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ORIGINAL ARTICLE

A passion fruit putative ortholog of the *SOMATIC EMBRYOGENESIS RECEPTOR KINASE1* gene is expressed throughout the in vitro de novo shoot organogenesis developmental program

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Abstract The *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1* (*SERK1*) gene is known to be related to early somatic and zygotic embryogenesis of numerous species. However, few studies have also shown the involvement of this gene during de novo shoot organogenesis developmental program. Here we report the cloning and characterization of the gene expression pattern of a *Passiflora edulis* putative ortholog, named *PeSERK1*, during DNSO-induction from hypocotyl and root explants. *PeSERK1* likely encodes a leucine-rich repeat receptor-like kinase showing high sequence similarity to other known SERK1 proteins. The results of in situ hybridization experiments evidenced a dynamic spatial expression pattern for *PeSERK1* throughout the DNSO pathway. *PeSERK1* transcripts were already detected at the initial hypocotyl and root explants, coincidental with provascular tissue differentiation. After 1 week of culture, *PeSERK1* expression was also observed in cells with an intense mitotic activity, at the site of callus initiation. The *PeSERK1* expression was observed mainly in actively dividing cells from which meristemoids, shoot-like structures or provascular elements were produced. At later stages of DNSO-induction, *PeSERK1* was preferentially expressed at the differentiating shoot apical meristem, including the leaf primordia, and in the procambium. These data indicate that *PeSERK1* might have a role during the organogenesis developmental program in *Passiflora,* apparently associated with the differentiation processes and with the maintenance of a cellular-competent state.

Keywords SERK · Shoot regeneration · Pluripotency · Cell division · In situ hybridization · *Passiflora*

Introduction

The ability of plants to generate new and adventitious organs throughout their whole life is the basis for the application of tissue culture techniques and for the establishment of plant regeneration systems. According to the recent review of Xu and Huang (2014), higher plants show three main types of regeneration systems: tissue regeneration, de novo organogenesis and somatic embryogenesis. The last two types of regeneration pathways are extensively used, either for research or for practical applications (Motte et al. 2014; Xu and Huang 2014). However, in contrast to somatic embryogenesis, the organogenic pathway is preferentially used in contemporary plant biotechnology because the culture conditions are relatively simple and the results are robust (Duclercq et al. 2011).

De novo organogenesis refers to the formation of shoots and roots from cultured explants, in in vitro conditions. This organogenic process is mainly influenced by the type of explant in combination with the use of growth regulators. The most common organogenic pathway is the de novo shoot organogenesis (DNSO) which, according to the classical finding of Skoog and Miller (1957), is induced in a high citokinin-to-auxin ratio condition for the majority of plant species. DNSO can be divided into three morphological

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stages: competence acquisition, induction and morphological differentiation (Duclercq et al. 2011). The acquisition of competence precedes the stage of induction where the initial competent cell or tissue becomes committed to form the induced organ (Wareing 1982; Duclercq et al. 2011). Although the regeneration pattern of some species does not strictly follow the established stages, the first step in the process (i.e. the acquisition of competence of a given cell or tissue to assume a new developmental fate) is certainly conserved (Duclercq et al. 2011) and is also a key step of the somatic embryogenesis pathway (Yang and Zhang 2010).

Many studies have suggested a link between the acquisition of the cellular-competent state and the expression of *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1* (*SERK1*) gene during embryogenic developmental program in plants (Kwaaitaal and de Vries 2007; Savona et al. 2012; Pilarska et al. 2015; Rocha et al. 2015). The *SERK* genes encode a transmembrane protein kinase belonging to the family of leucine-rich repeat receptor-like kinases (LRR-RLKs; Hecht et al. 2001). The *SERK* gene was initially isolated from carrot (*DcSERK*), and it was observed that it is specifically expressed in carrot embryogenic cell cultures (Schmidt et al. 1997). A functional ortholog of the *DcSERK* was afterwards identified in somatic embryogenic cultures of *Arabidopsis thaliana* (Hecht et al. 2001). Additional putative *SERK* genes have been identified in a wide range of plant species, including eudicots (Nolan et al. 2003; Thomas et al. 2004; Santos et al. 2005; Shimada et al. 2005; Sharma et al. 2008; Schellenbaum et al. 2008; Talapatra et al. 2013; Silva et al. 2014; Pilarska et al. 2015), monocots (Baudino et al. 2001; Hu et al. 2005; Pérez-Nuñez et al. 2009; Huang et al. 2010), and gymnosperms (Steiner et al. 2012). Although the vast majority of these studies suggested a putative function of the *SERK* genes in early stages of somatic embryogenesis, it has been recently suggested that the SERK proteins might have a broadened role in other developmental processes, including the DNSO pathway (Nolan et al. 2009; Savona et al. 2012; Li et al. 2015).

Although plant regeneration through both somatic embryogenesis and DNSO has long been described for passion fruit (see review by Otoni et al. 2013), the molecular basis of de novo morphogenesis has only recently deserved some attention (Rosa et al. 2013a, b; Rocha et al. 2015). We have recently described the cellular and molecular changes associated to somatic embryogenesis in *P. edulis* (Rocha et al. 2015) and here we contrast these results to the potential role of a *P. edulis* putative ortholog of *SERK1* during the organogenesis developmental program in *Passiflora,* where it is apparently associated with the differentiation processes and with the maintenance of a cellular-competent state.

