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Expression analysis of two *SOMATIC EMBRYOGENESIS RECEPTOR KINASE* (*SERK*) genes during in vitro morphogenesis in Spanish cedar (*Cedrela odorata* L.)

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Abstract

Somatic embryogenesis (SE) is one of the most important steps during regeneration, but the molecular mechanism of SE remains unclear for *Cedrela odorata*. SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK) is one of the genes associated with induction of SE and is considered a marker of cells competent to form somatic embryos. Our objective was to clone and characterize the *SERK1* and *SERK2* gene homologues and analyze their expression patterns during in vitro morphogenesis in Spanish cedar. Co*SERK1* and Co*SERK2* were isolated from cedar, both share domains characteristic of the *SERK* family, including leucine-rich repeats, a proline-rich motif, a transmembrane domain, and kinase domains. Embryogenic cultures were established from callus cultures induced on medium supplemented with 1 mg/L dicamba. Histological sections were studied to determine the embryogenic nature of the samples. The Co*SERK1* gene was highly expressed during the acquisition of embryogenic competence. The expression level of *SERK1* was lower in non-embryogenic tissues and organs than in embryogenic calli, and it was higher in 3-week old embryogenic calli. Co*SERK2* gene was highly expressed in leaves and shoots but no difference in expression was obtained between somatic and embryogenic tissues. These results suggest that the expression of Co*SERK1* is associated with somatic embryogenesis induction and could be used as a potential marker to monitor the transition from competent to embryogenic cells and tissues in Spanish cedar.

Keywords Co*SERK* · Kinase · LRR · 18S · Gene expression · Somatic embryogenesis

Abbreviations

SERK	Somatic embryogenesis receptor-like kinase
SE	Somatic embryogenesis
IZE	Immature zygotic embryo
MZE	Mature zygotic embryo
2,4-D	2,4-Dichlorophenoxyacetic acid
BAP	6-Benzylaminopurine
ZEA	Zeatin
cDNA	Complementary DNA
RT-PCR	Reverse transcription polymerase chain reaction

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Introduction

Somatic embryogenesis (SE) is considered the model system for plant regeneration, but the molecular mechanism of SE remains unclear for some species (Yang et al. 2011; Liu et al. 2018) including *Cedrela odorata*. Understanding the underlying molecular mechanisms of somatic embryogenesis is important to establish more efficient regeneration and genetic transformation protocols (Yang et al. 2011; Ma et al. 2012a). Many genes associated with the regulation of late stages of SE have been reported (Lotan et al. 1998; Stone et al. 2001; Zuo et al. 2002; Boutilier et al. 2002; Harding et al. 2003; Liu et al. 2018). The expression of the somatic embryogenesis receptor-like kinases (SERK) gene is a prerequisite for the molecular regulation of somatic embryogenesis because this gene

plays an important role in the signaling process during embryo formation (Talapatra et al. 2014). Therefore, expression analyses of this gene in recalcitrant species, such as Spanish cedar, are valuable for studying its use as a potential marker in somatic embryogenesis (Cameron 2010; Liu et al. 2018). SERKs belong to a small gene family of receptor-like kinases involved in signal transduction (Singh and Khurana 2017). *SERK* first isolated from carrot (*Daucus carota*) embryogenic cells, is considered a characteristic molecular marker for SE in carrot, *Dactylis glomerata*, and *Arabidopsis* (Schmidt et al. 1997; Singh and Khurana 2017). *SERK* genes have been reported to participate in the induction of SE, such is the case of pineapple (Ma et al. 2012a), coconut (Pérez-Núñez et al. 2009), cotton (Singla et al. 2008), *Arabidopsis* (Hecht et al. 2001), *Medicago truncatula* (Nolan et al. 2003), and carrot (Schmidt et al. 1997). *SERK* genes are regulated (up-regulated) by auxins in some plant species, and by auxins and cytokinins in others (Zhang et al. 2011). Zm*SERK1* and Zm*SERK2* are expressed in embryogenic and non-embryogenic cultures in maize (Baudino et al. 2001) and appear to play an important role in the maintenance of embryogenesis (Zhang et al. 2011).

Cedrela odorata L. (Spanish cedar, Meliaceae), is a species with a distribution from Southern Mexico to Northern Argentina and The Antilles (Patiño 1997), and in Costa Rica it grows on both the eastern and western slopes (Navarro et al. 2002). It is a highly valued tree in the timber industry for its strength and aroma, and it is used for construction and furniture (Cruz 2005). *C. odorata* is also used as a source of shade in coffee plantations, and in traditional medicine for the treating of malaria and diabetes (Martins et al. 2003). Despite its economic importance, this species has been categorized as vulnerable for several years (IUCN 2017). Overexploitation, along with the deterioration of both the environment and of its habitat, has contributed to its decline (Navarro et al. 2002). Establishment of plantations could relieve the pressure of the industry on the natural populations of this species. Microalpropagation techniques, which include all plant tissue culture methods, are widely used for in vitro vegetative propagation of the plants (Liao et al. 2006).

