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ORIGINAL ARTICLE

Molecular characterization and expression analysis of *SERK1* and *SERK2* in *Brassica napus* L.: implication for microspore embryogenesis and plant regeneration

Behzad Ahmadi[1] · Farhad Masoomi-Aladizgeh[1] · Mehran E. Shariatpanahi[1] · Pejman Azadi[1] · Mehdi Keshavarz-Alizadeh[1]

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Abstract

Key message **The** ***BnSERK1*** **and** ***BnSERK2*** **are involved in the process of microspore embryogenesis induction, development, and plantlet regeneration.**

Abstract Little is known about regulatory role of *somatic embryogenesis-related kinase* (*SERK*) genes family in the induction of microspore embryogenesis, development and plant regeneration. In this study, the expression of two *SERK* genes (*SERK1* and *SERK2*) was assessed during the microspore embryogenesis and plantlet regeneration in *Brassica napus* L. The *BnSERK1* was severely up-regulated 1–5 days following microspore culture and its expression drastically decreased in the globular-heart and also torpedo staged microspore-derived embryos (MDEs). In addition, high levels of *BnSERK1* transcript were detected in the MDE maturation phase and in the roots and shoots of the regenerated plantlets which indicates a broader role(s) of *BnSERK1* in the organ formation, rather than being specific to the embryogenesis. Results of partial sequencing indicated that the BnSERK1 shares a conserved serine-threonine kinase catalytic domain and exhibited 95 % similarity with AtSERK1, CsSERK1, BrSERK1, NaSERK1, and NbSERK1. A steady increase in the expression of *BnSERK2* was observed during the MDE initiation and development so that, the highest expression was noted in the MDE maturation phase i.e., late cotyledonary MDEs. Our results also indicated low amounts of *BnSERK2* transcript at the onset of rhyzogenesis but significantly higher expression in the developing roots. In contrast, the *BnSERK2* strongly up-regulated during the both initially and developed shoots. The BnSERK2 shares highly conserved LRR–RLK domain when compared with different species tested so that, high homology (100 %) was noticed with BrSERK2. Based on our findings, MDE formation and plantlet regeneration seem to be correlated with both *BnSERK1* and *BnSERK2* expression.

Keywords *Brassica napus* L. · DAPI · Microspore embryogenesis · qRT-PCR · *SERK*

Abbreviation

cDNA	Complementary DNA
DAPI	4′,6-Diamidino-2-phenylindole
qRT-PCR	Quantitative real-time PCR
SERK	Somatic embryogenesis-related kinase
LRR-RLK	Leucine-rich repeat transmembrane receptor-like kinase
MDE	Microspore-derived embryo

Introduction

Under normal conditions, microspores (in vivo) undergo gametophytic developmental pathway leading to mature pollen, however, under certain conditions, isolated microspores (in vitro) can change their developmental pathway toward embryogenesis (Touraev et al. 1997; Shariatpanahi et al. 2006). Isolated microspore culture is an important

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tool in plant breeding and biotechnology for rapid production of homozygous lines (doubled haploids), mutation and selection, establishing genetic maps and gene transformation (Liu et al. 2005; Brew-Appiah et al. 2013). Moreover, this system provides an excellent platform for embryogenesis mechanism(s) to be explored in a greater detail since the derived embryos are easy to be manipulated and can be produced in a large scale. Acquiring embryogenic competence requires the application of some type of external cue(s) leading to a stress(s) that preventing microspores from continuing their normal mode of gametophytic development and inducing sporophytic differentiation. In the genus *Brassica*, microspores are often induced by heat shock (Prem et al. 2005), anti-microtubular (Würschum et al. 2012), and mutagenic agents (Ahmadi et al. 2012), stress hormones (Ahmadi et al. 2014a) or polyamines (Ahmadi et al. 2014b).

