Rubber Science, **29**(2): 159-167, 2016 RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION AND EXPRESSION ANALYSIS OF A SOMATIC EMBRYOGENESIS RECEPTOR KINASE (*SERK*) GENE FROM *HEVEA BRASILIENSIS*

P. Kumari Jayasree, Soya Mary Jose, R. Supriya and Suni Annie Mathew

Rubber Research Institute of India, Kottayam - 686 009, Kerala, India

Received: 18 February 2016 Accepted: 08 April 2016

Jayasree, K.P., Jose, S.M., Supriya, R. and Mathew, S.A. (2016) Molecular characterization and expression analysis of a somatic embryogenesis receptor kinase (*serk*) gene from *Hevea brasiliensis*. *Rubber Science*, **29**(2): 159-167.

In many plant species, somatic embryogenesis receptor kinase (*SERK*) gene, belonging to the plant receptor kinase superfamily and which encodes a leucine-rich repeat receptor-like kinase, is closely related to induction of somatic embryogenesis. *SERK* gene having prominent role in somatic embryogenesis has been characterized from monocotyledonous and dicotyledonous plants. In the present study, a partial *SERK* gene was cloned and characterized from *Hevea brasiliensis* (clone RRII 105). The genomic DNA sequence encoding *SERK* gene in *H. brasiliensis* was amplified with gene specific primers designed based on the consensus sequence reported in other crops. Under optimum PCR conditions, a single band of approximately 0.6 kb size was amplified from genomic DNA and the amplicon was cloned and sequenced. The partial *HbSERK* gene obtained was 612 bp in length with one intron. Homology search and sequence analysis demonstrated a high degree of identity with *SERK* genes reported from other plant species. RT PCR analysis revealed higher expression of *HbSERK* gene in embryogenic callus, while no expression was detected in mature leaf indicating that *HbSERK* is associated with somatic embryogenesis induction. This is the first report of the characterization of a *SERK* gene in *H. brasiliensis* and the sequence was deposited to the GenBank under the accession number KJ451561.

Keywords: Embryogenic competence, Expression analysis, RT-PCR, *SERK* gene, Somatic embryogenesis

INTRODUCTION

Somatic embryogenesis is the process of formation of somatic embryos from somatic cells under *in vitro* conditions. This phenomenon has been reported for more than 200 plant species. During somatic embryogenesis, the transition of somatic to embryogenic state involves the genetic reprogramming of cells by the differential expression of a set of genes. Among the genes involved with the induction of somatic embryogenesis, *SERK* gene is playing an important role. *SERK* gene belongs to the large plant receptor kinases family, often termed receptor-like kinases (RLK), containing a single transmembrane domain with a cytosolic kinases domain. This gene is also characterized by an extra cellular domain and plays important role in controlling a broad range of plant developmental process (Cock *et al.*, 2002).

Correspondence: P. Kumari Jayasree (Email: kumarijaya@rubberboard.org.in)

The first member of the *SERK* gene family *(DcSERK)* was isolated from carrot suspension culture cells (Schmidt *et al.*, 1997). Since then, several *SERK* genes having a prominent role in somatic embryogenesis has been isolated from many crops including *Arabidopsis thaliana* (*AtSERK1*) (Hecht *et al.*, 2001), *Zea mays* (Baudino *et al.*, 2001), *Medicago truncatula* (Nolan *et al.*, 2003), *Helianthus annuus* (Thomas *et al.*, 2004), *Citrus unshiu* (Shimada *et al.*, 2005), *Triticum aestivum* (Singla *et al.*, 2008), *Vitis vinifera* (Maillot *et al.*, 2009), *Rosa hybrid* (Zakizadeh *et al.*, 2010) and *Ananas comosus* (Ma *et al.*, 2012). In addition to somatic embryogenesis, *SERK* genes which play important roles in disease resistance and defense signal transduction were also been isolated from rice and soybean (Hu *et al.*, 2005; Song *et al.*, 2008; Yang *et al.*, 2011).

