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CELL BIOLOGY AND MORPHOGENESIS

Characterization of three somatic embryogenesis receptor kinase genes from wheat, *Triticum aestivum*

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Abstract We report here the isolation and characterization of three *SOMATIC EMBRYOGENESIS RECEPTOR KINASE* (*TaSERK*) genes from wheat. *TaSERKs* belong to a small family of receptor-like kinase genes, share a conserved structure and extensive sequence homology with previously reported plant *SERK* genes. *TaSERK* genes are in general auxin inducible and expressed during embryogenesis in cell cultures. We show here that somatic embryogenesis in *Triticum aestivum* is associated with high *SERK* expression which could be enhanced with auxin application and is calcium dependent. *TaSERK* transcripts could also be enhanced by epibrassinolide and abscisic acid. *TaSERK1* and *TaSERK2* may have a role in somatic embryogenesis, whereas *TaSERK3* appears to be a brassinosteroid-associated kinase (BAK) lacking an SPP motif but shares a characteristic C-terminal domain with other SERK proteins. Also, the transcripts of all the three *TaSERK* genes could be induced in zygotic and somatic tissues. Although our analysis suggests them to be involved in somatic embryogenesis, they may have a broader role in acquiring embryogenic competence in wheat.

Keywords Auxin · Calcium · Somatic embryogenesis · Wheat

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Abbreviations

SE	Somatic embryogenesis
SERK	Somatic embryogenesis receptor-like kinase
RLK	Receptor-like kinase
2,4-D	2,4-Dichlorophenoxyacetic acid

Introduction

All living systems perceive and process information from impermeable chemical signals through cell surface receptors. Most animal receptor kinases have tyrosine kinase activity and only a few are serine/threonine kinases (Ullrich and Schlessinger 1990) or can phosphorylate serine/threonine in addition to tyrosine (Douville et al. 1992). Plant receptor-like protein kinases (RLKs) have been shown to autophosphorylate serine and/or threonine residues. Receptor-like kinases belong to a large gene family are plasma membrane-bound and play an important role in the perception and transmittance of external signals (Shiu and Bleecker 2001; Torii 2004). Plant receptor-like kinases are proteins with a predicted signal sequence, single transmembrane region, and a cytoplasmic kinase domain. There is increasing evidence that protein kinases play important roles in cellular signaling and metabolic regulation in plants (Krupa et al. 2006). The putative role of RLKs in developmental processes is to transduce environmental signals and/or information from neighboring cells to trigger specific responses, and one such response is somatic embryogenesis. Usually, auxin is required to induce somatic embryogenesis and subsequent auxin withdrawal or lowering is important for embryo maturation (Dudits et al. 1991; Mordhorst et al. 1997; Thibaud-Nissen et al. 2003; Feher et al. 2003; Mahalakshmi et al. 2003, 2007).

SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) is involved in the acquisition of embryogenic competence in plant cells. In carrot, *Dactylis glomerata* and *Arabidopsis*, *SERKs* were shown to be characteristic markers of embryogenic cell cultures and somatic embryos (Schmidt et al. 1997; Somleva et al. 2000; Hecht et al. 2001). Their subcellular location as a membrane protein (Shah et al. 2001a) and phosphorylation/dephosphorylation activities with kinase associated protein phosphatase (KAPP) (Shah et al. 2001b, 2002) was reported. SE is facilitated due to mutations in genes repressing meristematic cell divisions, such as *amp1* and the *clavata* mutants (Mordhorst et al. 1998). *AtSERK1* expression was considerably up-regulated in cultures derived from *amp1* mutant reflecting the natural tendency of this mutant to regenerate (Hecht et al. 2001). Simultaneously, ectopic expression of the genes *LEAFY COTYLEDON1 (LEC1*; Lotan et al. 1998) and *LEAFY COTYLEDON2* (*LEC2*; Stone et al. 2001), *BABY BOOM* (*BBM*; Boutilier et al. 2002) and *WUSCHEL* (*WUS*; Zuo et al. 2002) in *Arabidopsis* caused spontaneous formation of somatic embryos in intact plants or explants. The HAP3 subunit most closely related to *AtLEC1*, designated as *LEC1-LIKE*, has been shown to be important for normal embryo development in sunflower (Kwong et al. 2003; Fambrini et al. 2006). *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) and two in rice (Ito et al. 2005; Hu et al. 2005). Few *SERK*-like genes have also been reported; for example, eight in *Poa pratensis* (Albertini et al. 2005) and nine in rice (B. Singla, J. P. Khurana, P. Khurana, Structural characterization and expression analysis of the SERK gene family in rice (*Oryza sativa*), unpublished data). Ectopic expression of *AtSERK1* gene enhanced embryogenic cells in developing ovules, early embryos and in vascular tissues (Hecht et al. 2001). In *Zea mays, ZmSERK1* showed the strongest expression in microspores, while *ZmSERK2* expression was fairly uniform in all tissues investigated (Baudino et al. 2001). *OsSERK1* in rice was suggested to be involved in phytohormone signaling and its ectopic expression mediated defense signal transduction (Hu et al. 2005). In comparison, *OsSERK2* expressed in all organs with essentially similar levels (Ito et al. 2005). *SERK* genes in *M. truncatula* and sunflower have been identified with a predominant expression during somatic embryogenesis, rhizogenesis and shoot organogenesis (Nolan et al. 2003; Thomas et al. 2004).

We report here the characterization of three genes, leads for which were obtained from the expressed sequence tag (EST) clones isolated from *Triticum aestivum* cDNA library (Singla et al. 2007). Their sequence organization and characterization through temporal and spatial expression analysis in zygotic and somatic tissues is reported. The putative involvement of these three genes in auxin and brassinosteroid signaling pathway is also discussed.

