Characterization and expression analysis of somatic embryogenesis receptor-like kinase genes from *Phalaenopsis*

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ABSTRACT. Somatic embryogenesis receptor-like kinase (*SERK*) genes have been found to be involved in the somatic embryogenesis of several plant species. We identified and characterized 5 *PhSERK* genes in the *Phalaenopsis* orchid. The amino acid sequences of PhSERKs and other SERK proteins are highly conserved, with the highest homology observed in the leucine-rich repeat-receptor-like kinase domain. All 5 *PhSERKs* were expressed in all *Phalaenopsis* organs examined (root, leaf, shoot apical meristem, and flower), with the strongest expression, particularly for *PhSERK1* and *3*, in the shoot apical meristem of mature plants. Expression of all PhSERKs was downregulated during early floral bud development and was upregulated gradually until the semi-open flower stage was reached. All 5 *PhSERKs* were expressed during both seed germination and protocorm-like-body (PLB) development. In germinated seeds, quantitative real-time PCR revealed upregulation of all *PhSERK*s except *PhSERK4* at 1 week and downregulation after 4 weeks. The 5 *PhSERK*s were differentially expressed in the early stage

of PLB development and maintained substantial levels during PLB formation, with PhSERK1 and 5 upregulated 1 week after culture and PhSERK2, 3, and 4 downregulated over this period. Because physical wounding of PLB stimulates secondary PLB formation, the *PhSERK5* expression peak at week 3 coincided with visible and fully developed secondary PLBs. *PhSERK5* may be important in PLB induction and subsequent development. Our *PhSERK* expression analysis revealed that these genes have a broad role during orchid plant development.

Key words: *Phalaenopsis*; Leucine-rich repeat-receptor-like kinases; *SERK*; Protocorm-like body

INTRODUCTION

Somatic embryogenesis is a cellular differentiation pathway that converts totipotent embryogenic cells into somatic embryos during plant tissue culture and is important for plant propagation. To better manipulate plant regeneration via somatic embryogenesis, it is important to understand the molecular events underlying the transition from somatic to embryogenic cells (Yang et al., 2011). Both biochemical and morphological changes occur throughout the development of induced tissues during somatic embryogenesis (Santos et al., 2005). Genes involved in somatic embryogenesis include somatic embryogenesis receptor-like kinase (*SERK*; Schmidt et al., 1997), leafy cotyledon (Ledwon and Gaj, 2009, 2011), agamous-like 15 (Thakare et al., 2008), baby boom (Boutilier et al., 2002), and wuschel (Su et al., 2009).

The *SERK* gene is thought to play an important role in somatic embryogenesis (Schmidt et al., 1997; Sharma et al., 2008; Singla et al., 2008). It encodes a protein that belongs to the receptor-like kinase (RLK) family, which is highly conserved among plant species. It carries an external leucine-rich repeat (LRR) domain that is typically found in this class of protein (Santos et al., 2005). SERK genes play important roles in the response to external stimuli, cell-cell recognition during development, defense against pathogens, and self-incompatibility (Shiu and Bleecker, 2001). The first *SERK* gene, identified from carrot (*DcSERK*), was specifically expressed in competent cells during the globular stage (Schmidt et al., 1997). *DcSERK* is most closely related to *AtSERK1* in *Arabidopsis*, which is expressed in embryogenic cells and during early somatic embryogenesis (Hecht et al., 2001). Indeed, transgenic *Arabidopsis* plants overexpressing *AtSERK1* showed an enhanced capacity to initiate somatic embryogenesis during *in vitro* culture (Hecht et al., 2001). Moreover, *AtSERK1* and *2* play a role in male sporogenesis, particularly tapetum development (Albrecht et al., 2005; Colcombet et al., 2005). *ZmSERK1* and *2* of maize are similar to carrot and *Arabidopsis SERK* genes and are expressed in embryogenic calli as well as male and female reproductive tissues (Baudino et al., 2001). *OsSERK1* is thought to be partially responsible for mediating defense signal transduction and has a basic role in rice somatic embryogenesis (Hu et al., 2005). More homologs of *SERK* have been identified in several plant species, including *Dactylis glomerata* (Somleva et al., 2000), *Medicago truncatula* (Nolan et al., 2003, 2009), *Ocotea catharinensis* (Santa-Catarina et al., 2004), *Helianthus annuus* (Thomas et al., 2004), *Theobroma cacao* (Santos et al., 2005), *Citrus unshiu* (Shimada et al., 2005), *Triticum aestivum* (Singla et al., 2008), *Vitis vinifera* (Schellenbaum et al., 2008), *Solanum tuberosum* (Sharma et al., 2008), *Cocos nucifera* (Perez-Núñez et al., 2009), *Lactuca sativa* (Santos et al., 2009), *Rosa hybrida*

(Zakizadeh et al., 2010), *Musa acuminata* (Huang et al., 2010), *Secale cereale* (Gruszczyńska and Rakoczy-Trojanowska, 2011), *Glycine max* (Yang et al., 2011), *Cyclamen persicum* (Savona et al., 2012), *Ananas comosus* (Ma et al., 2012a,b), and *Cyrtochilum loxense* (Cueva et al., 2012). These reports all point to *SERK* as a specific marker of embryogenic competence during morphogenesis induction.

