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MOLECULAR CHARACTERIZATION AND EVOLUTION STUDIES OF A *SERK* LIKE GENE TRANSCRIPTIONALLY INDUCED DURING SOMATIC EMBRYOGENESIS IN *Phoenix Dactylifera* L v Deglet Nour

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A somatic embryogenesis receptor kinase like (*SERKL*) cDNA, designated *PhSERKL*, was isolated from date palm (*Phoenix Dactylifera* L) using RACE PCR. *PhSERKL* protein shared all the characteristic domains of the *SERK* family, including five leucine-rich repeats, one proline-rich region motif, a transmembrane domain, and kinase domains. Phylogenetic analyses using PHYLIP and Notung 2.7 programs suggest that the SERK proteins of some plant species resulted from relatively ancient duplication events. We predict an ancestor protein of monocots and dicots SERK using FASTML program. Somatic embryogenic cultures of date palm were established following transfer of callus cultures to medium containing 2, 4-dichlorophenoxyacetic acid. The role of *PhSERKL* gene during establishment of somatic embryogenesis in culture was investigated using quantitative real-time PCR. *PhSERKL gene* was highly expressed during embryogenic competence acquisition and globular embryo formation in culture. Overall, levels of expression of *PhSERKL* gene were lower in nonembryogenic tissues and organs than in embryogenic callus.

Key words: duplication, expression pattern, *Phoenix dactylifera, SERKL*

INTRODUCTION

Different genetic and physiological factors trigger somatic embryogenesis in vitro in several kinds of plant somatic cells. However, the molecular bases of genetic and biochemical mechanisms leading to somatic embryogenesis are not well understood. In the last 10 years, molecular and genetic studies focused in plant development have resulted in the identification of various genes involved in the regulation of this process.

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The first somatic embryo development *in vitro* was demonstrated by both REINERT (1958) and STEWARD *et al* (1958). Somatic embryogenesis (SE) is an *in vitro* regeneration system whereby an organized bipolar structure containing root and shoot apices, and morphologically resembling a zygotic embryo, is formed from one somatic cell. Somatic embryogenesis involves various molecular events including differential gene expression and various signal transduction pathways for activating or repressing numerous genes sets (CHUGH and KHURANA 2002). Genes involved in somatic embryogenesis are stage specific. The first somatic embryogenesis receptor like kinase (*DcSERKL*) is identified in early somatic embryogenesis and was originally isolated from embryogenic cells in suspension cultures of the dicot *Daucus carota* (SCHMIDT *et al.,* 1997). SERK belongs to the Leucine rich repeat, receptor-like kinases (LRR-RLKs), a subgroup of protein kinases characterized by an extracellular domain with at least five Leu-rich repeats (LRRs), a transmembrane domain, and an intracellular kinase domain. The putative role of RLKs in developmental processes is to transduce environmental signals and/or information from neighboring cells and to induce specific responses (BAUDINO *et al.,* 2001). The gene *ZmSERK* was isolated from maize, and its expression patterns were similar to *DcSERK* during SE, but it showed a more diverse expression pattern than that of *DcSERK* and *AtSERK1* (BAUDINO *et al.,* 2001). These results indicated that SERK plays an important role in SE, and it can be used as a marker of embryogenic competence.

Date palm (*Phoenix dactylifera* L. 2n = 36), famed for its sugar-rich fruits (dates) and cultivated by humans since 4000 B.C, is an economically important crop in the Middle East, Northern Africa and increasingly other places where climates are suitable. Despite a long history of human cultivation, the understanding of date palm genetics and molecular biology is rather limited, hindered by a lack of basic high quality data from genomics and transcriptomics. To ensure the renewal and extension of date palm groves, *in vitro* micropropagation techniques have been developed from zygotic embryos, axillary buds, immature leaves and mature flowers (TISSERAT, 1979; DRIRA *et al.,* 1983; MASMOUDI *et al.,* 2009). Date palm micropropagation through somatic embryogenesis has been previously reported (HUONG *et al.,* 1999; KRIAA *et al.,* 2012). The use of embryogenic suspension cultures improved the yields in the regeneration processes and allowed large-scale propagation of several date palm cultivars (FKI *et al.,* 2003). However, the efficiency of various somatic embryogenesis protocols described for date palm depends on the nature of the explants and the genetic characteristics to each cultivar, some of them being recalcitrant to *in vitro* culture (KRIAA *et al.,* 2012). During somatic embryogenesis, biochemical and morphological changes occur throughout the development of induced tissues (SHARP *et al.,* 1980), which are strongly related to modification of gene expression patterns (ZIMMERMAN *et al.,* 1993).

