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Full Length Research Paper

Isolation and molecular characterization of *RcSERK1*: A *Rosa canina* gene transcriptionally induced during initiation of protocorm-like bodies

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A somatic embryogensis receptor-like kinase (*SERK*) gene was isolated from protocorm-like bodies (PLBs) of *Rosa canina* by a rapid amplification of cDNA ends (RACE) approach and was designated as *RcSERK1*. The *RcSERK1* encodes a protein of 626 amino acid residues with a calculated molecular mass of 68.79 kDa and theoretical isoelectric point of 5.65. The amino acid sequence of *RcSERK1* shares all the characteristic features of a SERK protein, including the signal peptide (SP), the leucine zipper (LZ), the five leucine-rich repeats (LRRs), the pro-rich domain containing the so-called Ser-Pro-Pro (SPP) motif, the transmembrane domain (TM), the kinase domain and the C-terminal domain. The transcripts of *RcSERK1* were more enriched in PLBs than in rhizoids and callus, but not detected in leaflets (incubated under dark and before producing callus) and the regenerated shoots. Subcellular localization indicated that the fluorescence of RcSERK1-GFP was recorded in the plasma membrane. We argue that *RcSERK1* is a Leu-rich repeat receptor-like kinase (LRR-RLK) and plasma membrane localization protein.

Keywords: somatic embryogensis receptor-like kinase (*SERK*)1, protocorm-like bodies (PLBs), *Rosa canina*, RACE, *RcSERK1*.

INTRODUCTION

Somatic embryogensis receptor-like kinase (*SERK*) genes encode leucine-rich repeat receptor-like kinases (LRR-RLKs) (Schmidt et al., 1997; Hecht et al., 2001), and *SERKs* share the canonical structure of LRR-RLKs but have a limited number of leucine-rich repeat (LRR) motifs (Colcombet et al., 2005). The first *SERK* gene identified was reported in carrot (*Daucus carota*) suspension cultures where it was specifically expressed in cells which developed into somatic embryos (Schmidt et al., 1997; Nolan et al., 2009). *SERK* genes have been isolated from several plant species including *Arabidopsis thaliana* (Hecht et al., 2001), *Zea mays* (Baudino et al., 2001), *Medicago truncatula* (Nolan et al., 2003), *Helianthus annuus* (Thomas et al., 2004), *Ocotea catharinensis* (Santa-Catarina et al., 2004), *Dactylis glomerata* (Somleva et al., 2000), *Citrus unshiu* (Shimada et al., 2005), *Oryza sativa* (Hu et al., 2005) and *Theobroma cacao* (Santos et al., 2005). Ectopic expres-

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Abbreviations: EX, Extracellular domain; **LRRs,** leucine-rich repeats; **LRR-RLK,** leucine-rich repeat receptor-like kinase; **LZ,** leucine zipper; **PLBs,** protocorm-like bodies; **UTR,** untranslated region; **SE,** somatic embryogenesis; **SERK,** somatic embryogenesis receptor-like kinase; **SP,** signal peptide; **SPP,** serine-proline-proline; **TDZ,** thidiazuron; **TM,** transmembrane domain; **RACE,** rapid amplification of cDNA ends; **CaMV,** cauliflower mosaic virus; **PCR,** polymerase chain reaction; **RT-PCR,** reverse transcriptase-PCR.

sion of the full-length *AtSERK1* cDNA under the control of the cauliflower mosaic virus (CaMV) 35S promoter did not result in any altered plant phenotype. However, seedlings that overexpressed the *AtSERK1* mRNA exhibited a 3- to 4-fold increase in efficiency for initiation of somatic embryogenesis (Hecht et al., 2001). *AtSERK1* and *AtSERK2* receptor kinases function together as an important control point for sporophytic development controlling male gametophyte production (Colcombet et al., 2005). Fradin et al. (2009) indicated that tomato *SERK3/BAK1* physically associates with the RLP Ve1 to initiate verticillium immunity.