Phalaenopsis is one of the most important orchids for potted plant production in the global flower market. Mass production of orchids is achieved by *in vitro* germination of seeds, which develop into a structure called a protocorm and then into seedlings, or by induction of protocorm-like bodies (PLBs), which are equivalent to somatic embryos, and subsequent plantlet regeneration (Ishii et al., 1998; Mayer et al., 2010). A number of studies examining *Phalaenopsis* PLB induction and multiplication used explant sources, including flower stalk nodes and internodes (Reisinger et al., 1976; Lin, 1986), PLB sections (Young et al., 2000), leaves (Park et al., 2002), shoot tips (Tokuhara and Mii, 1993), and root tips (Park et al., 2003).

The developmental process of PLB formation and the underlying molecular events have not been explored in *Phalaenopsis*. Information regarding the function of *SERK* in regulating somatic embryogenesis in other plant species led us to investigate the contribution of *PhSERK* genes to PLB formation in *Phalaenopsis* by temporal and spatial expression analysis of protocorms and PLBs, in addition to their expression profile in various tissues. We found that the expressions of some *PhSERK* transcripts were related to cellular totipotency during *in vitro* development of PLBs of this orchid.

MATERIAL AND METHODS

Plant material and explant preparation

Potted plants of a hybrid, *Phalaenopsis* Sogo Vivien 'F858', were grown in the fan-and-pad greenhouse of National Pingtung University of Science and Technology (Pingtung, Taiwan) under 70% shade light and controlled temperatures ranging from 27°-30°C. Total RNA was extracted from root tip, leaf, shoot apical meristem (SAM), and inflorescence for real-time polymerase chain reaction (PCR) of the expression of *PhSERKs* in mature plants. Total RNA was extracted from floral buds of different sizes to analyze relevant development stages: buds of 0.2-0.29 cm (FB1), 0.3-0.39 cm (FB2), 0.4-0.49 cm (FB3), 0.5-0.59 cm (FB4), 0.6-0.79 cm (FB5), and 0.8-0.99 cm (FB6); semi-open flowers (FB7); and fully open flowers (FB8). RNA was also extracted from different floral parts of the fully open flower, including the sepal, petal, labellum, column, and pollinia (**Figure S1**).

Protocorms of *Phalaenopsis* were obtained from *in vitro*-germinated seeds. The seeds were surface-sterilized with 0.6% sodium hypochlorite for 10 min and rinsed with sterile water 3 times. Seeds were sown on germination medium of (in g/L) 3 Hyponex (7N-6P$_2$O$_5$-19K$_2$O), 1 bacto-peptone, 50 potato homogenate, 50 banana paste, 30 sucrose, 2 activated charcoal, and 8 agar (Sigma-Aldrich. St. Louis, MO, USA). The medium pH was adjusted to 5.6 before autoclave sterilization and pouring into Petri dishes.

PLBs were induced by culture of *in vitro* leaf segments on 1/4 MS medium, which includes 1/4-strength macro- and micro-elements of MS medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/L thidiazuron and 7 g/L agar (Sigma-Aldrich), and 30 g/L sucrose according to the method described by Hsu and Chen (2003). The basal half of primary PLBs was used for explants after removing the bisectioned apical top and was incubated on induc-

tion medium consisting of (in g l^{-1}) 3 Hyponex fertilizer (7N-6P$_2$O$_5$-19K$_2$O), 1 bacto-tryptone, 50 potato homogenate, 50 banana homogenate, 30 sucrose, 2 activated charcoal, and 8 agar (Sigma-Aldrich). The medium was prepared as described above. Explants were incubated in the dark at 25° ± 2°C, and 10 explants were included in each Petri dish as a replicate, with 4 replications for each cutting treatment. Primary PLBs developed from leaf sections after 4 months were used as explants for subsequent experiments.