In this study, we first isolated a SERKL cDNA (*PhSERKL*) from date palm. Few SERK-like genes have also been reported**.** We also investigated the molecular evolutionary history of plants SERK genes to gain further understanding of the evolutionary dynamics of nuclear gene families.

MATERIALS AND METHODS

In vitro culture conditions

It has been reported that SE can be induced in nonembryogenic callus of *Phoenix dactylifera* cultivar Deglet Nour when these cultures are incubated on MS medium containing 2,4-D (DRIRA and BENBADIS 1985). Non embryogenic calli were divided into two groups, one transferred to 2, 4-D-containing medium for SE induction, and the other maintained on 2,4-D-free

medium acting as negative control for real-time PCR. Batches of cultured callus were periodically used to extract total RNA for the relevant stage after 0, 10, 15, 25, 30, 35, 40, 45, 50 and 60 days incubation on 2,4-D containing medium. Negative control callus samples were taken from callus cultured without 2, 4-D at 0, 15, 25, 35, and 45 days. Various organs (root, stem, leaf, ovule and bract) were used to extract total RNA to detect *PhSERKL* expression level by real-time PCR.

RNA/DNA extraction and cDNA synthesis

Total RNA extraction was carried out using TRIZOL reagent (Takara). Poly (A) + RNA was isolated from total RNA using an mRNA Purification Kit (Takara). For first strand cDNA synthesis, 2 µg of Poly (A) + RNA was reverse transcribed in a final volume of 20 µl using oligo (dT) 18 and M-MLV reverse transcriptase (Invitrogen). Genomic DNA was extracted from tissues cultured *in vitro* using cetyltrimethyl ammonium bromide and treated with RNase A.

Cloning of PhSERKL cDNA

Degenerate primers (P1, P2, P3, and P4, listed in Table 1) designed based on conserved *SERK* regions were employed to amplify SERK fragments. The 5′ and 3′ sequences of *SERK* were amplified by rapid amplification of cDNA ends (RACE) using the 3′-Full RACE Core Set Ver.2.0 kit and 5′-Full RACE Core Set Ver.2.0 kit (Takara). The nested gene-specific primers for 3′ RACE were P5–P6 (Table1). Reactions were carried out following the PCR procedure: initial denaturation at 94 °C for 3 min, subsequent denaturation at 94 °C for 30 s, annealing at Tm °C for 30 s, extension at 72 °C for 1 min, 30 cycles, and the final extension at 72 °C for 10 min. The primers for 5′ RACE were P7–P8 (table1). The thermal cycle programs were: 94 °C for 5 min, 35 cycles at 94 °C for 45 s, Tm °C for 45 s, 72 °C for 1 min, followed by final extension at 72 °C for 10 min. Sequences were edited, aligned, and analyzed using DNAMAN and Clustal software tools. Specific primers P9 and P10 were designed according to the results of the RACE to amplify the full-length cDNA sequence and the genomic sequence.

Table 1. Details of primers used in the study

	Description Primer sequence (5′–3′)
Degenerate primers	P1 GAAGTT/CCATCTTGGCCAGC
	P2 ACCCAGTCC/AAGAAGCATGAC
	P3 AAGCTTGCCAACAYGGAAGGTGATGC
	P4 TCCATCCGCAAGCCTCCCT
3′ RACE primers	P5 CGATTGTGCTTTGGTTTCTATGG
	P6 CGAGATGGTTTCTGGGATCTG
5′ RACE primers	P7 GATTGTGCTTTGGTTTCTATGG
	P8 CGAGATGGTTTCTGGGATCTG
RT-PCR primers	P9 GATTGTGCTTTGGTTTCTATGG
	P10 GAGATGGTTTCTGGGATCTG
Real-time PCR primers	Upserk TTTGGTTTCTATGGCTGATGCT
	Dvserk GCTATAAAGTTCCAGGTATTGCAG
	*UpNAM1 GTGTCGATGGAGAGCTACGG
	DvNAM1 TTCAGACTTGCAGACACATACC