Materials and methods

Plant material

Seeds of *P. edulis* from the Maguary ''FB-100'' population were obtained from Flora Brasil, Ltda (Araguari, MG, http://www.viveiroflorabrasil.com.br). The seed coats were removed, and the seeds were surface sterilized and rinsed in sterile water. The seeds were subsequently transferred to 250 mL glass jars (5 seeds per jar; a total of 100 seeds) containing 40 mL half-strength MS medium (Murashige and Skoog 1962) supplemented with B5 vitamins complex (Gamborg et al. 1968), myo-inositol (0.01 % w/v), sucrose (3 % w/v), and Phytagel (0.25 % w/v) (Sigma Chemical Co., USA); the pH of the medium was adjusted to 5.7 ± 0.1. The jars were sealed with rigid polypropylene lids. All jars were kept in the dark for 15 days, until the seeds germinated. The seedlings were transferred to a temperature-controlled growth room (27 ± 2 °C) under 16-h photoperiod, photon lux density of 150 μmol m^{-2} s^{-1} (day-light fluorescent lamp) for 15 days.

Induction of de novo shoot organogenesis

In vitro DNSO-induction from *P. edulis* hypocotyl and root explants was performed according to Dornelas and Vieira (1994) and Silva et al. (2011), respectively. Hypocotyls and root segments (10–20 mm in length) from 30-day-old seedlings were excised and incubated in 90 × 15-mm polystyrene Petri dishes (J. Prolab, Brazil) containing 25 mL MS medium supplemented with B5 vitamins, myo-inositol (0.01 % w/v), sucrose (3 % w/v), Phytagel (0.25 % w/v), and 4.44 μM 6-benzyladenine; the pH of the medium was adjusted to 5.7 ± 0.1. The plates were sealed with Nexcare Micropore tape (3 M, Brazil) and kept in the same light and temperature conditions as described previously. A total of 15 plates for each type of explant (hypocotyl or root) were inoculated, with 10 explants in each plate.

Scanning electron microscopy (SEM)

Hypocotyl and root segments were collected after 0, 7, 14, 21, 28 and 35 days of culture in induction media, fixed in 4 % paraformaldehyde in 0.05 M phosphate buffer, pH 6.8, at 4 °C for 24 h. The samples were dehydrated in an ethanol series and critical point dried (CPD 030; Bal-Tec, Balzers, Liechtenstein). After mounting on aluminum stubs, the samples coated with colloidal gold (FDU 010; Bal-Tec, Balzers, Liechtenstein). Examinations and photography were performed at 10–20 kV under a JEOL JSM-5800 LV scanning electron microscope (JEOL Ltd. Tokyo, Japan).

Gene cloning and sequence analyses

We used a local BLAST tool (Altschul et al. 1997) and the *Arabidopsis thaliana* SERK1 (At1g71830) protein sequence as a bait, to search the PASSIOMA Expressed Sequence Tag (EST) database (Dornelas et al. 2006; Cutri and Dornelas 2012) in order to obtain putative *P. edulis SERK1* sequences. We found a single EST clone named PACEPE2105H12 showing high similarity (e-value = e−147) to the *Arabidopsis* SERK1 sequence and we chose this clone for further characterization. After transforming electro-competent *E.coli* DH5-alpha cells with the PACEPE2105H12 plasmid, ten positive clones were sequenced (3100 Genetic Analyzer, Applied Biosystems). The obtained sequences were processed using the CAP3 (Huang and Madan 1999) algorithm of the BioEdit software (Carlsbad, CA). The blastx tool of BLAST (Altschul et al. 1997) was used to compare the contigs to public databases at NCBI. Multiple sequence alignments of the deduced amino acid sequence of the *P. edulis* putative ortholog of SERK1 and other plant SERK orthologs were performed using CLUSTALX (Thompson et al. 1994). Neighbor-joining matrices (Saitou and Nei 1987) were used to obtain distance trees. Parsimony trees were obtained with MEGA4 (Tamura et al. 2007) using hand-corrected sequence alignments. Bootstrap values were obtained from 1000 replicates and visualized with TreeView (Page 1996). Pfam (Finn et al. 2010) was used to detect conserved protein motifs in the deduced protein sequences derived from the obtained sequences and the presence of a putative signal peptide was investigated using the server-based SignalP 4.1 (Petersen et al. 2011) and Signal-BLAST (Frank and Sippl 2008). Theoretical predictions of molecular weight and pI were performed using ExPASy (Artimo et al. 2012). We assessed the conservation of the protein structure by obtaining conservation scores as determined by the ConSurf webserver (Glaser et al. 2003; Landau et al. 2005; Ashkenazy et al. 2010), which were plotted onto the reported structure of the SERK1 extracellular domain (PDB:4LSC; Santiago et al. 2013), using as an input the same multiple sequence alignment used above for the distance trees.

In situ hybridization

In situ hybridization experiments were performed with hypocotyl and root explants collected at the same time-points of the DNSO-induction used for SEM analysis. The samples were fixed in 4 % paraformaldehyde at 4 °C overnight, and then dehydrated through a series of graded ethanol, as described above. The in situ hybridization protocol was essentially the one described by Rocha et al. (2015): A proteinase K pre-treatment (10 µg mL^{-1} in Tris–HCl, pH 7.5) was performed on deparaffinised slides at 37 °C for 10 min. Alternatively sense (control) or antisense DIG-labeled *PeSERK* (a fragment containing the last 1522 bp of the cDNA) RNA probes were used. The hybridization was performed at 42 °C for 16 h. To visualize the hybridization signal, anti-DIG antibodies (Roche, diluted 1:2000) conjugated to alkaline phosphatase were applied for 1 h at 37 °C and the hybridization signal was detected by reaction with NBT/BCIP (Pierce, USA). The hybridized slides were observed and documented using a Zeiss Axioskope microscope with AxioCam HRc digital camera.

