their biological roles. The published genome sequences of the orchids *D. officinale* (Yan *et al.* 2015) and *P. equestris* (Cai *et al.* 2014) are important resources, and have made possible to perform genome-wide identification and analysis of putative *SWEET* protein genes in these commercially important orchid species. The aim of this study was to identify genes encoding putative SWEETs in the *D. officinale* and *P. equestris* genome, phylogenetic analysis of the SWEETs, and determination the amino acid sequences of these proteins. In addition, we tried to analyze the exon-intron structures and expression patterns of *Phalaenopsis SWEETs* in various tissues and different abiotic stresses. Our findings will be useful for further research on the *SWEET* protein gene family in orchids.

Materials and methods

Detection of *SWEET* family genes in *D. officinale* and *P. equestris*: To identify SWEET proteins in *D. officinale* and *P. equestris*, a *BLASTP* (protein basic local alignment search tool) search was performed using the protein sequences of *Arabidopsis* SWEET proteins (Table 1 Suppl., He *et al.* 2010) and rice SWEET proteins (Table 1 Suppl., Yuan *et al.* 2014a) as queries against the *D. officinale* and *P. equestris* genome protein sequence databases (http://202.203.187.112/herbalplant; ftp://ftp.genomics.org.cn/from_BGISZ/20130120/; Cai *et al.* 2014, Yan *et al.* 2015) using the default search parameters. The predicted protein sequences were submitted to *Pfam* (http://pfam.xfam.org/) to confirm the presence of the SWEET domain using the default parameters. Sequences without the SWEET domain were discarded from further analyses.

Gene and protein structure, multiple sequence alignments, and phylogenetic analyses: The coding sequences and genomic sequences of *DoSWEET* and *PeSWEET* genes were analyzed using the *GSDS* (gene structure displayer server) web server *v. 2.0* (http://gsds.cbi.pku.edu.cn/) to identify the positions of introns and exons (Hu *et al.* 2015a). The deduced amino acid sequences of the DoSWEET and PeSWEET proteins were then submitted to *TMHMM* (hidden Markov model for predicting transmembrane helices in proteins) server *v.2.0* (http://www.cbs.dtu.dk/services/TMHMM/) in *FASTA* format using the defaut settings to predict the presence of protein domains. The multiple sequence alignment of DoSWEET and PeSWEET protein sequences was performed with *ClustalX 2.0* software using the default parameters (Larkin *et al.* 2007). The full-length DoSWEET and PeSWEET protein sequences along with SWEET protein sequences from *Arabidopsis* and rice were imported into *MEGA 5.0* software to construct phylogenetic trees by the neighbor-joining (NJ), minimum evolution (ME), and maximum likelihood (ML) methods with 1 000 bootstrap replicates, poisson model and complete deletion missing data treatment (Tamura *et al.* 2011).

Expression analysis of *SWEET* protein genes in *Phalaenopsis*: The expression RPKM (reads per kilobase per million reads) data for 16 *PeSWEET* genes in four tissues (flowers, leaves, stems, and roots) of *P. equestris* were from the study of Cai *et al*. (2015; Table 2 Suppl.). A heatmap of *PeSWEET* gene expression clusters from each tissue was constructed using *HemI 1.0* (Deng *et al*. 2014). The *Phalaenopsis equestris* (Schauer) Rchb.f. cv. Queen Beer Red Sky, which is one of the most popular cultivar in the Chinese Spring Festival, was grown in a greenhouse at the Chinese Academy of Forestry (Beijing, China) at day/night temperatures of 24/18 °C, an air humidity of

65 - 75 %, a 10-h photoperiod, and an irradiance of 700 μmol m^{-2} s^{-1}. The two-year-old seedlings were subjected to 4 °C (low temperature), 42 °C (high temperature), 100 mM NaCl (high salinity), and 100 mM PEG6000 (drought) for 12 h under the same other cultural conditions in the greenhouse. The control (CK) seedlings were irrigated with water. Each treatment had three biological replicates from different plants. The different floral organs (petal, sepal, column, and labellum) were collected 15 d after treatments, immediately frozen in liquid nitrogen, and stored at -80 °C for RNA isolation.