There are two reports of SE from zygotic embryo in *Cedrela*, one study applies 3 mg/L dicamba for SE induction (Peña-Ramírez et al. 2011), and the other report applies different concentrations of 2,4-D and BAP combined with a thermal shock (Cameron 2010). However, there are no studies on the molecular mechanisms of the SE in *C. odorata* (aan den Toorn 2015). The objective of this study was to characterize the homologues of the *SERK1* and *SERK2* genes, and to analyze their expression patterns during in vitro morphogenesis in *C. odorata*. The study of genes related to SE can lead to a further understanding of the embryogenic process in the species of interest and contributes to the improvement of regeneration protocols.

Materials and methods

Establishment of embryogenic cultures

Spanish cedar mature seeds were obtained from the Forest Seed Bank (BSF) of the Tropical Agricultural Research and Higher Education Center (CATIE). Two hundred seeds were disinfected with hand soap (15 min), sulfuric acid 20% (25 s), alcohol 70% (1 min) and chlorine 1.75% i.a plus four-drops/100 mL of Tween 20 (20 min) and washed with sterile distilled water after each disinfection step. Callus was induced in the dark using the root segments (1 cm) of 3-month seedlings germinated in vitro. The culture medium for germinating the seedlings consisted of MS salts (Murashige and Skoog 1962) at half ionic strength, 30 g/L sucrose, 2 mg/L glycine, and 100 mg/L myo-inositol. The pH of the medium was adjusted to 5.7, and 2.9 g/L phytagel was added as a jellifying agent. The culture medium was autoclaved at 120 °C with a pressure of 1 kg/cm^2 for 20 min. Cultures were maintained under a 12 h light/12 h dark photoperiod at 25 °C. Four seeds were placed in glass containers with 40 mL of culture medium for germination.

The culture medium for pro-embryogenic callus induction consisted of MS salts (Murashige and Skoog 1962) at full ionic strength, 30 g/L sucrose, 100 mg/L citric acid, 100 mg/L ascorbic acid, and 1 g/L of activated charcoal. The medium pH was adjusted to 7.5, jellified with 7 g/L agar, and supplemented with 1 mg/L dicamba (Gonzalez-Rodríguez and Peña-Ramirez 2007). The culture medium was autoclaved at 120 °C at 1 kg/cm^2 of pressure for 20 min. Cultures were maintained under a 12 h light/12 h dark photoperiod at 25 °C. Root segments were subcultured every 3 weeks on this medium until the formation of pro-embryogenic calli (PEC).

Culture medium for callus consisted of WPM salts (Lloyd and McCown 1980), 30 g/L sucrose, 100 mg/L citric acid, and 100 mg/L ascorbic acid. The medium was adjusted to 7.5 pH, jellified with 4 g/L gelrite, and supplemented with 3 mg/L TDZ and 0.01 mg/L zeatin (Gonzalez-Rodríguez and Peña-Ramirez 2007). The culture medium was autoclaved at 120 °C with 1 kg/cm^2 of pressure for 20 min. Cultures were maintained under a 12 h light/12 h dark photoperiod at 25 °C. Calli were subcultured every 3 weeks on this medium until RNA extraction.

Establishment of shoots

Shoots were induced from apical meristems (1 cm) of 4-week old plants germinated in vitro. The induction culture

medium consisted of WPM salts (Lloyd and McCown 1980) and 30 g/L of sucrose. The medium was adjusted to a pH 5.8, jellified with 2.9 g/L phytagel, and supplemented with 2.25 mg/L BAP. Each glass container with 20 mL of medium was autoclaved at 120 °C at 1 kg/cm^2 of pressure for 20 min. Three meristems were placed vertically in each container, and cultures were maintained under a 12 h light/12 h dark photoperiod at 25 °C for 4 weeks. Around 100 shoots obtained after 4 weeks (B0) were subcultured on the same culture medium, adding 2 mg/L of ZEA, for 4 weeks (B1).

Histology

Samples of 3-week old embryogenic calli were fixed with FAA [10% formaldehyde, 5% acetic acid, and 50% ethanol (v/v)] and were stored at 4 °C. They were dehydrated in a vacuum for 10 min with ascending concentrations of ethanol (10, 30, 50, 70, 85, 96, and 100%) for 1 h at 4 °C. Finally, tissues were later embedded in Paraplast plus paraffin (Leica Biosystems, Wetzlar, Alemania), and 5 μm thick sections were prepared with a microtome.