In *H. brasiliensis*, somatic embryogenesis is the most suitable *in vitro* system for genetic improvement through genetic transformation. During the past years, notable achievement has been made in transgenics. However, the low frequency of somatic embryos and plantlets, and the prolonged time requirement, limited the success in transgenic plant development. A promising alternative is to utilize pre-determined/ morphologically embryogenic cell cultures as the target tissue for transformation. Embryogenic callus having high population of totipotent cells is considered as the suitable target tissue for transformation. In many plant species, *SERK* gene was identified as a potential marker of embryogenic competent cells. In *H. brasiliensis*, this hypothesis is yet to be proved. The present study was an attempt to isolate and characterize *SERK* gene from the genomic DNA of *H. brasiliensis*, clone RRII 105 and to relate its association in embryogenic competence/induction of somatic embryogenesis.

MATERIALS AND METHODS

Induction of embryogenic callus

Embryogenic callus was produced from immature anther of *H. brasiliensis*, clone RRII 105, according to the protocol of Jayasree *et al.* (1999). Briefly, after sterilization, immature anther was cultured on callus induction medium. Callus obtained was sub-cultured for embryogenic callus formation and the emerging embryogenic callus was used for isolation of genomic DNA and RNA.

Isolation of DNA and RNA

Genomic DNA was isolated from one gram of embryogenic callus by the modified CTAB method (Doyle and Doyle, 1990). The quality of isolated DNA was checked using 0.8 per cent agarose gel and used for PCR amplification of the *SERK* gene. RNA was isolated from both embryogenic callus and mature leaf according to the procedure of Venkatachalam *et al.* (1999). Purified RNA was dissolved in DEPC treated water and after analysing the quality, DNA contamination was avoided by DNase treatment at 37 °C for 10 min.

PCR amplification of *SERK* gene

For the amplification of *SERK* gene, primers were designed from the consensus sequences (forward primers: F1, F2, F3 and reverse primers R1, R2, R3, R4) of the *SERK* gene of *Ricinus communis, Dimocarpus longan, Camellia nitidissima, Arabidopsis, Helianthus* sp., coconut and maize. These primers were screened in three combinations (F2R4, F3R3, and F3R4) for specific amplification of *H. brasiliensis SERK* gene (s) with expected size of approx. 450 bp, 300 bp and 400 bp, respectively. After analysing the result, the primer combination F3R4 (forward primer: 5'-GCT TGT ATA TCC CTA TAT GGC-3') and (reverse primer: 5'- CGA GCA AGA

TCA AAA GCC C-3') was selected. PCR reaction was carried out in a total volume of 20 µL containing 20 µg template DNA, 250 nM of each primer, 100 µM of each dNTPs, 0.5 U Taq DNA polymerase and 10x reaction buffer. Amplification was carried out in Perkin Elmer Thermal Cycler 480. The PCR conditions included an initial denaturation step at 94 °C for 3 minutes followed by 36 cycles of one minute denaturation at 92 °C, one minute annealing at 55 °C and one minute extension at 72 °C with a final extension at 72 °C for 10 minutes. PCR products were separated in 1.5 per cent agarose gel and DNA was purified.

Cloning and sequencing of *SERK* gene

Cloning of the purified PCR product was carried out with pGEM-T Easy Vector System cloning kit (M/s. Promega Corporation, USA) and transformed colonies were grown in antibiotic selective LB medium containing IPTG and x-gal. The ligated products were then transferred into competent *E. coli* DH5α cells and transformed cells were selected by blue white screening. Colony PCR was carried out as follows: 94 °C for 10 minutes, 28 cycles of denaturation at 92 °C for one minute, annealing at 55 °C for one minute, extension at 72 °C for one minute and a final extension at 72 °C for 10 minutes. White, transformed colonies were grown overnight in three mL liquid LB medium containing 100 µg mL^{-1} ampicillin and plasmid DNA was isolated using the Illustra Plasmid Prep Mini Spin Kit (M/s. GE Healthcare). Plasmid DNA recovered was also used for PCR amplification for the confirmation of the presence of insert. The sequencing of the cloned fragment was done using an automated DNA sequencer (M/s Macrogen, South Korea). The nucleotide sequence was compared through BLAST homology search tool in NCBI (National Centre for Biotechnology Information, USA). The isolated *SERK* gene sequence was aligned with the reported cDNA sequence of *AtSERK*1 from *Arabidopsis thaliana* using the multiple pair-wise sequence alignment software tool EMBOSS (European Bioinformatics Institute).

SERK gene expression analysis by RT-PCR

First strand cDNA was synthesised from RNA of embryogenic callus and mature leaf by reverse transcription reaction with oligo-(dT) primers using Improm-II Reverse Transcription System (M/s. Promega, USA) kit. Complementary DNA was synthesised by incubation at 42 °C for 1 min. The RT-PCR was performed using 100 ng of the cDNA with the above primer combination and cDNA was amplified through the same PCR conditions as described earlier with the inclusion of 32 cycles.