The following stages of protocorms and PLBs were used for real-time PCR analysis: P0 (mature dried seeds before transfer onto germination medium; P1, P2, P3, P4, P5, and P6 (protocorms developed from seeds by germination for 1, 2, 3, 4, 5, 8 weeks, respectively; PLB0-5 (the basal half of primary PLBs on induction medium for 0 to 5 weeks, respectively) (Figure 1).
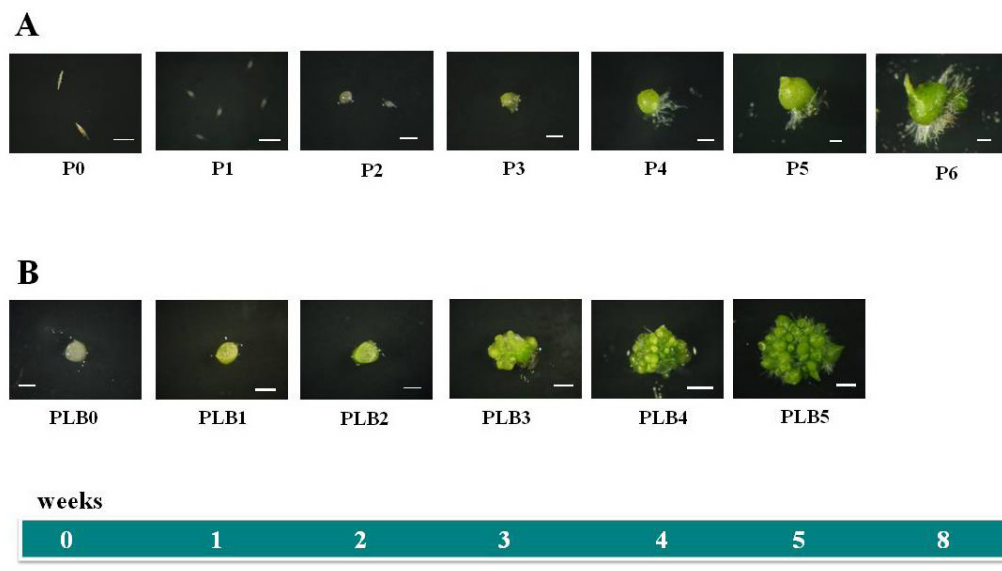
Figure 1. Protocorm development (**A**) and protocorm-like body (PLB) formation (**B**) in *Phalaenopsis*. P0-P6, seeds germinated into protocorm for 0, 1, 2, 3, 4, 5, and 8 weeks, respectively. PLB0-5, basal parts of primary PLB after cutting and growth on induction medium for 0, 1, 2, 3, 4, and 5 weeks, respectively. P0 (bar = 0.1 cm), P1-6 and PLB0-5 (bar = 0.2 cm).

RNA extraction and cDNA synthesis

Total RNA was isolated and purified using the CTAB reagent as described (Chang et al., 1993) with modification. RNA pellets were resuspended in DEPC-treated sterile water and then precipitated with 0.3X volumes 8 M LiCl. The total RNA solution was treated with RNase-free DNase (Promega, Madison, WI, USA) at 37°C for 30 min to remove residual genomic DNA. Purified RNA was quantified using a spectrophotometer (U2900, Hitachi, Tokyo, Japan), and aliquots were separated on a 0.8% agarose gel in 0.8X TE buffer to evaluate sample quality. Next, 2 μL RNA was used to synthesize single-stranded cDNA using the SMART cDNA PCR Synthesis Kit (Clontech, Mountain View, CA, USA). Synthesized sscDNA fragments were used as templates for real-time PCR.

Isolation of *PhSERKs*

Degenerate primers (**Table S1**) designed from SERK conserved domains were used to clone portion of the cDNA sequence. These primers were used for PCR amplification with cDNA from tight flower buds of *Phalaenopsis*. The amplified fragment was cloned into the pGEM-T Easy vector (Promega) and sequenced. Gene-specific primers (**Table S1**) were designed based on the known sequence of the *PhSERK1* fragment for rapid amplification of 5' and 3' cDNA ends (RACE) using the SMART RACE cDNA amplification Kit (Clontech). The 5'-RACE and 3'-RACE products were cloned into the pGEM-T Easy vector and sequenced. Based on RACE sequence data, the *PhSERK1*-F and *PhSERK1*-R primers (**Table S1**) were designed to amplify full-length cDNA using Ex Taq (Takara, Shiga, Japan) and designated *PhSERK1*. Other *PhSERK* sequences were retrieved from Orchidstra (http://orchidstra.abrc.sinica.edu.tw/none/) as partial expressed sequence tags (Su et al., 2009), with redundant sequences removed after multiple alignments using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), and were designated *PhSERK*2, *3*, *4*, and *5*. Specific primers were designed to amplify non-conserved regions of each gene using PCR for the following specific DNA fragments: *PhSERK2* (PATC125548), *PhSERK3* (PAVEGC012987), *PhSERK4* (PAALLC028299), and *PhSERK5* (PATC125663) (**Table S1**). To obtain the full-length for cDNA of *PhSERK2* to 5, the same RACE strategy was adopted as for *PhSERK1* using specific primer sequences (**Table S1**).

Characterization of PhSERKs and phylogenetic analysis

The signal peptide was predicted using the SignalP 4.0 Server tool (http://www.cbs.dtu.dk/services/SignalP/). The PSORT (http://psort.hgc.jp/) was used for protein prediction. To understand the phylogenetic relationship between PhSERK and known SERK genes of other plant species, multiple alignment was performed using Molecular Evolutionary Genetics Analysis (MEGA) 4.0 (Tamura et al. 2007). Sequence data were analyzed by neighbor-joining with the NEIGHBOR program in Phylogeny Inference Package (PHYLIP) and Unweighted Pair Group Mean Average methods in MEGA. Distances were calculated using DNADIST in PHYLIP. Bootstrapping and decay analysis involved neighbor-joining plots. Parsimony analysis and determination of clades involved use of MEGA.