Upon stress treatment and embryogenesis induction, many biochemical and morphological changes occur which is closely related to alterations in gene expression pattern (Tsuwamoto et al. 2007; Muñoz-Amatriaín et al. 2009). In the initial developmental stage, differentiated microspores acquire embryogenic competence either directly without a de-differentiation or through indirect mode of callusing. In the latter, the microspore initially losses its identity and perceives an appropriate stimulus bring competency for embryogenesis induction. Subsequently, MDEs enrolled into the maturation phase anticipating germination by desiccation and reserve accumulation (Seguí-Simarro and Nuez 2008; Soriano et al. 2013). Several genes are differentially expressed during the microspore embryogenesis induction, while others are expressed during the differentiation from MDE maturation stage up to full plant development (Seguí-Simarro and Nuez 2008; Hosp et al. 2014). Among the genes functionally involved in the embryogenesis process, the *SERK* genes have been claimed to play important roles (Schmidt et al. 1997; Krupa and Anamika 2006; Podio et al. 2014).

The *SERK* family, which encodes for leucine-rich repeat transmembrane receptor-like kinase (LRR-RLK) proteins, was first identified in carrot (*Daucus carota*) suspension cultures where it was specifically expressed in cells that developed into the somatic embryos (Schmidt et al. 1997). The plant LRR-RLKs are proteins with a predicted signal sequence, single transmembrane region, and a cytoplasmic kinase domain which transduce environmental signals and/or information from neighboring cells to trigger specific responses, and one such response is somatic embryogenesis (Krupa and Anamika 2006). The *SERKs* belong to a small gene family with at least five members in *Arabidopsis* and *Medicago truncatula* (Hecht et al. 2001; Nolan et al. 2003), three in *Zea mays* and grapevine (Baudino et al. 2001; Maillot et al. 2009), four in *Helianthus annuus* (Thomas et al. 2004) and two in *Oryza sativa* (Hu et al. 2005). The *SERK* family of receptor kinases is functionally diverse, involved in cell-to-embryo transition and controlling a number of other fundamental aspects of plant development. One of the best defined *SERK* genes in relation to somatic embryogenesis is the *A. thaliana SERK1* (*AtSERK1*) which its over-expression was shown to enhance embryogenic competence (Hecht et al. 2001). It has been proposed that the induction of somatic embryogenesis through the application of auxin, boron, and calcium-dependant signaling is also mediated by *SERK* pathway (Singla et al. 2008; Pandey et al. 2012). In addition to involvement in the somatic embryogenesis induction and development, *SERKs* also take part in pluripotency during the in vivo and in vitro organogenesis (Nolan et al. 2003; Thomas et al. 2004; Sharma et al. 2008). The *SERKs* are also known to be component of a brassinosteroid receptor complex and are involved in the brassinosteroid signaling which indicates that the functions of the members of the *SERK* family are not limited to embryogenesis, but they may play various roles depending on the gene and the system (Nolan et al. 2003; Singla et al. 2008). The objective of this study was to isolate, characterize, and analyze the expression of two *SERK* genes (*BnSERK1* and *BnSERK2*) during the microspore embryogenesis induction, development, and subsequent plantlet regeneration in *B. napus* L.

Materials and methods

Donor plant and growth condition

Rapeseed (*B. napus*) cv. 'Hyola 401' was used as the test plant. Donor plants were grown in a growth chamber at a day/night temperature of 15/10 °C with a 16-h photosynthetic photon flux density (PPFD) of 350 μE m^{-2} s^{-1} and relative humidity of 45–65 %. Donor plants were irrigated two times a week.

Isolated microspore culture and plantlet regeneration

Floral buds measuring 2.5–3.5 mm in length containing a mixed population of mid- to late-uninucleate microspores were harvested from the main and lateral branches of donor plants that had reached anthesis after about 90–110 days. These buds were surface sterilized by sodium hypochlorite (3 %) with gentle shaking for 10 min and washed three times (each 5 min) with cold sterile distilled water. Approximately, 180–200 sterilized buds were placed in a glass tube and were gently macerated into 25 ml of liquid NLN-13 (Lichter 1982) medium supplemented with 13 % (w/v) sucrose (Merck, Darmstadt, Germany) using a sterile