RESULTS AND DISCUSSION

Primary callus was induced from immature anther after 40 days of culture on callus induction medium. Subsequently, upon subculture to embryogenesis medium, brown callus clumps produced yellow, friable embryogenic callus and this callus was proliferated by frequent sub-culturing to the fresh medium with same composition at an interval of 50 days. This proliferated embryogenic callus was used for *SERK* gene amplification.

Good quality DNA could be isolated from embryogenic callus. Although different primer combinations were attempted, only with the primer combination F3R4 (expected size-400 bp) a good amplification of a single band with approx. size 0.6 kb was obtained (Fig. 1). Positive colonies containing the insert were amplified through colony PCR (Fig. 2) and the recombinant plasmid was isolated for

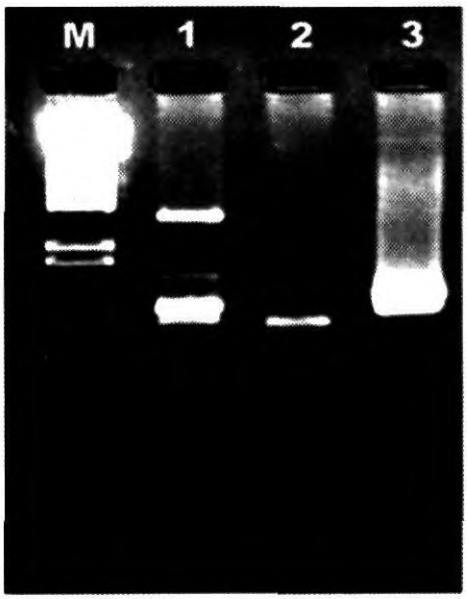

Fig. 1. PCR amplification of *SERK* gene from genomic DNA of embryogenic callus with different primers
Lane M - Marker
Lanes 1-3- Amplification of *SERK* gene with primer F2R4, F3R3, and F3R4

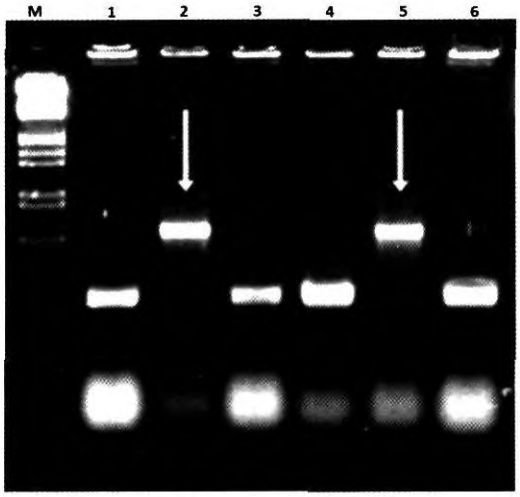

Fig. 2. Colony PCR of transformed colonies
Lane M - Marker
Lanes 1, 3, 4 and 6 - Non-recombinant colonies
Lanes 2 and 5 - Recombinant colonies carrying gene insert

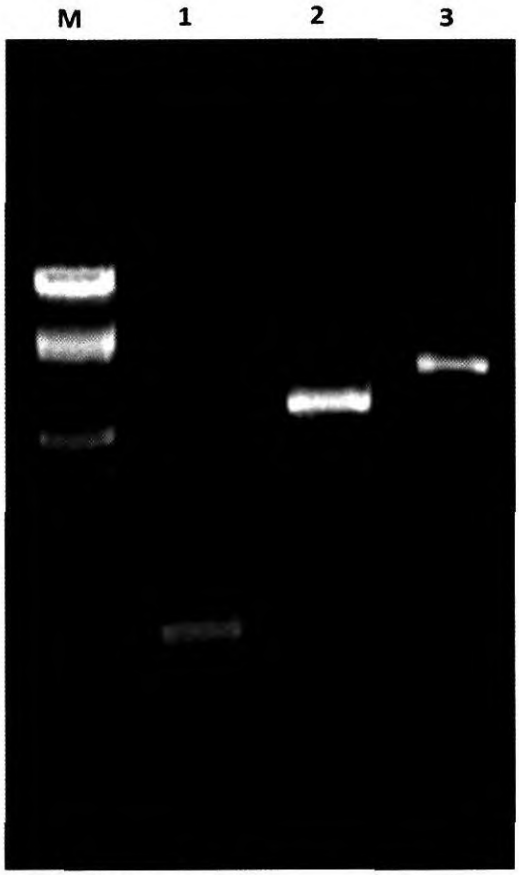

Fig. 3. Cloning of the gene
Lane M - Marker
Lane 1 - Purified PCR product
Lane 2 - Plasmid with insert
Lane 3 - Insert (← shows *SERK* gene released from plasmid)

