Current Plant Biology xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Current Plant Biology

journal homepage: www.elsevier.com/locate/cpb

# Isolation, phylogenetic analysis, and expression of a Somatic Embryogenesis Receptor like Kinase (*SERK*) gene in *Cattleya maxima* Lindl

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ARTICLE INFO

*Keywords:*
*CmSERK*
Somatic embryogenesis
Orchids
Leucine Rich Repeat
Molecular characterization

ABSTRACT

Orchidaceae is a central family of vascular plants in Ecuador because of its huge diversity and endemism. A prominent commercial native species well known for its richness of color is *Cattleya maxima* Lindl. Somatic embryogenesis is recognized as an important process for mass propagation of many ornamental crops including orchids. Somatic Embryogenesis Receptor like Kinases (*SERK*) genes, in particular, are highly expressed during the early phases of somatic embryogenesis and therefore, the study of their involvement in this process is of paramount importance to improve the commercial propagation of orchids. For this reason, we decided to isolate and characterize a *SERK* orthologue from *Cattleya maxima* L. The deduced amino acid sequence of a partial *CmSERK* cDNA shows the presence of all SERK typical domains, suggesting that it may be functionally active in *C. maxima*. Its role in somatic embryogenesis is further supported by its high expression level during embryogenesis and in protocorm-like bodies. Moreover, the basal expression of *CmSERK* in roots, leaves, and ovaries points to a broader developmental role of this gene.

## 1. Introduction

Plants Receptor Like Kinases (RLKs) are plasma membrane receptors responsible for the detection of extracellular signals, both biotic and abiotic, and for the sensing of endogenous stimuli [1–3]. RLKs typical organization includes an amino-terminal extracellular domain, a transmembrane domain, and an intracellular kinase domain [4].

SERKs proteins are a subgroup of RLKs involved in multiple plant-signaling pathways that govern important aspects of plant development such as growth, stomatal patterning, male sporogenesis, somatic embryogenesis, leaf senescence and are involved in the defense responses through the plant immune system [5–8]. A SERK protein consists of an N-terminal extracellular domain with five Leucine Rich Repeats (LRR1-5), a characteristic Proline-rich domain called the Ser-Pro-Pro motif (SPP), a single transmembrane domain and a cytoplasmic kinase domain, which can auto- and trans-phosphorylate Ser/Thr and Tyr residues [5].

In the model organism *Arabidopsis thaliana* five *SERK* paralogs have been identified (*AtSERK1-5*) [9], one of which (*AtSERK5)* has an unclear function or could even be a pseudogene [10]. Other *SERK* genes have been found in several dicot species such as *Populus trichocarpa* (*SERK1–4*), *Vitis vinifera* (*SERK 1-3*), *Gossypium hirsutu* (*SERK 1-3*), and others [11]. Multiple *SERK* paralogs have been reported also in monocots: *SERK1*, *SERK2* and *SERK3* for *Ananas comosus* [12,13], *Zea mays* [14], *Sorghum bicolor* [11] and *Triticum aestivum* [15], while only one *SERK* gene had been found in *Cocos nucifera* [16]. Aan den Toorn et al., [11] compared the aminoacid sequences of SERK proteins of monocot, dicot and non-vascular plants and found that SERK can be classified in four clades: non-vascular SERKs, monocots SERKs and two different clusters of dicots SERKs: one group containing SERK1 and SERK2, the other containing SERK3, 4 and 5. They also established that monocots SERKs are more closely related to members of the dicot SERK1-2 cluster.

*SERKs* were initially identified in carrot cell suspension as an embryogenic marker [17]. Somatic embryogenesis is the process through which embryos are formed from a somatic cell and is widely used for plant propagation since its discovery [18].

When multiple *SERK* paralogs are present, sub-functionalization occurs at some degree. Among the five *AtSERK* genes, for example, only *AtSERK1* was found to be expressed in small cell clusters during somatic embryogenesis, suggesting that *AtSERK1* is the principal component of the embryo signaling pathway for this species [19]. The *SERK1* gene of other species such as *Medicago truncatula*, *Solanum tuberosum* and *Citrus sinensis* is also highly expressed during somatic embryogenesis (See the revision of [8]), similarly to *AcSERK1* of *Ananas comosus* [13]. Contrarily, in *Zea mays* both *SERK1* and *SERK2* were reported to play a role

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https://doi.org/10.1016/j.cpb.2020.100139
Received 29 November 2019; Received in revised form 4 February 2020; Accepted 4 February 2020

in somatic embryogenesis.

Orchidaceae is the most diverse family of flowering plants [20]. Due to its particular and beautiful floral morphology, there is an increasing demand for species of this family for ornamental purposes, both as pot plants and cut flowers [21]. Orchids are one of the top horticultural traded plants and constitute 70 % of the species indexed in the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES) [22]. Somatic embryogenesis offers a tool to mass propagate orchids [23] allowing to meet the commercial demand of orchids as well as to conserve their biodiversity. *Cattleya maxima* Lindl. is a native epiphyte species distributed in the Andean and Coastal Ecuadorian regions [24].