* no apical meristem (NAM)

Bioinformatics analysis

The open reading frame (ORF) was predicted using the "ORF Finder" program (http://www.ncbi.nlm.nih.gov/gorf/gorf. html). The signal peptide was predicted by using the "SignalP3.0 Server" tool (http://www.cbs.dtu.dk/servicesSignalP/).The transmembrane region (TM) was predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM/). Multiple sequence alignment was performed with ClustalW. We predicted tertiary structure with ligand binding sites of the protein *PhSERKL* using the program 3D LigandSite (http://www.sbg.bio.ic.ac.uk/3dligandsite). We also used InterPro (http://www.ebi.ac.uk/interpro/) and ScanProsite (http://us.expasy.org/prosite/) to analyze the functional structure of the *PhSERKL* protein. Detection of potential recombination between mRNA sequences was carried out using RDP (Recombination Detection Program) version 3.0 (MARTIN *et al.,* 2010). Nucleotide diversity (π) and Tajima's D (TAJIMA, 1989) were calculated using DNAsp 5.10 (LIBRADO and ROZAS 2009). The phylogenetic relationships between the SERK genes for different species were analyzed using the maximum-likelihood (ML) method. For the ML analyses, we used the PROTML program of PHYLIP version 3.6 (FELSENSTEIN, 2000). We employed the JTT model of amino acid substitution. All indels were counted as missing. We performed ten random sequence addition searches using the J option and global branch swapping using the G option to isolate the ML tree with the best log-likelihood. In addition, we performed bootstrap analysis with 1000 replications. To infer the evolutionary events affecting the SERK genes, an analysis using Notung 2.7 (CHEN *et al.,* 2000) was performed. The ML tree with the highest log-likelihood was used for the gene tree. Both gene duplications and losses were considered to reconcile the gene tree with the species tree. *K*a (the number of nonsynonymous differences divided by the number of nonsynonymous sites) and *K*s (the number of synonymous differences divided by the number of synonymous sites) and Tajima's *D* (TAJIMA 1989) were calculated using DNAsp 5.10 (LIBRADO and ROZAS 2009). DNAsp uses the method of NEI and GOJOBORI (1986) to identify synonymous and nonsynonymous sites and a Jukes–Cantor correction is applied for multiple hits. Ancestral sequences were calculated for SERK protein of monocots and dicots using the FastMLweb server (http://fastml.tau.ac.il/).

Quantitative real-time PCR analysis

Real-time PCR has been used for gene expression studies in many species (CHAI *et al.,* 2010; MA *et al.,* 2010; QI *et al.,* 2010). The relative expression of *PhSERKL* was measured using Thunderbird SYBR qPCR Mix (Toyobo) performed on the iQ5 real-time PCR System (Biorad). A gene encoding a *Phoenix dactylifera* no apical meristem-related protein (NAM1) was used as the endogenous control (ADAM *et al.,* 2011). To prevent amplification of any contaminating genomic DNA, RNA preparations were treated with DNase I, and the probe primers were designed over an exon/intron boundary in the cDNA sequence. Specificity of the amplifications was verified by electrophoresis and at the end of the PCR run by melt curve analysis. Primer sequences used for *PhSERKL* and endogenous control are listed in table 1. Real-time PCR results were analyzed using the iQ5 System Software. Tissue samples were prepared as described in "*In vitro* culture conditions" section.

RESULTS AND DISCUSSION

Cloning of PhSERKL cDNA

PCR with degenerate primers designed from *SERKL* conserved domains was used to amplify potential *SERK* cDNA fragments from date palm. Assembly of these PCR fragments

generated a 1.7 kb contiguous fragment. Basic Local Alignment Search Tool (BLAST) searches with this fragment returned several hits matching *SERK* conserved domains, and the assembled sequence was putatively designated as a date palm *SERK* (*PhSERKL*) fragment. Specific primers designed from the putative *PhSERKL* fragment were used to amplify both 5′ and 3′ ends of *PhSERKL* cDNA by RACE PCR. A full-length cDNA sequence comprising 1890 bp, generated by specific primers designed from the sequences yielded by RACE, was defined as *PhSERKL* and has been submitted to GenBank (accession number: KJ956013). Using the same primers as for the cDNA, the full-length genomic sequence of *PhSERKL* comprising 6.345 bp was also isolated.