sequencing. Double digestion of the recombinant clone with *EcoRI* released the insert DNA fragment and on resolution in agarose gel, the insert of expected size was amplified (Fig. 3). The nucleotide sequence data confirmed the presence of the *SERK* gene in *H. brasiliensis* (Fig. 4) and the sequence was deposited in GenBank under the accession number KJ451561. The nucleotide sequence obtained was pair-wise aligned and

GCTTGTATATCCCTATATGGCAAATGGAAGTGTTGCATCATGTTTAAGAGGTAAGCATGCTCTTGTTTTACACATT
CATGGATATTTTAACTGCAACCTCACATAGCATGCTCTTGTTTTACACATTCATGGATATTTTAACTGCAAC
CTCACATATTGTTTTGATGATATGATTGGCTGATTGCATTTGTTTTTATCATTGACCTTCTGCTGCGTTTCA
TGGTTCTCATCAATCTCCTGATGGATGCTTGTAGAGCGCCCGCCATCTCAACCTCCTCTTGATTGGCCAACACGG
AAGCGAATTGCACTGGGATCTGCTAGGGGTCTATCTTATTTGCATGATCATTGTGACCCAAAGATTATTCATCGTGAT
GTTAAAGCTGCAAATATTTTATTGGATGAGGATTTTGAGGCTGTTGTTGGGGATTTTGGGTTGGCTAAGTTGATGGA
CTACAAGGATACTCATGTCACTACTGCCGTCCGTGGTACAATAGGGCATATAGCTCCAGAGTACCTCTCTACTGGAAA
ATCATCGGAAAAAACTGATGTTTTTGGGTATGGGATCATGCTTCTGGAGCTAATTACTGGACAGAGGGCTTTTGATC
TTGCTCG

Exon – 408 bp

Intron – 204 bp

Total - 612 bp

Fig. 4. Partial genomic nucleotide DNA sequence of *SERK* gene isolated from *H.brasiliensis*.

compared with *Arabidopsis thaliana SERK* gene (*AtSERK*1) using EMBOSS online (Fig.5). The cloned *HbSERK* sequence was 612 bp in length with one intron containing 204 base pairs. On comparison with previously reported sequences of *SERK* genes using Blast tool, the sequences of *H. brasiliensis* showing homology greater than 90 per cent are *Dimocarpus longan, Cyclamen persicum SERK*1 and *SERK*2 and maximum homology with *Ricinus communis* (93% identity).

As the expression of *SERK* gene, in general, is associated with somatic embryogenesis potential of cell cultures, we examined the expression of *SERK* gene using cDNA synthesised from embryogenic callus and leaf with the same primer combination. In the present study, reverse transcriptase (RT-PCR) results showed that with embryogenic callus cDNA, a single band with approx. size of 0.4 kb was amplified, while no amplification was observed for leaf DNA (Fig. 6a) revealing that *HbSERK* gene is expressed only in embryogenic callus and not expressed in leaf. These results showed that in *H. brasiliensis, SERK* gene might be playing a major role during the induction of somatic embryogenesis. In several monocot and dicot plant species, the *SERK* gene has been used as a marker of somatic embryogenesis and at the tissue level as a marker of cells competent to form somatic embryos (Schmidt *et al.*, 1997; Somleva *et al.*, 2000; Hecht *et al.*, 2001). In coconut and *Rosa hybrid,* higher expression of *SERK* gene occurred in embryogenic callus compared to non-embryogenic callus (Perez-Nunez *et al.*, 2009; Zakizadeh *et al.*, 2010). Similarly in pineapple, *AcSERK*1 was highly expressed during embryogenic competence acquisition and thus be a potential marker for embryogenic cells (Ma *et al.*, 2012). In the present study, the control gene, β actin, was amplified using actin primers and the expression was uniform in both embryogenic callus and leaf (Fig. 6b) indicating the constitutive expression of βactin in all stages of plant growth and development. The results of expression studied suggest that *HbSERK* gene has a vital role in the induction of somatic embryogenesis.