Despite the importance for the vegetative propagation of orchids, to date, *SERK* has been characterized in only two species of this family: *Cyrtochilum loxense* (ClSERK) [25] and *Phalaenopsis* orchid (5 *SERK* paralogs) [26]. The poor knowledge about genes involved in orchids somatic embryogenesis is especially surprising in the case of *Cattleya maxima*, due to its prominent commercial value. To overcome this gap, it was decided to isolate a *CmSERK* cDNA and analyze its expression profile.

## 2. Materials and methods

### 2.1. Embryogenic callus induction

Embryogenic calli were obtained as previously described [27]. Protocorms of *C. maxima* were subjected to saline stress (4 h in 0.3 M NaCl) and then cultured in the auxin induction medium (1/2 MS with 0,1 mg $L^{-1}$2,4-D) for 30 days. The embryogenic calli were subsequently used for RNA extraction.

### 2.2. RNA extraction and cDNA synthesis

Extraction of total RNA from *C. maxima* tissues was performed using the RNeasy plant kit (QIAGEN) following the manufacturer´s instructions, including DNA digestion. Each sample (5 µL, corresponding to roughly 1.7–1.8 µg RNA) was analyzed by 1% agarose gel electrophoresis, and concentration was measured in a NanoDropTM 1000 Spectrophotometer (Thermo Scientific).

The absence of genomic DNA was verified using aliquots (1 µL) of the extracted RNA as template in a PCR reaction with primers designed on the conserved region of available orchid actin gene sequences (Table1). cDNA was synthesized using 1 µg of total RNA with the High Capacity RNA to cDNA kit (Applied Biosystem) according to the manufacturer´s instructions.

### 2.3. Isolation of CmSERK by PCR amplification

The isolation of *C. maxima SERK* gene was obtained using the same approach previously used for the *ClSERK* gene [25]. Degenerated primers: S1 and S2 (Table 1) designed on conserved regions of SERK genes previously reported [14] were used in two rounds of PCR performed employing cDNA as template. The first PCR reaction was performed with an Applied Biosystem thermocycler in 20 µL final volume making use of Platinum TAQ Polymerase High Fidelity (INVITROGEN). The following PCR conditions were used: one cycle at 94 °C for 5 min, followed by 35 amplification cycles (94 °C for 30 s, 45 °C for 30 s, 68 °C for 2 min) and a final extension cycle of 10 min at 68 °C. The second, touch-up, PCR was performed with the same primers using 1 µL of the first PCR reaction as template. The touch-up PCR conditions were: one cycle of initial denaturation at 94 °C (5 min), 10 "touch up" cycles with denaturation at 94 °C for 30 s, an initial annealing temperature of 40 °C for 30 s (with an increase in the annealing temperature of 1 °C each two cycles), and elongation at 68 °C for 2 min, followed by 20 cycles with annealing temperature of 45 °C and a final extension step at 68 °C for 5 min. This second reaction produced a single band of 900 bp, that was subsequently purified with Wizard® SV Gel and PCR Clean-Up System (Promega) and cloned in pGEM®-T Easy vector (PROMEGA). The plasmids were transformed into *E. coli* and the insert sequenced. With the obtained sequences a search Gen Bank and EMBL databases applying the BLAST algorithm were performed [29].

### 2.4. Rapid amplification of 5´untranslated end

To obtain the complete sequence of *CmSERK*, the 5′ untranslated region (UTR) was amplified with a Rapid Amplification of 5′cDNA Ends using the 5′RACE system by Invitrogen, according to the manufacturer's instructions except that dNTPs were added after heating the PCR mix to 80 °C. The first-strand cDNA was synthesized from mRNA (~4 µg of total RNA) extracted from embryogenic calli using a gene-specific