Somatic embryogensis receptor-like kinase (*SERKs*) form a sub-group among LRR-RLKs, which comprise the largest sub-family of RLKs in plants, and are involved in key plant developmental processes. *SERK* genes were isolated in several plant species suggesting the ubiquitous presence of a small *SERK* gene family in all plants, and moreover, their functional conservation with a specific role in embryogenesis, and possibly other developmental processes (Nolan et al., 2009).

We have successfully established a high efficiency somatic embryogenesis (SE) system of *Rosa canina*, which is an important ornamental plant widely grown in the world, whose protocorm-like bodies (PLBs) were induced from rhizoids by thidiazuron (TDZ) using leaflets of tissue cultured seedlings. PLBs were also considered to be somatic embryos, with an intermediary callus formation, which sometimes is a prerequisite for PLBs formation (Tian et al., 2008). We have studied the development process, morphological characteristics and microscopic structure of PLBs when compared with that of somatic embryos from other plants, and we found that PLBs were embryo aggregates, which were coated by cellular tissues (Tian et al., 2008).

In this study, we isolated and characterized a LRR-RLK gene, designated as *RcSERK1*. And our analyses suggested that *RcSERK1* was orthologous to other plant *SERKs*, and the possible role of *RcSERK1* would be crucial for the explanation of somatic embryogenesis (SE) of *R. canina*.

MATERIALS AND METHODS

Plant materials

Leaflets, callus, rhizoids, PLBs and the regenerated shoots of *R. canina* were collected separately. All samples of plants were frozen immediately in liquid nitrogen and stored at -80℃ for RNA extraction.

Isolation of the *RcSERK1* gene

Polymerase chain reaction (PCR) was performed to obtain a partial sequence of *RcSERK1* by using the first strand cDNA of *R. canina* as a template. With multiple sequence alignment of related *SERK* base sequences, two degenerate primers corresponding to the amino acid sequences, FKS1 sequence (5'-GTGAAY(C/T) CCTTGCACATGGTTY(C/T)CATGT-3') and RKS1 sequence (5'-ATGGAR(A/G)TACAAGGAR(A/G)ACCCAR(A/G)GTH(A/T/C)ACA-3') were used for the PCR of partial sequence. The AUAP primer (5'-GGCCACGCGTCGACTAGTAC-3') and another primer corresponding to the FKS2 sequence (5'-TAATGGCAGCGTT GCCTCAGTT-3') were used for 3' RACE. Primers for 5' RACE were: AAP sequence, 5'-GGCCACGCGTCGACTAGTA CGGGII GGGIIGGGIIG-3' and RK5-1 sequence, 5'-TGGTGCTGCTTTA CTATTTGCTGCC-3', for the first polymerase chain reaction (PCR). And the second PCR primers were: AUAP and RK5-1.

The RACE reactions were performed according to the manufacturer's protocol (Invitrogen RACE cDNA amplification kit, USA). We obtained a single full-length cDNA sequence by combining the 5'-RACE fragment, a partial fragment and C-terminal fragment. Finally, a pair of primers (FK1: 5'-TGGGGTGGTGGT GAGAACAGGCTTTGG-3' and RK1: 5'-ACCGCCGCAAATGA TACAAC TTGC-3') were then designed from the putative 5' and 3' untranslated region (UTR) of the full-length cDNA sequence. An 1881bp putative *RcSERK1* fragment was generated. The nucleotide sequences of *RcSERK1* reported in this paper have been submitted to the GenBank under accession numbers: HM802242. The resultant DNA fragments and RACE products were gel purified and cloned into the pMD18-T vector (Takara) and sequenced (Invitrogen, Beijing).