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glass rod. The crude suspension was filtered through a 40-µm metal mesh (Damavand Tes Sieve Ltd. Tehran, Iran), collected into two 15-ml centrifuge tubes, and the volume was adjusted with fresh NLN-13 medium 12 ml. The filtrate was centrifuged at $100\times g$ for 4 min. The supernatant was decanted and the pellet was rinsed in fresh NLN-13 medium. This procedure was repeated twice. Finally the plating density was adjusted to 6×10^4 microspores ml^{-1} using a hemocytometer (Precicolor, Germany). Microspore suspension (5 ml) was dispensed into 6-cm sterile plastic Petri dishes (Farazbin, Tehran, Iran); then, the cultures were incubated at 30 ± 0.5 °C in the dark for 14 days. Once MDEs were visible to the naked eye, the Petri dishes were transferred onto a rotary shaker in the dark at 30 rpm at 25 °C. Fully developed MDEs were transferred onto the B5 medium (Gamborg et al. 1968) containing 0.1 mg l^{-1} gibberellic acid (GA3, Fluka, Buchs, Switzerland), 2.5 % sucrose, pH 5.8, and 0.7 % agar (Duchefa Biochemie) and were maintained at 25 ± 1 °C under a 16-h photoperiod with light intensity of 40 µE m^{-2} s^{-1} for 2 weeks. Then, the distal half of the cotyledons was sliced off and the MDEs were transplanted onto the hormone-free B5 medium containing 2 % sucrose, pH 5.8, and 0.6 % agar for plantlet development (Ahmadi et al. 2012).

Determining MDE developmental stage

Developmental stages of embryogenic microspores, multicellular structures and the developing pro-embryos were detected using 4′,6-diamidino-2-phenylindole (DAPI) nucleic acid staining, which preferentially stains doubled-stranded DNA (Vergne et al. 1987). 1 ml of cultured microspores was transferred to 1.5 ml vials and centrifuged at $150\times g$ for 4 min. The supernatant was decanted and microspores were fixed with Carnoy reagent (ethanol: glacial acetic acid, 3:1, v/v) for 15 min. Then, the suspension was centrifuged at $150\times g$ for 4 min; the supernatant was decanted and 300 µl of fresh ethanol:water (1:1, v/v) was added to the pellet using gentle shaking. Finally, the suspension was centrifuged and microspores were stained with DAPI:glycerol (3:1, v/v) solution for 24 h. Samples were observed using an inverted microscope (Nikon Eclipse TE 2000-S) with fluorescent illumination and confocal laser scanning microscope.

RNA extraction and cDNA synthesis

Total RNA was extracted using TRIZOL reagent (Invitrogen, Karlsruhe, Germany) according to manufacturer's instructions. A total quantity of 50–100 mg of embryogenic or sporophytic tissues was used. The RNA samples were then treated with RNase-free DNaseI (Promega Corporation, Madison, WI) to eliminate DNA contamination and RNA quantification was performed using the Thermo Scientific NanoDrop 2000pc. The absorbance ratios of the RNA samples at 260/280 nm and 260/230 nm were between 1.8 and 2.0. Quality of RNA samples was verified on 1 % agarose gel. In order to complementary DNA (cDNA) synthesis, 1.0 µg of total RNA extracted from either embryogenic or sporophytic tissues was reversely transcribed into the cDNA using iScriptc DNA Synthesis Kit (Bio-Rad Laboratories Inc., Hercules, California, USA).

Quantitative real-time PCR

The qRT-PCR was conducted in a 12.5 µl reaction volume with the use of LightCycler FastStart DNA Master SYBR Green I (Bio-Rad), 0.5 µl of forward and reverse primers (10 pmol) and 1 µl of cDNA (50 ng μl^{-1}). The primers used for qRT-PCR reactions are listed in Table 1. The iCycleriQreal-time PCR detection system (Bio-Rad) was used with the following reaction conditions: denaturation one repeat of 95 °C for 4 min, followed by 40 cycles of 20 s at 95 °C, 20 s at 58 °C, and 30 s at 72 °C. The *18s-rRNA* as housekeeping gene was used as internal control. Relative quantitation of the target gene expression level was performed using $2^{-\Delta\Delta CT}$ method (Pfaffl 2001) relative to 0-day-old (freshly) cultured microspores as control. The entire experiment was repeated three times.