Quantification of transcript levels by real-time PCR assay

Quantitative real-time PCR and data analysis involved the ABI PRISM 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with SYBR Green PCR Master Mix (Applied Biosystems). Total RNA was isolated from *Phalaenopsis* tissues, and 2 μL was used for first-strand cDNA synthesis by priming with 10 μM oligo $(dT)_{12-18}$ Primer (Invitrogen, Carlsbad, CA, USA) and reverse transcribed with the SuperScript first-strand synthesis system (Invitrogen). Specific primers used for *PhSERK* gene expression analysis (Table 1) were designed to amplify specific regions of individual genes. The amplification program was 95°C for 10 min, 1 cycle; 95°C for 15 s, 60°C for 1 min, 40 cycles. The *PhActin* housekeeping gene was used as an internal control (Table 1). The optimal concentrations of target genes and the internal control *PhActin* were determined according to Chen et al. (2005).

Three replicates of each reaction were performed, and data were calculated according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Table 1. Full-length *PhSERK* cDNAs identified in *Phalaenopsis*.

Gene name	Full length (bp)	ORF (bp)	5' UTR (bp)	3' UTR (bp)	Protein length (aa)
PhSERK1	2265	1899	245	121	633
PhSERK2	2512	1887	256	369	629
PhSERK3	2388	1848	283	257	616
PhSERK4	2292	1848	101	343	616
PhSERK5	2104	1869	51	184	623

ORF = open reading frame; UTR = untranslated region.

RESULTS

Identification of *PhSERKs* from *Phalaenopsis* orchid

Partial *PhSERK1* gene fragments from *Phalaenopsis* were obtained using degenerate primers designed from the conserved domain and the full-length sequence by RACE. Assembly of PCR fragments generated a full-length sequence of 2049 bp (Table 1). To identify additional SERK sequences in *Phalaenopsis*, we searched the Orchidstra database for potential cDNA clones. Four clones were found to be unique among 19 SERK sequences and were obtained by PCR with specific primers designed from SERK cDNA sequences (Orchidstra accession Nos. PATC125548, PAVEGC012987, PAALLC028299, and PATC125663). Full-length PhSERK cDNA for these 4 ESTs were obtained using 5' and 3' RACE. All 5 PhSERKs contained both 5' and 3' untranslated regions (UTR) of 51 to 369 bp. Sequence information for the 5 cloned PhSERKs is shown in Table 1. The deduced length of proteins ranged from 616-633 amino acids.

Except for PhSERK3 and 4, the PhSERK proteins showed features of the protein kinase superfamily because they contained domains typical of SERK proteins found in other species, including signal peptide, leucine-zipper, and 5 LRR domains, as well as a serine-proline-proline motif, a hydrophobic transmembrane domain, and a Ser/Thr kinase domain comprising 11 sub-domains of conserved amino acid sequences (Figure 2). The signal peptides of PhSERK1, PhSERK2, and PhSERK5 contained a potential cleavage site between 24 and 29 amino acids as predicted by the SignalP 4.0 Server tool (http://www.cbs.dtu.dk/services/SignalP/) (**Figure S2**). The Ser/Thr protein-kinase active-site signature was located from 319-601 aa, the PhSERK transmembrane region was at 256-294 aa, and a cytoplasmic tail spanned 295-650 aa (Figure 2). PhSERK3 and 4 lacked the signal peptide, which was also observed for the DcSERK protein involved in plant embryogenesis (Schmidt et al., 1997).

Phylogenetic relationships between *Phalaenopsis* SERK proteins

Full-length amino acid sequences for the 5 PhSERKs and other SERK proteins were used for phylogenetic analysis. Compared to other SERK proteins from monocots and dicots, PhSEKK1 and 2 showed the highest similarity with coconut CnSERK and pineapple AcSERK1 and 2 as well as *Cyrtochilum loxense* ClSERK, rice OsSEK1, alfalfa MtSERK1, maize ZmSERK1 and 2, *Arabidopsis* AtSERK1 and 2, and potato StSERK1 (Figure 3 and