Histological sections of 3-week old embryogenic callus were hydrolyzed with periodic acid (PAS) for 15 min, washed with tap water, and air-dried. Sections were stained with Schiff reagent in the dark for 10 min. They were washed with water until the runoff was colorless, and then were air-dried. Sections were stained with 1% naphtol blue in 7% acetic acid (30 s), washed with water and air-dried (Fisher 1968). Stained sections were observed under a light microscope to determine the embryogenic nature of the samples.

Primer design

Primers to amplify the *CoSERK* gene were designed from the *SERK1* gene sequence from *Citrus clementina* (Phytozome accession number Ciclev10027231m): F1: 5′-TTCCTAGTG CTCTTGGGAATCTG-3′, R1: 5′-GCAACACTTCCATTG GCCATGTA-3′, F2: 5′-GGATCTCCACCATTTTCTCCTCC-3′, and R2: 5′-CCAAAAACATCCGTTTTCTCTGA-3′ to amplify Co*SERK1*. Sequences from the *SERK2* gene of the plant species *Citrus sinensis* (accession NM_001288871.1), *Citrus sinensis* (orange 1.1g007020m.g), *C. clementina* (Ciclev10000597m.g) and *Azadirachta indica* (obtained in CLC Main Workbench 7 from the local alignment of public contigs of *A. indica* (accession GCA_000439995.3) with the *SERK2* sequence of *Citrus sinenensis*) were utilized to design the following primers: F 5′-CTAGCTTGGTGAGCTTGG ATCT-3′ y R 5′-CCGTAGTGACATGGGTATCC-3′. Primers used in quantitative PCR *SERK1* F 5′-CTTCGCTTACGC GGTTTCTG-3′, R 5′-CGGCCAATCAAGAGGAGGTT-3′; *SERK2* F 5′-GCACATTGGTGGCAGTGAAA-3′, R 5′-CAA AAGCCGCGTAAACGGAG-3′; *18S** (Brunner et al. 2004) F

5′-AATTGTTGGTCTTCAACGAGGAA-3′, R 5′-AAAGGG CAGGGACGTAGTCAA-3′.

Amplification and sequencing of *SERK* genes

The primers described above were used for PCR following the directions of the manufacturer of the PCR Master Mix (Thermo Fisher Scientific). A cDNA template synthesized with the Maxima H Minus First Strand cDNA Synthesis (Thermo Scientific) kit using the (dT)18 primer was used for PCR. The PCR products were sequenced with the Applied Biosystems 3130 genetic analyzer or sent to external Company (Macrogen, Republic of Korea).

SERK gene expression analysis through qRT-PCR

Total RNA was extracted with the NucleoSpin RNA II (Macherey–Nagel) kit, following the directions from the manufacturing company. The RNA was extracted from at least three biological samples of mature zygotic embryos, and the next in vitro obtained tissues: 3-week old roots, leaves, and stems, non-embryogenic callus, pro-embryogenic callus, 3-, 6- and 9-week old embryogenic callus, and shoots. Reverse transcription reactions (cDNA synthesis) were performed with the Maxima H Minus First Strand cDNA Synthesis (Thermo Scientific) kit, using the *Random Hexamer Primers* from 25 ng of total RNA, following the instructions of the manufacturing company.

Co*SERK1*, Co*SERK2*, and *18S* fragments with expected sizes of 126 bp, 127 bp, and 74 bp, respectively were amplified. Gene expression was measured with the StepOne Real-Time PCR System equipment (Thermo Scientific). The reaction was 20 μL: 10 μL of Power SYBR Green PCR Master Mix (Applied Biosystems), 2 μL (10 pmoles/μL) each Primer, 2 μL cDNA, 4 μL ultrapure water. Measurements were made in triplicate with the thermocycler program: 1 cycle 95 °C (10 min), 40 cycles 95 °C (15 seg)–55 °C (1 min)–72 °C (20 seg), 1 cycle (melt curve stage) 95 °C (15 seg)–60 °C (15 seg)–95 °C (15 seg).

Expression data for each gene was normalized [$\Delta C_T = Ct$ (gene of interest) − Ct (reference gene)]. Relative quantification was calculated ($RQ = 2^{-\Delta(\Delta C_T)}$) using root as a reference sample [$\Delta\Delta C_T = \Delta C_T$ (sample) − ΔC_T (reference)] (Livak and Schmittgen 2001). Data obtained for relative expression (RQ) were Log_{10} transformed and then analyzed with an analysis of variance (ANOVA), then statistical differences between treatments were analyzed with a Tukey test.

Results

Embryogenic cultures establishment

The root segments in dicamba showed a visible response 3 weeks after induction. Nevertheless, it was approximately 16–20 weeks after the induction of embryogenic callus that we observed the formation of a cream colored friable callus with cells of globular appearance (Fig. 1a). Once subcultured on the embryogenesis culture medium, the callus appearance did not vary, except in color. Unlike previously reported (González-Rodríguez and Peña-Ramírez 2007), we did not observe the presence of somatic embryos. We also obtained a compact and white callus in the culture medium supplemented with dicamba, which was considered non-embryogenic.