Materials and methods

Plant material and growth conditions

Seeds of hexaploid wheat (*T. aestivum*) were obtained from the Directorate of Wheat Research, Karnal. Seeds were surface sterilized with 4% sodium hypochlorite for 30 min. Seedlings were grown on cotton saturated with RO water at 28°C, under a daily photoperiodic regime of 16 h/8 h light/dark cycle. Light was provided by fluorescent tubes (Philips TL 40W/54, 6,500 K) with a fluence rate of 70 μmol m^{-2} s^{-1}, as per experimental requirements. The 13-day-old seedlings were employed for the present investigation and were raised as described earlier (Mahalakshmi et al. 2003, 2007). Zygotic tissue samples were collected from field-grown wheat plants. Wheat calli were raised as previously described (Khurana et al. 2002) and for auxin and brassinosteroid induction experiments, procedure was followed as described by Singla et al. (2006).

Identification of *SERK* homologues in wheat (*T. aestivum*)

To identify *SERK* homologues in wheat (*T. aestivum*), the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nim.nih.gov/BLAST/nr/EST), and The Institute for Genomic Research (TIGR) database (http://www.tigrblast.tigr.org/euk-blast) resources were used. The amino acid sequence of the known SERK proteins was used to search for their homologues in wheat using the TBLASTN program; the redundant sequences were removed after multiple alignments by the Clustal_X program (version 1.83), and partially ESTs recovered. The search was limited to the identification of the SPP domain and the C-terminal domain in these ESTs to avoid retrieving other plant receptor kinase sequences (Schmidt et al. 1997).

Completion of 5′ untranslated region by rapid amplification of 5′cDNA ends

Completion of the 5'untranslated region (UTR) was done using the rapid amplification of 5′cDNA ends (5′ RACE) kit (Invitrogen) according to the manufacturer's instructions. The first strand cDNA was synthesized from mRNA

Table 1 Sequence of primers used in the experiments

TaSERK1_R	5′-CGGGTGGTGTTGGAGGATTGAATG-3′
TaSERK1_NR	5′-CGAGCCAGTTGATGGAACCGCTC-3′
TaSERK2_R	5′-TCATCTTGGGCCAGACAGTTCCACGGC-3′
TaSERK2_NR	5′-CTCCTGAGAGGTTGTTGTTTGAGAGATC-3′
TaSERK3_R	5′-CAGATGGCACTAAGATAGCCGTAAAGC GGTTAAC-3′
TaSERK3_NR	5′-CTGTTTGTGTTGTACAGAACCCAATC-3′
mod_M13F	5′-CCCAGTCACGACGTTGTAAAACG-3′
mod_M13R	5′-AGCGGATAACAATTTCACACAGG-3′

(~5 μg) isolated from total RNA (~1 mg) extracted from 1-day auxin induced leaf bases using gene-specific primers (GSP, R) and gene specific nested primers (GSP, NR) (Table 1). In brief, the first strand cDNA synthesis was done with Superscript^{TM} II RT, RNA template removed by RNase mix, purified in the SNAP columns, followed by homopolymeric tailing of cDNA and then amplification of the target cDNA. The PCR product was cloned in pGEMT-easy vector and sequenced using M13 modified primers (Table 1).

RNA isolation and northern analysis

Total RNA was extracted using the TRIzol reagent (Sigma) and resolved on a 1.2% agarose gel containing 1.1% formaldehyde, at 120 V. After alkaline blotting to Hybond-N$^+$ membrane (Amersham), hybridization was carried out as mentioned previously (Mahalakshmi et al. 2007). RNA isolation and quantitative real-time PCR analysis was followed as described previously (Singla et al. 2006).

DNA isolation and Southern hybridization/analysis

Genomic DNA was isolated from 13-day-old wheat seedlings, according to the method of Dellaporta et al. (1983). An aliquot of 20 μg of wheat DNA was digested by the restriction endonuclease *Bgl*II and the digested DNA resolved on a large 0.8% agarose gel. The resolved DNA fragments were blotted onto a Hybond-N$^+$ (Amersham, UK) membrane, pre-hybridization and hybridization carried out for 5 and 24 h, respectively, in the Express^{Hyb} buffer (BD Biosciences, Clontech) along with 80 μg of denatured herring sperm DNA at 60°C. The kinase and the C-terminal region and 3′UTR of *TaSERK1* was used as a radiolabeled (α^{32}P-dATP) probe. Post-hybridization washing was performed thrice successively for 20, 10 and 10 min, in 0.1% SSC/0.5% SDS at room temperature. The membrane was exposed to X-ray film for autoradiography in a light-proof hypercassette (Amersham, UK) with an intensifying screen for 72–96 h at −80°C.

Results

Sequence analyses of *TaSERK* genes

In monocots, presence of the basal meristem in the leaf base makes it an ideal material for studies on somatic embryogenesis. In an earlier study from our laboratory, Mahalakshmi et al. (2003) showed that wheat leaf base segments (5 mm) of the second and third leaf can rapidly respond to short-term auxin (2,4-D) treatment (24 h) for induction of somatic embryogenesis. With the eventual aim to elucidate the molecular events underlying SE, *T. aestivum SERK* gene (*TaSERK3*) was identified from the auxin induced cDNA library (Singla et al. 2007). In an attempt to identify more SERK protein coding genes in wheat, the BlastN and TBlastX search of cDNA clones or ESTs of wheat available at NCBI and TIGR (NR and EST) was performed. After multiple alignments by Clustal_X program, the redundant sequences were removed and two ESTs recovered (accession numbers TC252811 and TC253788). The strategy adopted to obtain complete clones used a modified 5′ RACE for these two ESTs (accession numbers TC252811 and TC253788). Upon BLAST analysis of *TaSERK* sequences using the NCBI database, it was observed that TC252811 (from TIGR) corresponds to BT009426 (in the NCBI), which has been completed in the present study and designated as *TaSERK1*. The sequences were then verified by obtaining full-length cDNAs in a single PCR reaction, independently, and resequencing.