Total RNA was isolated from the flower samples using an *EASYspin Plus* RNA extraction kit (*Aidlab*, Beijing, China) following the manufacturer's instructions. First-strand cDNA was synthesized from 2 μg samples of total RNA using the *PrimeScript* 1st strand cDNA synthesis kit (*Takara*, Dalian, China) according to the manufacturer's instructions. Real-time quantitative (q) PCR assays were performed using the *qTOWER* PCR system (*Analytik Jena AG*, Jena, Germany). Reactions were performed in a 10 mm^3 volume containing 0.25 mm^3 of cDNA, 200 nM of each primer (Table 3 Suppl.) and 5 mm^3 of *SYBR Premix Ex Taq II* (*Takara*). The following conditions were used for amplification: 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and 60 °C for 30 s. The specificity of the amplicon for each primer pair was verified by melting curve analysis. All real-time qPCR experiments were performed with three biological replicates, and each replicate was measured in triplicate. The relative expressions were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001), and the *Actin4* gene from *Phalaenopsis* was used as an internal reference to normalize the expression of *PeSWEET*s, which has been evaluated to be the most stable reference genes for vegetative and reproductive tissues of *Phalaenopsis* (Yuan *et al.* 2014b) and also stably expressed in the different flower organs and stressed seedlings in this study. The amplification efficiency ($0.95 < E < 1.05$) and correlation coefficient ($R^2 > 0.99$) of each primer pair in this study have been estimated for the future analysis (Table 3 Suppl.).

Results

The SWEET proteins were identified based on *BLASTP* searches against the *D. officinale* and *P. equestris* genome protein sequence databases. After the conserved domain searching against the *Pfam* database and manual annotation processes, 22 DoSWEET and 16 PeSWEET sequences were obtained. The putative SWEET proteins were named according to their homologous genes in *A. thaliana* (Table 4 Suppl.). Each homologous *AtSWEET* gene corresponds to approximately one to six *DoSWEET* genes, and one to four *PeSWEET* genes. All of the putative SWEET proteins contain conserved 7-TM except for Dendrobium_GLEAN_10039399 with 5-TM being detected (Table S4); although its non-full length sequence have been detected from the *D. officinale* genome database, the putative sequence had high homology with AtSWEET5 and several DoSWEET proteins and been named as DoSWEET5b. The predicted full length DoSWEET amino acid sequences range from 218 to 527 amino acids (aa) in length, whereas the lengths of the predicted PeSWEET proteins range from 235 to 298 aa.

To study the evolutionary relationships among SWEET proteins, we constructed unrooted phylogenetic trees by three methods (NJ, ME, and ML) using predicted SWEET protein sequences from *D. officinale*, *P. equestris*, rice, and *Arabidopsis* (Fig. 1, Fig. 1 Suppl.). All the three approaches produced similar tree topologies and phylogenetic distributions into four clades: clade I contained 16 DoSWEETs and eight PeSWEETs, clade II contained two DoSWEETs and no PeSWEET, clade III contained four DoSWEET and seven PeSWEET, and clade IV contained no DoSWEET and one PeSWEET proteins.

Clade I is a large clade, and some members share a high degree of protein sequence similarity; DoSWEET4a-e, DoSWEET5a-e, DoSWEET7a-f, and PeSWEET7a-d, form separate subclades, indicating that they may have originated from common ancestors by frequent gene duplication, and that there might be functional redundancy among the group members, similar to previous reports for soybean (Patil *et al.* 2015). Based on the phylogenetic tree, several putative paralogous (PeSWEET5a/b, PeSWEET7a/b, PeSWEET10/14, and DoSWEET9/10) and orthologous (PeSWEET15/ DoSWEET13, PeSWEET4/DoSWEET4c) proteins were identified with high bootstrap support (> 98 %).

The phylogenetic tree also shows that both clades I and III contain orchid, rice, and *Arabidopsis* SWEET proteins, indicating that the members of each clade shared a common ancestor prior to the divergence of monocot and dicot lineages, and that the expansion of the clades occurred after the divergence of orchids, rice, and *Arabidopsis*. Additionally, this further illustrates that these genes are evolutionarily conserved, and that they could perform fundamental functions. The specific absence of PeSWEET proteins in clade II, and of DoSWEET proteins in clade IV may indicate that the expansion of these clades occurred before the divergence of orchids, rice, and *Arabidopsis*, and that these SWEET proteins could have lost their functions during evolution and subsequently disappeared.

Additionally, two proteins (DoSWEET5a and PeSWEET16) do not show close affinities to any subclades in the phylogenetic tree and had low homology with other SWEET proteins (Fig. 1), indicating that they may play different roles in physiological processes.