Table S2). PhSERK3 and 4 were grouped with the other SERK proteins lacking the signal peptide. PhSERK5 clustered with PpSERK1 and 2, with 64-65% similarity for both proteins (Figure 3 and Table S2).
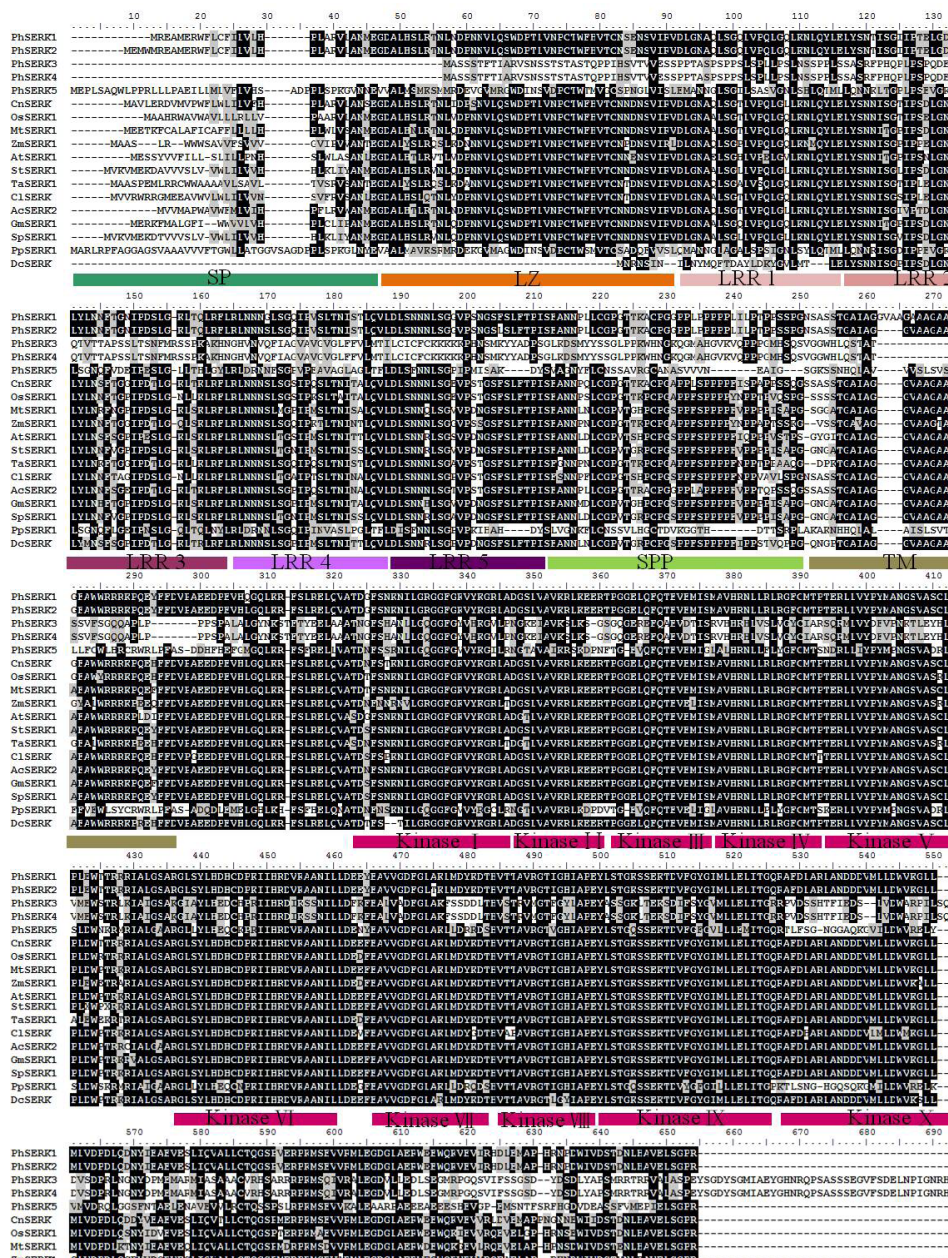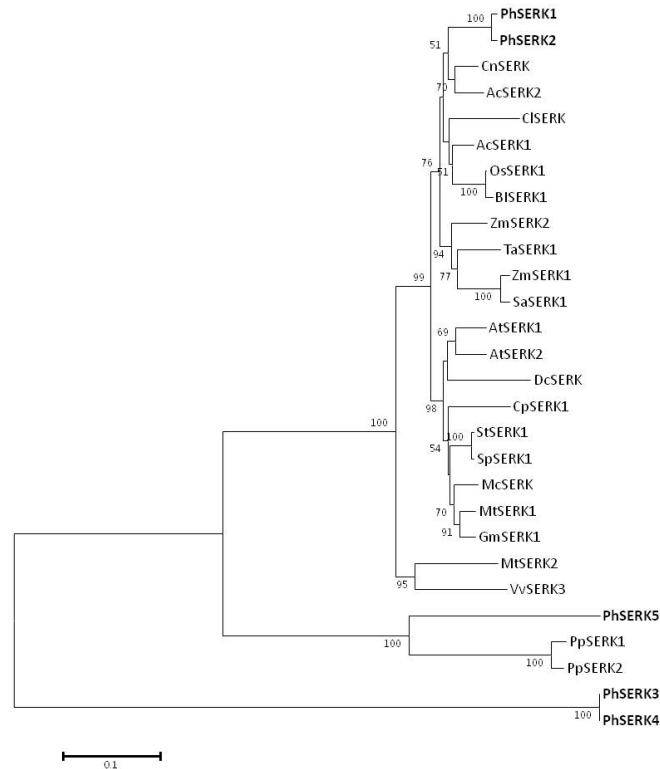

Figure 2. Alignment of predicted amino acid sequences of SERK family proteins. Identical amino acids are shaded in black. Different domains are indicated above the corresponding sequences.