The mRNA sequence of *TaSERK1* (2,298 bp) and *TaSERK2* (2,310 bp) showed an overall identity of 70 and 90% at the nucleotide and protein level, respectively. The 3′UTR is 284 and 276 bp and 5′UTR is 130 and 390 bp, with a predicted amino acid length of 628 and 547 for TaSERK1 and TaSERK2, respectively. *TaSERK3* mRNA is 2,529 bp in length, with 585 and 365 bp long 5′ and 3′UTR, respectively, and a predicted amino acid length of 527. The predicted molecular mass of TaSERK1, TaSERK2 and TaSERK3 is 69, 60 and 58 kDa, respectively. Surprisingly, TaSERK2 and TaSERK3 lack the signal peptide and the leucine-zipper domain, which are also absent in the DcSERK protein (Schmidt et al. 1997) and the similarity between both the proteins is 80%. At the protein level, TaSERK1 and TaSERK2 showed highest identity with OsSERK2 and ZmSERK2, respectively (Supplementary Table 1) (Baudino et al. 2001; Ito et al. 2005). It is clear from the amino acid sequence alignment that both the *TaSERKs* align closely with *SERK* genes from

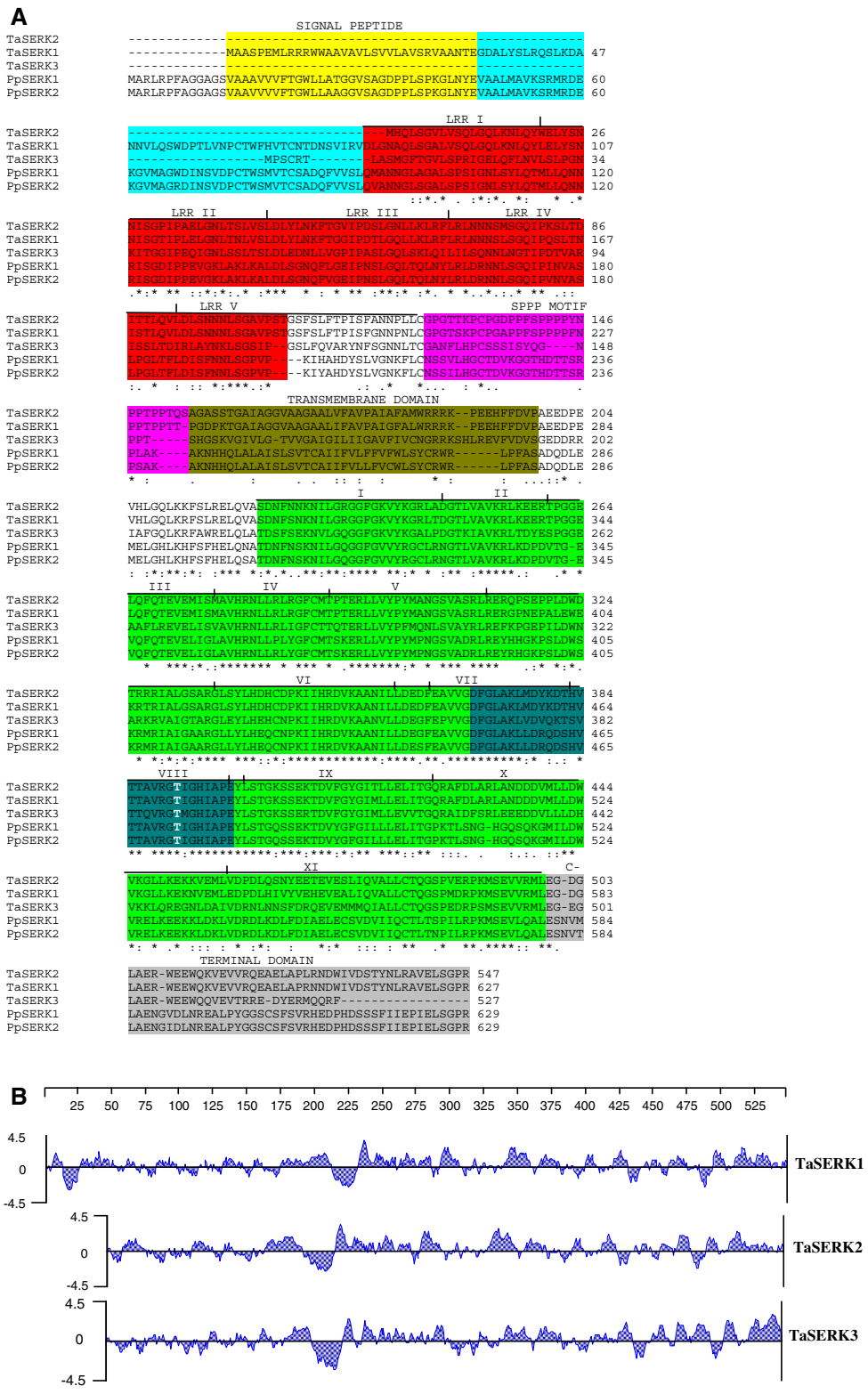

Fig. 1 **a** Alignment of amino acid sequences of TaSERK1, TaSERK2, TaSERK3 with PpSERK1 and PpSERK2. The different domains are shown by colors, which are labeled on the top of each domain. **b** Hydropathy profile of TaSERK1-3 proteins made according to the Kyte and Doolittle method

other species (Supplementary Fig. 1). More detailed analysis was done using PSORT and Prosite for the identification of regions characteristic of SERK proteins (Nakai and Kanehisa 1992; Hofmann et al. 1999).

