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RESEARCH ARTICLE

IDENTIFICATION OF SOMATIC EMBRYOGENESIS RECEPTOR KINASE (*SERK*) GENES IN SORGHUM THROUGH *IN SILICO* ANALYSIS

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ABSTRACT

Somatic embryogenesis receptor kinases (*SERKs*) constitute a small gene family and are functionally conserved in plants with a specific role in embryogenesis and possibly in other developmental processes. The present investigation was aimed at identifying the SERK gene (s) present in sorghum genome through *in silico* studies. Here we report two *SERK* genes (*SbSERK1,* and *SbSERK2*) from sorghum (*Sorghum bicolor* (L.) Moench.) by the comparative analysis of known *SERK* cDNA sequences from sorghum and other plants and their chromosomal location on sorghum. The sequences of *SbSERK1* and *SbSERK2* were more similar to that of *ZmSERK1* and *ZmSERK2* genes of maize. A putative *SbSERK3* was also identified which was more similar to the rice *OsSERK1*.

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INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench.) is a leading cereal, ranking fifth in importance after wheat, rice, maize, and barley. It is a self-pollinated diploid (2n=2x=20) C4 grass widely adapted to diverse agricultural environments around the world. As sorghum has a smaller genome size of 730 Mbp and is fully sequenced, it makes an attractive model for functional genomics of C4 grasses (Paterson *et al.,* 2009). Somatic Embryogenesis (SE) is an *in vitro* asexual reproduction process in which somatic cells gives raise to somatic embryos under favorable experimental conditions (Madhu *et al*., 2015). In tissue culture, differentiated somatic cells acquire embryogenic competence and proliferate as embryogenic cells during the induction phase (Dodeman *et al*., 1997 and Parameswari Namasivayam., 2007). The events that take place during the period in which plant cells undergo the transition from somatic to embryogenic cell are poorly understood (De Jong *et al*., 1993). Genes like *LEC1* (Lotan *et al*., 1998), *LEC2* (Stone *et al*., 2001), *BBM* (Boutilier *et al*., 2002), *WU* (Zuo *et al*., 2002) and *AGL15* (Harding *et al*., 2003) are involved in the somatic embryogenesis process but in the later stages of SE. These genes inturn helps in increasing the overall frequency of SE and prolongs the process of SE. However out of all the genes that have been isolated and analyzed during SE, somatic embryogenesis receptor kinase (*SERK*) gene, is the only one that has been shown successfully to be a specific marker distinguishing individual embryo forming cells in carrot suspension cultures (Schmidt *et al*., 1997). *SERKs* constitute a small gene family and are functionally conserved in plants with a specific role in embryogenesis and possibly other developmental processes. In addition to play as a key role in embryogenesis process, *SERK* gene is also involved in the transduction of extracellular signaling processes as diverse as plant development, disease resistance or self incompatibility (Baudino *et al*., 2001; Krupa *et al*., 2006).

SERK belongs to Leucine-rich repeat, receptor like kinases (LRR-RLKs), a subgroup of protein kinases. The predicted *SERK* protein contains an N-terminal Leucine zipper (LZ) domain followed by 5 LRRs, a serine and proline rich SPP domain, a transmembrane domain and an intracellular serine /threonine kinase domain (Figure 1). The SPP domain is a unique feature of the *SERK* family of receptor kinases (Schmidt *et al*., 1997; Hecht *et al*., 2001). A unique feature of *SERK* protein is the presence of proline-rich region between the extracellular LRR domain of *SERK* and the membrane-

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spanning region, which is a conserved feature of plant cell wall proteins known as extensions (Varner and Lin 1989). Upto now, the ubiquitous presence of this small *SERK* gene family has been identified, isolated and characterized in almost all the plant species. So in this current study, based on the c-DNA sequence information obtained from the close relatives of sorghum like maize and rice, attempt was made to predict the loci, structure and copy number of *SERK* genes on sorghum genome using *in silico* studies.

MATERIALS AND METHODS

To predict the presence of *SERK* genes in sorghum, Phytozome *v* 10.3 is used as a basic platform which serves as the best plant comparative genomic portal. Nucleotide sequences were compared to EMBL and GenBank databases using the BLASTN (nucleotide query to nucleotide db) algorithm. Clustal Omega tool is used for the protein sequence analysis which inturn uses seeded guide trees and HMM profile-profile techniques to generate alignments between three or more protein sequences. NCBI database is used for identification of c-DNA and its relative protein sequence information of Maize, Rice, Arabidopsis and Medicago. Phylogenetic trees (Figure 2) were constructed using ClustalW of EMBL database.