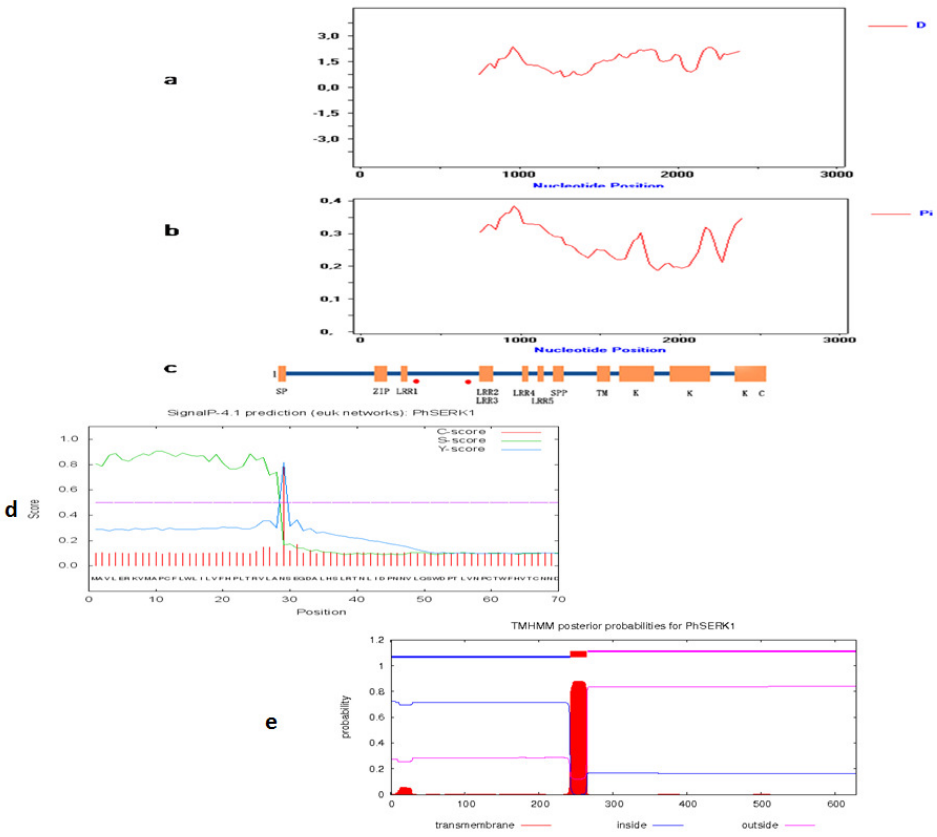

Figure 1. PhSERKL sequence analysis. a: Sliding-window analysis of Tajima's D for the 30 SERK mRNA sequences plotted against nucleotide position; b: Sliding-window analysis of nucleotide diversity for the 30 SERK mRNA sequences plotted against nucleotide position; c : Structural features of the SERKL gene: exon–intron structure ; d: Prediction of signal peptide sequence. e: The transmembrane region predicted by TMHMM tool.

A BLASTN search of this sequence resulted in several *SERK* similarity hits from other plant species. Nucleotide diversity (π) and Tajima's D were calculated for different regions of *SERK* mRNA from various species of plants (Fig.1a and Fig.1b). This figure shows that the value of π is very high in the first part of the sequence (up to 1000 bp) in the ClustalW alignment of SERK mRNA, with gaps that corresponds to the first domains of SERK proteins (SP, LZ, SPP, and TM). The kinase domain (1000 -2000 bp) shows a very low π value and a strong positive Tajima's D, underscoring the high degree of conservation of the SERK gene in the kinase domain (822 bp).