Results

De novo shoot organogenesis from hypocotyl and root explants

In order to study the complete time course of DNSO pathway in passion fruit (*P. edulis*) obtained from both hypocotyl and root explants, the morphogenetic responses were followed from the initial explant until the shoot-like structures became visible to the naked eye.

Regeneration of shoots from hypocotyl explants occurred, primarily, at the cut surfaces of the explants (Fig. 1). The first morphological changes were recorded during the first week of culture, when the hypocotyl explants became slightly swollen (Fig. 1a) and an intense cell proliferation, observed at the cut explants surface, gave rise to the callus (Fig. 1b). The callus growth progressed and after 12-14 days of culture, the first meristemoids (organogenic sectors) were observed as small structures emerging on the surface of the explant (Fig. 1c,dD). These structures continued their development originating shoot buds (Fig. 1e–g). Completely regenerated shoots were observed after 25–30 days of culture (Fig. 1h–j).

The regeneration of shoots from root explants initiated at the cut surfaces of the explants, involving callus formation, as described above for hypocotyl explants (Fig. 2a–j). Nonetheless, regeneration was also observed in regions located far from the cut surface of the explant (Fig. 2k–n). At the cut surfaces, callus formation started during the first week after culture initiation (Fig. 2a, b). After a proliferation stage where calli increased in volume (Fig. 2c), the formation of meristemoids was observed after 14 days of culture (Fig. 2d, e). The meristemoids gradually developed into shoot meristems (Fig. 2f–h). After 28–35 days, the cut surface of the root explants was covered with regenerated shoot buds (Fig. 2i, j). On the other hand, some organogenic structures were also observed differentiating directly from within the root explant (Fig. 2k, l) after 2 weeks of culture. These structures arose

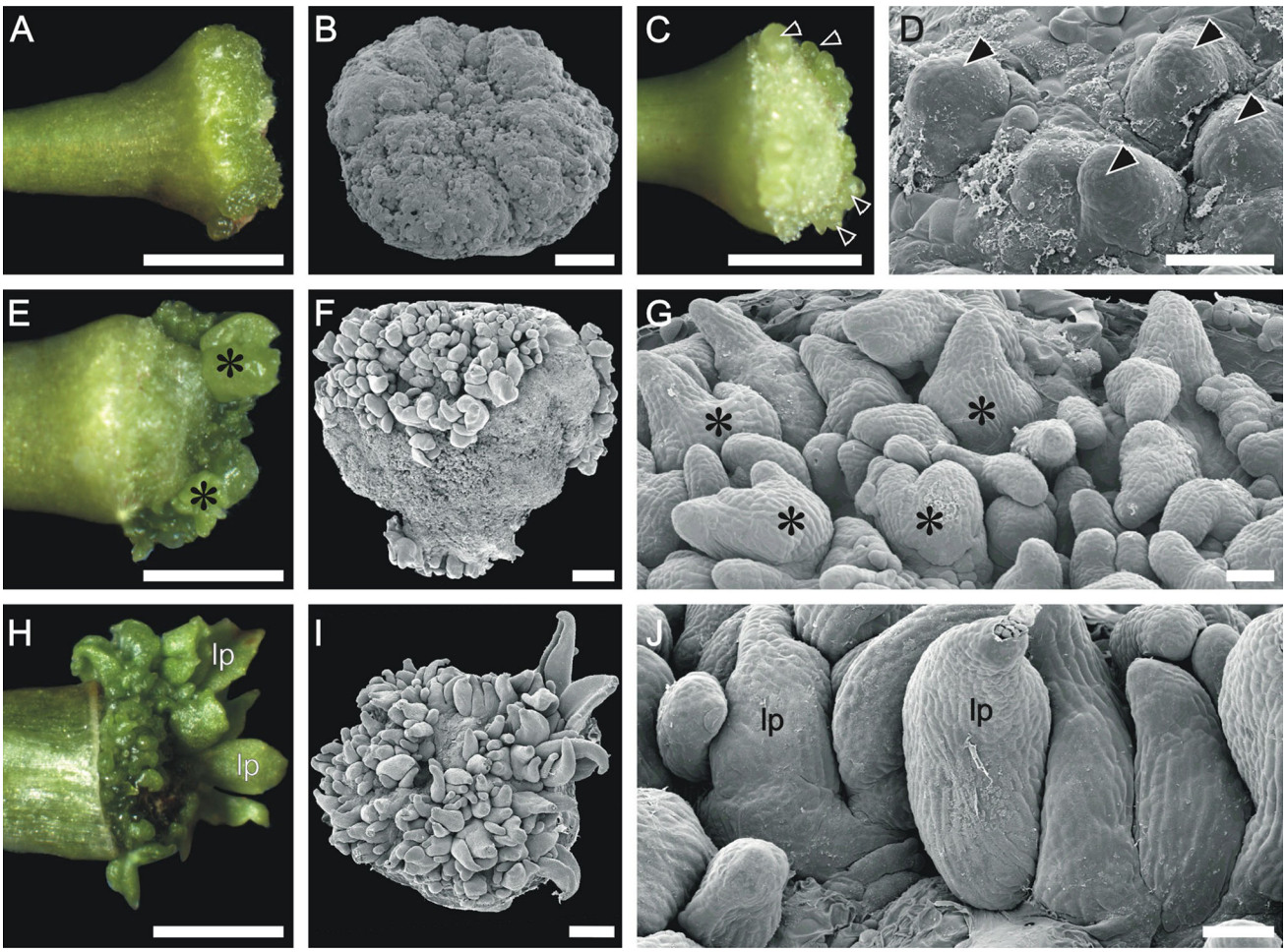


Fig. 1 De novo shoot organogenesis from hypocotyl explants. Macroscopic view (**a**, **c**, **e**, **h**) and scanning electron microscopy (**b**, **d**, **f**, **g**, **i**, **j**). **a**, **b** Explants after 7 days of culture showing the beginning of callus formation at the cut surface. **c**, **d** Explants after 14 days of culture showing the development of meristemoids (*arrowheads*) at the callus surface. **e**, **g** Explants after 21 days of culture showing the differentiation of the firsts organogenic structures (*asterisk*). Note the development of shoot meristems in early developmental stages (**g**). **h**, **j** After 28 days of culture, developing shoots producing leaf primordia could be observed (**i**, **j**). lp, leaf primordium. *Bars* = a–c, e–f, h–i = 500 µm; d, g, j = 100 µm