A hydrophobic amino acid signal peptide sequence with a possible cleavage site between positions 30 and 31 is present in TaSERK1, which is conserved in rice, maize, *Arabidopsis* and *Medicago* (Fig. 1a). This is followed by a

leucine-zipper sequence and five LRRs in TaSERK1 and TaSERK2, as defined earlier by Hecht et al. (2001) for AtSERK in *Arabidopsis*. Between the LRRs and the single transmembrane region is a SPP motif, characteristic of SERK proteins (Schmidt et al. 1997). The TaSERK3 protein shows the presence of characteristic five LRRs, followed by the transmembrane domain and the C-terminal region but lacks the SPP motif. The intracellular kinase domain contains 11 sub-domains of conserved amino acid sequences in all the three TaSERK proteins, as described by Hanks et al. (1988). A putative protein kinase ATP-binding signature region is present in the kinase domain at position 311–333 in TaSERK1, 231–253 in TaSERK2 and 229–251 in TaSERK3. The 29-amino acid residue activation loop (A-loop) in subdomains VII and VIII, which was shown to be the active site of AtSERK1 (Shah et al. 2001c), is present with 100% identity in TaSERK1, TaSERK2, and with some changes in the amino acid residues in TaSERK3 (Fig. 1a). Threonine at position 468, shown to be essential for AtSERK-mediated signaling and the target for phosphorylation, is highly conserved in all the three TaSERKs, as in other SERK proteins (Hecht et al. 2001; Nolan et al. 2003). TaSERKs contain an active site signature of Ser/Thr protein kinases at positions 428–440, 348–360 and 346–358 in subdomain VI in TaSERK1, TaSERK2 and TaSERK3, respectively, which are indicative of their function as a serine/threonine kinase. The 15 invariant amino acids found in almost all eukaryotic protein kinases (Shah et al. 2001b) are present in TaSERK1, TaSERK2 and TaSERK3. Following the kinase region is a Leu-rich C-terminal domain as described by Schmidt et al. (1997), conserved in TaSERK1 and TaSERK2. This domain may be involved in mediating the protein–protein interaction necessary for transmission of an intracellular phosphorylation cascade (Shah et al. 2001a). There are six putative *N*-glycosylation sites, two in LRR2, two in LRR4, one in LRR5 and one in domain V of the kinase region in almost all the known SERKs, including TaSERK1 and TaSERK2 but only two in TaSERK3 (one in LRR2 and one in LRR5). In LRR4 of TaSERK2, the second glycosylation site is lost (also in OsSERK1) as asparagine is replaced by aspartic acid and alanine in wheat and rice, respectively. In LRR5, the glycosylation is *O*-linked instead of *N*-linked in TaSERK1 and TaSERK2 as shown in *Arabidopsis* and *Medicago* (Shah et al. 2001b; Nolan et al. 2003). In extensins also, usually all prolines in the SPP repeat are hydroxylated and are considered to be targets for *O*-linked glycosylation (Schmidt et al. 1997). TaSERK3 does not share one of the characteristic features of the SERK proteins, i.e. it lacks SPP motif but contains the C-terminal region which has also been suggested to be a SERK-specific feature (Schmidt et al. 1997; Baudino et al. 2001; Albertini et al. 2005) (Supplementary Fig. 1c). The hydrophilicity plots show the signal peptide sequence in TaSERK1 and the transmembrane domain in all the three TaSERK proteins to be hydrophobic in nature (Fig. 1b).

The BLAST results suggested the identification of two TaSERK proteins, thus to confirm further, Southern hybridization using a sequence coding for part of the kinase domain along with the C-terminal region of *TaSERK1* as a probe, was carried out on *T. aestivum* DNA digested with *Bgl*II, which does not have a restriction site within the *TaSERK1* sequence. The results show four bands apparent in the *Bgl*II digested genomic DNA, suggesting presence of more members in *TaSERK* family (Supplementary Fig. 2a). To perform a specific Southern hybridization, a probe from the 3′-untranslated region of *TaSERK1* indicated that the strongly hybridizing band (Supplementary Fig. 2b) corresponds to the *TaSERK1* gene. Phylogenetic analysis revealed that the three TaSERKs grouped along with monocots and dicots, formed a separate cluster. TaSERK1 is closest to OsSERK2 and TaSERK2 to ZmSERK1 as is evident from the phylogenetic tree while TaSERK3 was grouped with the SERK proteins which lack the SPP motif, i.e. PpSERK1 and PpSERK2 (Fig. 2).

Differential expression of *TaSERK* genes

The expression profile of *TaSERK* genes was also analyzed for various wheat tissues and in vitro raised cultures. The *TaSERK* transcripts of the three genes were detectable in leaves but levels were quite low in seedlings and roots (Fig. 3). The expression was relatively high in the auxin induced basal segment derived calli (BSH) for *TaSERK1* and *TaSERK2*. To determine the abundance of *TaSERK* genes in the zygotic tissue, real-time PCR was performed with total RNA isolated from ovaries, anther, milky stage of seed (MSS), developing seed (DS) and mature seed (MS). Significant differences were found in the transcript abundance of *TaSERK* genes as they showed complexity of specific and overlapping expression patterns in the various tissues/organs analyzed (Fig. 3). *TaSERK1* and *TaSERK2* showed essentially similar expression profiles in the zygotic tissues, suggesting they may perform redundant functions. The *TaSERK3* expression was highest in the developing seed, thereby suggesting its role in both zygotic and somatic embryogenesis.