The PhSERKL ORF and relationship to other family members

Somatic embryogenesis is an effective way for plant propagation and genetic transformation (KUMAR *et al.,* 2005; WU *et al.,* 2008). An understanding of the molecular mechanism of a *SERK* gene, *PhSERKL* gene will be a benefit for the establishment of a more effective regeneration system and valuable for the study of cell differentiation and embryo development in palm date. A comparative alignment of the predicted *PhSERKL* coding regions with the corresponding genomic sequence revealed that it consisted of 11 exons interspersed by 10 introns (Fig.1c). The highly conserved exons and exon/intron structure points to the functional significance of this gene family (SHARMA *et al.,* 2008). The *PhSERKL* protein of *Phoenix Dactylifera* L v Deglet Nour showed an overall high identity with others PhSERK of palm species (Table 2) (REKIK *et al.,* 2013) and with other plants SERKs at the protein level, such as *Ananas comosus* (AcSERK1), *Cocos nucifera* (CnSERK), *Oryza sativa Japonica* (OsSERK1). Structural analysis suggested that *PhSERKL* encodes a distinct SERK protein (629 aa) belonging to the LRR-RLK family (WALKER 1994), sharing all the characteristic domains of the SERK family. The main difference between SERK proteins and other RLKs is the existence of Pro-rich domain containing the SPP motif between the LRRs and the transmembrane domain (ALBRECHT *et al.,* 2008). The Pro-rich domain has been predicted to act as a "hinge," providing flexibility to the extracellular part of the receptor or as a region for interaction with the cell wall (HECHT *et al.,* 2001). The other two SERK-specific features (ZIP and C- terminal) are also present in *PhSERKL*. The ZIP region has been shown to be involved in oligomerization of AtSERK1 proteins (SHAH *et al.*, 2001a). It is suggested that the C-terminal leucine-rich domain plays a role in protein–protein interactions (SCHMIDT *et al.,* 1997). The five LRRs comprised of 24 amino acids are located extracellularly and contain all the conserved amino acid residues of other SERKs. The single transmembrane region divides the extracellular domains, containing the SP, ZIP, LRRs, and SPP regions, with the intracellular region containing the kinase and C- terminal domains. The SP domain contains a hydrophobic amino acid signal peptide sequence is predicted by SignalP software at the amino terminus; with a possible cleavage site between position 28 and 29 (Fig.1d). The intracellular kinase domain contains the 11 subdomains of conserved amino acid sequences from position 305–592 as described by HANKS *et al.,* (1988). The position of the TM helix (Fig.1e) predicted by TMHMM is from 243 to 265 aa. A putative protein kinase ATP-binding region signature is present at position 311 to 333 in the kinase domain. The 29-amino acid residue activation loop (A-loop), containing the active site of *AtSERK1* (SHAH *et al.,* 2001a, b), is also presented in *PhSERKL* at position 421 to 450 with 100% identity to *CnSERK1*. A serine/threonine protein kinase active-site signature was located from position 428 to 440.

Table 2. SERK proteins of date palm identity matrix

Seq->	PhSERK1	PhSERK2	PhSERK3	PhSERKL
PhSERK1	ID	0;674	0;471	0;813
PhSERK2	0;674	ID	0;452	0;727
PhSERK3	0;471	0;452	ID	0;481
PhSERKL	0;813	0;727	0;481	ID

Divergence at Synonymous and Nonsynonymous Sites of PhSERKL with plants SERKs mRNA

To explore whether Darwinian positive selection was involved in driving gene divergence after duplication, we calculated the nonsynonymous/synonymous substitution ratio (ka /ks) for the coding region of some recently duplicated paralogs using a sliding window of 20 amino acids. Generally, ka: ks>1 indicates positive selection, ka:ks< 1 indicates negative or purifying selection, and a ratio= 1 indicates neutral evolution (WANG *et al.,* 2005).

‘ For the SERK paralogs, ka:ks ratios were always near zero for kinase domain, the conserved SERK domain, suggesting strong purifying selection on this motif. In contrast, many higher ka/ks ratios (ka:ks>1) were generally found in the regions outside the kinase domain, especially in the intermotif regions. Such positive selections have also been observed in the RLK/Pelle gene family in Arabidopsis and rice (SHIU *et al.,* 2004). A higher proportion of new exons had ka:ks>1 and a higher frequency of insertions/deletions (indels) than did the old exons, implying that positive selection played an important role in the evolution of new domains (WANG *et al.,* 2005). Therefore, positive selection is one of the major driving forces for the emergence of new motifs/functions in protein after gene duplication.