To analyze the basic characteristics of the *DoSWEET* and *PeSWEET* genes, we mapped their gene structures including exons and introns. All *SWEET* family genes

from *D. officinale* and *P. equestris* were predicted to contain introns in their genomic sequences, similar to what has been found for other plant *SWEET* family genes such as those from soybean (Patil *et al.* 2015) and tomato (Feng *et al.* 2015). *DoSWEET5c*, *11* and *PeSWEET6* contain six introns each, *DoSWEET2a*, *2b*, *4c*, *4e*, *5d*, *7f*, *9*, *10, 13* and *PeSWEET9-16* contain five introns each, *DoSWEET5a*, *5e*, *7b* and *PeSWEET4*, *5a* and *5b* contain four introns each, and *DoSWEET4a, 4b, 7a, 7c-e* and *PeSWEET7a-d* contain three introns each, and *DoSWEET4d* has two introns (Table 4 Suppl.).

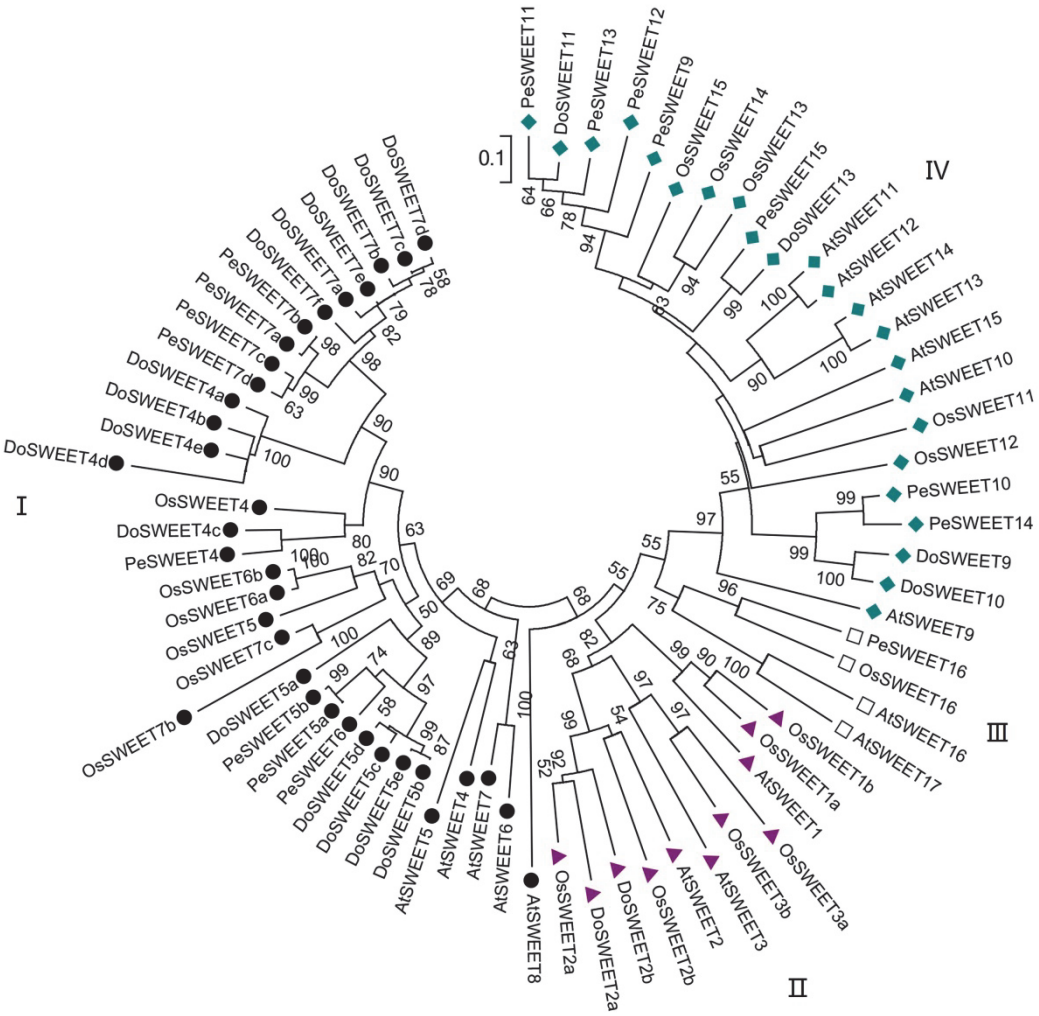

Fig. 1. An unrooted neighbor-joining phylogenetic tree of predicted SWEET proteins from *Dendrobium officinale*, *Phalaenopsis equestris*, *Arabidopsis thaliana*, and *Oryza sativa***.** The numbers at the nodes represent bootstrap percentage values based on 1 000 replications, poisson model, and complete deletion missing data treatment. The *scale bar* represents 0.1 substitutions per amino acid position. AtSWEET and OsSWEET protein sequences were used as references to categorize the DoSWEETs and PeSWEETs.