Gene specific probes were made exclusively of the UTR (5′ or 3′) to allow the expression patterns to be distinguished for the three *TaSERK* genes. In *T. aestivum* cv CPAN 1676 culture system used in this study, leaf base explants treated with 2,4-D for 24 h rapidly followed by 10 days on MS basal medium, in dark, form somatic embryos (Mahalakshmi et al. 2003). Both 2,4-D or IAA used for the induction of somatic embryogenesis in the leaf

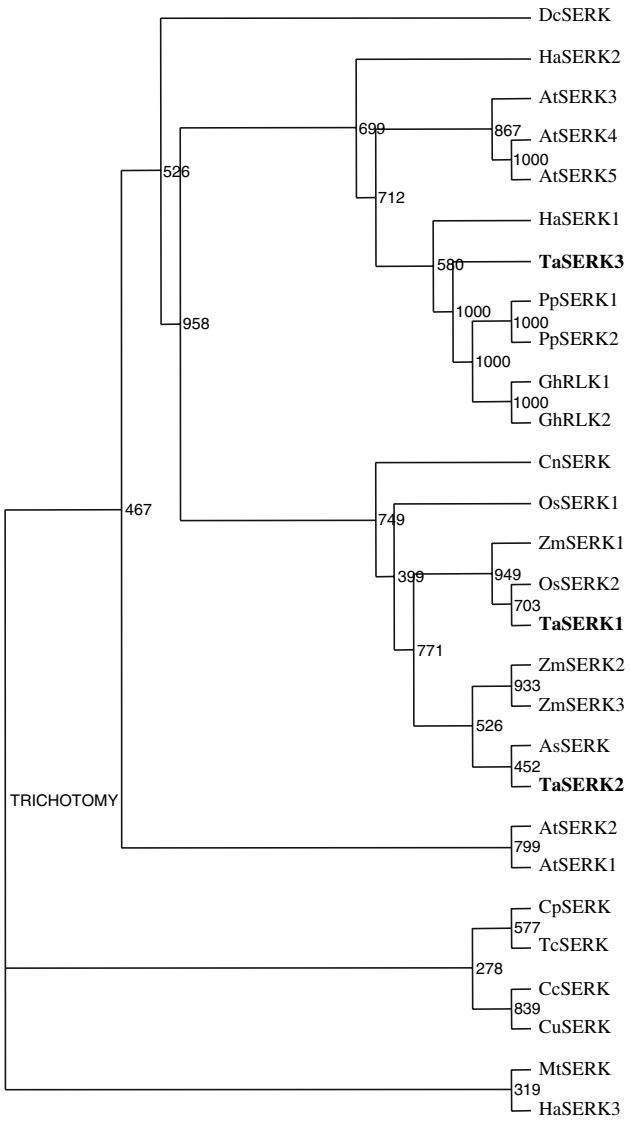

Fig. 2 Unrooted tree generated using Clustal_X program by neighbour-joining method indicating phylogenetic relationship among the wheat SERK, *Arabidopsis* (AtSERK1 to AtSERK5; CAB42254, AAK68073, AAK68074, AAD28318 and AAD28319), *Zea mays* (ZmSERK1, ZmSERK2 and ZmSERK3; CAC37638, CAC37639 and CAC37642), *Helianthus annuus* (HaSERK1 to HaSERK3; AAL93161, AAL93162 and AAL93163), *Oryza sativa* (OsSERK1 and OsSERK2, AK103038 and AK09777), *Medicago truncatula* (MtSERK1, AAN64293), *Daucus carota* (DcSERK, AAB61708), *Theobroma cacao* (TcSERK, AAV03482), *Citrus unshiu* (CuSERK BAD32780), *Coffea canephora* (CcSERK, ABN42681), *Cocus nucifera* (CnSERK, AAV58833), *Cyclamen persicum* (CpSERK, ABC71312), *Avena strigosa* (AsSERK, CAF33340), *Poa pratensis* (PpSERK1 and PpSERK2; AJ841693 and AJ841697) and *Gossypium hirsutum* (GhRLK1 and GhRLK2; AAT64017 and AAT64032) proteins. Bootstrap values from 1,000 replicates are indicated at each node