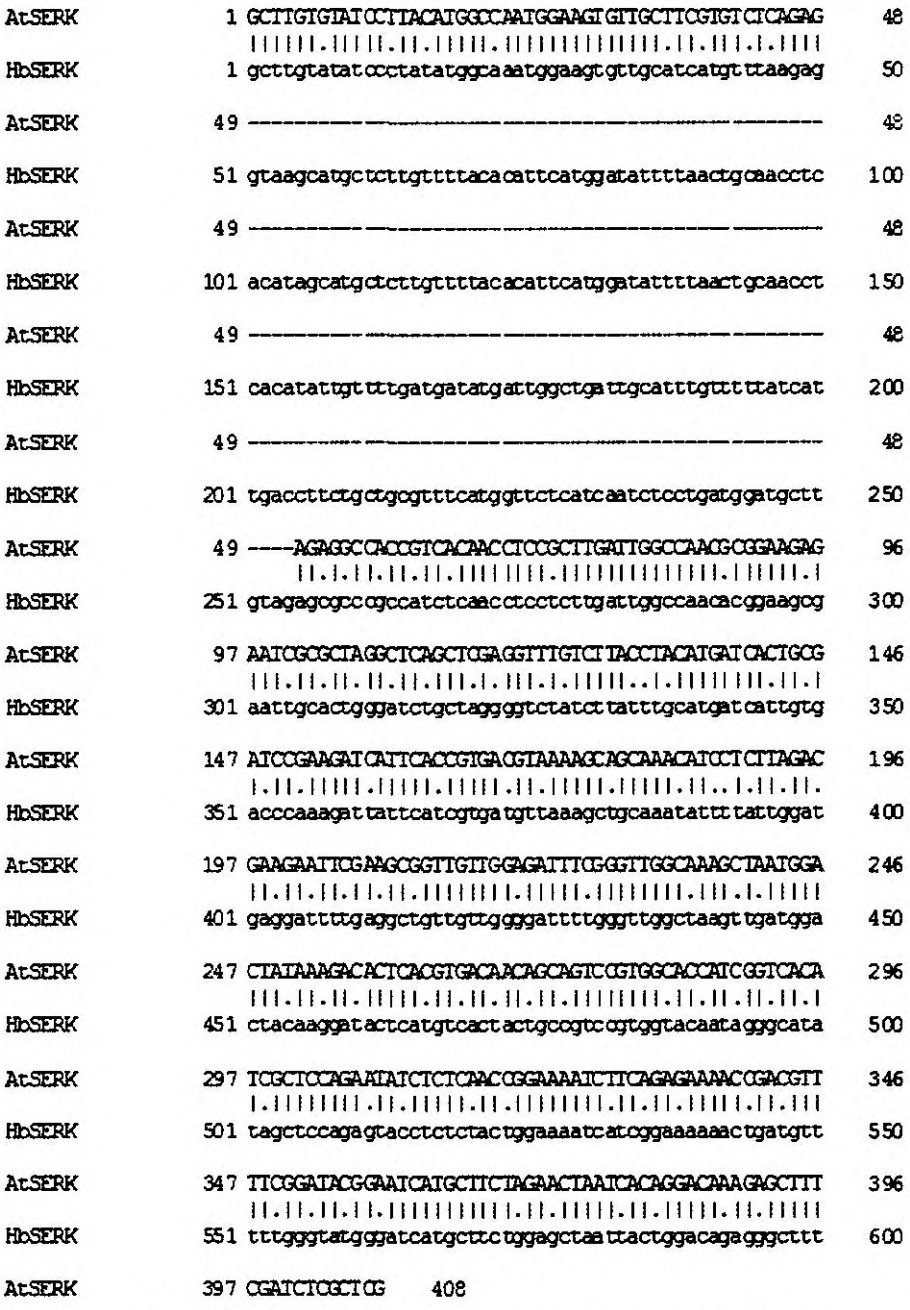

Fig. 5. EMBOSS gene sequence alignment of the isolated partial *SERK* gene sequence of *Hevea* with cDNA of *AtSERK*1 (Gaps show the position of the introns within the genomic sequence of *Hevea SERK* gene)