Multiple alignments of DoSWEET and PeSWEET protein sequences showed that the MtN3/saliva domain is highly conserved in the intracellular regions and it is relatively conserved in the transmembrane regions (Fig. 2 Suppl.). There is a predicted serine phosphorylation site in each of the highly conserved second MtN3/saliva domain regions and also in many of the first MtN3/saliva domains. One protein (PeSWEET9) has a threonine instead of a serine in the first MtN3/saliva domain region, while PeSWEET5a, b, 6, and DoSWEET5a, c-e have alanine residues and PeSWEET10 has an asparagine instead of serine in the first MtN3/saliva domain. One protein (DoSWEET5b) do not have a predicted phosphorylation site in the first MtN3/saliva domain. This implies that all of these proteins could potentially be phosphorylated at the predicted phosphorylation sites except for PeSWEET10 because of the substitution of asparagine for serine.

To quantify *PeSWEET* gene expression in four different tissues, we constructed a heatmap using the calculated RPKM values from the RNA-seq data (Fig. 2, Table 3 Suppl. and Cai *et al.* 2014). The expression patterns of the 16 *P. equestris SWEET* genes were observed in flower, leaf, stem, and root tissues. Three

genes, *PeSWEET7b*, *7d*, and *14* were not expressed in any of the four tissues. Seven genes were specifically not expressed in certain tissues: five genes in the leaves, one gene in the stems, and six genes in the roots. Expression of six genes was detected in all four tissues, *PeSWEET4*, *5a*, *6*, *11*, *13*, and *16*; although only three (*PeSWEET4*, *11*, and *13*) showed high expression, and *PeSWEET4* and *13* were highly expressed in flowers (RPKM > 200) and stems (RPKM > 100). Expressions of *PeSWEET11* and *13* was relatively high (RPKM ~100) in leaves, and all genes showed low expressions in the roots.

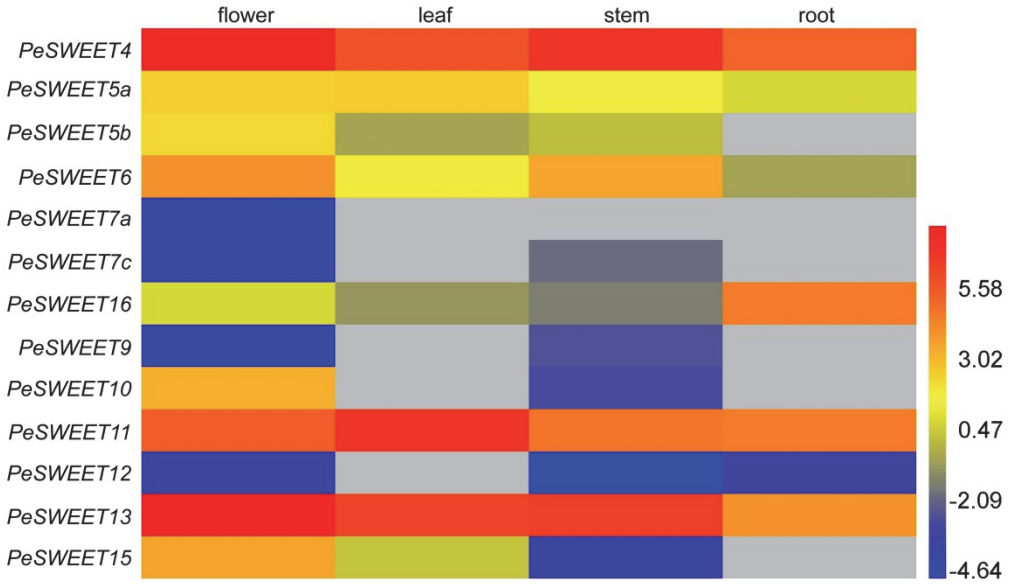

Fig. 2. A heatmap showing the relative expression of 16 *PeSWEET* genes in four tissues (flowers, leaves, stems, and roots). The *color scale* represents RPKM-normalized counts which have been transformed to log format. *Blue* indicates a low expression, *red* indicates a high expression, and *gray* indicates no expression.