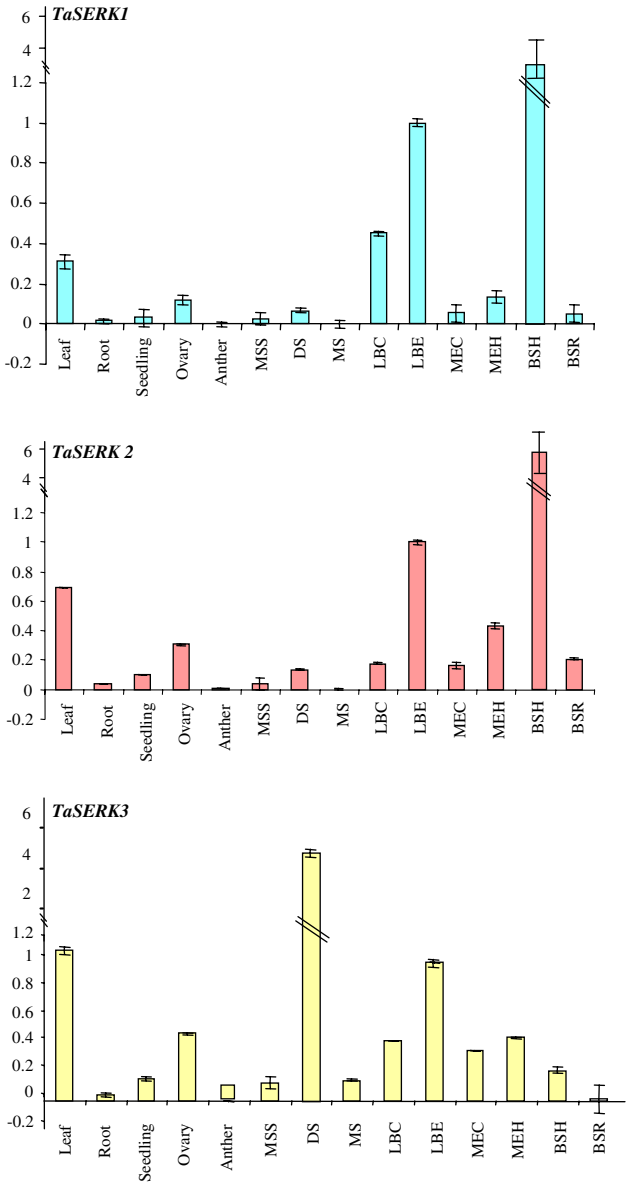

Fig. 3 Real-time PCR expression profiles of individual *TaSERK* cDNAs normalized to housekeeping gene, *ACTIN*, in different tissues (leaf, root, seedling, ovary, anther, milky stage of seed (*MSS*), developing seed (*DS*), mature seed (*MS*), control (*LBC*), embryogenic (*LBE*) leaf bases and mature embryos, calli on control (*MEC*), hormone (10 μM 2,4-D, *MEH*), basal segments on hormone (10 μM 2,4-D, *BSH*) and regeneration (*BSR*) medium, respectively. The relative mRNA levels were normalized with respect to the housekeeping gene, *ACTIN*. The *error bars* represent mean ± SD of two biological replicates, each analyzed with three technical replicates

base explants appeared equally potent for *TaSERK* expression (data not shown), therefore further experiments were restricted to 2,4-D treatment. The expression of all the three *TaSERKs* could be upregulated with short-term 2,4-D treatment (2 h) as revealed by real-time PCR analysis (Fig. 4). Besides 2,4-D, the transcript levels of *TaSERK3*, which seems to be an orthologue of BAK, showed enhanced expression in BR-treated leaf base tissue.

Ca^{2+}/calmodulin signaling system has an essential role in sensing and transducing environmental stimuli (Yang and Poovaiah 2003). Ca^{2+}/calmodulin binding protein,

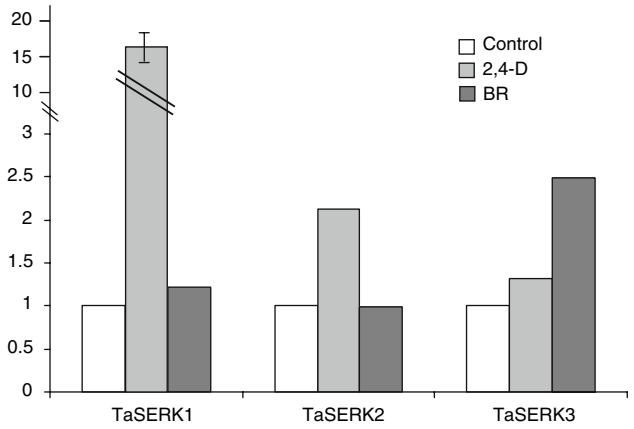

Fig. 4 Real-time PCR expression levels of three *TaSERK* cDNAs in control, 2,4-D (2 h, 10 μM) and epibrassinolide (2 h, 10 nM, *BR*) treated leaf bases of 13-day-old wheat seedlings. The relative mRNA levels were normalized with respect to the housekeeping gene, *ACTIN*. The *error bars* represent mean ± SD of two biological replicates, each analyzed with three technical replicates

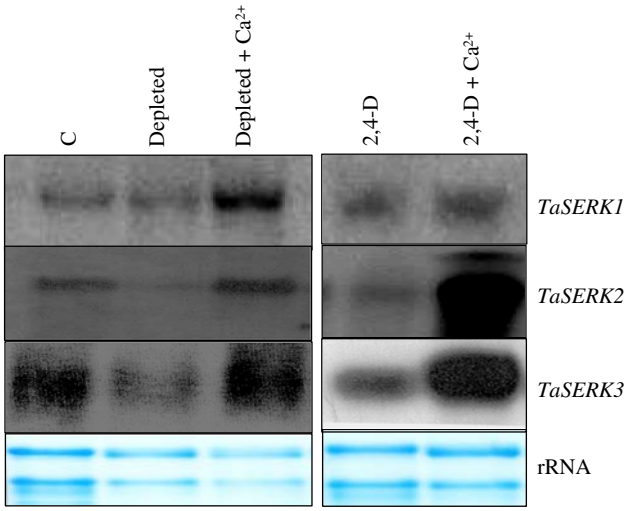

Fig. 5 Northern blot analysis to study the transcript abundance of the three *TaSERKs* after auxin depletion in KPSC buffer for 14 h, treatment with 10 μM 2,4-D and/or 320 μM $CaCl_2$. Control indicates RNA from wheat leaf bases before auxin depletion. Each *lane* was loaded with 20 μg of total RNA and methylene blue stained rRNA represents the equal loading

DWF1, has been found to be critical for brassinosteroid synthesis and plant growth (Du and Poovaiah 2005). Ca^{2+} ions have been shown to regulate the transcript abundance of early auxin-inducible *Aux/IAA* genes (Singla et al. 2006) and are involved in the induction of SE in wheat leaf base system (Patnaik and Khurana 2005; Mahalakshmi et al. 2007). This implies a strong connection between auxin, calcium and somatic embryogenesis. To study the effect of Ca^{2+} ions on *TaSERK* expression, the wheat leaf bases were first depleted of their endogenous auxin (Singla et al. 2006) and calcium was supplied either alone or in combination with 2,4-D. The *TaSERKs* expression decreased upon auxin depletion but the transcripts corresponding to all the three *TaSERK* genes could be detected in the presence of exogenous calcium and auxin (Fig. 5).