Results

Sequence analyses of *BnSERK* genes

The cDNA sequences of *A. thaliana*, *B. rapa*, and *Camelina sativa* from National Center for Biotechnology Information (NCBI) were aligned using CLUSTALW (www.ebi.ac.uk/tools/msa/clustalw2) and primers were designed at the most conserved SERK regions. Primers were used to amplify a 281 bp fragment for *BnSERK1* and 235 bp *BnSERK2* fragment from *B. napus* cDNA which was cloned and sequenced. The *BnSERK1* had 92 % similarity with *BrSERK1* (XM_009106643.1), 81 % with *CsSERK1* (XM_010429728.1), and 80 % with *AtSERK1* (NM_105841.4). The *BnSERK2* exhibited 97 % similarity with *BrSERK2* (XM_009122484.1), 91 % with *BrSERK1* (XM_009106643.1), 91 % with *AtSERK* (NM_122117.3), and 87 % with *CsSERK2* (XM_010456127.1).

To compare the similarity between BnSERKs and SERKs from other plant species, a phylogenetic tree was constructed based on the amino acid sequence of the putative BnSERK and those of selected plants. The translated amino acid sequences of BnSERK1and BnSERK2 were compared to BrSERK1 and BrSERK2, respectively,

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Table 1 Primer pairs used for relative expression analyses of *BnSERK1* and *BnSERK2* and *18s-rRNA* (internal control) genes during the microspore embryogenesis initiation, development, and plantlet regeneration in *Brassica napus* L.

Gene	Forward primer	Reverse primer	Size	References
BnSERK1	5′-CGACCACTGCGACCCTAAGAT-3′	3′-CCCTTTCACCCAGTCAAGCA-5′	281	This study
BnSERK2	5′-GAGCCTCATCAGCTTGGATCT-3′	3′-GAAGTGTTCTTACAAGGTCACCCC-5′	235	This study
18s-rRNA	5′-GAGCCTCATCAGCTTGGATCT-3′	3′-GAAGTGTTCTTACAAGGTCACCCC-5′	169	Unfried et al. (1989)

Fig. 1 Alignment of amino acid sequences of BnSERK1 using CLC Sequencer (ver. 6.4). Comparison of the BnSERK1 sequence with AtSERK1, CsSERK1, BrSERK1, NbSERK1, and NaSERK1

to analyze the conservation levels of the desired domains. Both BnSERKs represented partial fragments of the complete protein. While, the BnSERK1 had 95 % similarity with all amino acid sequences from AtSERK1, CsSERK1, BrSERK1, *Nicotiana benthamiana* SERK1, and *N. attenuate* SERK1 (Fig. 1), the BnSERK2 exhibited 100 % homology with BrSERK2, 88 % with CsSERK2, 88 % with AtLRR, 87 % with *Tarenaya hassleriana* SERK2 and 84 % with AlLRR. Therefore, the phylogenetic tree was constructed for BnSERK2 (Fig. 2). Based on our findings, we assigned the accession of *BnSERK1* and *BnSERK2* and registered them in NCBI (accession number: KT281978 and KR869962, respectively).

The results of alignment indicated that the BnSERK1 sequence started at the consensus sequence amino acid 449 and finished at consensus amino acid 491which represents a conserved catalytic domain of the serine-threonine kinase while, the BnSERK2 sequence started at the consensus sequence amino acid 142 and finished at the consensus sequence amino acid 218 that is responsible for LRR-RLK domain.

Expression analysis

The *BnSERK1* was severely up-regulated (Fig. 3) during the embryogenesis induction and initially MDE developmental stages (Fig. 4a–d) so that, high expression level was observed in the 5-day-old pro-embryos. However, its expression was drastically decreased (Fig. 3) in the globular-heart and torpedo-staged MDEs (Fig. 4e, f). High amounts of *BnSERK1* transcript were also detected (Fig. 3) when the torpedo-staged MDEs developed into the early and late cotyledonary MDEs (Fig. 5a, b). Fully developed cotyledonary MDEs were transferred onto the B5 regeneration medium for plantlet development. Few cotyledonary albino MDEs (Fig. 5c, about 0.5 % of total MDEs produced) were appeared in the regeneration medium which exhibited slower growth rate, development and failed to regenerate normally into the complete plantlet. Compared with early and late cotyledonary MDEs, significantly lower *BnSERK1* expression was observed in the albino MDEs. Expression of *BnSERK1* was also considered in the primary and developed shoots and roots of the regenerated plantlets (Fig. 5d, e). When compared with primary shoots, significantly higher expression was noted in the primary roots at the onset of plantlet regeneration. However, its expression was significantly increased later in the developing shoots.