To further investigate the roles of *SWEET* genes in various floral organs, the expression patterns of selected *PeSWEET* genes were determined by real-time qPCR in the *P. equestris*. We designed gene-specific primer pairs for seven *PeSWEET* genes (Table 3 Suppl.). As shown in Fig. 3, *PeSWEET11* was highly expressed in the labellum, followed by the column and the sepal. *PeSWEET13, 15,* and *16* had the highest expression in the column, and *PeSWEET13* and *16* showed similar expression in the four floral organs.

Previous studies have shown that *SWEET* genes might be involved in diverse biotic stress responses (Chong *et al.* 2014, Manck-Götzenberger and Requena 2016). To investigate the roles of *SWEET* genes in responses to abiotic stresses, we examined the relative expression profiles of the seven *PeSWEET* genes after low temperature, high-temperature, salinity, and drought treatments by real-time qPCR in seedlings of *P. equestris*. As shown in Fig. 4, *PeSWEET11* and *13* showed similar expression patterns under several different abiotic stresses, and both were significantly upregulated in response to high temperature treatment. Expression of *PeSWEET16* was also increased significantly in response to high temperature. Expression of *PeSWEET7b* was significantly increased under low temperature, whereas expression of *PeSWEET12* was dramatically suppressed at low temperature, suggesting that these two genes could play positive or negative roles under this condition, respectively. Expressions of *PeSWEET5a* and *15* did not show significant difference under these different abiotic stresses, indicating that these two genes might not participate in the response to them.

Discussion

Unlike mammals, chordates, bacteria, and yeast, which have only one type of *SWEET* gene, plant genomes contain multiple *SWEET*-type genes that together comprise a *SWEET* gene family, which is indicative of the importance of these genes to plants (Hamada *et al.* 2005, Saier *et al.* 2006, Yuan and Wang 2013), There are 17 predicted *SWEET* genes in *Arabidopsis*, 21 in rice (Chen *et al.* 2010), 17 in grapevine (Chong *et al.* 2014), 35 in potato (Manck-Götzenberger and Requena 2016), and 29 in tomato (Feng *et al.* 2015). Compared to these five species, *P. equestris* had only 16 *PeSWEET* family genes and 22 *DoSWEET* family genes were identified in *D. officinale* in this study. However, these might not be representative of all of the *SWEET* gene family members in *D. officinale* and *P. equestris* due to the incomplete genome sequences (Cai *et al.* 2014, Yan *et al.* 2015).

Phosphorylation is one of the most important post-translational protein modifications, because it is related to the regulation of many cellular processes. Our analysis indictes that the intracellular region of SWEET family proteins is likely to be an important functional area or an active regulatory region, and its regulation is likely subjected to reversible phosphorylation/dephosphorylation controlled by one or more protein kinases/phosphatases. These predicted phosphorylation sites have also been identified in plant SWEET family proteins from other species such as tomato (Feng *et al.* 2015).

