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Short communication

Molecular characterization of somatic embryogenesis receptor-like kinase (*SERK*) genes from plum (*Prunus salicina*) and peach (*Prunus persica*)

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ABSTRACT

Isolation, cloning and molecular characterization of somatic embryogenesis receptor kinase genes (*SERK*) from plum (*Prunus salicina*) and peach (*Prunus persica*) is reported in this paper. Two *SERK* genes were isolated from peach and one gene was isolated from plum. SERK 1 consisted of 822 bp in *Prunus persica* and 828 bp in *Prunus salicina*. The SERK 2 primer was able to generate an 1881 bp fragment from peach and it was found to code 626 amino acids. The conserved domains of these genes were that of catalytic domain of protein kinases.

Key words: *Prunus* spp., *SERK* genes, protein kinases, gene isolation, cloning.

Somatic embryogenesis in many plant species is one of the most viable pathways for plant regeneration and *in vitro* propagation. Past and present research in somatic embryogenesis continues to provide insights from newly studied species and the application of modified methodologies. However, the molecular processes that govern the properties of embryonic competence in plant cells remain elusive (Santa-Catarina *et al*., 7). In order to efficiently regulate plant formation *via* somatic embryogenesis, it is important to understand the molecular mechanisms that underlie the transition from a somatic cell to an embryogenic cell (Fehé, 1). During somatic embryogenesis, biochemical and morphological changes occur throughout the development of induced tissues, which is closely related to alterations in gene expression. Several genes are differentially expressed during somatic embryogenesis induction. The employment of various molecular techniques has led to the identification of several embryogenesis-related genes such as LEA (*Late Embryogenesis Abundant*), SERK (*Somatic Embryogenesis Receptor-like Kinase*), AGL15 (*Agamous-like15*), BBM (*Baby Boom*), LEC1, FUS3 (*Fusca3*) and ABI3 (*ABA Insensitive 3*) (Ikeda *et al*., 3). Some of these genes are useful as markers for somatic embryogenesis since this process is dependent on several factors including genotype. Hence, as a first step to identification of markers it is essential to isolate the genes related to somatic embryogenesis. Among the genes involved in the induction of somatic embryogenesis, the *SERK* gene is claimed to have an important role. To date, a large number of *SERKs* have been characterized from monocotyledonous and dicotyledonous plants and have been shown to play an important role in somatic embryogenesis (Schmidt *et al*., 8; Hecht *et al*., 2; Nolan *et al*., 5). In carrot, it is reported that the *SERK* gene is specifically expressed in embryogenic tissues and not in non-embryogenic tissues and hence serve as markers for embryogenesis. The aim of the present study was to isolate, clone, sequence and compare the *SERK* genes in plum and peach with other reported *SERK* sequences.

Total RNA was isolated from 100 mg of fresh tender leaves of Japanese plum (Acc. No. 98032) and peach (Acc. No. XVII 8D) (which were collected from the growing apex of these trees from the field) using the NORGEN-Biotek kit (Norgen Biotek Corp., Canada) according to the instructions of the manufacturer. RNA samples were treated with DNase I (Invitrogen, Burlington, ON, Canada) prior to the synthesis of cDNA to remove any traces of genomic DNA. The first strand cDNA was synthesized from 5 µg of total RNA using the RevertAid™ Premium First Strand cDNA Synthesis Kit (Fermentas, Burlington, ON, Canada). Primers were first selected from the already reported SERK genes from a related species *Rosa sinensis* (Zakizadeh *et al*., 10) belonging to the family *Rosaceae*. With these sequences the peach genome site was probed and the primers were designed based on these sequences. The PCR primers used were (*SERK* 1 Forward primer- ATGGCTGTCCACCGCAATCTGC; Reverse- CCTGGGACCGGACAACACATCG and SERK 2 Forward primer –ATGGAGAGCAAGGTAGGGAATTCA; Reverse- CCTTGGACCAGATAATTCAACTGC). RTPCR reactions were carried out using 0.5 µl platinum *Taq* DNA polymerase (Invitrogen, Burlington, ON, Canada), 1 X reaction buffer, 1.5 mM $MgCl_2$, 0.2 µM forward and reverse primers, and 1 µl of cDNA