A steady increase in the *BnSERK2* expression was detected (Fig. 6) during the MDE initiation and development so that, the highest expression was observed in the MDE maturation phase i.e., late cotyledonary MDEs. In contrast to the early and mature cotyledonary MDEs, significantly lower *BnSERK2* expression was noticed in the albino MDEs. According to our results, low level of *BnSERK2* transcript was detected at the onset of in vitro

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Fig. 2 Alignment of amino acid sequences of BnSERK2 using CLC Sequencer (ver. 6.4). Comparison of the BnSERK2 sequence with BrSERK2, CsSERK2, AtLRR, AlLRR, and ThSERK2 and phylogenetic tree construction for partial sequences

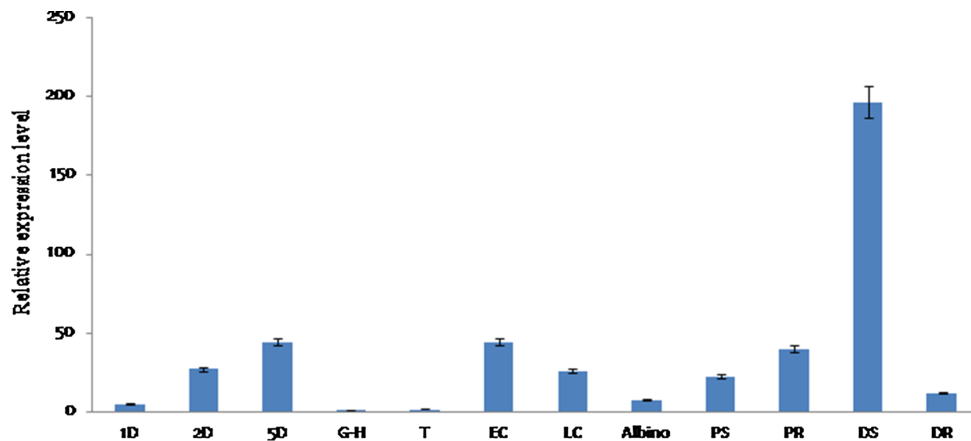

Fig. 3 Expression of *BnSERK1,* relative to 0-day-old (freshly) cultured microspores as control, during the microspore embryogenesis initiation, development, and plantlet regeneration in *Brassica napus* L. 1D: 1 day after microspore culture; 2D: 2 days after microspore culture; 5D: 5 days after microspore culture; *G-H* globular-heart staged MDEs, *T* torpedo-staged MDEs, *EC* early cotyledonary MDEs, *LC* late cotyledonary MDEs, *Albino* albino MDEs, *PS* primary shoots, *PR* primary roots, *DS* developed shoots, *DR* developed roots. The error bars represent mean ± SD of three biological replicates, each analyzed with three technical replicates

rhyzogenesis i.e., primary roots, but severely expressed later in the developing roots while, it was strongly up-regulated during the both initially and developed shoots.

Discussion

Isolated microspore culture technique provides a useful model system for embryogenesis induction and embryo development to be studied in a greater detail. In this work, two different members of the *SERK* family (*BnSERK1* and *BnSERK2*) were identified, and their expression during the MDE initiation, formation, and subsequently plantlet regeneration was investigated. Our results indicated that the expression of *BnSERK1* was significantly increased in the early steps of MDE formation i.e., 1–5 days following microspore culture. Using semi-automatic cell tracking, Schmidt et al. (1997) noted that upon 2, 4-D treatment, embryogenic cells were appeared on the seed-derived carrot hypocotyl explants cultured under in vitro condition. Surprisingly, *DcSERK* expression was found in the all morphologically discernible single cell types that were present in an embryogenic cell culture but its expression was never encountered in the non-embryogenic cultures. The *SERK1* gene is highly expressed in embryogenic tissues, being considered a molecular marker for somatic