RNA isolation, DNase treatment and semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) assay

Total RNA from various *R. canina* tissues was extracted using RN09-EASY spin Kit (Biomed, Beijing, China) according to the manufacturer's instructions. Total RNA preparations were subjected to an on-column DNase digestion, while simultaneously performing RNA clean-up, using a Qiagen RNase-Free DNase-Set and Qiagen RNeasy RNA Clean-up Midi Kit (Qiagen, Germany). The first strand cDNA was synthesized with 1 µg total RNA and 1 µl superscriptII enzyme (Invitrogen, USA) according to the manufacturer's protocol. As a control, the 18s rRNA gene (Genbank accession number: FM164424.1) was amplified from various *R. canina* tissues. The primers used for detecting *RcSERK1* gene expression were: forward primer 5'-CGTCGCTCATCCCTTATGGATCAT-3' and reverse primer 5'-AGAATTCGGATGAGGAGCTAATTC-3'. The PCR was performed as follow: pre-denaturation at 94℃ for 5 min, followed by 35 cycles of 45 s at 94℃, 45 s at 55℃, 2 min at 72℃ for *RcSERK1*, 28 cycles for 18s rRNA and a final extension of 10 min at 72℃. The amplified products were resolved on a 1.2% agarose gel and then detected by agarose gel electrophoresis. All RT-PCR experiments were repeated at least three times.

Sequence alignment and phylogenetic tree analysis

The sequence alignment of RcSERK1 and other SERK amino acid sequences were compared by DNAMAN (ver 5.2.2) and the phylogenetic tree was constructed by neighbor-joining method with MEGA program (ver 4.0).

Subcellular localization

The *RcSERK1* open reading frame (ORF) were cloned into the *Hind* III and *Sma*I sites of the pSAT6-GFP-N1 vector. This vector contains a modified red-shifted (green fluorescent protein, GFP) at *Nco*I-*Xba*I sites. The *RcSERK1*-GFP construct was transformed into onion epidermal cells by particle bombardment as described earlier (Wang and Fang, 2002). The transient expression of the *RcSERK1*-GFP fusion protein was observed using confocal microscopy.