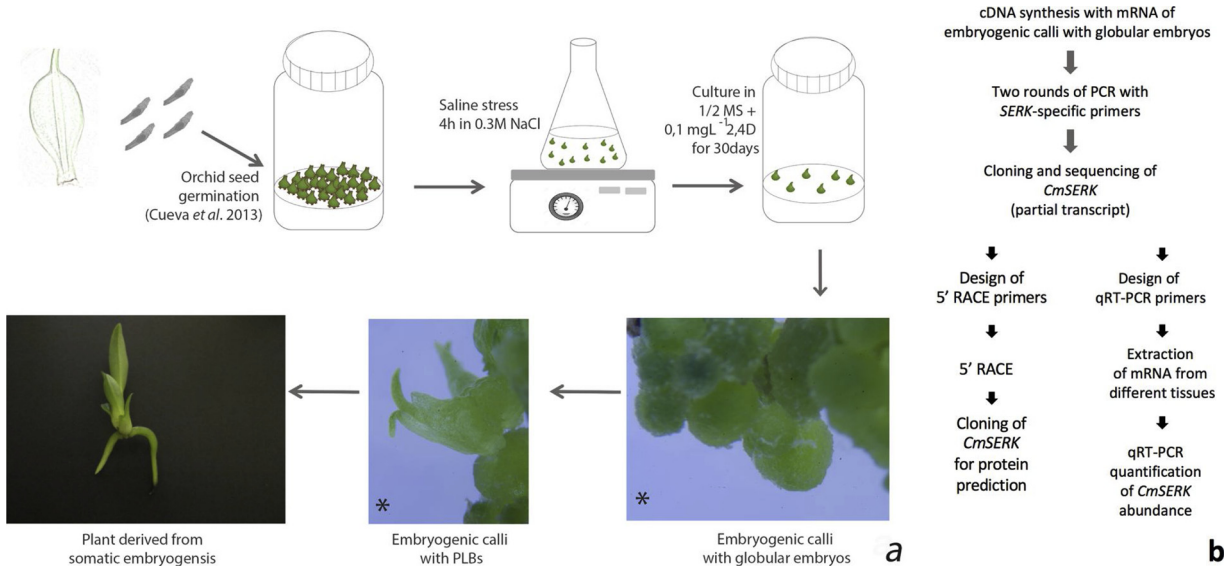



**Fig. 1.** Flowchart of the methods used. a) Process of somatic embryogenesis induction. After germination, protocorms were treated with saline stress and then cultured for 30 days in induction medium to induce calli formation. Further culturing of the induced calli generates gobular embryos that subsequently develop into Protocorms Like Bodies (PLBs). The tissues used for mRNA extraction employed for *CmSERK* cloning and qRT-PCR are indicated with an asterisk (*) b) Steps of the molecular protocol applied in this study.

**Table 1**
Details of primers used in this study.

| Primer | Sequence (5´to 3´) | Purpose | Reference |
|---|---|---|---|
| *S2* | CGRTGMACWGCCATRCTIATCAT | *CmSERK* isolation | [14] |
| S3 | GTGAAYCCTTGCACATGGTTYCATGT | *CmSERK* isolation | [14] |
| CmSERK-GSP1 | ACGGCGACAAGTGAACCATC | 5´RACE | |
| CmSERK-GSP2 | CCTTTCCAAATCCACCTCTGC | 5´RACE | |
| CmSERK-GSPN3 | TAGGAATGGTGCCCGTCAAG | 5´RACE | |
| ∝ Tubulin Fw | GGATTAGGCTCTCTGCTGTTGG | *CmSERK* espression | [28] |
| ∝ Tubulin Rv | GTGTGGATAAGACGCTGTTGTATG | *CmSERK* espression | [28] |
| SERK1_Cm Fw | TTTAATCCTCCGGTTACAGTCT | *CmSERK* espression | |
| SERK1_Cm Rv | AATGTTCTTGTGGCTTACGACG | *CmSERK* espression | |

primer (*CmSERK-GSP1*, Table 1). A first PCR 5′UTR amplification was performed in an Applied Biosystem Thermocycler employing gene-specific nested primers (*CmSERK-GSP2,* Table 1). A nested PCR was performed with a second gene-specific primer (*CmSERK-GSPN3*, Table 1) annealing downstream of the UTR. Amplification products were then purified with Wizard® SV Gel and PCR Clean-Up System (Promega), cloned in pGEM-T Easy (Promega), and sequenced.

### 2.5. Sequence analysis and motif prediction

The targeting of the protein inferred from the cDNA sequence was predicted utilizing PSORT (Prediction of Protein Sorting Signals and Localization Sites in Amino Acid Sequences) (http://psort.nibb.ac.jp/; [30]) and Scan Prosite [31].

The protein sequence was also analyzed by searching for conserved motifs in CCD database [32]. Signal P3.0 was used to confirm the presence of a signal peptide [33].

### 2.6. Amino acid sequence alignments and phylogenetic analysis

CLUSTAL X version 2.0.11 [34] was used to perform the alignment of the amino acid sequences of CmSERK and other monocots, dicots, and non-vascular SERK. Parameters for the alignment were: Gonnet series as protein weight matrix, gap extension penalty equal to 0.2, gap, open penalty equal to 10, and divergence sequences delayed at 30 %. The phylogenetic analysis was inferred using the Neighbor-Joining (NJ) method [35]. Dayhoff method [36] was used to compute distances and are in the units of the number of amino acid substitutions per site. The values of replicates for the tree are based on 1000 bootstrap tests [37]. The analysis was conducted in MEGA X [38].