Gene duplication of SERK genes in plants

The palm family emerged ~80 million years ago and as such it represents one of the lineages that radiated early in monocot evolution (WILSON *et al.,* 1990; DUVALL *et al.,* 1993). The comparative analysis of *PhSERKL* protein of date palm with others species presents an ideal opportunity to investigate the dynamics of angiosperm gene family evolution, and in particular, to expand our understanding of the evolution of the *SERK* gene family.

Duplicated genes arise frequently in eukaryotic genomes through local events that generate tandem duplications, large-scale events that duplicate chromosomal regions or entire chromosomes, or genome-wide events that result in complete genome duplication (DUJON *et al.,* 2004). Indeed, the existence of multigene families is evidence of the repeated gene duplication that has occurred over the history of life. One of the examples of the comprehensive analysis of gene duplication events in plants is the study of the *SERK* gene family presented here. This study revealed the complicated evolution of the *SERK* gene family that occurred during the course of plant diversification. In our study, the phylogenic tree resulting from GeneTree analysis showed that some *SERK* genes in plants (Table 3) evolved in a complex manner that included several

duplication events (Fig.2). We observe that multiple independent duplication events have occurred throughout the evolutionary history of the *SERK* family. Based on the reconciled phylogeny (Fig.2), we estimate 15 independent gene duplication, 1 coduplication and 50 gene loss events during the diversification of this gene family. The clade I and clade II arose from at least one major duplication events to obtain the *SERKL1 and SERKL2* of *Pentapetalae (Eudicots)* and *SERK* of monocots with *SERK3* of Eudicots (Fig.2). The ancestor *SERK* protein of monocots and dicots is shown in Figure 3. The clade III is composed of *SERKL1 of Acrogymnospermae* diverged from ancestral duplications (Fig.2).

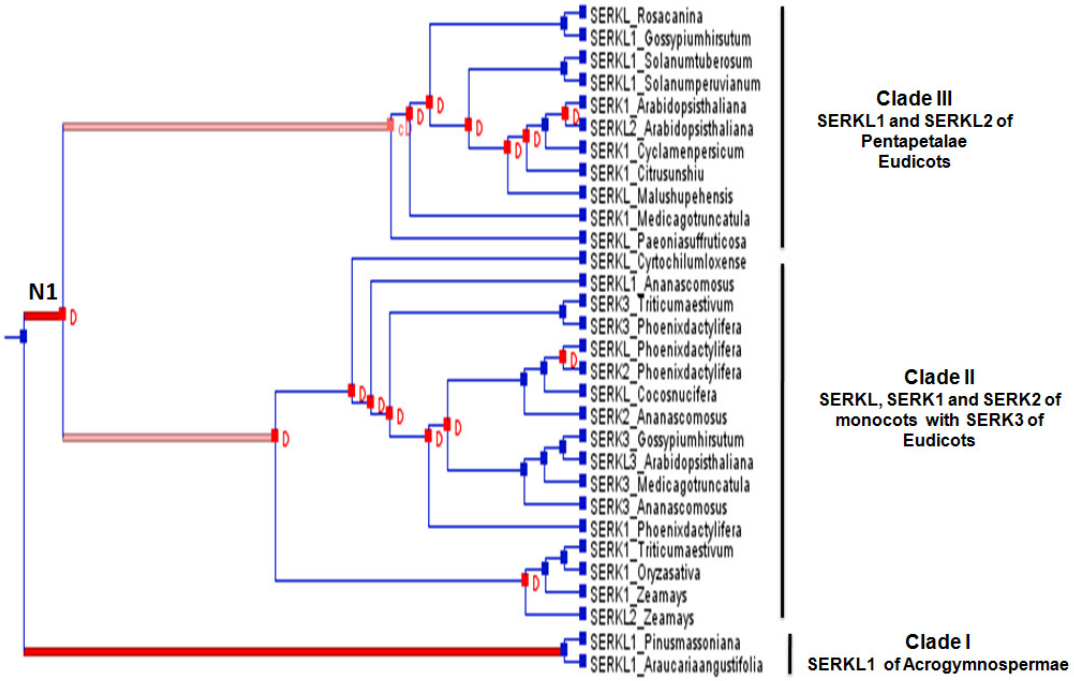

Figure 2. Reconciled tree for the SERK plants family**.** The reconciled tree involves 15 gene duplications; 1 coduplication and 50 gene losses. The solid boxes indicate gene duplications that were inferred on the basis of mismatches between the gene tree and the species tree. The red boxes indicate those duplications that required multiple copies of SERK genes within the same species.