endogenously and, as they developed into adventitious buds, the peripheral cell layers (epidermis and cortex) were disrupted exposing the inner tissues of the explant (Fig. 2k, m). Completely formed shoot buds were observed after 25–30 days of culture (Fig. 2k, n) and they were connected to the vascular cylinder of the root explant (Fig. 2n), confirming the direct organogenic regeneration pattern.

PeSERK1 encodes a putative passion fruit SERK1 homolog

The alignment of all sequences obtained from the sequencing of the *P. edulis* PACEPE2105H12 cDNA clone generated a single 2588 bp-long consensus. This included a fragment of a 17 bp-long 3′ poly-A tail, suggesting the presence of a complete 3′-UTR sequence. Accordingly, the BLAST results indicated the presence of a complete coding sequence and additional 332 bp 5′-UTR and 367 bp 3′-UTR. Based on the results of sequence similarity produced by BLAST, we deposited this sequence in GenBank under the accession number KT373980 naming it *Passiflora edulis SERK1* (*PeSERK1*).

The *PeSERK1* deduced protein sequence (628 amino acids-long) showed the presence of a predicted 29 amino acids-long putative signal peptide (Supplementary Figures 1 and 2), 5 leucine-rich repeats (LRR), a proline-rich domain, a transmembrane domain and a serine/threonine kinase domain (Fig. 3a). These domains are found in all members of the SERK family (aan den Toorn et al. 2015). The degree of sequence conservation was not uniform along the deduced PeSERK protein sequence, ranging from 79 to 94 %, when compared to other family members. The sequences of the signal peptide, around the transmembrane domain and at the C-terminal were the most divergent.