### 2.7. CmSERK expression analysis

The specific primers used for the analysis of *CmSERK* expression are SERK1_Cm_RT_Fw and SERK1_Cm_RT_Rv (Table 1). These primers amplify a fragment of 151 bp and were designed to overlap the boundary of two adjacent exons to prevent the amplification of any unwanted genomic DNA. RNA extraction and cDNA synthesis were performed as explained above from different tissues: pollinia, ovary, leaves, leaf tips, roots, root tips, calli with globular embryos, calli with protocorms like bodies, protocorms in MS + sorbitol and protocorms in MS + 2,4-D. Three biological replicates of each sample were used with two technical replicates each. The relative expression was quantified with an Applied Biosystems 7500 Fast Real-Time PCR System using Fast SYBR® Green Master Mix (Applied Biosystem) following the manufacturer's instructions. The PCR was performed using the next cycling conditions: one cycle at 95 °C for 10 min and 45 cycles at 95 °C/10 s, 58 °C/15 s, and 72 °C/30 s. RT-qPCR expression levels were normalized to the ∝-tubulin level for each tissue with the primers designed on the sequence of *Oncidium* cv "Gower Ramsey" following the specification of Hou and Yang [28] (Table 1). PCR efficiency of each run was determined with the LinRegPCR program http://LinRegPCR.nl [39]. To highlight difference of expression an Analysis of variance (ANOVA) with Tukey posterior test was performed, using R statistical environment [40]

## 3. Results

### 3.1. Isolation of C. maxima SERK (CmSERK)

Degenerated primer designed on the basis of conserved regions of the zipper domain (S3) and a reverse primer situated at the conserved regions of the kinase domain (S2) of *Arabidopsis* and carrot SERK genes [14] were used to amplify the *CmSERK* sequence. The first round of PCR with S2/S3 primer pair did not yield a single band detectable by agarose gel electrophoresis (not shown). On the contrary, a second, nested touch-up PCR using the same primers, gave a single band of 900 bp.

Besides, we used a 5´RACE assay to extend the sequence to1060 bp. This partial sequence was registered as *CmSERK* gene in the EMBL database (EMBL accession number HE587946).

### 3.2. Sequence analysis and motif prediction

The deduced polypeptide, truncated at the C-terminus, consists of 356 amino acids; the analysis of the amino acid sequence confirmed that it belongs to the Protein Kinases Superfamily (Cl09925 E-value: $2{,}23^{e-07}$) [32].

The protein sequence shows several SERK typical domains: a SPP motif (36 aa), the hallmark of SERK proteins, which was proposed to act as a "hinge" that gives flexibility to the extracellular domain of the protein [9]; a 28 amino-acids Signal Peptide (SP), with a possible cleavage site between amino acids 25 and 26, that was confirmed by Signal P3.0 [41] with and a; a Leucine zipper (ZIP) domain (45 aa) involved in the oligomerization of AtSERK1 [42]; five LRR domains (for a total of 119 aa) which are associated with the protein-protein interaction [43] and are necessary to have a correct target of the protein to the plasma membrane [42]; the transmembrane domain (TM, 43 aa) with a transmembrane region involving amino acids 240–246 and the cytoplasmic tail from the amino acid residue 263; and, finally a truncated Kinase domain (83 aa) starting from amino acid 288 (E-value: $5{,}4^{e-15}$).

The truncated CmSERK protein is 96 % identical to ClSERK and 93 % identical to *Dendrobium officinale SERK* (DoSERK) sequence annotated in the NCBI database. From the other monocots, the more related is AcSERK (87 %). The Signal Peptide and SPP domains show the lowest homology compared to the other considered proteins (Fig. 2).

### 3.3. Relation of CmSERK to other LRR-RLKs

To investigate the position of *CmSERK* within the LRR-RLK family, we performed an additional analysis of the canonical sequences of the Leucine Repeats of *CmSERK* and other plants LRR-RLK sequences. For this purpose, the sequences of the five LRRs of *C. maxima* with