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in a total of 50 µl. The PCR products were run on a 1.5% agarose gel and the amplification obtained was documented. The ligation of the amplicon was carried out as per the user's manual provided with the pGEM-T Easy vector (Promega, Nepean, ON, Canada). *Escherichia coli* DH5 alpha were used as the host cells. The ligation mixture was prepared by adding 2 µl of 2X rapid ligation buffer, 1 µl of vector, 3µl of insert and 1µl of T4 DNA ligase. Final volume was made upto 10 µl and the ligation reaction was set up at 4°C. Competent cells were prepared and transformed as described by Sambrook *et al*. (6). Transformation was once again confirmed by carrying out a PCR with M13 reverse primer and forward primers of *SERK*. The recombinant plasmid was isolated using the Qiagen mini prep kit (Qiagen, Canada) and sequenced. These sequences thus obtained were annotated and compared with database sequences using the BLAST program of NCBI (http://blast.ncbi.nlm.nih. gov/Blast. cgi). The sequences were also analysed using the conserved domain finder in the NCBI site.

The first primer, *i.e. SERK* 1 could generate a 828 bp contiguous genomic fragment from *Prunus salicina* and a 822 bp fragment from *Prunus persica*. These fragments were complete with a start codon and a stop codon. They were designated as plum *SERK* (Ps *SERK*1) and peach *SERK* (Pp *SERK* 1). These sequences showed a 92% homology to each other. The annotated sequence results and the amino acid translations are given in Fig. 1. Pp *SERK*1 sequences

>**Ps-SERK1 (828 bp)**
ATGGCTGTCCACCGCAATCTGCTTCGTCTGCGTGGATTTTGCATGACACCAACAGAAAGGCTGCTTGTTTATCCTTATATGGCTAATGGAAGTGTAGCATCAT-
GTTTAAGAGATCGTCCAGAAGCACAGCCTCCACTTGATTGGGAAATAAGAAAACGCATATCATTGGGATCTGCAAGAGGCCTTGCTTATTTGCATGATCATT-
GTGATCCGAAAATTATTCACCGTGATGTCAAAGCTGCAAACATATTGTTAGATGAGGAATTTGAAGCAGTTGTTGGAGACTTTGGATTGGCTAAACTCATGGAT-
TACAAAGATACGCATGTTACCACAGCTGTACGTGGCACAATTGGTCATATAGCACCAGAGTATCTCTCAACTGGAAAGTCGTCAGAGAAAACTGATGTTTTTG-
GATATGGAGTCATGCTTCTTGAACTTATCACTGGGCAGAGGGCTTTCGATCTTGCTCGGCTTGCAAATGATGATGATGTGATGTTGCTTGATTGGGTCAAAGGATT-
GTTGAAAGATCGGAGATTGGAAGCTCTTGTTGATGCTGATCTAAATGGTAACTATAACGACGACGAAGTGGAGCAGCTGATTCAAGTAGCTCTACTCTGCACG-
CAAGGCACCCCAGGGGAAAGGCCCAAGATGTCAGAGGTTGTCAGAATGCTTGAAGGGGATGGTTTGGCGGAAAGATGGGAGGAGTGGCAGAAAGAGGAGAT-
GTTCCGCCAAGACTTCAACCCTATTCAACATGCAAATTCGAACTGGATCATGGACTCCAGTTCTCAAATTCCACCCGATGTGTTGTCCGGTCCCAGGATTCAC**TAG**

>**Ps-SERK1-aa (275 aa)**
MAVHRNLLRLRGFCMTPTERLLVYPYMANGSVASCLRDRPEAQPPLDWEIRKRISLGSARGLAYLHDHCDPKIIHRDVKAANILLDEEFEAVVGDFGLAKLMDYKDTHVT-
TAVRGTIGHIAPEYLSTGKSSEKTDVFGYGVMLLELITGQRAFDLARLANDDDVMLLDWVKGLLKDRRLEALVDADLNGNYNDDEVEQLIQVALLCTQGTPGERPKMSEV-
VRMLEGDGLAERWEEWQKEEMFRQDFNPIQHANSNWIMDSSSQIPPDVLSGPRIH