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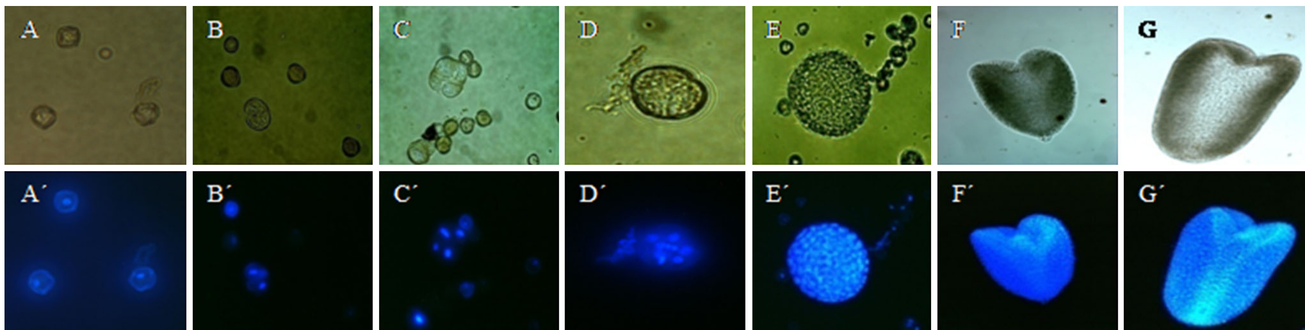

Fig. 4 Microspore-derived embryo (MDE) development in *Brassica napus* L. **a** Freshly isolated microspore culture containing mid- to late- uninucleate microspores; **b** microspores (1 day after culture); **c** microspores (2 days after culture); **d** multi-cellular structure (5 days after culture); **e** globular MDE; **f** heart-staged MDE; **g** torpedo-staged MDE. **a**'–**g**' The DAPI staining of corresponding MDE developmental stages

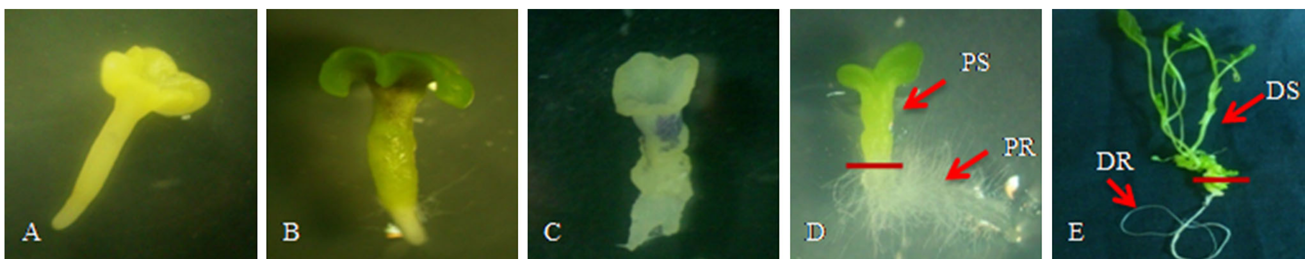

Fig. 5 Microspore-derived embryo (MDE) regeneration in *Brassica napus* L. **a** Early cotyledonary MDE; **b** mature MDE; **c** albino MDE; **d** primary shoot (PS) and root (PR); **e** developed shoot (DS) and root (DR)