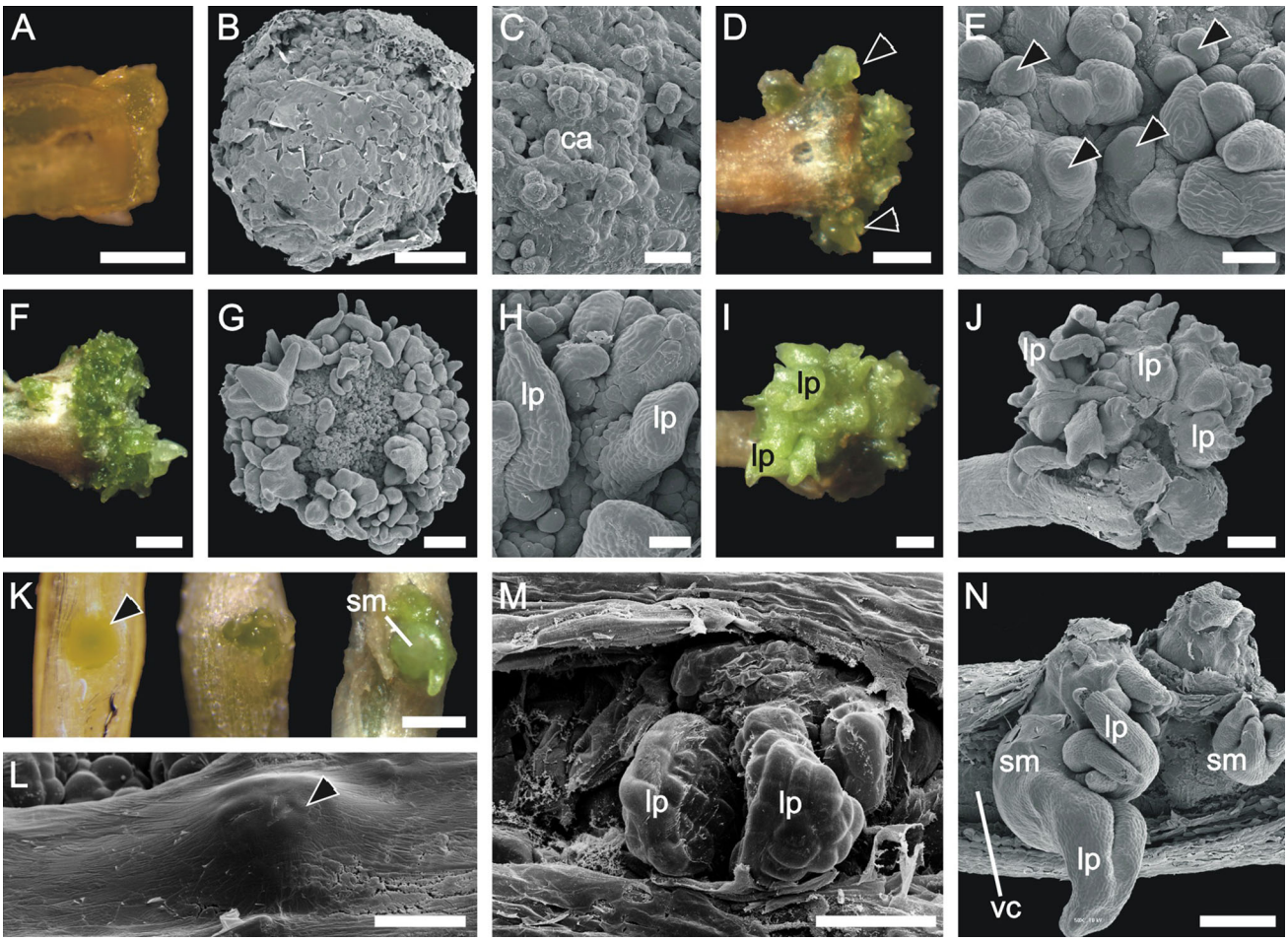
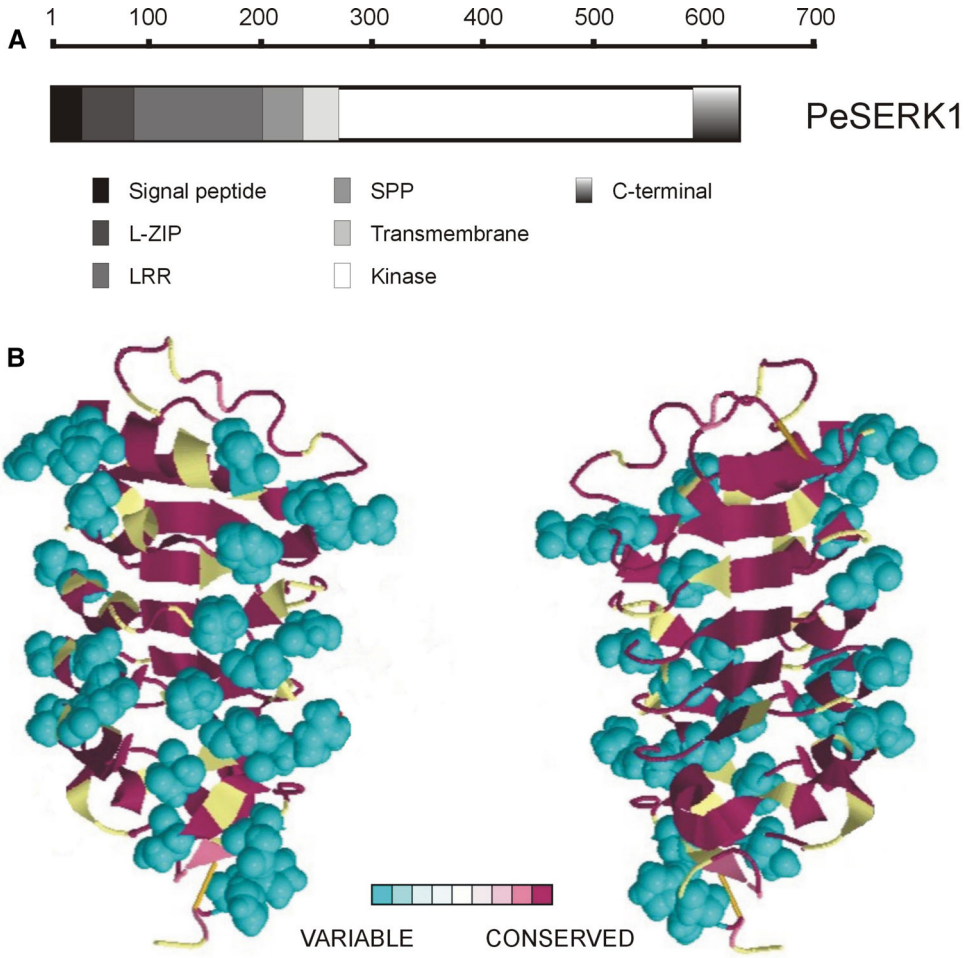


Fig. 2 De novo shoot organogenesis from root explants. Macroscopic view **(a**, **d**, **f**, **i**, **k)** and scanning electron microscopy **(b**, **c**, **e**, **g**, **h**, **j**, **l**, **n)**. **a-j** Shoot regeneration at the cut surface of the explant via callus formation. **a–c** Explants after 7 days of culture showing the beginning of callus formation at the cut surface. Note the granular aspect of the callus **(c)**. **d–e** Explants after 14 days of culture showing the differentiation of organogenic structures (*arrowheads*). **f–h** Explants after 21 days of culture showing shoot meristems with leaf primordia in early stages **(h)**. **i**, **j** After 28 days of culture, regenerated shoots were evident. **(k–n)** Direct shoot organogenesis in regions far from the cut explants surface. **k** From *left to right*: Macroscopic view of organogenic responses after 14, 21 and 28 days of culture. Note the early stages of meristemoid differentiation (*arrowhead*). **l** Explants after 14 days of culture showing the early stages of meristemoid differentiation and their endogenous origin (*arrowheads*). **m** Disruption of the cortex and epidermis caused by the development of regenerating shoots. **n** Regenerated shoot bud. Note the vascular connection with the vascular cylinder of the explant. *ca* callus, *lp* leaf primordium, *sm* shoot meristem. *Bars* = a–b, d–g, i–k, n = 500 μm; c, h, l–m = 100 μm

Nevertheless, some motifs were consistently conserved, including the cysteine pair preceding the SPP domain, typical of SERK Dicot S1/2 Class (aan den Toorn et al. 2015) and the residues at the extracellular domain, considered essential for the interaction with BRI1 orthologs (Roux et al. 2011; aan den Toorn et al. 2015; See Supplementary Fig. 3). Accordingly, the convex (also named "solvent-exposed" side, Santiago et al. 2013) is less conserved than the concave side of the extracellular domain (Fig. 3b), as shown by the conservation scores obtained using the ConSurf (Ashkenazy et al. 2010) algorithm. These results are consistent with the molecular mechanism of SERK1 activation and with other analyses performed with both dicot and monocot SERK proteins (Santiago et al. 2013; aan den Toorn et al. 2015).