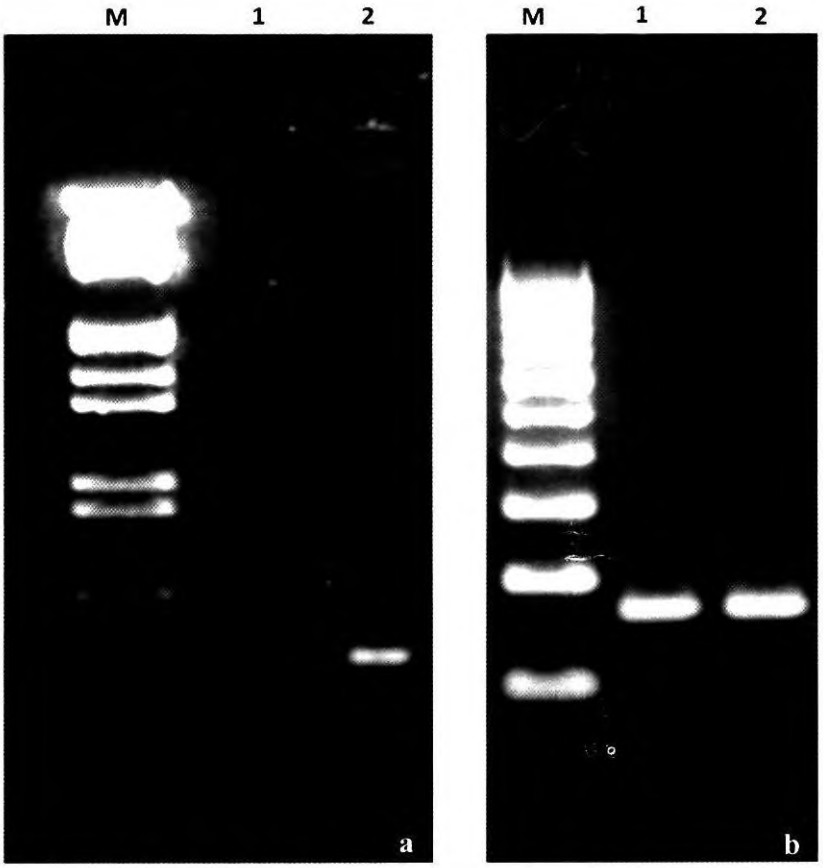

Fig. 6a-b. RT PCR of *SERK* (a) and â-actin gene (b) from cDNA
6a. Lanes M – Marker (ë DNA double digest)
1 – Mature leaf
2 – Embryogenic callus
6b. Lanes M - Marker (ë DNA double digest)
1 – Mature leaf
2 – Embryogenic callus

CONCLUSION

The present study reports the molecular characterization of a partial gene sequence encoding *HbSERK* with a size of 612 bp containing 408 base pair length exon and 204 base pair length intron, for the first time. Sequence homology search indicated 93 per cent identity of *HbSERK* with *SERK* gene of *Ricinus communis*. RT PCR expression analysis revealed a close relationship of *SERK* gene with induction of somatic embryogenesis in *H. brasiliensis* and thus could be used as a potential marker for identifying embryogenic competent cells. In the light of this information, further attempt on full length sequence characterization of *SERK* gene through cDNA cloning

should address more on the mechanism of somatic embryogenesis which could improve the regeneration efficiency and plant recovery from transformed cell lines in *H. brasiliensis*.

ACKNOWLEDGEMENT

The authors express their sincere thanks to Dr. A. Thulaseedharan, Joint Director, Biotechnology Division of RRII, for providing the facilities to carry out the study.

REFERENCES

Baudino, S., Hansen, S., Brettschneider, R., Hecht, V.F.G., Dresselhaus, T., Lorz, H., Dumas, C. and Rogowsky, P.M. (2001). Molecular characterization of two novel maize LRR receptor-like kinase, which belong to the *SERK* gene family. *Planta*, **213**: 1-10.

Cock, J.M., Vanoosthuyse, V. and Gaude, T. (2002). Receptor kinase signalling in plants and animals. Distinct molecular systems with mechanistic similarities. *Current Opinion in Cell Biology*, **14**: 230-236.

Doyle, J.J. and Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue BRL. *Focus*, **12**: 13-15.