Figure 3. Phylogenetic tree of known somatic embryogenesis receptor-like kinases (SERKs) based on predicted full-length amino acid sequences. The 23 other plant SERKs and accession numbers in GenBank are *CnSERK* (AY791293.2), *AcSERK1* (HM236375.2), *AcSERK2* (HM236376.1), *ClSERK* (FN994192.1), *OsSERK1* (AB188247.1), *OsBISERK1* (AY463361.1), *ZmSERK1* (NM_001111662.1), *ZmSERK2* (NM_001111663.1), *TaSERK1* (JF808017.1), *SaSERK1* (GQ283907.1), *AtSERK1* (NM_105841.4), *AtSERK2* (NM_103144.4), *DcSERK* (U93048.1), *CpSERK1* (EF672247.2), *StSERK1* (EF175216.1), *SpSERK1* (EF623824.1), *McSERK* (JX863894.3), *MtSERK1* (AY162176.1), *MtSERK2* (HM_640001.1), *GmSERK1* (EU869193.1), *VvSERK3* (XM_002262662.1), *PpSERK1* (AJ841698.1), and *PpSERK2* (AJ841697.1).

Expression pattern of *PhSERK*s in *Phalaenopsis* tissue

PhSERK transcripts were detected in all tissue examined, although at varying levels of expression. The expression pattern in SAM was similar among the 5 *PhSERK* genes and was higher than in root tips, leaf, and inflorescence (Figure 4). The expression in SAM was higher for *PhSERK1* and *3* than the other *PhSERK*s (Figure 4). In floral buds, expression of all 5 *PhSERK*s was downregulated with development stage, was maintained at relatively low levels, and was greatly reduced in the stage 6 floral bud (FB6) of 0.8-0.99 cm. Expression of all *PhSERK*s was increased in the semi-open and fully open flower stage (FB7-8). The *PhSERK4* transcript was most abundant in the first flower bud stage (FB1) and was maintained at relatively high levels in subsequent floral stages. In floral parts of open flowers, *PhSERK*s showed similar expression patterns, except the level was higher for *PhSERK4* than other *PhSERK*s in pollinia (Figure 4, PL).
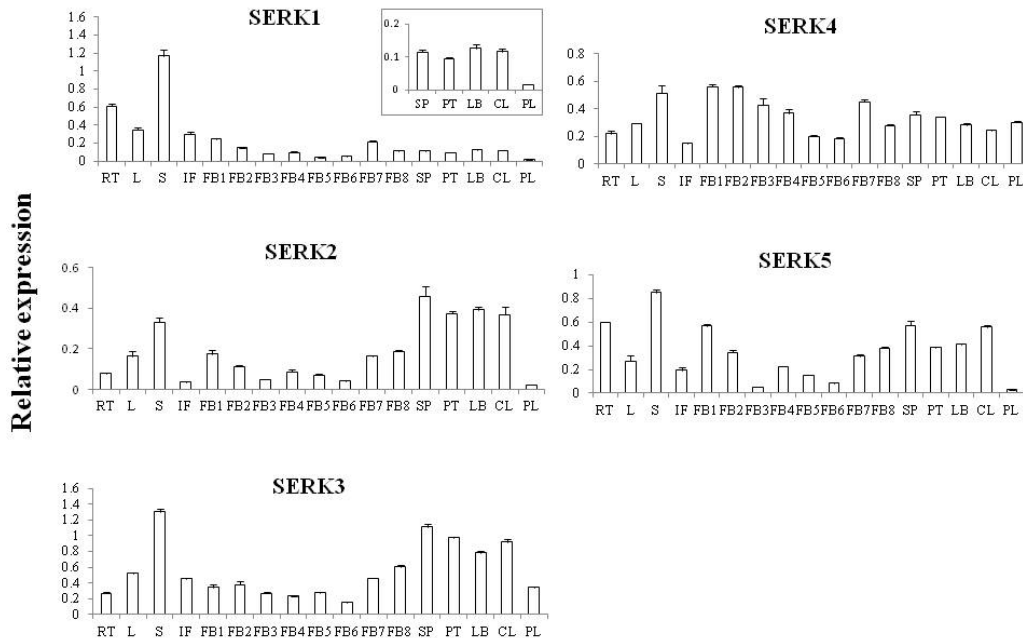
Figure 4. Real-time PCR of *PhSERKs* in different tissues and flower developmental stages of *Phalaenopsis* Sogo Vivien. Relative mRNA levels were normalized to that of the housekeeping gene *PhActin*. RT = root tip; L = leaf; S = shoot apical meristem; IF = inflorescence; FB = floral bud; FB1 = 0.20-0.29 cm; FB2 = 0.30-0.39 cm; FB3 = 0.40-0.49 cm; FB4 = 0.50-0.59 cm; FB5 = 0.60-0.79 cm; FB6 = 0.80-0.99 cm; FB7 = semi-open flower; FB8 = fully open flower; SP = sepal; PT = petal; LB = labellum; CL = column; PL = pollinia.