>**Pp-SERK1 (822 bp)**
ATGGCTGTCCACCGCAATCTGCTTCGTCTGCGCGGATTTTGCATGACACAAACAGAAAGGCTGCTTGTATATCCTTATATGGCTAATGGAAGTGTAGCATCAT-
GTTTAAGAGATCGTACAGAAGCACAGCCTCCACTTGATTGGGAAAAAAGAAAACGCATCGCATTGGGATCTGCAAGAGGCCTTGCTTATTTACATGATCACT-
GTGATCCGAAAATTATTCACCGTGATGTGAAAGCTGCAAACATATTGTTAGATGAGGAATTCGAAGCAGTTGTTGGAGACTTTGGATTGGCCAAACTCATGGAT-
TACAAGGATACACACGTTACCACAGCTGTACGTGGCACAATGGGTCATATAGCGCCGGAGTATCTCTCAACTGGAAAGTCATCAGAGAAAACTGATGTTTTTG-
GATATGGAGTCATGCTTCTTGAACTTGTCACTGGGAAGACGGCTTTTCATCTTGCTCTGCTTGCAAATAATGATGATGTCTTGTTATTTGATTGGGTCAAAGGAC-
TATTGAAAGATCGGAGATTGGAAGCATTTGTTGATCCTGATCTAAAGGGTTACTATATTGACGAGGAGGTGGAGCAGCTGATCCAAGTAGCGCTACTCTGCACA-
CAAGGCTCCCCAGGGAAACGGCTCAAGATGTCTGAGGTTGTCCAAATGCTTGGAGGTGATGGTTTGGCTGAAAGATGGGAGGCCTGGCAGAAAGAGGAGAT-
GTTCGACCAAGACTTCAACCCTATTCAACATGCAAGTACGAATTGGATCATGGACTCTAGTTCTCAAATTCCACCCGATGTGTTGTCCGGTCCCAGG**TGA**

>**Pp-SERK1-aa (273 aa)**
MAVHRNLLRLRGFCMTQTERLLVYPYMANGSVASCLRDRTEAQPPLDWEKRKRIALGSARGLAYLHDHCDPKIIHRDVKAANILLDEEFEAVVGDFGLAKLMDYKDTHVT-
TAVRGTMGHIAPEYLSTGKSSEKTDVFGYGVMLLELVTGKTAFHLALLANNDDVLLFDWVKGLLKDRRLEAFVDPDLKGYYIDEEVEQLIQVALLCTQGSPGKRLKMSEV-
VQMLGGDGLAERWEAWQKEEMFDQDFNPIQHASTNWIMDSSSQIPPDVLSGPR