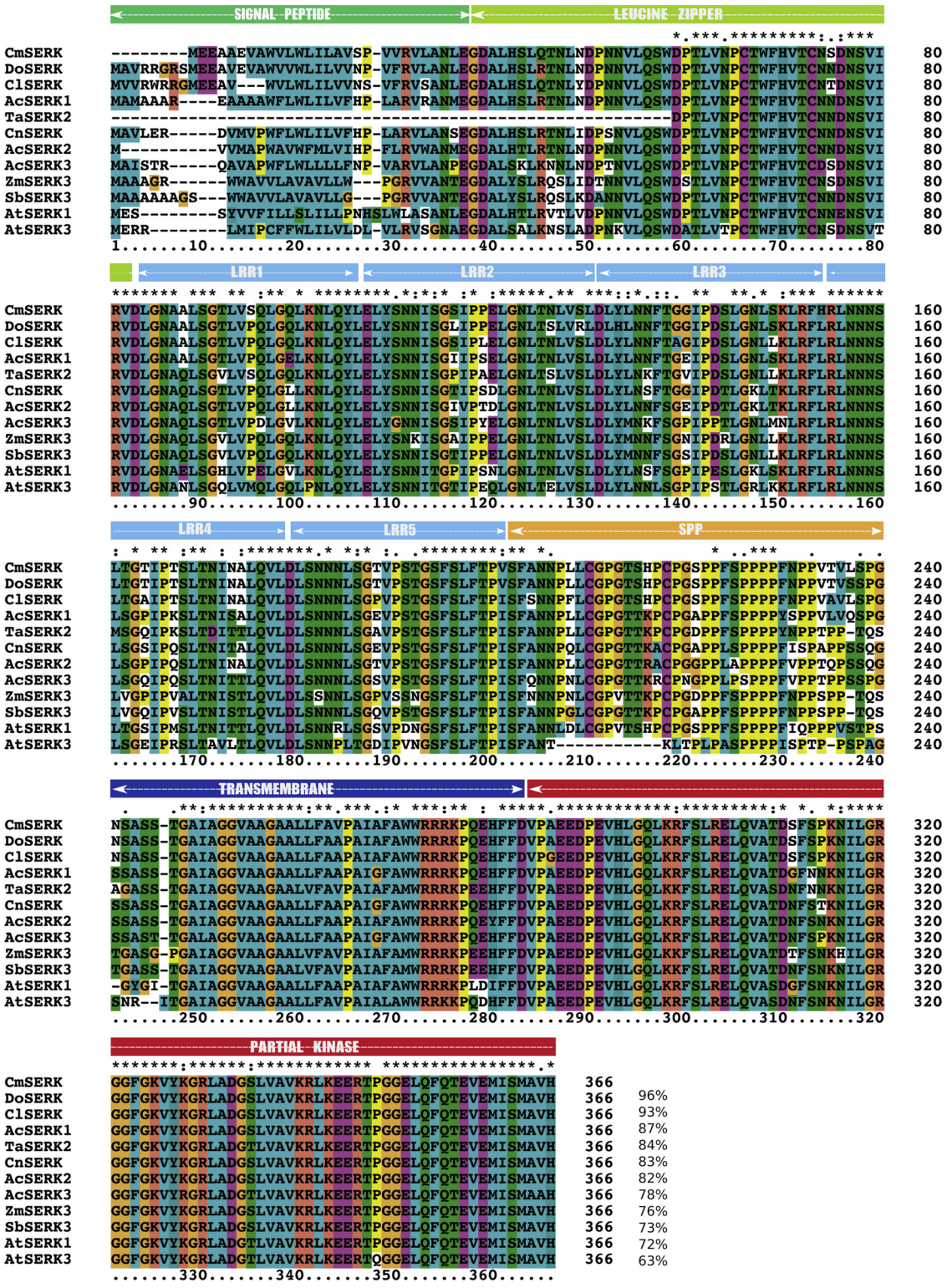
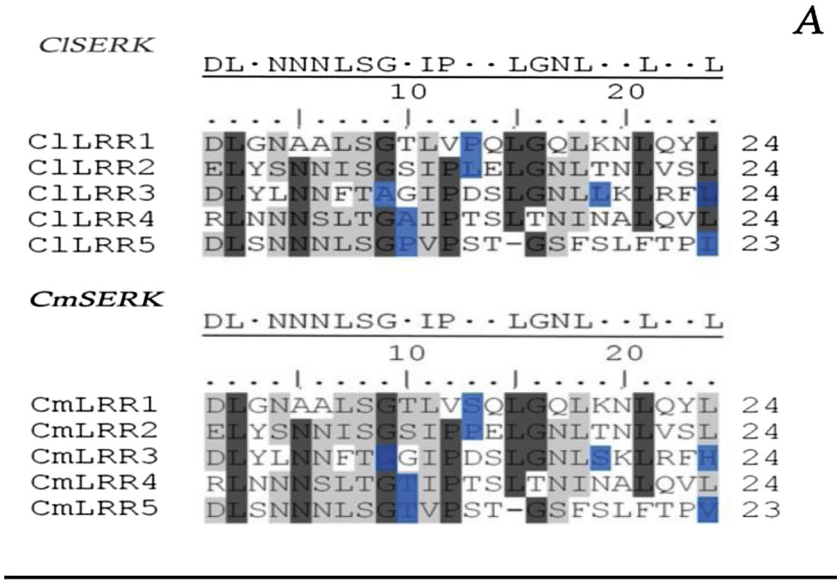



**Fig. 2.** Alignment of the partial predicted amino acid sequences of CmSERK and other SERKs family protein kinases. Alignment was performed using ClustalX 2.0.11. Clustal consensus are showed. Percentages at the end of the alignment indicate amino acid identity among considered CmSERK.

CLUSTAL (Fig. 3a) were aligned to obtain a consensus sequence. A separate alignment with the five LRRs of ClSERK [25], yielded a second consensus. The analysis showed that LRR of the two Orchids' SERK are very similar, in both species the LRR 1–4 show 24 residues while LRR5 has 23 residues.