Table 3. Taxa , source and list of accession numbers used in this study

Phylogenetic group family species	SERK locus and accession numbers
Spermatophyta	
Pinidae	
Araucaria angustifolia	AaSERKL1 : ACY91853.1
Pinus massoniana	PmSERKL1 :ACZ56417.1
Mesangiospermae	
Poales	
Ananas comosus	AcSERKL1 : AEC46975.1 ; AcSERK2 : AEC46976.1 ; AcSERK3:AEC46977.1
Poaceae	
Zea mays	ZmSERK1 : NP_001105132.1 ; ZmSERKL2 : NP_001105133.1
BEP clade	
Triticum aestivum	TaSERK3 : AEP14553.1 ; TaSERK1 : AEP14551.1
Oryza sativa	OsSERK1 : AAU88198.1
Arecaceae	
Phoenix dactylifera	PhSERKL : KJ956013
Cocos nucifera	CnSERKL : AAV58833.2
Cyrtochilum loxense	ClSERKL : CBV98085.1
Pentapetalae	
Paeonia suffruticosa	PsSERKL : AHI59639.1
asterids	
Cyclamen persicum	CpSERK1 : AEB40066.1
Solanum	
Solanum tuberosum	StSERKL1 : ABO14173.1
Solanum peruvianum	SpSERKL1 : ABR18800.1
rosids	
malvids	
Citrus unshiu	CuSERK1 : BAD32780.1
Arabidopsis thaliana	AtSERK1 : NP_177328.1 ; AtSERKL2 : NP_174683.1 ; AtSERKL3AAK68074.1
Gossypium hirsutum	GhSERKL1 : ADR00582.1 ;GhSERK3: AEG25668.1
fabids	

Rosaceae	
Malus hupehensis	MhSERKL : AFP25205.1
Rosa canina	RcSERKL : ADM94278.1
Medicago truncatula	MtSERK3 : ADO15298.1 ; MtSERK1 : AAN64293.1

Figure 3. Ancestral protein of monocots and dicots using FASTML program in the N1 indicate in Figure 2

Expression Analysis

According to DRIRA & BENBADIS (1985), SE was induced by transferring nonembryogenic callus generated from the leaf base by 60 days of incubation on a medium without 2,4-D, to the medium containing 2,4-D. *PhSERKL* expression was analyzed by real-time PCR, and tissue samples were derived as described in the "Plant Material" Section. The results obtained showed that *PhSERKL* is expressed during SE with two peaks of expression at different times (Fig.4). The first peak of expression occurred at 15 days of culture in 2,4-D-containing medium. This coincided with the appearance of the cytological features of calli with embryogenic potential, and embryogenic cells can be found in these calli (HE *et al.,* 2010).

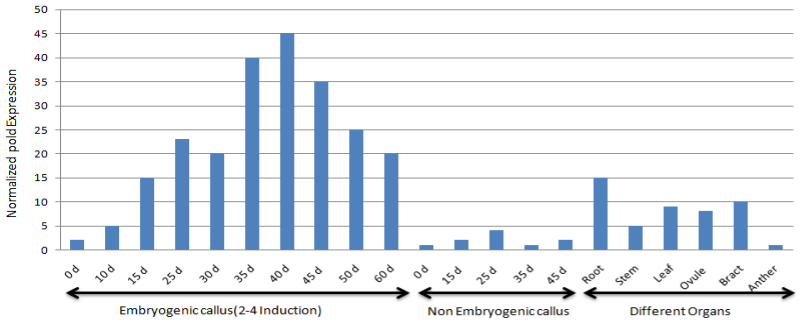

Figure 4. Relative expression of *PhSERKL* measured using real-time PCR Embryogenic callus shows *PhSERKL* expression in callus cultured on 2;4-D containing medium; Nonembryogenic callus shows *PhSERKL* expression in callus cultured on medium without 2;4-D used as negative control; Different organs show *PhSERKL* expression in different organs