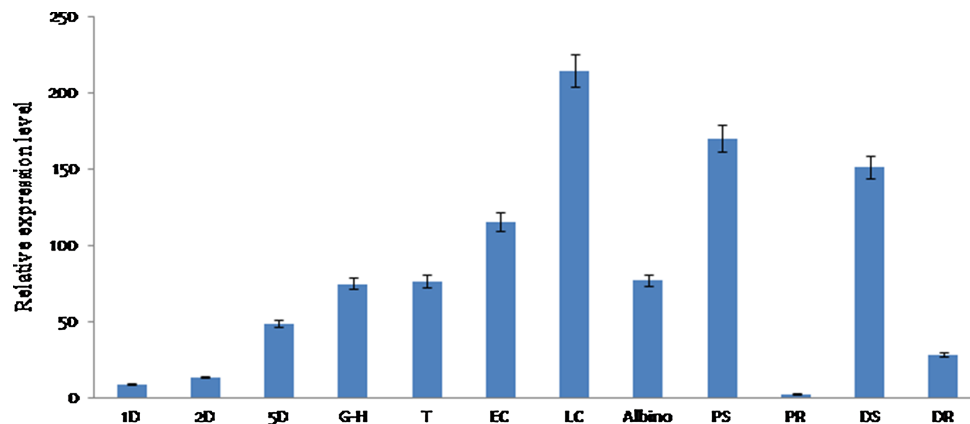

Fig. 6 Expression of *BnSERK2,* relative to 0-day-old (freshly) cultured microspores as control, during the microspore embryogenesis initiation, development, and plantlet regeneration in *Brassica napus* L. 1D: 1 day after microspore culture; 2D: 2 days after microspore culture; 5D: 5 days after microspore culture; *G-H* globular-heart staged MDEs, *T* torpedo-staged MDEs, *EC* early cotyledonary MDEs, *LC* late cotyledonary MDEs, *Albino* albino MDEs, *PS* primary shoots, *PR* primary roots, *DS* developed shoots, *DR* developed roots. The error bars represent mean ± SD of three biological replicates, each analyzed with three technical replicates

embryogenesis (Schmidt et al. 1997). Also, *AtSERK1* was highly expressed during embryogenic cell formation in culture and during early embryogenesis in *A. thaliana* (Hecht et al. 2001). Ectopic expression of *AtSERK1* conferred sustained embryogenic competence under in vitro conditions which suggests that the *AtSERK1* plays an essential role in the acquisition of embryogenic competence (Hecht et al. 2001). In wheat (*T. aestivum* L.), the expression of *TaSERK1* was induced rapidly after 24 h of auxin treatment, long before the first appearance of somatic embryos. There seems to be a strong correlation between the increase in *TaSERK1* expression and induction of

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somatic embryogenesis in the embryogenic calli developed from wheat basal leaf segments where the expression of the *TaSERK1* was high (Singla et al. 2008).

Our results indicated that, the expression of *BnSERK1* was drastically decreased in the globular-heart and also in the torpedo-staged MDEs. According to Kurczynska et al. (2007) the *AtSERK1* was expressed before fertilization during both male and female sporophytic and gametophytic development and after fertilization in the developing embryo until the heart stage. Working on anther culture in grapevine (*Vitis vinifera* L.), Schellenbaum et al. (2008) also observed high expression of *VvSERK1* induced during the early stages of embryogenesis before embryos were visible on the calli but when embryos were visible, very low expression of *VvSERK1* was detected in the detached embryos. To gain a deeper insight, a *SERK1* promoter-driven *GUS* (*prSERK1::GUS*) expression construct was transformed into the tissue of the embryogenic seedlings, 2HA, of *M. truncatula* using *Agrobacterium rhizogenes*- and *A. tumefaciens*-mediated transformation (Nolan et al. 2009). Expression analysis indicated that *prSERK1::GUS* was strongly up-regulated in the newly developed embryos but not in further embryo developmental stages providing evidence of the association of *SERK1* expression with the switch to embryogenic pathways and cell reprogramming (Nolan et al. 2009). However, our results indicated a sharp increase in *BnSERK1* transcript levels in the early and late cotyledonary MDEs. Regarding the embryogenic tissues in cacao (*Theobroma cacao* L.), Santos et al. (2005) found that *TcSERK* is highly expressed in the initially induced embryogenic calli and interestingly, the *TcSERK* expression was found in mature somatic and zygotic embryos, suggesting that this gene plays a role during the development and maturation of cacao embryos, while it was expressed only up to globular stage in carrot (Schmidt et al. 1997; Somleva et al. 2000), heart stage in *A. thaliana* (Hecht et al. 2001), and *Hieracium* (Tucker et al. 2003). Few albino MDEs were produced when MDEs were transferred onto the regeneration medium. Compared with early and late cotyledonary MDEs, lower level of *BnSERK1* transcript was detected in the albino MDEs.