We then compared the two consensus sequences with those obtained from other LRR-RLK proteins described in the literature. For this analysis, a representative protein for five of the fourteen LRR subfamilies were used [44]. All the conserved Leu residues present in the considered plant LRR canonical sequences are also present in both ClSERK and CmSERK (Fig. 3b).



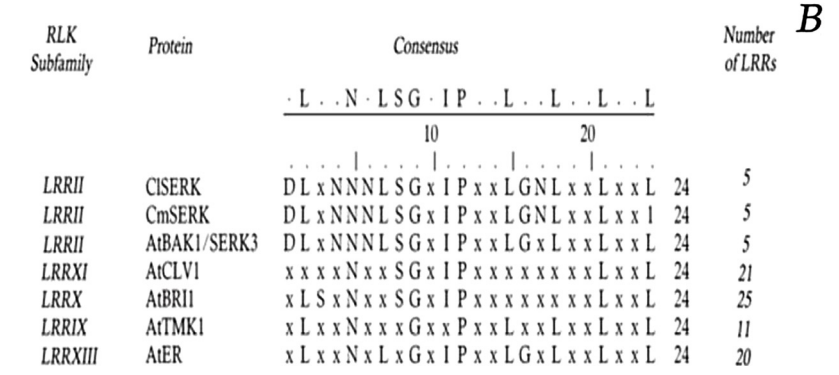




**Fig. 3.** Analysis of the Relation of Orchid SERKs to other LRR-RLKs LRR A. Consensus sequences of two orchid SERKs (CmSERK and ClSERK) LRRs 1 to 5, analyzed with the Clustal algorithm. B. Alignment of the consensus sequences of the LRRs of RLK subfamily proteins. LRR consensus sequences of ClSERK and CmSERK were obtained with the Clustal algorithm, while the consensus and number of other LRRs were obtained from the literature. X represents any amino acid. Apart the orchid ones, all considered proteins are from *A. thaliana*: AtBAK1/SERK3, BRI1 associated kinase or Somatic Embryogenesis Receptor like Kinase 3; AtCLV1, CLAVATA1 protein; AtBRI1, Brassinosteroid Intensive gene product; AtTMK1, transmembrane protein; and AtER, ERECTA protein.

### 3.4. Relation of CmSERK to other SERKs

A phylogenetic analysis of *CmSERK* was performed to compare the deduced amino acid sequence with those of 37*SERK* genes: 16 from monocots species, 13 from dicot species and 8 from non- vascular plants. The resulting phylogenetic tree (Fig. 4) revealed four clusters: Monocot SERK, Dicot SERK1/2, non-vascular plants SERKs and Dicots SERKs 3/4/5. Monocots SERKs fell in the same group as Dicots SERK1/2.

### 3.5. Expression analysis

To assess whether this gene plays multiple roles in orchids as in other organisms, we analyzed the expression of *CmSERK* gene in different tissues, including both embryogenic and not embryogenic. For not embryogenic tissue we analyzed: pollinia, ovary, leaves, leaf tips, root and root tips. For the embryogenic tissues we analyzed two different stages of embryogenic calli development, calli with globular embryos or calli with PLB, and finally in protocorms previously exposed to sorbitol or 2,4-D, two treatments known to stimulate somatic embryogenesis in this species [27]. *CmSERK* expression was observed in all the analyzed tissues. The expression was significantly higher ($p < 0,02$) in calli with globular embryos when compared to the non embryogenic tissue and protocorms exposed to sorbitol. As shown in Fig. 5, the highest expression level was found in embryogenic calli, particularly those characterized by the presence of globular embryos at a stage immediately preceding the appearance of PLBs, which are in fact the equivalent of somatic embryos in orchids ([23] and [45]). This finding confirms the role of *SERK* in promoting somatic embryogenesis and is consistent with the expression pattern previously observed in the case of the orchid *C. loxense* [25]. The second highest expression level was observed in calli with PLBs, pointing at a function of *SERK* in maintaining the somatic embryogenesis pathway in orchids.

## 4. Discussion

The SERK genes encode for leucine-rich repeat receptor-like kinases (LRR-RLKs) [9] of the LRRII subfamily. In the genome of *A. thaliana*, there are 220 LRR-RLKs proteins, five of them are SERKs [5]. Following the first discovery of carrot SERK gene [17], many other SERK genes had been characterized for several species. Orchid SERK genes have been reported in three species so far: one in the Andean orchid *C. loxense* [25], five in *Phalaenopsis* [26] and one in *Dendrobium officinale* (available at the NCBI database under the accession AKN89445). The respective predicted proteins show specific amino acid residues typical of the LRRII subfamily, D in position 1, N in position 4 and 6, and G in position 16, all of which are only occasionally present in the consensus sequences of other plant LRRs. The number of repeats is similar to those of the SERK family members and considerably lower than those present in the majority of other LRR-RLK proteins (Fig. 3).