Shoot establishment

With 2.25 mg/L BAP an average of three shoots per explant in the apical meristems of Spanish cedar were obtained. We observed two morphogenic responses when the B0 shoots were subcultured on 2 mg/L ZEA: the formation of both a compact callus on the bottom edge of the explants and a nodular callus on the whole explants (apparently embryogenic). Both calli were produced in equal proportions. In this case, explant B1 showed characteristics of a compact callus that is highly propagative (data not shown).

Histology

We analyzed histological sections of 3-week old embryogenic calli, confirming the presences of small cells densely stained that denote high metabolic activity (Fig. 1b), these cells began to divide, forming proembryos that emerged on the surface of the callus. Histological analysis of previous stages showed that calli begin with small, isodiametric cells showing prominent nuclei and nucleoli, dense cytoplasm, small vacuoles and thin cell walls.

CoSERK1 and CoSERK2 sequence analysis

The homologous sequences of SERK in *C. odorata* were obtained using primers of *SERK* from *Citrus clementina, C. sinensis,* and *Azadirachta indica*. The sequences obtained were of 925 bp and 763 bp. An alignment against the *SERK* protein of *Daucus carota* (AAB61708.1) was performed. The sequence with the highest similarity to the AAB61708.1 protein was named Co*SERK1* (925 pb), and the other one Co*SERK2* (763 pb). Sequences were deposited in GenBank with the accession numbers MF631766 and MF631767 for Co*SERK1* and Co*SERK2*, respectively. The DNA sequences were translated in silico and subjected to a pBLAST. Co*SERK1* had the highest similarity with SERK1 of *Citrus unshiu* (98%, BAD32780.1), SERK2 of *Carica papaya* (98%, XP_021896015.1), SERK of *Carica papaya* (98%, ABS32228.1 and 98%, ABS32233.1), and the precursor of SERK of *Citrus sinensis* (98%, NP_001275800.1). On the other hand, CoSERK2 had the highest similarity with SERK of *Paeonia suffruticosa* (92%, ABW74475.1 and 92%, AHI59639.1), three isoforms of SERK2 of *Manihot esculenta* (89%, XP_021630232.1, 89%, XP_021630231.1 and 89%, XP_021630230.1), and two isoforms of SERK2 of *Hevea brasiliensis* (89%, XP_021679462.1 and 89%, XP_021679461.1). The Co*SERK1* sequence comprises exons 4–10, while the Co*SERK2* sequence comprises exons 4–9 (Fig. 2a). These exons code for LLR2–3 (exon 4), LRR4 (exon 5), LRR5 (exon 6), SPP (exon 7), TM
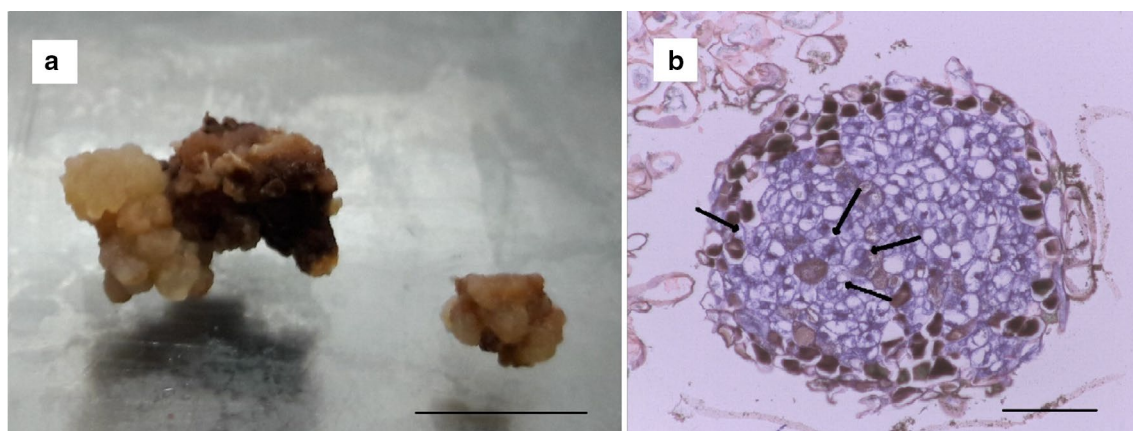

Fig. 1 Embryogenic culture initiation and histology of embryogenic tissue. **a** Pro-embryogenic callus obtained from the root after 20 weeks of culture using 1 mg/L dicamba. **b** Histological section of 3-week old embryogenic callus. Arrows show cells with high metabolic activity. Bars: **a** 1 cm, **b** 50 μm