>**Pp-SERK2 (1881 bp)**
ATGGAGAGCAAGGTAGGGAATTCACTTTGTCTCTGGTTGATCTTAGTGGCTCATCCACTATGGATGACAATGGTGCTTGCTAATATGGAAGGTGATGCTTTG-
CATAGTCTAAGGACCAATTTGGAGGACCCTAACAATGTCCTGCAAAGTTGGGATCCTACCCTTGTCAACCCTTGTACATGGTTTCATGTCACATGCAACAAT-
GAAAATAGCGTCATAAGAGTTGACCTTGGAAATGCACTCTTGTCGGGTCAACTTGTTCCACAGCTCGGCCTTCTTAAGAATTTACAATATTTGGAACTCTA-
CAGTAATAACATAAGTGGACCAATTCCTAGTGAACTGGGGAACCTAACCAGCTTGGTGAGCTTGGATCTTTATTTGAATAGTTTTGCGGGTCTAATCCCAGA-
CACCTTGGGCAAGCTGTCAAAACTGCGATTCCTCCGACTTAACAACAACAGCTTGGTGGGTCCGATCCCTATGTCATTGACTAATATCTCCTCACTTCAAGTACTG-
GATCTGTCAAATAATCACCTCTCTGGAGAAGTTCCGGACAATGGCTCCTTCTCTTTATTCACTCCCATAAGTTTTGCTAACAACTTGAATCTATGTGGCCCAGTA-
ACTGGTCGCCCCTGCCCAGGATCTCCTCCATTTTCACCTCCCCCACCTTTTGTCCCACCACCCCCCATTTCAACACCAGGAGGGAATAGTGCCACTGGGGCTATC-
GCTGGTGGAGTTGCTGCTGGTGCTGCTTTACTATTTGCTGCCCCTGCAATTGCATTTGCATGGTGGCGACGGAGAAAGCCGCAAGAATTTTTCTTTGATGTACCT-
GCTGAGGAGGATCCTGAAGTACATCTTGGGCAGCTTAAGAGGTTTTCTTTGCGAGAATTACAAGTTGCAACAGATAGTTTTAGCAACAAAAACATTCTGGGGAGAG-
GTGGGTTTGGTAAGGTCTATAAAGGGCGACTAGCAGATGGTTCACTGGTCGCTGTGAAAAGACTGAAAGAAGAGCGCACCCCTGGTGGGGAGTTGCAGTTTCAAA-
CAGAAGTAGAGATGATCAGCATGGCCGTGCATCGAAATCTTCTTCGGTTACGTGGGTTCTGTATGACACCAACTGAGCGATTACTTGTTTATCCTTATATGGCTAATG-
GCAGTGTTGCCTCATGTTTAAGAGAACGCCCACCATCCCAACCACCTCTTGATTGGCCAACTCGGAAGCGAATTGCATTGGGATCTGCAAGGGGTCTTTCTTATTT-
GCACGATCATTGTGACCCGAAGATTATCCACCGTGATGTGAAAGCTGCAAACATTTTGCTGGATGAGGAGTTTGAGGCTGTGGTTGGAGACTTTGGGTTGGCTA-
AACTTATGGACTACAAAGACACCCACGTCACTACTGCCGTACGTGGCACAATTGGTCATATAGCTCCAGAGTACCTGTCTACTGGGAAGTCTTCTGAGAAAACT-
GATGTTTTCGGTTACGGCATTATGCTTCTGGAGCTTATTACTGGTCAGAGGGCTTTTGATCTTGCTCGGCTTGCCAATGATGATGATGTCATGTTGCTTGATTGGGT-
GAAAGGACTACTCAAAGAGAAAAAGCTAGAAATGCTGGTTGATCCTGATCTCCAGAACAATTATGTAGAAGCTGAGGTAGAGCAGCTAATTCAAGTTGCACTGCTCT-
GCACACAAGGTTCTCCAATGGACCGGCCTAAGATGTCAGAAGTGGTGAGAATGCTAGAAGGTGATGGCTTAGCAGAGCGATGGGATGAGTGGCAAAAGGTC-
GAAGTACTCCGCCAGGAAGTGGAGTTAGCTCCTCACCCAAATTCTGATTGGATTGTTGACTCAACAGAAAATTTGCATGCAGTTGAATTATCTGGTCCAAGG**TGA**

>Pp-SERK2-aa (626 aa)
MESKVGNSLCLWLILVAHPLWMTMVLANMEGDALHSLRTNLEDPNNVLQSWDPTLVNPCTWFHVTCNNENSVIRVDLGNALLSGQLVPQLGLLKNLQYLELYSNNIS-
GPIPSELGNLTSLVSLDLYLNSFAGLIPDTLGKLSKLRFLRLNNNSLVGPIPMSLTNISSLQVLDLSNNHLSGEVPDNGSFSLFTPISFANNLNLCGPVTGRPCPGSPPF-
SPPPPFVPPPPISTPGGNSATGAIAGGVAAGAALLFAAPAIAFAWWRRRKPQEFFFDVPAEEDPEVHLGQLKRFSLRELQVATDSFSNKNILGRGGFGKVYKGRLADGSL-
VAVKRLKEERTPGGELQFQTEVEMISMAVHRNLLRLRGFCMTPTERLLVYPYMANGSVASCLRERPPSQPPLDWPTRKRIALGSARGLSYLHDHCDPKIIHRDVKAANIL-
LDEEFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTDVFGYGIMLLELITGQRAFDLARLANDDDVMLLDWVKGLLKEKKLEMLVDPDLQNNYVEAE-
VEQLIQVALLCTQGSPMDRPKMSEVVRMLEGDGLAERWDEWQKVEVLRQEVELAPHPNSDWIVDSTENLHAVELSGPR

Fig. 1. Nucleotide and amino acid sequences for *SERK* genes from plum and peach.