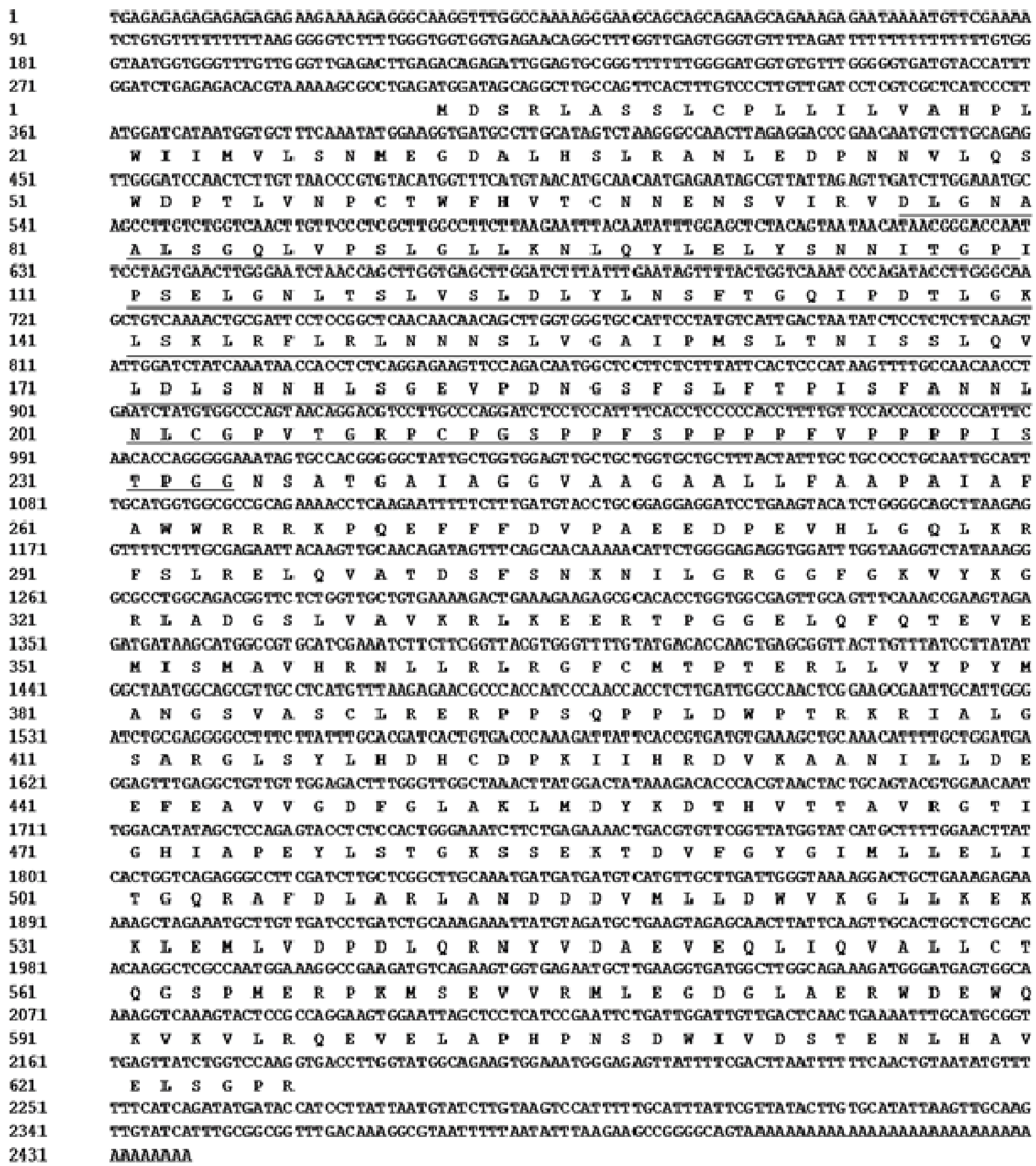

Figure 1. Nucleotide and deduced amino acid sequences of *RcSERK1* (GenBank No. HM802242). The canonical structure of the RcSERK1 proteins is the presence of the extracellular SPP motif in combination with precisely five LRRs, which is underlined.

RESULTS

Isolation of the *RcSERK1* gene from PLBs of *R. canina*

Using the homologous regions of the *SERK1* genes, a partial cDNA was isolated from PLBs of *R. canina* by using degenerate PCR. The full-length cDNAs were obtained by employing rapid amplification of the 3'-cDNA end (3'-RACE) and the 5'-cDNA end (5'-RACE), and were designated as *RcSERK1* (Genbank accession No. HM802242). Sequence analysis showed that the *RcSERK1* cDNA was 2438 bp in length, including a complete ORF of 1881 bp flanking with a 5'-UTR of 301 bp and a 3'-UTR of 256 bp (Figure 1). The predicted protein of *RcSERK1* comprises 626 amino acids with a calculated