Fig. 2 Graphic representation of exons size and distribution and conservation of in silico translated *CoSERK* proteins. **a** Schematic comparison between Co*SERK1* (925 bp) and Co*SERK2* (763 bp) partial sequences and the 11 exons of *SERK1* (1878 bp) of *Arabidopsis thaliana*. **b** Alignment of *CoSERK1* (MF631766), *CoSERK2* (MF631767), *AtSERK1* (NM_105841), and *AtSERK2* (NM_001333091) (118–477 amino acids) performed in MUSCLE. Asterisks indicate conserved regions
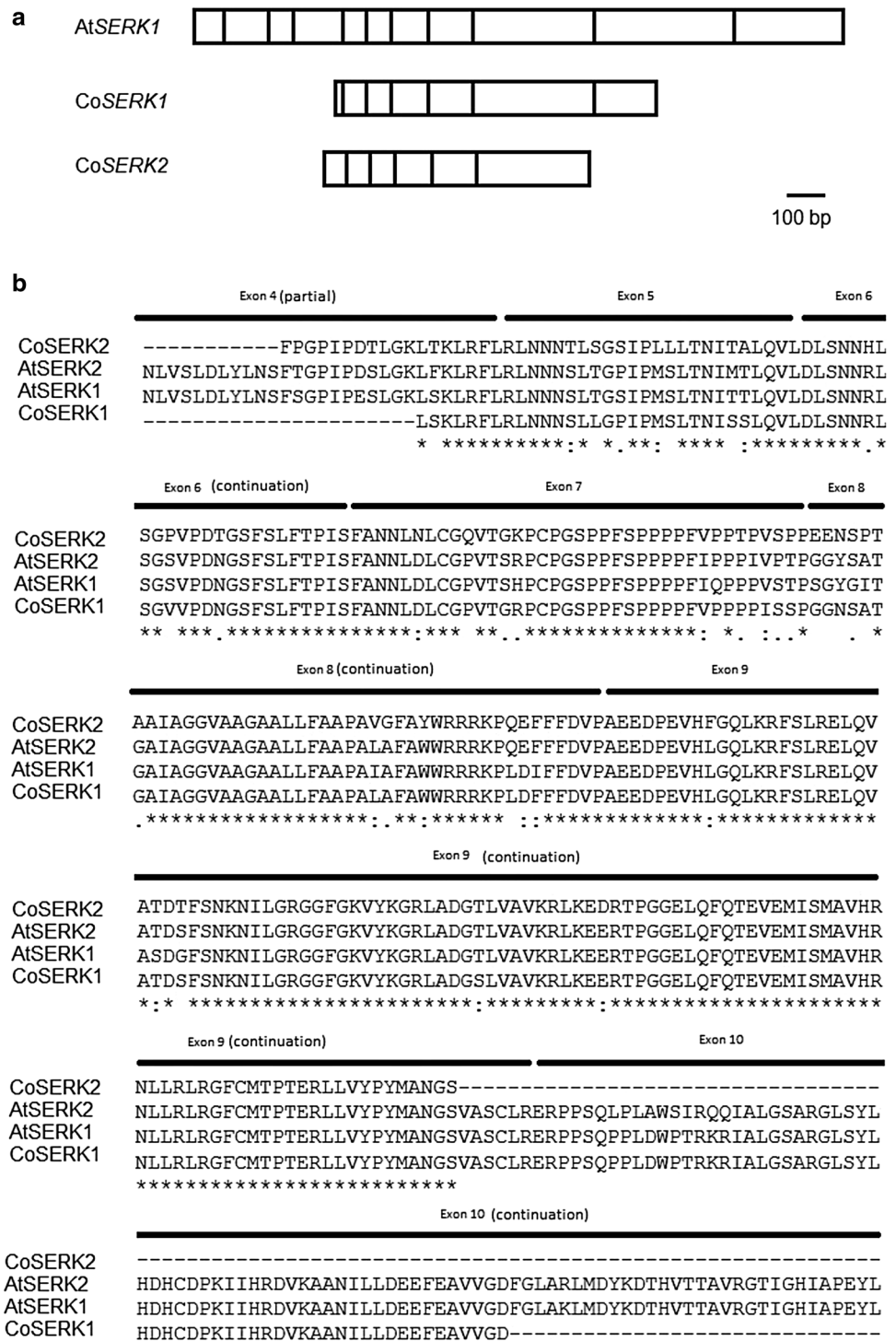
(exon 8), kinase I–V (exon 9), and kinase VI–IX (exon 10). The SPP domain, encoded by exon 7, contains two cysteine residues characteristic of the SERK1/2 subfamily. The translated sequences align with the residues 18–447 of SERK1 and SERK2 proteins of *Arabidopsis thaliana* (Fig. 2b).