A phylogenetic analysis was performed based on a multiple sequence alignment, including the entire amino-acid sequence of PeSERK1 and its counterparts from other plant species (Fig. 3c). PeSERK1 was included in a clade known as SERK Dicot S1/2 Class (aan den Toorn et al. 2015), together with other known dicot SERK1 orthologs. Proteins belonging to the SERK Dicot S1/2 Class have been related to developmental processes (Schmidt et al. 1997; Hecht et al. 2001; Albrecht et al. 2005; Lewis et al. 2010; aan den Toorn et al. 2015). The analysis revealed a monocot-specific SERK1 group and the SERK sequences


A
1
100
200
300
400
500
600
700
PeSERK1
Signal peptide
SPP
C-terminal
L-ZIP
Transmembrane
LRR
Kinase
B
VARIABLE
CONSERVED
C
CpSERK1
CpSERK2
SlSERK1
PtSERK1
PtSERK2
PeSERK1
VvSERK1
GmSERK1
MtSERK1
AtSERK1
AtSERK2
VvSERK2
DcSERK1
ZmSERK1
OsSERK
ZmSERK2
OSbiSERK
AtSERK4
AtSERK5
AtSERK3
SlSERK3
SmSERK1
SmSERK2
MpSERK
SERK Dicot S1/2
SERK Monocot
SERK Dicot S3/4
SERK Non-vascular plants

◀**Fig. 3** *Passiflora edulis* PeSERK1 protein structure and relationships with other SERK homologs. **a** Graphic representation of PeSERK1 protein structure, showing the position of the typical SERK subdomains. *L-ZIP* Leucine-zipper domain, *LRR* Leucine-rich domain, *SPP* Proline-rich domain, *Transmembrane* single-pass hydrophobic transmembrane domain, *Kinase* intracellular kinase domain. The *scale* represents protein length, in number of amino acids. **b** The amino acid conservation score, as determined by the ConSurf algorithm (Ashkenazy et al. 2010) was plotted onto the SERK1 extracellular domain (PDB: 4LSC; Santiago et al. 2013). Highly conserved residues are plotted in magenta, whereas lower scores are depicted by other *color shades*, with *cyan* depicting more variable residues. On the *left-handed side* of the figure is the convex or solvent-exposed side and on the *right-handed side* of the figure, the concave side of the SERK extracellular domain. **c** Parsimony-based tree of SERK homologs from diverse plant species. Branch lengths were calibrated according to Dayhoff matrix-based distances. Only bootstraps above 75 % are shown. The sequences used to construct the alignment are from GenBank: *Arabidopsis thaliana* (AtSERK1, Q94AG2; AtSERK2 Q9XIC7; AtSERK3, Q94F62; AtSERK4, Q9SKG5; AtSERK5, Q8LPS5), *Cyclamen persicum* (CpSERK1, A7L5U3 and CpSERK2, E5D6S9), *Daucus carota* (DcSERK1, O23921), *Glycine max* (GmSERK1, C6ZGA8), *Marcantia polymorpha* (MpSERK, BAF79935), *Medicago truncatula* (MtSERK1, Q8GRK2), *Oryza sativa* (OsbiSERK, Q6SF1; OsSERK, Q5Y8C8). *Populus trichocarpa* (PpSERK1, B9MW41; PpSERK2, B9IQM9), *Selaginella moellendorfii* (SmSER1, D8SBB8; SmSERK2, D8S0N3), *Solanum lycopersicon* (SlSERK1, G0XZA3; SlSERK3, G0XZA5), *Vitis vinifera* (VvSERK1, D7TXV2; VvSERK2, A5BIY4) and *Zea mays* (ZmSERK1, Q93W70; ZmSERK2, Q94IJ5)

from non-vascular plants also grouped together, in a more basal position in relation to other angiosperm SERK3/4 orthologs (Fig. 3c), similar to what was observed by aan den Toorn et al. (2015).

Our results thus indicate that PeSERK1 likely is the *P. edulis* homolog of SERK1, and it is the first described *Passiflora* homolog of the SERK family member of leucine-rich repeat receptor-like kinases.

PeSERK1 is expressed in *P. edulis* during in vitro organogenesis program

In situ hybridization experiments were performed to establish the spatial/temporal distribution of *PeSERK1* transcripts during in vitro shoot organogenesis from hypocotyl (Fig. 4) and root (Fig. 5) explants. A faint *PeSERK1* hybridization signal was associated to the vascular tissues of the initial hypocotyl explant (Fig. 4a). The onset of the first morphogenetic responses became evident after 1 week of culture with an intense cell proliferation at the cut surface of the explant. In these areas, *PeSERK1* transcripts were weakly detected (Fig. 4b). After 14 days of culture numerous meristemoids, consisting of small clusters of cells with meristematic features, were observed at the periphery of the callus and showed *PeSERK1* expression (Fig. 4c, d). As the meristemoids developed and differentiated into adventitious buds, strong *PeSERK1* expression was confined to subepidermal apical region of these organogenic structures (Fig. 4e). After 28 days, the *PeSERK1* transcripts were detected in the meristem cells of the regenerated shoots, as well as in the leaf primordia and procambium tissue (Fig. 4f).