Discussion

Somatic embryogenesis is a notable illustration of plant totipotency, involves a broad repertoire of gene expression, regulated by various factors which are either independent or interdependent (Nomura and Komamine 1995; Chugh and Khurana 2002; Feher et al. 2003). Differential display of mRNA to identify genes specifically expressed in embryogenic carrot cells was first reported by Schmidt et al. (1997). One such gene encoded a leucine-repeat receptor protein kinase and was designated as a somatic embryogenesis receptor like kinase (*SERK*). Hecht et al. (2001) proposed that *AtSERK1* is a component of the embryogenesis-signaling pathway in *Arabidopsis* based on ectopic expression studies in culture. The aim of the work presented here was to determine if *SERK*-mediated signaling pathway is operative during zygotic and somatic embryogenesis in wheat. To achieve this, we first isolated *TaSERK3* gene from an auxin induced cDNA library of wheat and then the other closely related *SERK* genes, *TaSERK1* and TaSERK2.

The *TaSERK* genes

The predicted amino acid sequences of TaSERKs, like other SERKs, indicate that they could function as LRR-RLK (Walker 1994). RLKs exist as monomers until binding of an extracellular signal molecule induces receptor dimerization. This brings the intracellular kinase domains of individual monomers into close proximity, allowing transphosphorylation, which activates the kinase domains and causes the regulation of cellular response (Becraft 2002). Among the SERK proteins identified to date, the *Arabidopsis* AtSERK1 has been most extensively studied, with elucidation of some of the roles played by the different regions of the protein (Hecht et al. 2001; Shah et al. 2001a–c, 2002). The sequence similarity (80%) of TaSERK1 with AtSERK1 suggests that the corresponding regions in TaSERK1 may play a similar role. We note that *TaSERK1* and *TaSERK2* are closely related and it would be of interest to know whether they have similar or different functions to perform. *TaSERK3* could probably act as a BAK, although further detailed studies would be required to delineate its precise functions.

Expression of *TaSERKs* is linked with embryogenesis

During the initial phase of embryogenesis, somatic cells embark on a progression of developmental events referred to as dedifferentiation, entailing competence acquisition, induction and determination. In carrot, *SERKs* mark single cells that are competent to regenerate through SE (Schmidt et al. 1997). In the present instance, there was not much difference in the expression of different *TaSERKs* in the leaf bases treated with auxin (at induction stage), except that *TaSERK1* showed maximal expression. The expression of all the three *TaSERKs* was induced rapidly, after 24 h of auxin induction, long before the first appearance of somatic embryo, which are first visible after 10–12 days of culture in dark. The non-embryogenic wheat leaf bases treated with auxin did not show any increase in *TaSERK* transcript levels. Thus, there seems to be a strong correlation between the increase in *TaSERKs* expression and induction of somatic embryogenesis in leaf base explants. A similar correlation was also observed in the embryogenic calli developed from basal segments where the expression of the *TaSERK1* and *TaSERK2* was high. A correlation between *SERK* expression and SE was also demonstrated in cultured tissues of *Dactylis glomerata* (Somleva et al. 2000) and *Arabidopsis thaliana* (Hecht et al. 2001), suggesting an identical role for *SERK* during SE among these plants. In contrast, the maize *ZmSERK1* and *ZmSERK2* genes are expressed in both embryogenic and non-embryogenic callus cultures (Baudino et al. 2001).

There are diverse reports on the probable role of SERKs in various aspects of plant development. The present study has revealed that *TaSERK* transcripts (*TaSERK3* specifically) could be involved both in zygotic and somatic embryogenesis. In contrast, *MtSERK1* is expressed throughout the plant as revealed by the analysis of ESTs originating from libraries obtained from stem, leaf, root and flower tissue (Nolan et al. 2003). The RT-PCR and microarray analyses have shown that *AtSERK1* and *AtSERK2* are expressed in all aerial organs (Colcombet et al. 2005; Albrecht et al. 2005) as well as in stamen and ovules (Shah et al. 2001b; Colcombet et al. 2005). In *Citrus*, homologous clones for *SERK* genes were obtained from an EST catalog of a cDNA library from an ovary tissue at anthesis (Shimada et al. 2003, 2005). The transcript levels of *TaSERKs* were moderate in ovaries for all the three *TaSERKs*, but the reason for their low abundance in anthers is unusual. As in wheat, in *Poa pratensis* too, *PpSERK1* and *PpSERK2* expression was relatively higher in leaves than in roots (Albertini et al. 2005).