Expression of *PhSERK*s during protocorm development and PLB formation

All 5 *PhSERK* genes showed transcriptional activity during 8 weeks of seed germination with development into protocorms. Except for *PhSERK4*, most *PhSERK*s were upregulated during the first week of germination (Figure 5), when the embryo swelled (Figure 1, P1). At week 2, *PhSERK2* expression was increased 2- to 3-fold as compared to the initial stage during protocorm development, with the embryo emerged from the seed coat (Figure 5, P2) The *PhSERK4* transcript level reached a maximum during week 3, with approximately 3-fold higher expression than during week 2 (P3) and then decreased to half of the level in control dry seeds (Figure 5, P0) during the last 3 weeks (P4-P6). All *PhSERK* genes showed reduced expression during protocorm development after 4 weeks until the first leaf formed in the tip and the absorbing hairs became elongated in the base (Figure 5, P4-P6).

To investigate the association between *PhSERK* gene expression and secondary PLB formation, we analyzed sectioned primary PLB explants during 5 weeks of culture. The mRNA levels of *PhSERK1* and *5* slightly increased by week 1 after PLBs were wounded (Figure 1 and 5, PLB1). Expression of *PhSERK5* was present beginning in week 1, peaked at week 3 (PLB3), and decreased thereafter (PLB4). Additionally, upregulation of the 5 *PhSERK*s at week 2 (PLB2) coincided with active cell division on wounded primary PLBs at this time, showing an approximately 6-fold increase in the level of *PhSERK2*. After 4-5 weeks, the

wounded primary PLB explants could regenerate more secondary PLBs, but the transcriptional activity of *PhSERK2* to 5 decreased (Figure 1 and 5, PLB4-5). However, expression of *PhSERK1* peaked 4 weeks after wounding (Figure 5, PLB4); at this time, many secondary PLBs became apparent and formed from epidermal layers proximal to the wound site (Figure 1, PLB4). The expression pattern of *PhSERK3* and *PhSERK4* were similar during PLB development. For example, the level of both was decreased at week 1 (Figure 5, PLB1), increased at week 3 (PLB3), and then decreased (PLB4-5).
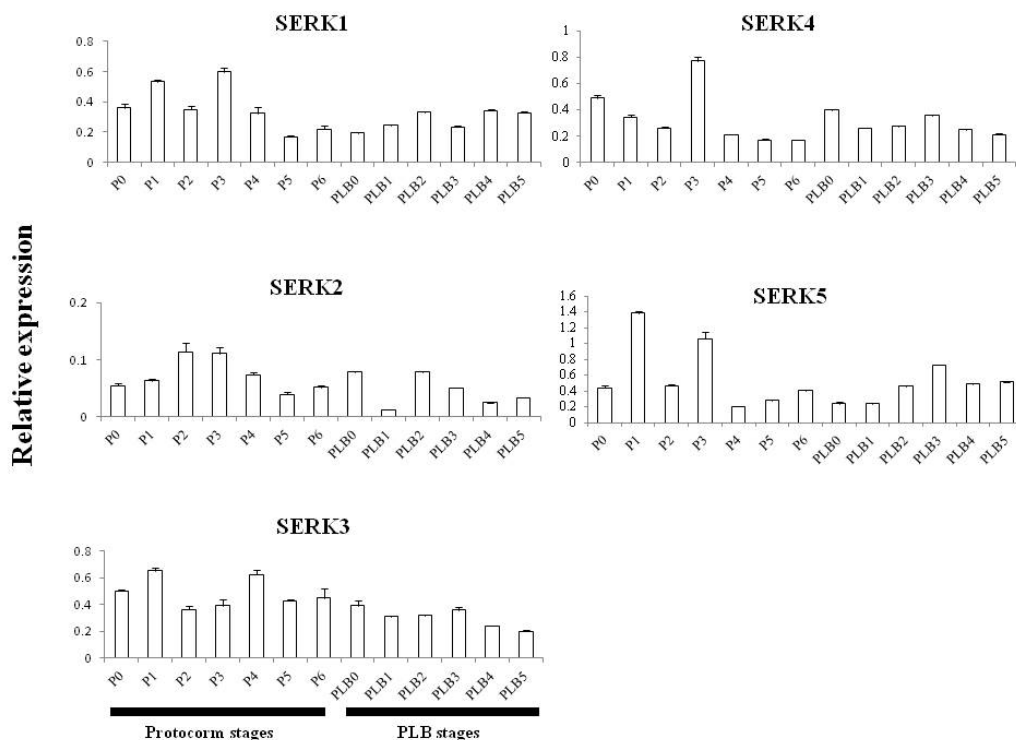
Figure 5. Expression of *PhSERK* genes during protocorm (P) and protocorm-like body (PLB) development in *Phalaenopsis* Sogo Vivien. Relative mRNA levels were normalized to that of *PhActin*. P0-P6 indicates weeks after seed germination. PLB0-PLB5 indicates culture time (weeks) on induction medium for the PLB explant after cutting out the apical part.