Here we present the isolation and characterization of a cDNA from the orchid *Cattleya maxima*, *CmSERK*, whose predicted amino acid sequence shows all SERK specific domains (Fig. 2). Using the predicted amino acid sequence of CmSERK a phylogenetic comparison was performed (Fig. 4) with SERKs of 37 other species, including orchid SERKs with the exception of *Phalaenopsis* SERKs because not publicly available.

All the monocots SERK protein sequences were grouped in a cluster,

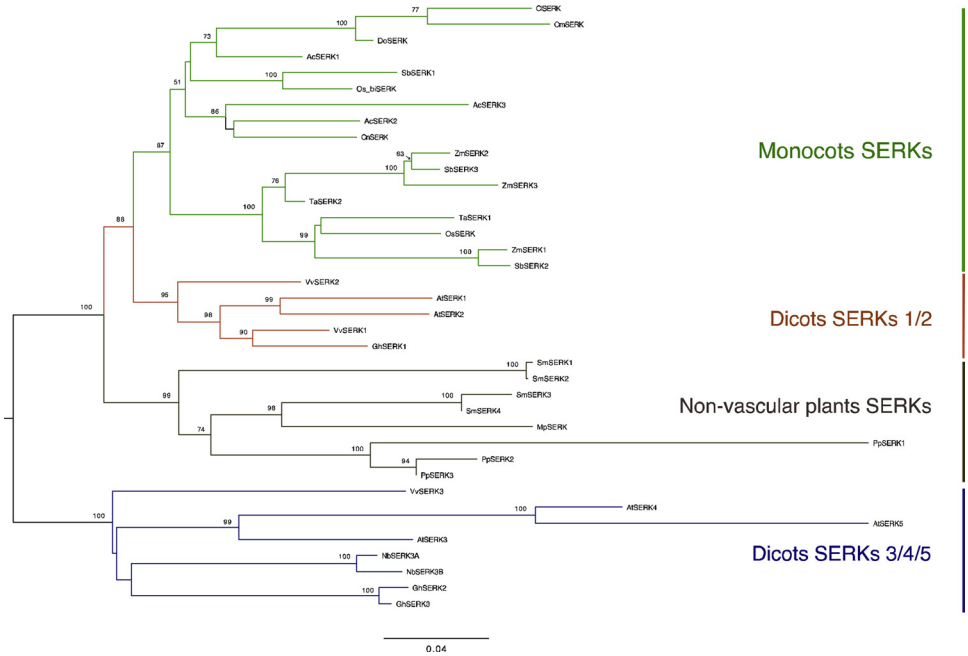



**Fig. 4.** Phylogenetic tree showing the relationships between the deduced amino acid sequence of *Cattleya maxima* SERK compared to SERK proteins of other species. The multiple alignment of *Cattleya maxima* CmSERK protein (HE587946), *Cyrtochilum loxense* ClSERK (FN994192), *Dendrobium officinale* DoSERK (KP009862), *Ananas comosus* AcSERK1 (AEC46975), AcSERK2 (AEC46976), AcSERK3 (AEC46977), *Cocos nucifera* CnSERK (CnSERK-Q5S1N9), *Sorghum bicolor* SbSERK1 (XM_002443894), SbSERK2 (XM_002447912), SbSERK3 (XM_002454009), *Oryza sativa* OsSERK (AAU88198), Os-biSERK (AAR26543), *Zea mays* ZmSERK1 (AJ277702), ZmSERK2 (AJ277703), ZmSERK3 (BT042695), *Triticum aestivum* TaSERK1 (JF808017), TaSERK2 (JF808018), *Arabidopsis thaliana* AtSERK1 (NM_105841), AtSERK2 (NM_1031144), AtSERK3 (NM_119497), AtSER4 (NM_126955), AtSERK5 (NM_126956), *Vitis vinifera* VvSERK1 (XM_002270811), VvSERK2 (XM_002276378), VvSERK3 (XM_002262662), *Gossypium hirsutum* GhSERK1 (HQ621831), GhSERK2 (JF430801), GhSERK3 (JF800909), *Nicotiana benthamiana* NbSERK3A (HQ332144), NbSERK3B (HQ332145), *Selaginella moellendorffii* SmSERK1 (XM_002980668), SmSERK2 (XM_002976930), SmSERK3 (XM_002978263), SmSERK3 (XM_002978263), SmSERK4 (XM_002966218), *Marchantia polymorpha* MpSERK (AB306524), *Physcomitrella pattens* PpSERK1 (XM_001769715), PpSERK2 (XM_001767626) and PpSERK3 (XM_001759070) was performed by ClustalX 2.0.11 software and the Phylogenetic tree was constructed by the Neighbor-Joining method and evaluated by 1000 bootstrap analysis (MEGA X).