In root initial explants, *PeSERK1* hybridization signal was also observed in the 4–5 layers of parenchymatic cells adjacent to the vascular region, including the pericycle (Fig. 5a). After one week of culture, a series of cell divisions in the pericycle region gave rise more layers of parenchymatic cells that also showed *PeSERK1* hybridization signal (Fig. 5b). In the periphery of the proliferation zone, cells became meristematic and produced meristemoids (Fig. 5c). These meristemoids were observed after 14 days of culture, both at the periphery of the explant and internally, far from the cut surface. At this stage, a strong *PeSERK1* hybridization signal was associated with the meristemoids both at peripheric and internal regions (Fig. 5c, d). In internal regions of the explant, the development of meristemoids resulted in the disruption of the cortex and root epidermis (Fig. 5c); subsequently, these meristemoids differentiated into shoot buds. In developing shoots meristems, *PeSERK1* transcripts were detected in meristematic cells and in the leaf primordia (Fig. 5e, f). No hybridization signal was observed with the sense probe (Figs. 4g, 5g).

Discussion

Despite the fact that the expression of *SERK* genes is historically associated to embryogenic pathways (both somatic and zygotic), some reports have demonstrated a broader role for these genes in different plant developmental programmes (Thomas et al. 2004; Nolan et al. 2009), including de novo organogenesis (Savona et al. 2012; Li et al. 2015). Here we described the cloning and characterization of a putative passion fruit (*P. edulis*) ortholog of the *SERK1* gene and its spatial expression pattern during the induction of DNSO from two different explants; hypocotyls and roots. DNSO is the prevailing mode in vitro plant regeneration for the genus *Passiflora* (Dornelas and Vieira 1994; Faria and Segura 1997; Garcia et al. 2011; Silva et al. 2011; Otoni et al. 2013; Vieira et al. 2014), although somatic embryogenesis has already been established for some of species (Silva et al. 2009; Paim-Pinto et al. 2011; Rosa et al. 2015).

The organogenic responses formed on the hypocotyl and root segments of *P. edulis* showed a similar tissue origin. From hypocotyl explants, they initiated by cell divisions at the tissues surrounding the vascular bundle and near the cut surfaces of the explants. In root segments, the morphogenic

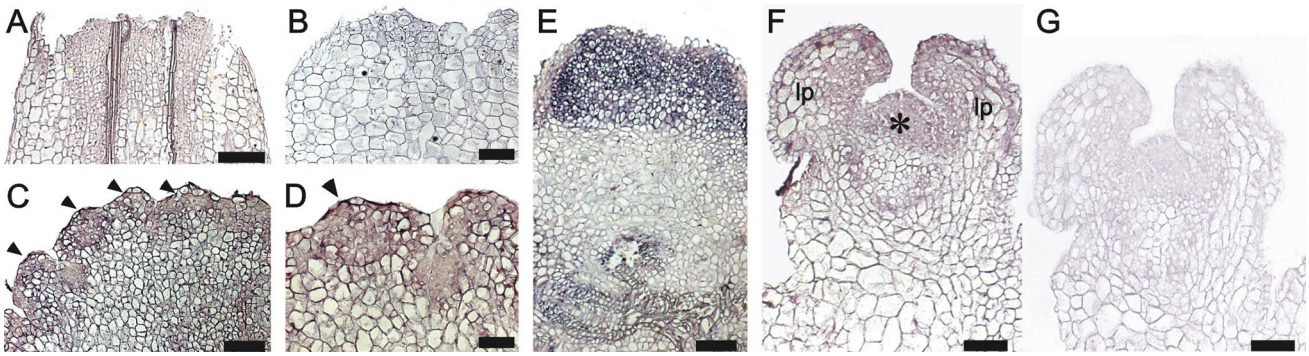


Fig. 4 Expression patterns of the *P. edulis PeSERK1* gene during de novo shoot organogenesis from hypocotyl explants. All sections are longitudinal in relation to the original explant. Positive hybridization signal is *pink/purple*. **a** A faint *PeSERK1* hybridization signal was associated to the vascular tissues of the initial hypocotyl explant. **b** After 1 week of culture, *PeSERK1* transcripts were weakly detected in dividing cells at the cut surface of the explants. **c**, **d** After 14 days of culture, *PeSERK1* expression could be detected in meristemoids (*arrowheads*). **e** Strong *PeSERK1* expression was confined to subepidermal apical region of the organogenic callus portions after 21 days of culture. **f** After 28 days, the *PeSERK1* transcripts were detected in the meristem cells of the regenerated shoots, as well as in the leaf primordia and procambium tissue. **g** A section parallel to the one shown in **f** was hybridized with the sense probe. No signal above background was detected. *Bars* = a, c, e = 100 μm; b, d, f, g = 50 μm