when compared with reported sequences using the BlastX programme of NCBI revealed that they showed maximum similarity to the receptor kinase 1 precursor of *Ricinus communis* and receptor kinase of *Vitis vinifera*. Ps *SERK* 1 showed a maximum similarity to receptor kinase of *R. communis* and *Gossypium hirsutum.* The conserved domain of these sequences were analysed using the conserved domain finder service at the NCBI site and it showed a homology to the catalytic domain of protein kinase. Fig. 2 & 3 show the conserved domains of Pp *SERK*1 and Ps *SERK*1. The SERK 2 primer was able to generate an 1881 bp fragment from peach and after annotation it was found to code 626 amino acids. These nucleotide sequences showed 93% similarity to the already reported *SERK* genes of *Rosa canina* and *V. vinifera.* The analysis of amino acid sequences using clustal W programme also showed 95%, similarity to reported SERK sequences of *R. canina* and *Carica papaya*. Somatic embryogenesis receptor kinases are members of plant receptor like kinases (RLKs). These RLKs have been shown to auto-phosphorylate serine or threonine residues. Receptor-like kinases are plasma membrane bound and plays an important role in the perception and transmission of external signals, cell-cell recognition process during development, symbiosis, defence against pathogen and self incompatibility (Shiu and Bleecker, 9). Plant receptor-like kinases are proteins with a predicted signal sequence with single transmembrane region and a cytoplasmic kinase domain. There are evidences that protein kinases plant an important role in cellular signalling and metabolic regulation in plants (Krupa *et al*., 4). In this study also the conserved domain analysis of Pp *SERK* 2 gene revealed a common structural feature of a leucine zipper on the N terminal side and a leucine repeat region and a catalytic domain of protein kinase (Fig. 4). The N terminal domain with leucine rich repeats (LRRs) is proposed to act as a protein binding region in the LRR receptor kinase and this is a hallmark of SERK genes. The annotated sequence results and the amino acid translations were registered with NCBI and they were assigned accession numbers JX308798 for PsSERK1, JX308799 for PpSERK1 and JX308800 for PpSERK2 (http://www.ncbi.nlm.nih.gov/Genbank). This study opens up the prospects of expression studies during embryogenesis and identification of markers linked to somatic embryogenesis in these *Prunus* species.

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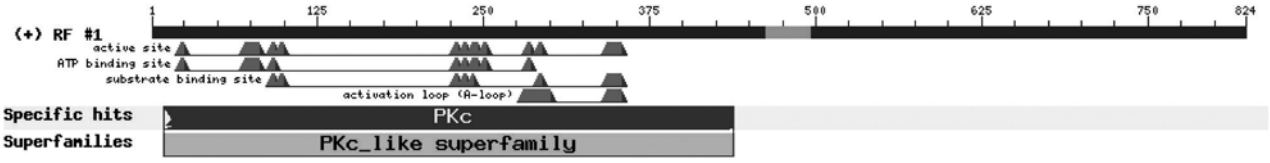

Fig. 2. Conserved domains of Pp *SERK*1.

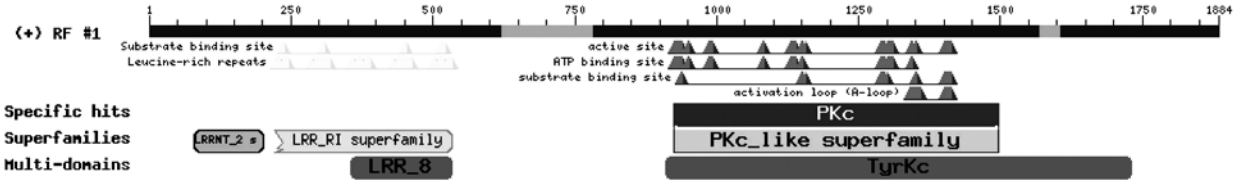

Fig. 3. Conserved domains of Ps *SERK*1.

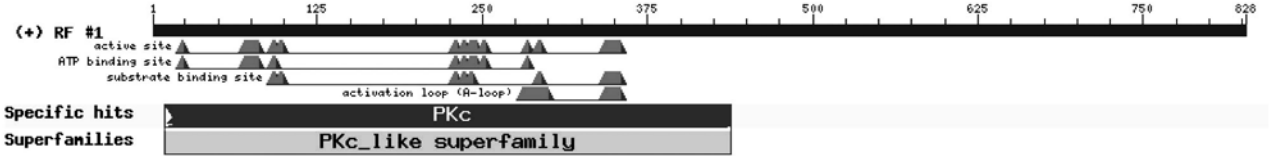

Fig. 4. Conserved domain of Pp *SERK*2.

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