RESULTS AND DISCUSSION

Based on the *SERK* gene c-DNA sequence information from other crop plants like maize and rice, genome wide comparative analysis was performed to identify the loci and copy number of *SERK* genes in sorghum. The main referral gene sequences used in this study are *ZmSERK1* (EMBL accession number AJ400868), *ZmSERK2* (EMBL accession number AJ400869) and *OsSERK1* (EMBL accession number AB188247).

SERK gene family

SERK belongs to a small gene family with at least five members in Arabidopsis (Hecht *et al*., 2001), three in maize (Baudino *et al*., 2001), five in *Medicago truncatula* (Nolan *et al*., 2003), four in *Helianthus annuus* (Thomas *et al*., 2004), two in rice (Ito *et al*., 2005; Hu *et al*., 2005) and at least three in wheat (Singla *et al*., 2008). A correlation between *SERK* expression and somatic embryogenesis was demonstrated in cultured tissues of carrot (Schmidt *et al*., 1997), *Dactylis glomerata* (Somleva *et al.,* 2000), *Arabidopsis thaliana* (Hecht *et al.,* 2001), *Medicago truncatula* (Nolan *et al.,* 2003), sunflower (Thomas *et al.,* 2004), rice (Hu *et al.,* 2005), cocoa (Santos *et al.,* 2005), and *Triticum aestivum* (Singla *et al.,* 2008).

The *SbSERK* family

The *SERK* gene family in sorghum has at least three members. These three genes were identified during a screen of *SERK* c-DNA sequences of maize and rice with sorghum. *ZmSERK 1* and *2* helped in identifying two *SERK* genes in sorghum and one by *OsSERK1*. These three genes are precisely described here at their nucleotide and protein levels.

SbSERK1

SbSERK1 gene was identified while analyzing *ZmSERK1* gene using BLASTN algorithm along the sorghum genome and hits a maximum similarity on chromosome (*chr*) 6 in sorghum. The total length of this functional gene is 5285 bp in which coding region occupies 1869 bp. The first portion of the gene starts with 284bp of 5'UTR and ends with 241 bp of 3'UTR and between these two UTR's it is occupied by 11 exons and 10 introns. When analyzed at the protein level using SIM module of ExPASy tools *SbSERK1* having 622 amino acids (aa) is showing the highest similarity of 86.7% with the peptide sequence of *ZmSERK1* gene containing 622 aa. The similarities of protein sequences between *SbSERK1*, *ZmSERK1*, *OsSERK1*, *AtSERK1* and *MtSERK1* are shown in Figure 3.