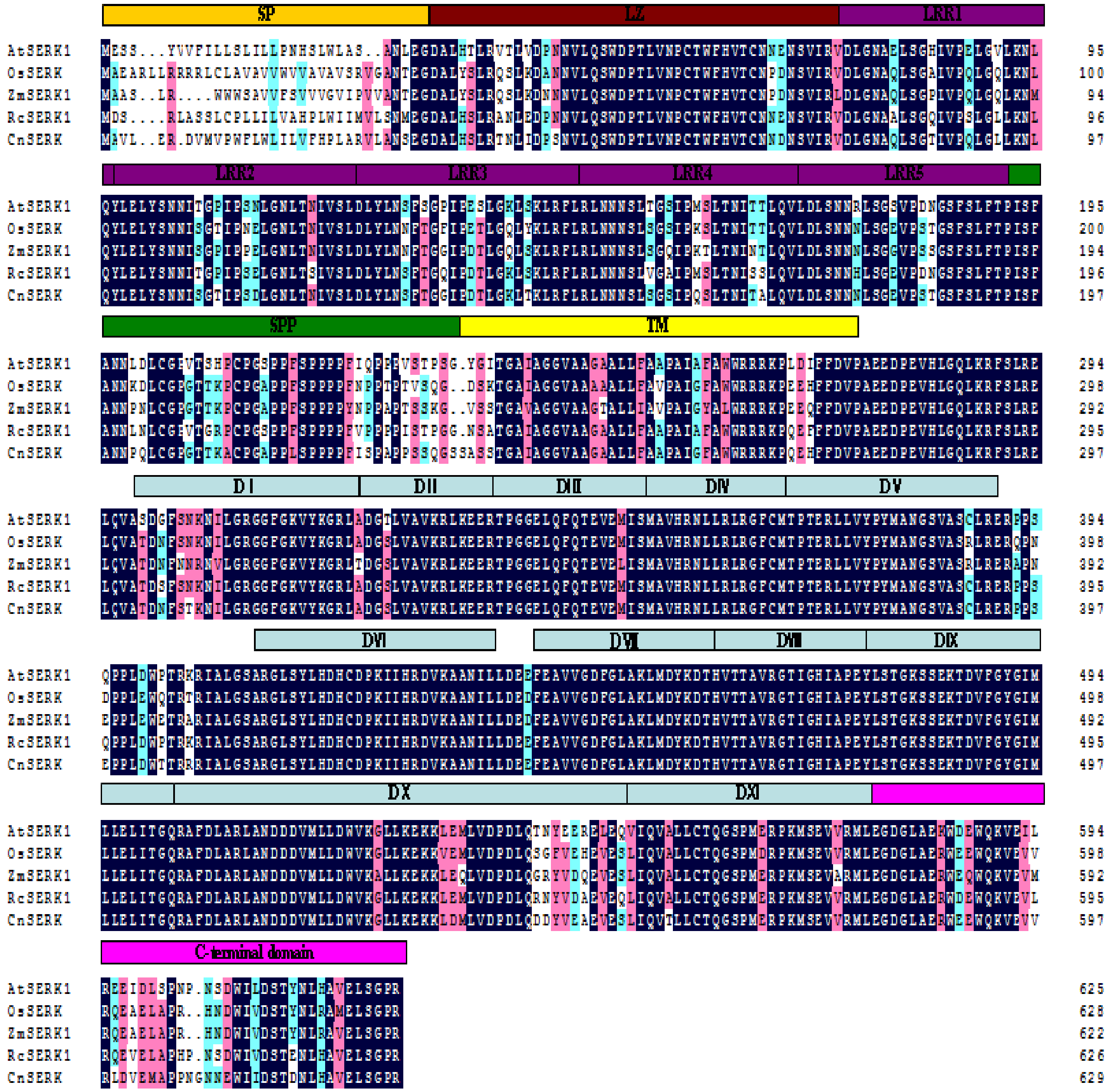

Figure 2. The alignment of the identified RcSERK1 with other plant SERK proteins including the SP, LZ, five LRRs, SPP motif, TM, kinase domain (DI to DXI) and the C-terminal domain. Positions containing identical residues are shaded in navy blue, while conservative residues are shaded in pink.

molecular mass of 68.79 kDa and its theoretical isoelectric point was 5.65.

As shown in the Figure 2 alignment, *RcSERK1* belongs to the LRR-type cell surface RLKs, which possess a number of characteristic domains. These include an extracellular domain (EX) containing a variable number of LRR units immediately followed by a single transmembrane domain (TM) and an intracellular kinase domain responsible for phosphorylating downstream proteins (Hecht et al., 2001). The amino acid sequence of *RcSERK1* shows a high percentage of identity with *AtSERK1* (88.52%) and *DcSERK* (77.32%). The predicted protein structure of the RcSERK1 protein shares all the characteristic features of that protein, and starts at the N-terminus with a signal peptide (SP) followed by a LZ, the five LRRs, a pro-rich domain called the serine-proline-proline (SPP), a single TM, the 11 conserved subdomains of a Ser-Thr kinase and a C-terminal leu-rich domain (Hanks et al., 1988). The hallmark of the SERK proteins is the presence of the extracellular Ser-Pro-Pro motif in combination with precisely five LRRs (Albrechta et al., 2005). Upon comparison of