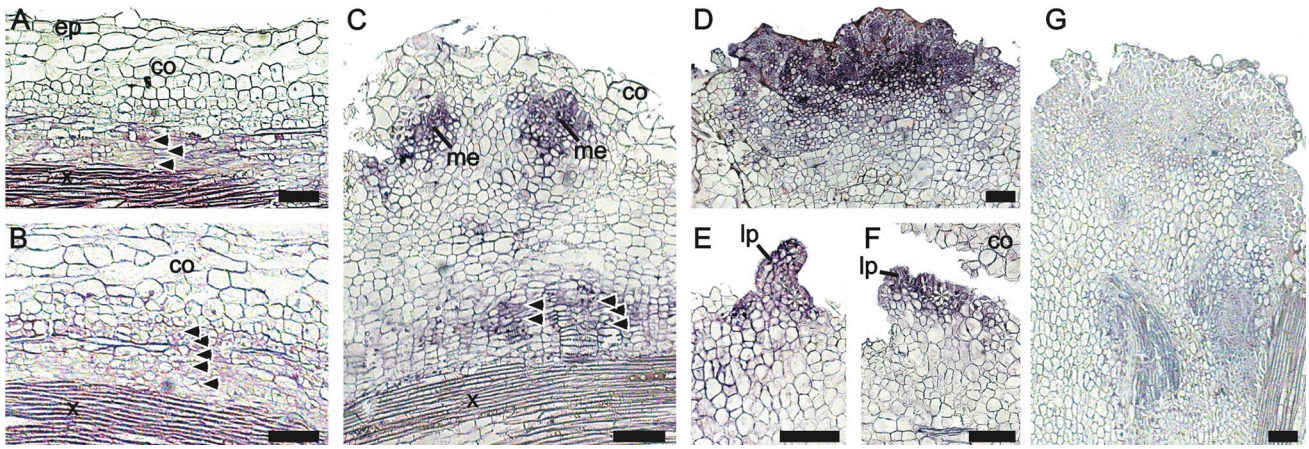


Fig. 5 Expression patterns of the *P. edulis PeSERK1* gene during de novo shoot organogenesis from root explants. All sections are longitudinal in relation to the original explant. Hybridization signal can be observed as *pink/purple*. **a** *PeSERK1* hybridization signal was also observed in 4–5 layers of parenchymatic cells adjacent to the vascular region, including the pericycle (*arrowheads*). **b** After 1 week of culture, a series of cell divisions in the pericycle region gave rise more layers of parenchymatic cells that also showed *PeSERK1* hybridization signal (*arrowheads*). **c** After 14 days of culture, a strong *PeSERK1* hybridization signal was associated with the formation of meristemoids (me) and cell proliferation associated with the pericycle (*arrowheads*). **d–f** After 21 days of culture, *PeSERK1* transcripts were detected in meristem cells (*asterisks*) and in the leaf primordia (lp) of regenerating shoot buds. **g** A section parallel to the one shown in **d** was hybridized with the sense probe. No signal above background was detected. *co* cortex cells. *Bars* = 100 μm

responses also originated from the parenchyma cells associated to the vascular bundle and the pericycle. These observations are in agreement with the regeneration pattern described in previous histological studies for hypocotyl- and root-derived DNSO (Dias et al. 2009; Rocha et al. 2012, respectively). Additionally, both systems showed the same cellular mechanism were shoots originated from cells exhibiting meristematic features and the activity of *PeSERK1* gene, suggesting that a single organogenic programme might be being activated, and that it initiates from a (otherwise undefined) meristemoid stage.

The *P. edulis* homolog of *SERK1* belongs to the SERK Dicot S1/2 clade, presenting high sequence identity to other dicot sequences. All conserved protein structural features and residues (including those involved with ligand-binding receptors such as BRI1) expected to be present in *buona fide* SERK1 ortologs were observed in PeSERK1.

Although the biological functions described in the literature for the members of the SERK Dicot S1/2 clade are associated to somatic embryogenic processes (Schmidt et al. 1997; Hecht et al. 2001; Santos et al. 2005; Sharma et al. 2008; Steiner et al. 2012; Talapatra et al. 2013; Silva

et al. 2014), according to our in situ hybridization results, *PeSERK1* transcripts were observed throughout the passion fruit organogenic pathway obtained from the in vitro culture of hypocotyl and root explants. However, *PeSERK1* expression of was not only observed during the regeneration of shoots. The accumulation of the *PeSERK1* transcripts was also observed in vascular tissues, callus and shoots, besides those involved in DNSO. Before the establishment of the organogenic culture, *PeSERK1* transcripts were detected in the vascular tissues of both hypocotyl and root explants. The expression in these tissues was associated to provascular tissue development. Accordingly, *AtSERK1* was similarly expressed in the procambium and root pericycle of *Arabidopsis* (Hecht et al. 2001; Kwaaitaal and de Vries 2007; Nolan et al. 2009).

At 1 week of culture, *PeSERK1*expression was detected in dividing cells committed to form the initial callus tissue at the cut surface of the explants (both root- and hypocotyl-derived explants) and at the proliferating zone in regions far from the cut surface in root explants. The expression of this gene continued throughout the DNSO process in masses of small dividing cells (meristemoids) prior to the de novo differentiation of shoots. The close relationship between the expression of *SERK* genes and cellular proliferation activity seems to be conserved in different plant species (Nolan et al. 2009; Pérez-Nuñez et al. 2009; Savona et al. 2012; Li et al. 2015). These findings are consistent with a more broad view of the actual SERK1 function, which would be associated to switches in developmental cell fate (Nolan et al. 2009; Savona et al. 2012). The expression of *PeSERK1* in callus cells, as observed here, indicates a putative role for this gene in the organization of an organogenic callus. It is also in agreement with the involvement of *SERK1* in cellular reprograming and trans-/differentiation processes, as suggested by Nolan et al. (2009).

At later stages of DNSO, *PeSERK1* was also detected in cells at the meristem and early leaf primordia of regenerated shoots. This expression pattern was confirmed in other few reports of *SERK* expression during organogenesis (Nolan et al. 2009; Thomas et al. 2004; Savona et al. 2012; Li et al. 2015). However, how the spatial expression pattern of this gene relates to the mechanism(s) controlling meristematic cell identity is poorly understood.

In summary, here we reported the cloning and characterization of a *P. edulis* gene encoding a putative ortholog of a leucine-rich repeat receptor-like kinase, *PeSERK1* including its expression pattern during DNSO-induction from hypocotyl and root explants. We expect that these results might contribute to the understanding the molecular mechanisms underlying developmental processes involved in plant morphogenesis.

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