SERK gene expression analysis through qRT-PCR

Figure 3 supports the assumption that the 18S gene was expressed in equal quantities in all the tissues in study. The expression of Co*SERK1* was similar in somatic tissues and in non-embryogenic callus (approximately 1 compared to the root) (Fig. 4). The stem tissues presented the lowest
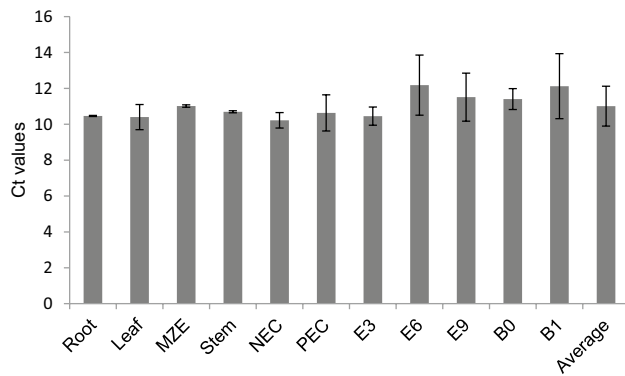
Fig. 3 C_t (cycle threshold) values for 18S transcripts in the 11 studied tissues. The last bar shows the mean value of the 66 values shown in the rest of the bars. Error bars represent the standard deviations. *MZE* mature zygotic embryo, *NEC* non-embryogenic callus, *PEC* pro-embryogenic callus, *E3* 3-week old embryogenic callus, *E6* 6-week old embryogenic callus, *E9* 9-week old embryogenic callus, *B0* shoot obtained in BAP, *B1* B0 placed in ZEA

Fig. 4 Expression of Co*SERK1* (above) and CoSERK2 (below) in embryogenic and somatic tissues of Spanish cedar. *MZE* mature zygotic embryo, *NEC* non-embryogenic callus, *PEC* pro-embryogenic callus, *E3* 3-week old embryogenic callus, *E6* 6-week old embryogenic callus, *E9* 9-week old embryogenic callus, *B0* shoot obtained in BAP, *B1* B0 placed in ZEA. Different letters above bars indicate significant differences from a Tukey test ($p < 0.05$)
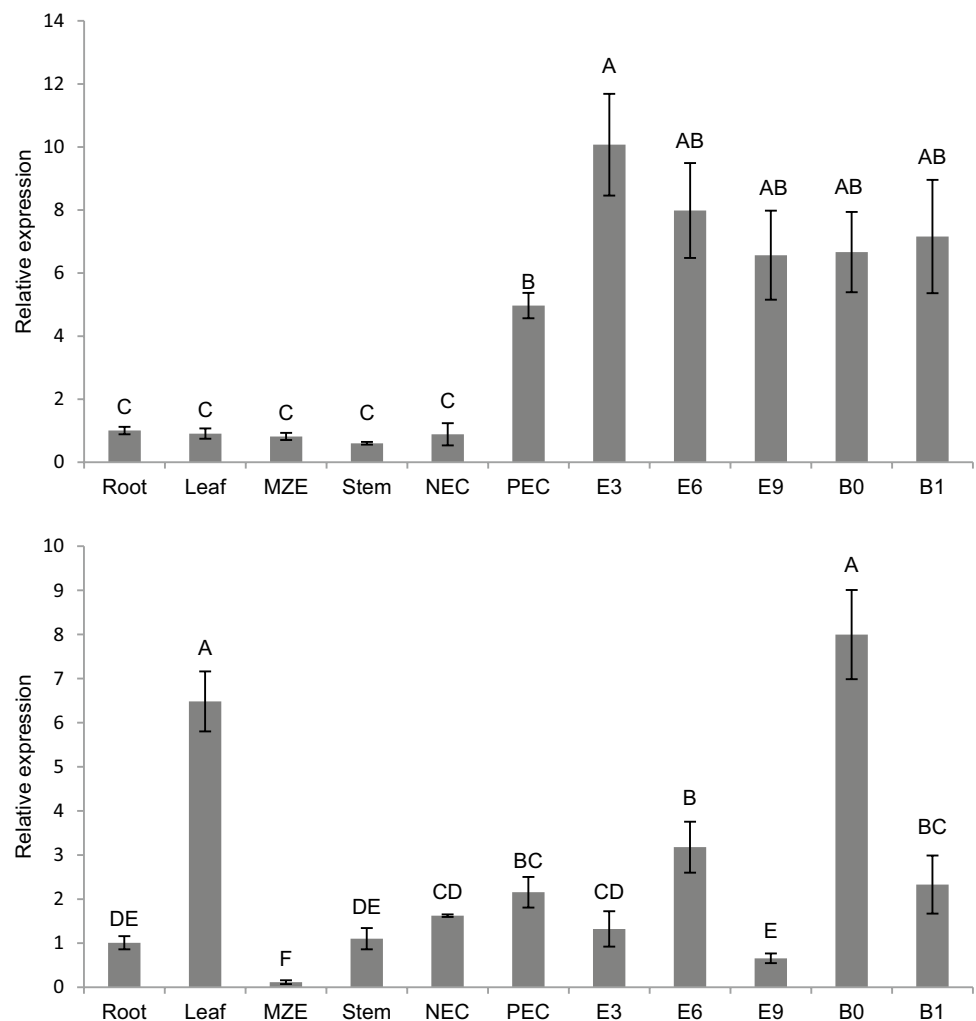
expression (0.6-fold, Fig. 4). We also observed that the expression of this gene increased in the pro-embryogenic callus, showing the highest expression (tenfold) in the 3-week old embryogenic callus and decreased slightly in the 6-week old and in the 9-week old embryogenic callus (eightfold and 6.5-fold, respectively). The expression of Co*SERK1* was similar in the 6- and 9-week old embryogenic callus and in the evaluated shoots. In the case of Co*SERK2*, Fig. 4 demonstrates a higher expression in leaves and in B0 (6.5- and eightfold, respectively), with lower expression in MZE and in 9-week old embryogenic callus (0.11- and 0.65-fold, respectively). No difference in expression was obtained between somatic and embryogenic tissues Co*SERK2* gene.