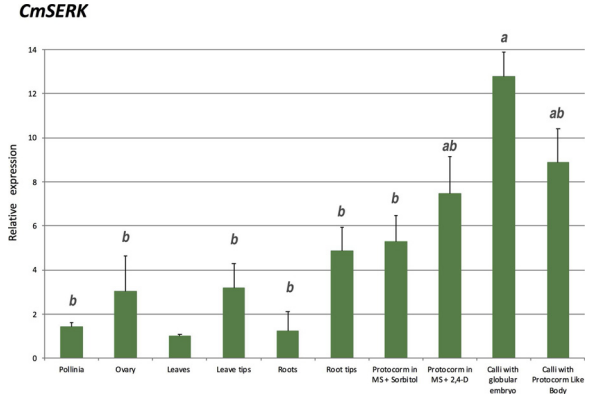



**Fig. 5.** Relative expression of *CmSERK* gene as determined by reverse transcription-qPCR analysis. Results shown are means ± standard error of three independent samples calibrated to the expression of the leaf. Same letters above the bars are not significantly different ($p > 0.05$) according to Tukey´s test. Some of the tissues used in the analysis are reported in the Fig. 1.

within which CmSERK was closely linked to the other orchid SERKs (ClSERK and DoSERK) thus forming a sub-cluster. This finding is likely to reflect the evolutionary history of the major lineages of monocots [46], in which Asparagales (Orchidaceae) is a separated group. All dicots SERK1 and SERK2 are grouped in the same clade (Dicots SERK1/2), that is relatively close to the monocots cluster. The same phylogenetic structure was found by Aan den Toorn et al., [11], confirming that monocots SERK genes are more similar to SERK1/2 of dicots. Supernumerary SERK proteins of analyzed dicots species that possess more than two paralogues, like *A. thaliana*, *Gossypium hirsutum,* and *Nicotiana benthamiana*, were grouped in a distinct branch from SERK1 and 2. This result is in accordance to that reported by Aan den Toorn et al., [11] who concluded that the duplication event from which the divergence originated is dicot-specific and occurred after the split between monocot and dicot plants. Summarizing, the phylogenetic distance between SERK proteins mirrors the evolutionary history of the species considered. Whether these large-scale duplications were followed by some form of sub-functionalization between SERK paralogues, as occurred for members of other *Arabidopsis* gene families in [47], remains an open question.

In general, SERK genes display broad functional plasticity, controlling multiple plant pathways of biotechnological interest. It was early predicted that SERK genes are involved in somatic embryogenesis, hence the name. However, it is now clear that SERK genes control many other plant developmental processes like microsporogenesis, brassinosteroid- and phytosulfokine-dependent growth, immune responses and cell death [5].

In Orchids, the expression of *SERK* genes and the consequent establishment of the somatic embryogenesis developmental process can be triggered by environmental factors. In the case of *C. maxima,* somatic embryogenesis has been induced by osmotic stress followed by cellular proliferation [27]. In this work, a high level of *CmSERK* transcripts was observed also in protocorms cultivated in either MS plus sorbitol or in MS plus 2,4-D, indicating that both these factors can induce somatic embryogenesis independently. In addition to expression in calli and in response to inducing factors, we also observed *CmSERK* expression in roots, leaves, and ovary, suggesting that its role is not limited to somatic embryogenesis. Further studies will be necessary to elucidate the role of SERK genes in orchids and other monocots.

The questions if monocots SERKs, which are not related to dicots SERK3/4, can accomplish the same functions as SERK 3/4, such as plant immune responses, need more studies to be fully clarified.

## 5. Conclusion

Orchids are a plant family of economic importance. Somatic embryogenesis, the generation of embryo from sporophytic tissue, can be employed to facilitate Orchids propagation. Embryogenesis Receptor like Kinases (*SERK*) genes are involved in the onset of somatic embryogenesis. Here *CmSERK* was identified, the *SERK* homolog in *Cattleya maxima*, a prominent Orchid species. Both the analysis of conserved protein domains and the phylogenetic position relative to *SERK* genes previously known are consistent with the putative role of *CmSERK*. Direct quantification of *CmSERK* expression confirmed that it is highly

expressed in SE-competent tissues, either proliferation calli or protocorms exposed to with SE-inducting treatments, such as osmotic stress or proliferation hormones.

Therefore, *CmSERK* represent the ideal molecular target for the activation of SE pathway, paving the way for the application of this technology to the large-scale propagation of this important species.

## Author contributions

Cueva-Agila A, Cella R and Concia L conceived and designed research. Cueva-Agila A and Concia L conducted experiments and analyzed data. Alberca-Jaramillo N. conducted part of the experiments. Cueva-Agila A and Concia L wrote the manuscript. All authors read and approved the manuscript.

## Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Acknowledgements

This work was financed by the Universidad Técnica Particular de Loja, LOja, Ecuador (UTPL) Through the project “Estudio de caracterización y expresión del gen *SERK* en orquídeas” and partially supported by a fellowship of the Secretaría Nacional de Ciencia y Tecnología, Ecuador (SENESCYT) awarded to A. Cueva-Agila.

The authors express their gratitude to Omar Malagón A. for reading of the manuscript.

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