The second peak of expression is observed at 40 days of culture in 2,4-D- containing medium; at that time, globular embryos were already visible in the callus. *PhSERKL* gene expression was relatively stable in the nonembryogenic callus cultured on the 2,4-D-free medium, which was used as negative control (Fig.4). *PhSERKL* may play a role in inducing the process of embryogenesis of date palm callus. Expression of *PhSERKL* gene occurred at lower levels in all the organs examined than the expression levels in embryogenic callus (Fig.4). The expression of *PhSERKL* gene in the ovule was similar to the expression in somatic tissues such as leaf and stem.The lowest expression level of *PhSERKL* gene was detected in anther; it was only about 1/8th of that in ovules (Fig.4). This result was the same with *Ananas comosus* (JUN MA *et al.,* 2012) and different with those obtained from carrot (SCHMIDT *et al.,* 1997), Arabidopsis (HECHT *et al.,* 2001), and banana (HUANG *et al.,* 2010), where SERK was only expressed in embryogenic callus. However, the high expression level of *PhSERKL* during the acquisition of embryogenic

competence and the development of the globular embryo is similar to the SERKs above. This is suggestive of a broader role of *PhSERKL* in cell reproduction and organ formation, rather than embryogenesis alone in date palm, similar to the cases of *AcSERK1* (JUN MA *et al.,* 2012), *ZmSERK1* (BAUDINO *et al.,* 2001) and *StSERK1* (SHARMA *et al.,* 2008) in potato. The transcript levels of *PhSERKL* in somatic organs and ovules were moderate, and the lowest abundance was found in anthers. This result coincided with the expression pattern of *TaSERK* in *T. aestivum* (SINGLA *et al.,* 2008).

CONCLUSION

In this study, the full length cDNA of a date palm SERKL gene (*PhSERKL*) has been isolated from a cDNA library prepared from induced callus and the sequence of the *PhSERKL* protein has been predicted. Based on structural and functional similarity, *PhSERKL* is likely an orthologue of *AcSERK1* and *CnSERK*, and acts as a key component mediating somatic embryogenesis. Our phylogenetic and genetic evolutionary analysis confirmed that *PhSERKL* belongs to a gene family in some of the studied species and resulted from a relatively ancient duplication event. These studies could further reveal the function of date palm *SERK*s and help the establishment of a highly effective *in vitro* propagation and transformation system of date palm.

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MOLEKULARNA KARAKTERIZACIJA I EVOLUCIONA ISPITIVANJA *SERK* LIKE GENA INDUKOVANIH U TOKU TRANSKRIPCIJE U TOKU SOMATSKE EMBRIOGENEZE kod *Phoenix Dactylifera* L v Deglet Nour

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Izvod

Izvršena je izolacija cDNA receptor kinase *like* (*SERKL*), označene kao *PhSERKL*, u toku somatske embriogeneze datule-urme (*Phoenix Dactylifera* L.) primenom RACE PCR. *PhSERKL* protein deli sve karakteristike domena *SERK* familije, uključujući pet repetitivnih sekvenci bogatih leucinom, jedan motiv region bogat prolinom, transmembranski domen i domene kinaze. Filogenetske analize korišćenjem PHYLIP i Notung 2.7 programa sugerišu da SERK protein nekih biljnih vrsta rezultiraju iz relativno starih događaja duplikacije. Predvideli smo zajedničkog pretka SERK proteina kod monokotiledonihi i dikotiledonih biljaka koisteći FASTML program. Somatske embriogene kulture datule su dobijene transferom kulture kalusa u medium koji je u sastavu imao 2, 4-dichlorofenoskiacetik kiselinu. Uloga *PhSERKL* gena u toku pripreme somatske embriogeneze u kulturi je ispitivana primenom kvantativnog *real-time* PCR. *PhSERKL* gen je imao visok nivo ekspresije u toku sticanja embriogene kompetentnosti i formiranja globularnogembriona u kulturi. Globalno, nivoi ekspresije *PhSERKL* gena su bili niži u neembrionalnim tkivima i organima u odnosu na embrionalne kaluse.

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