Discussion

SERK genes have been isolated in different species and a variable number of members of this gene family have been observed. Six members of this family have been identified in *Medicago truncatula* (Nolan et al. 2003, 2011),

five members have been identified in *Arabidopsis thaliana* (Hecht et al. 2001), four members in *Rosa hybrida* (Zakizadeh et al. 2010), three members in *Vitis vinífera* (Schellenbaum et al. 2008), two members in *Ananas comosus* (Ma et al. 2012a, b), and one member in species such as *Daucus carota* (Schmidt et al. 1997) and *Cocos nucifera* (Pérez-Núñez et al. 2009). In the present study, we isolated two partial sequences which are homologous to *SERK*. The isolated sequences showed an 87% similarity for hypothetical proteins. Major differences corresponded to conservative substitutions (Fig. 2b). Both sequences include domains that belong to the SERK family, including the SPP domain (exon 7), which is characteristic of this family (Hecht et al. 2001) (Fig. 2a), with two cysteine residues, which are characteristic of the SERK1/2 subfamily (aan den Toorn et al. 2015).

We used the 18S gene to normalize the Co*SERK1* and Co*SERK2* expression data. The number of transcripts per cell was approximately the same in all tissues, as the mean values of C_t ranged between 10.22 and 12.18 (Fig. 3, total mean 11, standard deviation 1.1). A similar assumption was made by Zakizadeh et al. (2010) with the actin gene to normalize the expression of *SERK* genes in *Rosa hybrida*. These authors obtained Ct values between 27.3 and 30.3. Additionally, the 18S gene has previously been used in qRT-PCR to normalize the expression of *SERK* in *Theobroma cacao* (Santos et al. 2005), *Lactuca sativa* L. (Santos et al. 2009), *Cocos nucifera* L. (Pérez-Núñez et al. 2009), *Musa* spp. (Huang et al. 2010), *Gossypium hirsutum* (Shi et al. 2012, 2014), *Triticum aestivum* L. (Delporte et al. 2013), and *Curcuma alismatifolia* Gagnep (Sucharitakul et al. 2014). In the root tissue, the number of Co*SERK1* and Co*SERK2* transcripts per *18S* transcript is 7.87×10^{-7} ($2^{-20.2766}$), and 4.32×10^{-7} ($2^{-21.1416}$), respectively, this is a proportion of 1:0.55.

Expression of the Co*SERK1* gene in leaves, stem, mature zygotic embryo, and non-embryogenic callus was similar to its expression in the root normalized to 1 (Fig. 4). Furthermore, expression of the Co*SERK1* gene was higher in embryogenic tissues, with a maximum in 3-week old embryogenic callus, with respect to normalized expression in the root (Fig. 4). Therefore, these results and other studies, demonstrate that the *SERK* gene has a higher expression in early stages of the embryogenic callus. For example, the *SERK* gene reaches its expression peak in embryogenic cultures of *Momordica charantia* at 28 days (Talapatra et al. 2014), in *Glycine max* at 15 days (Yang et al. 2011), in *Triticum aestivum* L. at 8 days (Delporte et al. 2013), in *Cyclamen persicum* at 7 days (Savona et al. 2012), in *Cyrtochilum loxense* at 20 days (Cueva et al. 2012), and in *Ananas comosus* at 40 days (Ma et al. 2012a). In grapes, Vv*SERK1* have a higher gene expression level in callus after (6 weeks later) being transferred to embryogenic induction media (Schellenbaum et al. 2008). Expression peaks coincide with the gain of embryogenic competence in some species and with somatic embryo formation in others. Our results show agreement with the evidence that the expression level of *SERK1* is lower in somatic and non-embryogenic tissues compared with embryogenic tissues (Yang et al. 2011; Ma et al. 2012a; Talapatra et al. 2014). In contrast, Zakizadeh et al. (2010) suggest *SERK3* and *SERK4*, instead of *SERK1*, as markers for somatic embryogenesis in *Rosa hybrida*, since the highest expression of *SERK1* was obtained in roots and stamens. Moreover, the highest expression of *SERK1* in *Solanum tuberosum* (Sharma et al. 2008) was found in seeds and microtubers instead of embryogenic tissues. Even though pro-embryogenic and non-embryogenic calli were obtained in the same culture medium (1 mg/L dicamba), expression was different for both genes, which suggests that the presence of auxin is not enough to induce the expression of *SERK* genes. Ta*SERK1* and Ta*SERK2* play a role in SE in cotton (Singla et al. 2008).