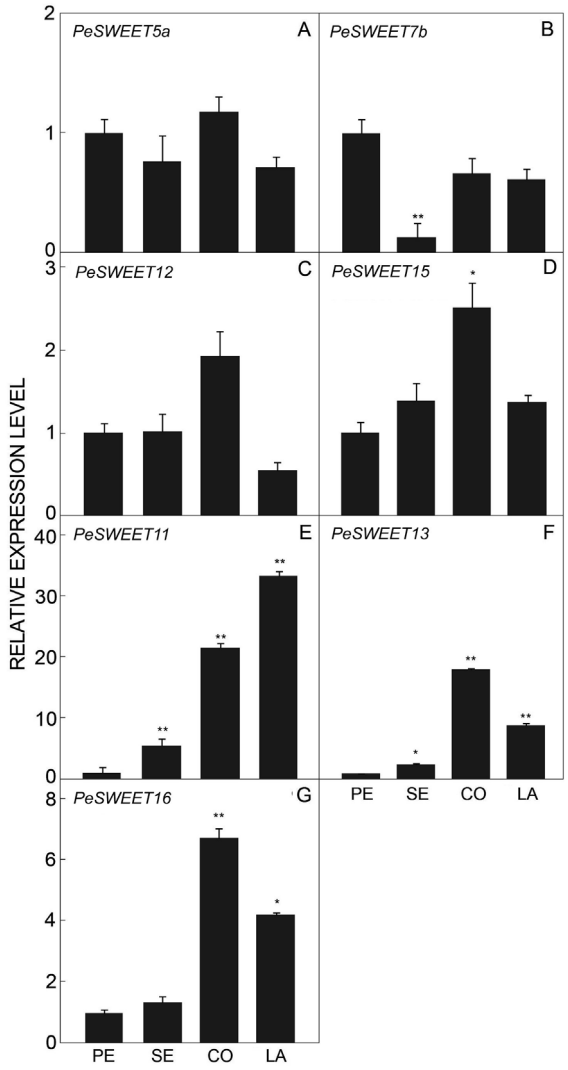

Fig. 3. Relative expressions of *PeSWEET* genes in different floral organs (PE - petal, SE - sepal, CO - column, LA - labellum). The expressions were normalized to the expression of *Actin4*, and expression in the petal was set at 1.0. Means ± SDs, $n = 9$ (three biological replicates and three technical replicates). * - $P < 0.05$, ** - $P < 0.01$ (*ANOVA* calculated using *SPSS*).

AtSWEET17 shows significant expression in the root tonoplast, and is responsible for the transfer of fructose to maintain the fructose balance in the root cytoplasm (Guo *et al.* 2014). As a paralog of *AtSWEET17*, *AtSWEET16* is also significantly expressed in root, but it is responsible for the transfer of sucrose, glucose, and fructose (Klemens *et al.* 2013). In this study, *PeSWEET16* was grouped with *AtSWEET16/17* in clade IV, and gene expression analysis showed that *PeSWEET16* was highly expressed in *Phalaenopsis* roots (Table 2 Suppl.), suggesting that it might have a similar function to *AtSWEET17* in maintaining the fructose balance in the root cytoplasm.

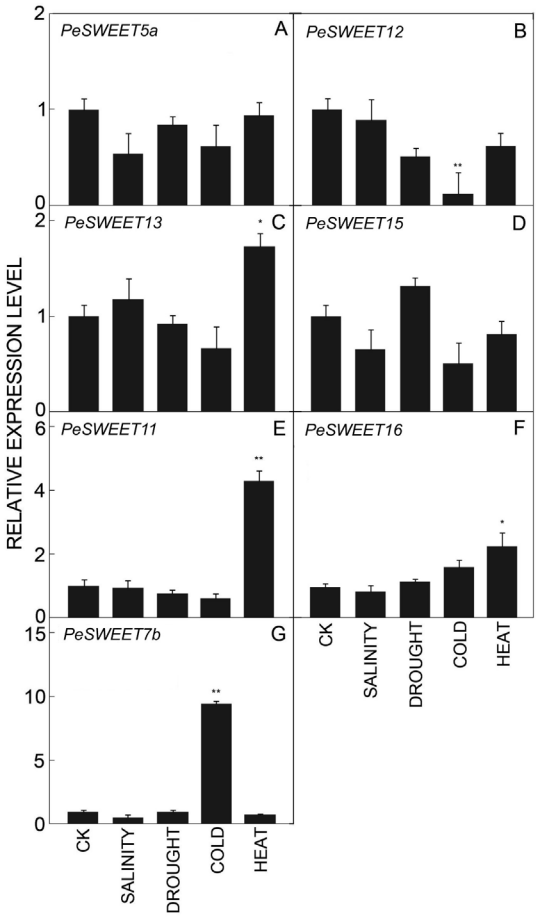

Fig. 4. Relative expressions of *PeSWEET* genes in flowers under different abiotic stresses lasting 12 h (CK - control, SALINITY - 100 mM NaCl, DROUGHT - 100 mM PEG6000, COLD - 4 °C, HEAT - 42 °C). Gene expression was normalized to that of *Actin4*, and the expression in the control was set at 1.0. Means ± SDs, $n = 9$ (three biological replicates and three technical replicates). * - $P < 0.05$, ** - $P < 0.01$ (*ANOVA* calculated using *SPSS*).