Figure 1. Structural features of SERK genes in sorghum

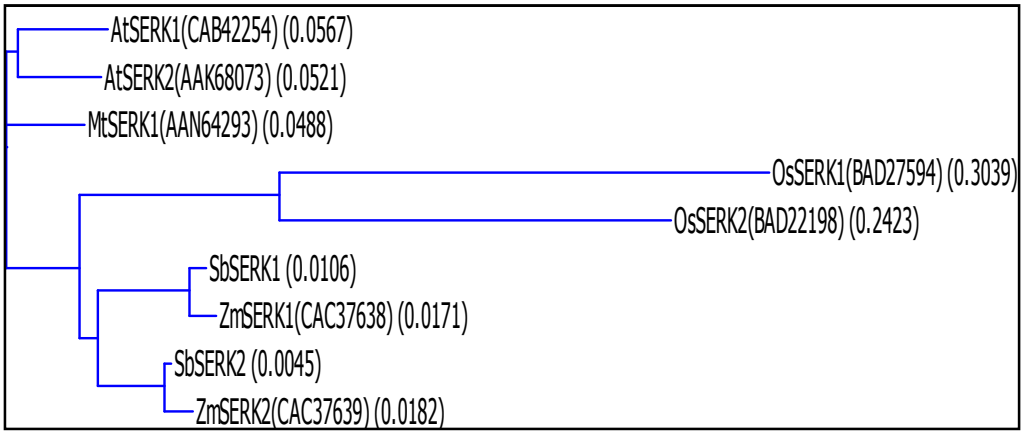

Figure 2. Phylogenetic relationship of SERK1 and SERK2 Proteins

Figure 3. Multiple sequence alignment of Sorghum SERK1

Figure 4. Multiple sequence alignment of Sorghum SERK2

SbSERK2

This specific *SbSERK2* gene was identified on *chr* 4 in sorghum genome which shows the highest similarity with *ZmSERK2* of maize. The total predicted length of this gene is 6152 bp having 353 bp of 5'UTR and 457 bp of 3'UTR between this occupied by a total of 11 exons and 10 introns. The CDS (coding sequence) sequence consists of 1881 bp which codes for 626 amino acids. The similarity of *SbSERK2* peptide sequence when analyzed with SIM module of ExPASy tools with the peptide sequence of *ZmSERK2* (626 aa) is 97.1%, which shows the close relative protein functions between sorghum and maize. The similarities of protein sequences between *SbSERK2*, *ZmSERK2*, *OsSERK2*, and *AtSERK1* are shown in Figure 4.

SbSERK3

SbSERK3 gene was identified while analyzing *OsSERK1* gene using BLASTN algorithm along the sorghum genome and hits a maximum similarity on *chr* 7 in sorghum. The total length of this functional gene is 6597 bp in which coding region occupies 1896 bp. The first portion of the gene starts with 470 bp of 5'UTR and ends with 611 bp of 3'UTR and between these two UTR's it is occupied by 11 exons and 10 introns. When analyzed at the protein level using SIM module of ExPASy tools *SbSERK1* having 631 amino acids (aa) is showing the highest similarity of 94.2% with the peptide sequence of *OsSERK1* gene containing 620aa.

The available literature and expression studies still do not provide adequate information to differentiation between the several *SERK* genes in each species. The protein sequences among sorghum, maize and rice share sequence similarities up to 86.7-94.2% is well interpreted as essential for a common function. *ZmSERK1* is preferentially expressed in male and female reproductive tissues while *ZmSERK2* was relatively uniform in expression in the tissues investigated (Baudino *et al*., 2001). The suppression of *OsSERK1* by RNA interference resulted in an inhibition and *SERK1* over expression resulted in induction of shoot regeneration from callus. Over expression of *OsSERK1* resulted in an increased resistance to blast fungus (Hu *et al.,* 2005).

In *Arabidopsis*, at ovule maturity, all cells of the embryo sac express *AtSERK1,* the *SERK* gene of *Arabidposis* (Hecht *et al.*, 2001). The *AtSERK1* gene is expressed during megasporogenesis and in all cells of the embryo sac up to the stage of fertilization. After fertilization, *AtSERK1* promoter-driven GUS activity is found in all cells of the developing embryo up to the heart stage. The expression of the *AtSERK*1 homolog gene *PpSERK1* was studied by Albertini *et al.* (2005) who revealed by *in Situ* hybridization that *PpSERK* is expressed in the megaspore mother cell of sexual genotypes, but not in that of apomictically reproducing *Poa pratensis* plants. Partial cDNA fragments showing homology to *SERK* and LRR-Kinase genes of *Arabidopsis* have also been isolated in buffel grass by subtractive hybridization (Dwivedi *et al*., 2005). It had very low abundance (100 times less) in apomicts while it is over-expressed in its close sexual relative (F_2 segregant). Over expression of SERK1 in Arabidopsis did not result in any obvious plant phenotypes, but gave a 3 to 4-fold increase in embryogenic competence, which indicates that SERK1 not only marks embryogenic competence, but also promotes the transition of somatic cells to an embryogenic state (Hecht *et al.,* 2001). The identification of SERK genes in sorghum is expected to throw more light on its function in somatic embryogenesis, embryo development and other phenomena. The role of SERK genes in megasporogenesis (Albertini *et al.,* 2005; Dwivedi *et al*., 2005) and microsporogenesis (Hecht *et al.,* 2001) may be further investigated to utilize them in development of apomictic and hybrid cultivars.

SP- Signal peptide domain; ZIP- N-terminal Leucine zipper domain; LRR- leucine rich repeat-Receptor Like kinases; SPP- serine and proline rich domain; TM- transmembrane domain; Kinase- an intracellular serine / threonine kinase domain; C- C terminal domain. Proline-rich SPP domain between LRR and TM is a unique feature of the SERK family of receptor kinases. The phylogenetic tree with the SERK family in sorghum (Sb), Arabidopsis (At), alfalfa (Mt), maize (Zm) and rice (Os) generated using ClustalW using amino acid sequence data.

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