In pineapple, Ac*SERK2* is not specifically associated with SE, and could play an important role in morphogenesis (Ma et al. 2012b). Vv*SERK2* stays relatively constant during the SE process (Schellenbaum et al. 2008). Expression of the *SERK* gene was used as a marker of embryogenic competence during the induction of morphogenesis (48 h) in *Helianthus annuus* L. (sunflower) from IZE (Thomas et al. 2004). In *Cyclamen persicum, SERK1* and *SERK2* were shown to be markers of pluripotency, since the activity of both genes was observed in initial cells and derivatives in organogenesis and embryogenesis, and this activity was found to decrease with the formation of organs and embryos (Savona et al. 2012). Both genes were also shown to be markers of totipotency in the formation of embryos (Savona et al. 2012).

The expression of Co*SERK1* in shoots is approximately sevenfold when compared to the root, a similar expression pattern was observed for Ta*SERK1* (Singh and Khurana 2017). Because the shoots used in this study could follow an organogenic or embryogenic pathway, we believe that the Co*SERK1* transcript is being accumulated in reaction zones (where adventitious shoots or embryos will appear), as well as in vascular tissue and foliar primordia, as has been described by Thomas et al. (2004) in *Helianthus annuus* L, these authors found that the expression peak of *SERK* in IZEs is similar (around 10) regardless of whether the culture medium was embryogenic, highly embryogenic, or organogenic, indicating the accumulation of *SERK* transcript in vascular tissue and foliar primordia, as well as in reaction zones (where adventitious shoots or somatic embryos will emerge).

It is clear that the expression pattern of the Co*SERK2* gene is different from that of Co*SERK1* (Fig. 4), especially in 3-week old embryogenic callus, and 9-week old embryogenic callus where *CoSERK2* has lower or similar expression with the root normalized to 1 (Fig. 4).

Particularly, the expression of Co*SERK2* in MZE is lower than in the root. Co*SERK2* presented an expression pattern similar to Gh*SERK2* (*Gossypium hirsutum*) with same levels in non-embryogenic and embryogenic callus (Liu et al. 2018). Thus, it is possible to discard a redundancy of functions in somatic embryogenesis. Furthermore, the pattern of Co*SERK2* suggests a wider set of functions compared to Co*SERK1* (Ma et al. 2012b), for example Ta*SERK2* and Ta*SERK1* have a similar expression pattern with higher levels during embryogenic callus development in *Triticum* (Singh and Khurana 2017). In other species, a prolonged decrease in the expression of *SERK2* as the age of the embryogenic culture increases has been observed (Savona et al. 2012; Ma et al. 2012b). This pattern was not observed in our results. Zakizadeh et al. (2010) did not find a relation between the expression of *SERK2* and embryogenic cultures of *Rosa hybrida*. They reported that the *SERK2* gene had a higher expression in stems (three-fold) and was equally expressed in petals and embryogenic callus (twofold).

Conclusions

The expression pattern of the Co*SERK1* gene suggests that the function of this gene is related to somatic embryogenesis, while the expression pattern of Co*SERK2* suggests that this gene is not as clearly related to somatic embryogenesis. Based on these results, we suggest that the expression pattern of Co*SERK1* could be used as a maker for somatic embryogenesis in Spanish cedar. The complete sequence of the *CoSERK1* gene must be obtained because it is necessary to design a strategy to improve regeneration in *Cedrela*. It is necessary to establish the effect of overexpression of *CoSERK1* in somatic tissues in vitro. To demonstrate if it is possible to use it to induce embryogenic cells or to monitor the expression of the gene through the embryogenesis process as well as to determine the participation of related genes that allow improving the embryogenic response and the regeneration of *Cedrela odorata*.

Compliance with ethical standards

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