DISCUSSION

SERKs are thought to be important in several aspects of plant development in addition to somatic embryogenesis; these SERKs include AtSERK1 (Hecht et al., 2001), TcSERK (Santos et al., 2005), StSERK (Sharma et al., 2008), TaSERKs (Singla et al., 2008), GmSERK1 (Yang et al., 2011), and AcSERKs (Ma et al., 2012a,b). Somatic embryogenesis offers an advantage in plant propagation because many somatic embryos can be obtained in a short amount of time. Formation of PLBs in orchids is thought to be similar to somatic

embryogenesis (Ishii et al., 1998; Mayer et al., 2010). Despite several studies of PLBs in *Phalaenopsis* (Young et al., 2000; Park et al., 2002), no studies have examined the link between *SERK* genes and PLB development in orchid. According to previous studies of other plants, *SERK*s are LRR-RLK factors and involved in somatic embryogenesis. We identified 5 *PhSERK* genes in the *Phalaenopsis* orchid. Three of these, *PhSERK1*, *2*, and *5*, showed typical SERK protein structural features, as reported for other plant species. The high sequence similarity of PhSERK1 and 2 with CnSERK (Pérez-Núñez et al., 2009), AcSERK1 and 2 (Ma et al., 2012a,b), ClSERK (Cueva et al., 2012), OsSERK (Ito et al., 2005), MtSERK1 (Nolan et al., 2003), ZmSERK1 (Baudino et al., 2001), and AtSERK1 (Hecht et al., 2001) suggests that *PhSERK1* and 2 are involved in PLB formation or somatic embryogenesis, as has been proposed for orthologs from other species. Clustering of PhSERK5 with PpSERK1 and 2 (Figure 3) also points to a similar function in embryogenesis (Albrecht et al., 2005). However, PhSERK3 and 4 lack signal peptides and thus may have major functions in processes other than somatic embryogenesis (**Figure S2**). Signal peptide-lacking SERKs *DcSERK* (Schmidt et al., 1997) and *TaSERK2* (Singla et al., 2008) were proposed to have a role in somatic embryogenesis. However, *TaSERK3* showed the highest expression in developing seeds and appeared to be a brassinosteroid-associated kinase lacking a signal peptide, although it shares a characteristic C-terminal domain with other SERK proteins (Singla et al., 2008).

We analyzed the expression of the 5 *PhSERK* genes in different tissues, flower bud stages, seed germination, and PLB formation in *Phalaenopsis* orchids. Tissue-preferential expression of these *PhSERK*s in SAM was detected at high levels but at low levels in other tissues such as leaves, root tips, and inflorescence (Figure 4). The expression pattern of *PhSERK*s is reminiscent of their association with plant organ development as reported in other species. Moreover, AtSERK1 and 2 were reported to have redundant functions in sporophytic development and controlled male gametophyte production (Albrecht et al., 2005; Colcombet et al., 2005). Interestingly, *PhSERK4* was preferentially expressed in pollinia as compared with other *PhSERK*s, and its expression peaked at the FB7 stage, when the anther was readily differentiated (Figure 4). *PhSERK4* may have a role in sporophytic development.

Previous studies revealed that *SERK* transcripts are involved in both zygotic and somatic embryogenesis. Upregulation of *PhSERKs*, except for *PhSER2*, early during protocorm development, suggests that these *PhSERK*s are involved in morphogenesis of the *Phalaenopsis* orchid during seed germination. Schmidt et al. (1997) detected *DcSERK* mRNA only in early embryos up to the globular stage. *PhSERK4* expression increased rapidly within 3 weeks of seed germination and decreased when seedlings were fully developed (Figure 5). This finding is consistent with those in *PpSERK*s (Albrecht et al., 2005) and *GmSERK1* (Yang et al., 2011), which were also expressed in early plant embryogenesis, with decreased expression in later stages. Therefore, *PhSERK4* appears to be a marker for normal protocorm development in this orchid.

Schellenbaum et al. (2008) observed that *VvSERK1* and *3* were highly expressed during somatic embryo induction, before the visible appearance of embryos. *VvSERK2* appears to be stably expressed and differs from the 2 other *VvSERK* genes (Schellenbaum et al., 2008). In wheat, *TaSERK1* and *2* transcript levels were enhanced in embryogenic calli and may have a role during somatic embryogenesis, whereas *TaSERK3* expression was the highest in developing seeds (Singla et al., 2008). *StSERK1* was upregulated during the induction of somatic embryogenesis in potato and was continuously repressed during the embryo-transition phase (Sharma et al., 2008). *CnSERK* was detected in embryogenic tissues during somatic embryo-

genesis, with expression greatly decreasing during somatic embryo development (Pérez-Núñez et al., 2009). When wounded explants were cultured in our PLB induction medium, *PhSERK5* was highly expressed during the third week, when secondary PLBs became apparent (Figures 1 and 5). *PhSERK5* transcription may be closely associated with the acquisition of embryogenic competence and cell proliferation during orchid PLB formation. Decreased levels of *PhSERK2*, *3*, *4*, and *5* during later stages of PLB formation suggest that they may be critical for PLB maturation (Figure 5).

In summary, the expression pattern observed of all 5 *PhSERK*s during PLB formation suggests their involvement during *in vitro* cellular differentiation. Our results agree well with those of previous studies of other species showing the expression of *SERK*s during early stage of somatic embryogenesis and detection until somatic embryo formation.

Supplementary material

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