Many *SWEET* family genes have been putatively associated with sterility in plants. *OsSWEET5*, *11*, and *14* have been shown to be differentially expressed in rice flowers during panicle development, and are required for normal reproductive development (Chu *et al.* 2006, Antony *et al.* 2010, Wang *et al.* 2010, Yuan *et al.* 2014a). *AtSWEET8* is highly expressed in the microsporophyt and the tapetum during anther development (Guan *et al.* 2008), the expression of *AtSWEET5* is the highest in the male gametophyte three days before flowering (Bock *et al.*

2006), and *AtSWEET7* is preferentially expressed during pollen development (Bock *et al.* 2006). Moreover, the tobacco *TOBC023B06* gene (Quiapim *et al.* 2009) and the tomato *LeSTD1* gene (Salts *et al.* 2005) have also been reported to be involved in reproductive development. In *Phalaenopsis*, *PeSWEET4* and *13* was most highly expressed in flowers, with RPKM > 240, which was much higher than expression in the other three vegetative organs (Fig. 2 and Table 2 Suppl.). *PeSWEET11, 13, 15,* and *16* were highly expressed in the column relative to the expression in sepals and petals (Fig. 3); the gynostemium (column) is a reproductive organ composed of fused male (stamens) and female (pistil) parts in *Phalaenopsis* and other orchid species. These results indicate that the four *PeSWEET* genes could be involved in reproductive development of *Phalaenopsis.*

Abiotic stresses (low and high temperature, salinity, and drought) can induce cell dehydration; therefore, improving the content of osmotically active compounds to maintain water balance in plants is of vital importance. Soluble sugars (sucrose, glucose, fructose, *etc*.) are important osmolytes. Increase in content of soluble sugars in plants experiencing stress can reduce the water potential of the cell and enhance its water-retaining capacity to maintain normal growth under adverse conditions (Yamada *et al.* 2010). Thus, SWEET proteins, as sugar transporters, are involved in sugar accumulation and transfer in plants to promote their ability to resist various kinds of stress.

Recently, many *SWEET* genes in different plants have been shown to be induced by abiotic stresses at the transcriptional level, suggesting that these genes might be associated with the plant responses to these stresses. For example, transcription of the *AtSWEET15* gene is induced by salinity and drought (Seo *et al.* 2011), while expression is down-regulated in response to cold stress (He *et al.* 2008). The *AtSWEET16* gene, similar to the homologous gene *AtSWEET17*, is also involved in the stress response. However, plants overexpressing *AtSWEET16/17* show reductions in fructose or glucose content in leaves under cold stress, and expression of *AtSWEET16* is down-regulated under cold and osmotic stress (Klemens *et al.* 2013). In contrast, overexpression of the pepper *SWEET* family gene *CaPIF1* enhances tolerance to cold stress in tomato (Seonga *et al.* 2007).

There is a positive correlation between the soluble sugar content and cold resistance in many plant species (Graham and Patterson 1982). In the *Phalaenopsis* , there is a significant negative correlation between low temperature and soluble sugar content (Liu *et al.* 2007). Expression of *PeSWEET7b* was extremely significantly up-regulated under low temperature, suggesting that as a sugar transporter protein, it might participate in the transport and accumulation of soluble monosaccharides inside the cell to improve tolerance and adaptation to low temperature. On the contrary, the expression of *PeSWEET12* was significantly down-regulated in response to low temperature.

When *Phalaenopsis* seedlings are treated with day/night temperature of 40/30 ^{o}C for 6 d the soluble sugar content increases continuously (Yang and Chen 2009). Expressions of *PeSWEET11, 13,* and *16* were significantly up-regulated, suggesting that these genes might participate in sugar transport to regulate resistance to high temperature.

There were no significant differences in expression in the seven *PeSWEET* genes in response to salinity and drought. A possible explanation could be that stresses were rather mild or that these *PeSWEET* genes may not be involved in the response to drought or salt stress.

In this study, we conducted a detailed analysis of the *SWEET* gene family in two orchid species (*D. officinale* and *P. equestris*), including genome-wide identification, phylogeny, gene structure, conserved transmembrane domain, and expression pattern analyses. To lay foundation for improving the ornamental features and stress resistance of ornamental orchid species by genetic engineering methods, we have analyzed the *SWEET* genes expression in the different floral organs and in response to abiotic stresses. These results will form the basis for future gene-cloning and functional analysis by using comparative genomics approaches to unravel the role of *SWEET* genes in the floral development and response to abiotic stress in the other orchids.

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