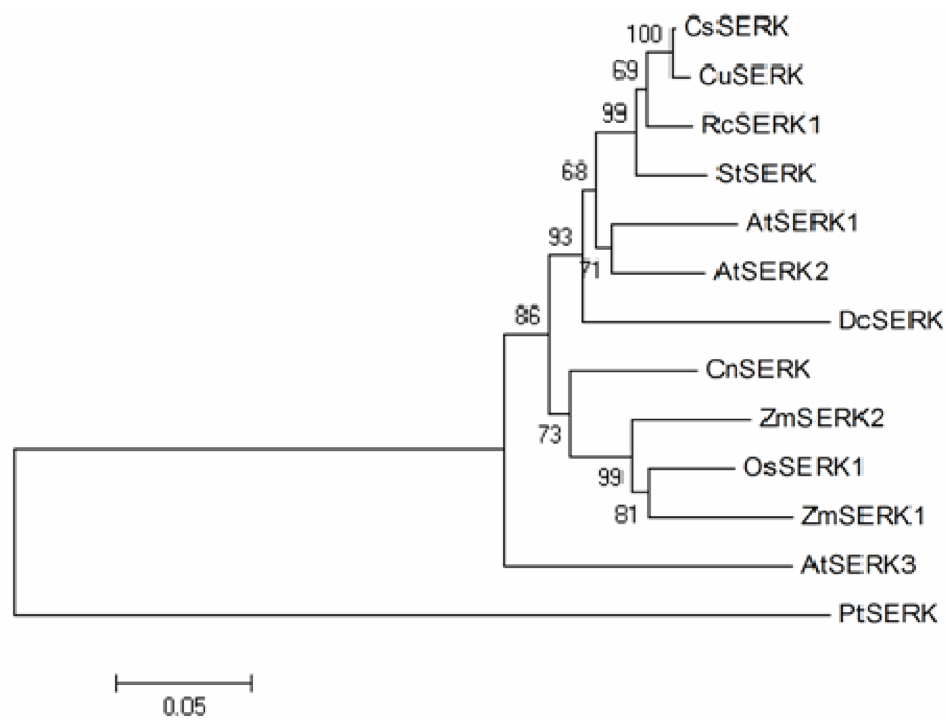

Figure 3. Phylogenetic tree analysis of RcSERK1 and other plant SERK proteins. The tree was constructed by neighbor-joining method with MEGA program (ver 4.0). Branch numbers represent percentage of bootstrap values in 1000 sampling replicates and scale indicates branch lengths. The accession numbers are as follows: CsSERK (*Citrus sinensis*) (FJ851422), CuSERK (*Citrus unshiu*) (AB115767), RcSERK1 (*R. canina*) (HM802242), StSERK (*Solanum tuberosum*) (EF175215), AtSERK1 (*Arabidopsis thaliana*) (NM_105841), AtSERK2 (*A. thaliana*) (AF384969), AtSERK3 (*A. thaliana*) (AF384970), DcSERK (*Daucus carota*) (DCU93048), CnSERK (*Cocos nucifera*) (AY791293), ZmSERK1 (*Zea mays*) (NM_001111662), ZmSERK2 (*Z. mays*) (NM_001111663), OsSERK1 (*Oryza sativa*) (AY652735) and PtSERK (*Populus tomentosa*) (DQ680855).

the amino acid sequences with other species, it was found that RcSERK1 had very high identity including the LZ, LRRs, SPP motif, TM, kinase domain and the C-terminal domain, except for the SP (Figure 2).