Hecht, V., Vielle-Calzada, J.P., Hartog, M.V., Schmidt, D.L., Boutilier, K., Grossnicklaus, U. and de Vries, S.C. (2001). The *Arabidopsis* somatic embryogenesis receptor kinase I gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. *Plant Physiology*, **127**: 803-816.

Hu, H., Xiong, L. and Yang, Y. (2005). Rice *SERK1* gene positively regulates somatic embryogenesis of cultured cell and host defense response against fungal infection. *Planta*, 222: 803-816.

Jayasree, K.P., Asokan, M.P., Sobha, S., Sankariammal, L., Rekha, K., Kala, R.G., Jayashree, R. and Thulaseedharan, A. (1999). Somatic embryogenesis and plant regeneration form immature anthers of *Hevea brasiliensis* (Muell. Arg.). *Current Science*, **76**: 1242-1245.

Ma, J., He, Y., Wu, C., Liu, H., Hu, Z. and Sun, G. (2012). Cloning and molecular characterization of a *SERK* gene transcriptionally induced during somatic embryogenesis in *Ananas comosus* cv. Shenwan. *Plant Molecular Biology Reporter*, **30**: 195- 203.

Maillot, P., Lebel, S., Schellenbaum, P, Jacques, A. and Walter, B. (2009). Differential regulation of *SERK*, LEC- like and pathogenesis related genes during indirect secondary somatic embryogenesis in grapevine. *Plant Physiology and Biochemistry*, **47**: 743-752.

Nolan, K. E., Irwanto, R. R. and Rose, R. J. (2003). Auxin up regulates *MtSERK1* expression in both *Medicago truncatula* root forming and embryogenic cultures. *Plant Physiology*, **133**: 218-230.

Perez-Nunez, M.T., Souza, R., Saenz, L., Chan, J.L., Zuniga-Aguilar, J.J. and Oropeza, C. (2009). Detection of a *SERK*-like gene in coconut and analysis of its expression during the formation of embryogenic callus and somatic embryos. *Plant Cell Reports*, **28**: 9-11.

Schmidt, E.D.L., Guzzo, F., Toonen, M.A.J. and de Vries, S.C. (1997). A leucine-rich repeat containing receptor-like kinase marks somatic plant cells competent to form embryos. *Development*, **124**: 2049-2062.

Shimada, T., Hirabayashi, T., Endo, T., Fujii, H., Kita, M. and Omura, M. (2005). Isolation and characterization of the somatic embryogenesis receptor - like kinase gene homologue (*CtSERK1*) from *Citrus unshiu* Marc. *Scientia Horticulturae*, **103**: 233-238.

Singla, B., Khurana, J.P. and Khurana, P. (2008). Characterization of three somatic embryogenesis receptor kinase genes from wheat, *Triticum aestivum*. *Plant Cell Reports*, **27**: 833-843.

Somleva, M.N., Schmidt, E.D.L. and de Vries, S.C. (2000). Embryogenic cells in *Dactylis glomerata* L. (Poaceae) explants identified by cell tracking and by *SERK* expression. *Plant Cell Reports*, **19**: 718-726.

Song, D., Li, G., Song, F. and Zheng, Z. (2008). Molecular characterization and expression analysis of *OsBISERK1*, a gene encoding a leucine rich repeat receptor- like kinase during disease

resistance responses in rice. *Molecular Biology Reports*, **35**: 275-283.

Thomas, C., Meyer, D., Himber, C. and Steinmetz, A. (2004). Spatial expression of a sunflower *SERK* gene during induction of somatic embryogenesis and shoot organogenesis. *Plant Physiology and Biochemistry*, **42**: 35-42.

Venkatachalam, P., Thanseem, I. and Thulaseedharan, A. (1999). A rapid and efficient method for isolation of RNA from bark tissues of *Hevea brasiliensis*. *Current Science*, **77**: 635-637.

Yang, C., Zhao, T., Yu, D. and Gai, J. (2011). Isolation and functional characterization of a *SERK* gene from soybean (*Glycine max* (L.) Merr.). *Plant Molecular Biology Reporter*, **29**: 334-344.

Zakizadeh, H., Stummann, B.M. Liitken, H. and Muiller, R. (2010). Isolation and characterization of four somatic embryogenesis receptor - like kinase (*RhSERK*) genes from miniature potted rose (*Rosa hybrida* cv. Linda). *Plant Cell Tissue Organ Culture*, **101**: 331-338.