Expression of *TaSERKs* as influenced by calcium and brassinosteroid

BR treatment leads to altered endogenous auxin levels and/or enhanced auxin sensitivity (Sasse 1999; Nakamura et al. 2003, 2006; Li et al. 2005) suggesting potential crosstalk between BR and auxin signaling pathways, although further work is required to validate this assumption Also, BRI1-BAK1 (AtSERK3) receptor complex interactions have been shown (Li et al. 2002; Russinova et al. 2004). Cultures of wheat leaf bases treated with auxin or BR alone show that the *TaSERKs* expression is clearly induced by both for AtSERK3 in particular, although BR alone could not induce somatic embryo formation (data not shown) when applied for 24 h. In *D. glomerata* cultures, there were many more *SERK*-expressing cells than the number of somatic embryos, suggesting that embryo development arrested after *SERK* expression in some of these cells (Somleva et al. 2000). In *M. truncatula*, no significant difference were observed in *MtSERK1* expression between the highly embryogenic 2HA and non-embryogenic *M. truncatula* cv Jemalong, which showed little or no embryo formation (Nolan et al. 2003). AtSERK3/BAK1 is known to be a component of the brassinosteroid receptor complex and involved in the brassinosteroid signaling in *Arabidopsis* (Li et al. 2002; Nam and Li 2002). These results suggest that the functions of the members of the *SERK* family are not limited to embryogenesis, but they may play diverse roles depending on the gene and the system. The competence to somatic embryogenesis in a BAP-dependent manner is either organogenic or embryogenic culture conditions in sunflower and correlated to accumulation of *SERK* transcripts during the initial 48 h. Cells could simultaneously be competent for rhizogenesis and SE in *Medicago truncatula* and *MtSERK1* gene up-regulated in both embryogenic cultures and root forming cultures (Nolan et al. 2003; Thomas et al. 2004). Expression and mutant analysis of other *SERK* genes also suggest that their functions are not limited to embryogenesis (Baudino et al. 2001; Nam and Li 2002; Li et al. 2002; Nolan et al. 2003; Thomas et al. 2004; Ito et al. 2005).

Although no detailed work has been done but some earlier reports do indicate the involvement of calcium in SE in carrot (Jansen et al. 1990; Overvoorde and Grimes 1994), sandalwood (Anil and Rao 2000), *Medicago truncatula* (Davletava et al. 2001) and wheat (Patnaik and Khurana 2005, Mahalakshmi et al. 2007). We have shown earlier that calcium could enhance somatic embryogenic response in the wheat leaf bases which could be reversed by calcium chelator, EGTA (Mahalakshmi et al. 2007). Similarly, *TaSERKs* could also be induced in the presence of exogenous calcium suggesting its importance in the

somatic embryogenic pathway of the wheat leaf base system.

The genes involved in the somatic embryogenic pathway

Auxin is known to be one of the most important plant growth regulators in the induction of SE and is the basic trigger for the expression of genes associated with embryogenesis. The auxin inducible genes, *Aux/IAA*, the best characterized class of early auxin-responsive genes (Abel et al. 1995) have been shown to be associated with SE. For example, *IAA30*, involved in the maturation process, has been implicated in conferring competence for SE (Braybrook et al. 2006). Ca^{2+} ion is an important second messenger and an integral part of the cellular signaling system in plants (Yang and Poovaiah 2003). Ca^{2+}/calmodulin system has an essential role in sensing and transducing environmental stimuli (Snedden and Fromm 2001; Yang and Poovaiah 2003; Du and Poovaiah 2005). Interestingly, in an earlier study, we also observed that the expression of the three genes encoding calcium-regulated proteins in *T. aestivum*, i.e. calmodulin binding protein kinase (TaCBK), calcium dependent protein kinase (TaCDPK), and putative calcium binding protein (TaCaBP) to be involved in SE (Mahalakshmi et al. 2007). At the same time, similar results could be obtained for the *TaSERKs* expression providing strong evidence that calcium plays an important role in SE in the wheat leaf base system as well. Recently, *TaIAA1* (an early auxin-responsive gene) transcripts have been shown to be up-regulated by calcium and reversed completely by EGTA, suggesting a link between auxin, calcium and somatic embryogenesis (Singla et al. 2006).

In *Arabidopsis*, *Leafy Cotyledon* (*LEC*) genes, *LEC1*, *LEC2*, and *FUSCA3* (*FUS3*), were identified originally as loss-of-function mutations resulting in defects in both embryo identity and seed maturation processes (Harada 2001). Temporal and spatial patterns of auxin distribution during SE induction using transgenic *Arabidopsis* plants, indicated that the loss of embryogenic potential in the *lec2* mutants is not related to the distribution of exogenously applied auxin and *LEC* genes likely function downstream in auxin induced SE (Gaj et al. 2005). Expression of *knotted* (*kn*) and *ZmLEC1*, a maize homologue of the *Arabidopsis LEAFY COTYLEDON1* (*LEC1*), was detected in a small group of cells and entire somatic embryos at globular stage, respectively, during in vitro SE in maize (Zhang et al. 2002). The carrot homolog of *Arabidopsis LEC1* (*C-LEC1*) was expressed in embryogenic carrot cells and in somatic embryos developing from them, but not in cells from a non-embryogenic culture (Yazawa et al. 2004). Post-embryonic expression of *LEC1* is sufficient to induce SE in transgenic plants although somatic embryo formation is more robust with *LEC2*. *LEC2* probably acts in concert with other transcriptional factors, including *ABI1* and *FUS3*, to regulate the maturation phase (Braybrook et al. 2006). A sunflower gene *HaL1L*, homologous to the *AtL1L* gene (Kwong et al. 2003), has been shown to be involved in zygotic and somatic embryo development. The ectopic accumulation of *HaL1L* suggests that it might be involved in switching somatic cell fate toward embryogenic competence via the production of plant hormones and/or by increasing the sensitivity of the cell to these substances (Fambrini et al. 2006). Thus, besides *SERKs*, other genes have also been shown to confer competence to the somatic cells, thereby switching on the somatic embryogenic pathway.

The present study thus presents evidence to indicate the dependence on *TaSERK* transcripts for the 2,4-D mediated induction of somatic embryogenesis in the wheat leaf base system. BR and Ca^{2+} ions could up-regulate *TaSERK* expression in the wheat leaf base system. The signaling of 2,4-D-mediated induction of somatic embryogenesis in wheat is thus mediated by the *SERK* pathway. Further elucidation of the mechanism of stimulus–response coupling and identification of the different participants within the signaling pathway(s) would provide valuable insight into the process of plant embryogenesis.

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