We chose herbaceous and woody dicots and monocots, with different families and genera, to investigate the evolutionary relationship among plant SERK proteins. A phylogenetic tree (Figure 3) was constructed using the neighbor-joining method with the full-length amino acid residues. The results showed that RcSERK1 was tightly clustered with CsSERK and CuSERK, and StSERK were grouped into a cluster, and henceforth, designated as RcSERK1, whereas AtSERK1 and AtSERK2 formed another cluster.

Expression analysis of *RcSERK1*

The expression profiles of *RcSERK1* gene in various *R. canina* tissues were investigated using a semi-quantitative RT-PCR assay. Various tissues were respectively collected, as described in materials and methods. *RcSERK1* mRNA was detected in PLBs, callus and rhizoids, but not in leaflets and the regenerated shoots, and mainly in the PLBs but weak in the callus and rhizoids (Figure 4).

Localization of *RcSERK1* in the plasma membrane

To examine subcellular localization of RcSERK1 protein, the RcSERK1-GFP fusion protein was introduced into onion epidermal cells by particle bombardment. As shown in Figure 5, the RcSERK1-GFP fusion protein was recorded in the plasma membrane, whereas the control GFP alone was distributed throughout the cytoplasm. These results show that the RcSERK1 protein is a plasma membrane localized protein.

DISCUSSION

In this study, a LRR-RLK gene *RcSERK1* has been isolated from PLBs of *R. canina*, and its expression has been investigated in leaflets, callus, rhizoids, PLBs and the regenerated shoots. To our knowledge, this is the first

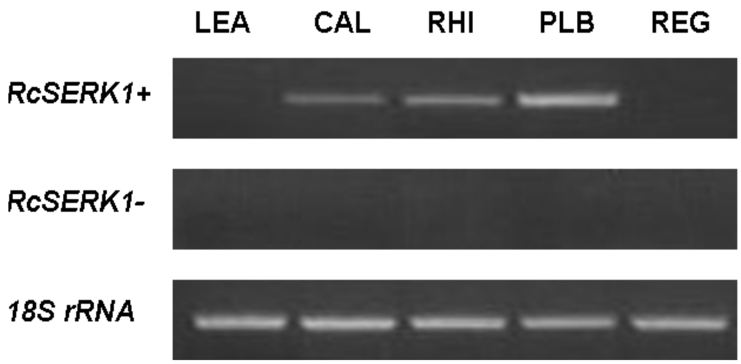

Figure 4. Expression patterns of RcSERK1 in different tissues. Expression patterns of *RcSERK1* in leaflets (LEA), callus (CAL), rhizoids (RHI), PLBs (PLB) and the regenerated shoots (REG). Ethidium bromide staining of PCR products using *RcSERK1*-specific primers with (top) and without (middle) prior reverse transcription and the RT-PCR products with 18S rRNA-specific primers (bottom).

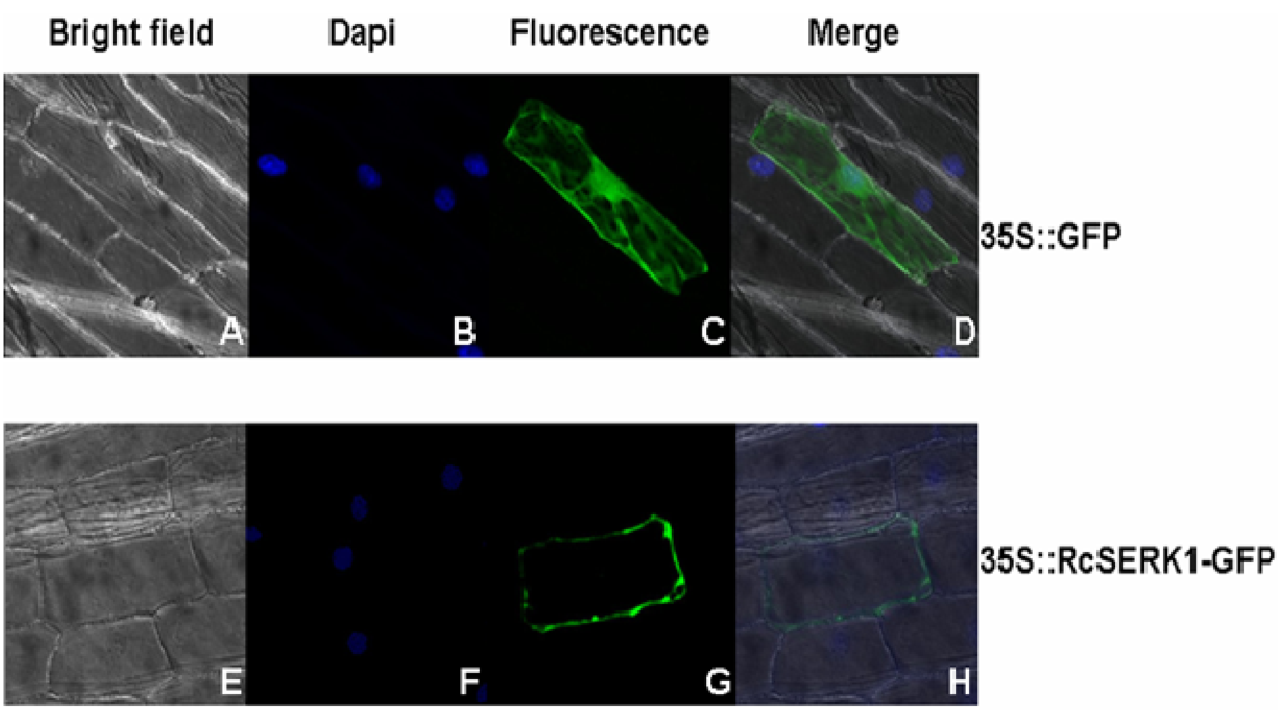

Figure 5. *RcSERK1* localizes to the plasma membrane. Onion epidermal cells were transformed with 35S::GFP and 35S::RcSERK1-GFP. Transformed cells in the bright light (A and E), in the dark for cell nucleuses stained with DAPI (B and F), in the dark for the GFP-fusion proteins (C and G) and the merge of A, B and C (D) / E, F and G (H) were visualized, after incubation for 20 h.

Report of cloning of a *SERK* gene in *R. canina* and the study of its expression.

Sequence analysis of *RcSERK1* revealed high levels of similarity to other plant species of *SERKs*, and it contains the signal peptide (SP), the leucine zipper (LZ), the five leucine-rich repeats (LRRs), the pro-rich domain containing two tandemly repeated SPP sequences, the TM, the kinase domain and the C-terminal domain. Its subcellular location was verified as a membrane protein, consistent with previous reports (Shah et al., 2001), and implied the role of *RcSERK1* as a functional gene. The sequence alignment and phylogenetic tree analysis both were consistent with *RcSERK1* being a functional *SERK* orthologue, for the hallmark of the SERK proteins is the presence of the extracellular SPP motif in combination with precisely five LRRs.

Furthermore, RcSERK1 clustered most closely with *SERK* gene family members such as *CsSERK1*, *CuSERK1*, *AtSERK1* and *StSERK*, which is implicated in evoking somatic embryogenesis. Monitoring of *SERK1* expression during progression of *R. canina* SE revealed *RcSERK1* expression not only in PLBs, but also in callus and rhizoids, however, not detected in leaflets and the regenerated shoots. It showed that *RcSERK1* was expressed at the beginning of the callus of leaves *in vitro*, but under artificial stress-induced, such as dark and 2,4-dichloro-phenoxyacetic acid (2,4-D), and then disappeared at the stage of protocorm-like bodies (PLBs). So expression analysis suggests that the isolated *RcSERK1* could be a functional *SERK* orthologue and an important embryogenic correlation factor. Transformation of the *RcSERK1* into plants and further analysis should reveal its possible functions in SE of *R. canina*.

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