In addition to expression during the MDE formation, our results also revealed that the *BnSERK1* was also severely up-regulated post-embryonically in the shoots and roots of the regenerated plantlets which suggest a broader role(s) of *BnSERK1* in the organ formation, rather than being specific to the embryogenesis *per se*. The *SERK1* has been indicated to play an important in pluripotency during the in vitro shoot and root formation in *M. truncatula* and sunflower (Nolan et al. 2003; Thomas et al. 2004). In rice, a significant reduction in shoot number has been observed in transgenic embryogenic calli with RNA interference for *OsSERK1* (Hu et al. 2005). In order to investigate the expression pattern of *SERK1* in *A. thaliana*, Hecht et al. (2001) fused the *AtSERK1* promoter and the *Escherichia coli*-glucuronidase (*GUS*) gene. The *GUS* expression in the cotyledons, primary leaves, hypocotyl, and roots and also in other organs of the mature plant, such as the pedicel and the petals was detected. In potato (*Solanum tuberosum* L.), *SERK1* was highly expressed in microtubers which can develop into the complete plants through the organogenesis (Sharma et al. 2008). The *SERK1* is expressed in the pluripotent cells of the vascular procambium, and it was speculated that *SERK1* functioned in maintaining the pluripotent nature of procambial cells (Kwaaitaal and de Vries 2007). Given that procambial cells are the origin of somatic embryos in carrot, it makes sense in a developmental context that procambial cells, which already exhibit a stem cell capacity, given the correct signals, could acquire a totipotent nature and develop into somatic embryos (Guzzo et al. 1994, 1995; Kwaaitaal and de Vries 2007). According to our results, when compared with the developing roots, significantly higher *BnSERK1* transcripts were observed in the developing shoots.

The *SERK2* has also been claimed to be involved in the acquisition of embryogenic competence in plant cells and also in the regulating vegetative and generative development however, information about its regulatory role(s) is rather limited (Colcombet et al. 2005; Singla et al. 2008; Podio et al. 2014). Regardless of severity of its expression, our results indicated that the *BnSERK2* was constitutively expressed in the all steps of MDE initiation, development, and plantlet regeneration as well. Working on somatic embryogenesis in *Z. mays*, Baudino et al. (2001) noted a relatively uniform expression of *ZmSERK2* in the embryogenic and non-embryogenic cultures. Similar results were also observed by Ito et al. (2005) and Schellenbaum et al. (2008) in *O. sativa* and *V. vinifera,* respectively. Expression analysis also indicated that the *BnSERK2* was highly expressed in the MDE maturation phase (late cotyledonary MDEs). Moreover, the *BnSERK2* was severely up-regulated during the shoot initiation and development and significantly lower amounts of *BnSERK2* transcripts were detected during the in vitro rhizogenesis. Working on apomictic *Paspalum notatum,* Podio et al. (2014) also observed a relatively low expression of *PnSERK2*in roots, but a strong representation in leaves, and reproductive tissues (Podio et al. 2014).

Results of partial sequencing indicated that the BnSERK1 shares a conserved serine-threonine kinase catalytic domain i.e., 95 % similarity with AtSERK1, CsSERK1, BrSERK1, NbSERK1, and NaSERK1. The crucial function of serine-threonine kinase domain is recognition of an extracellular ligand, which leads to activation of the intracellular kinase domain and subsequent transduction of downstream signaling pathways (Torii 2004). Serine-threonine kinases are known to play an

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important role(s) in a multitude of cellular processes, including division, proliferation, apoptosis, differentiation, and somatic embryogenesis (Torii 2004; Afzal et al. 2008). Our results indicated that the BsSERK2 shares highly conserved LRR-RLK domain when compared with different species so that a 100 % homology was noticed with *BrSERK2*. LRRs have been found in a variety of proteins with diverse functions, from yeast to flies, humans, and plants, and are implicated in protein–protein interactions. According to Jones and Jones (1997), the plant LRR domain has the consensus sequence L– –L– –L– –L–L– –N–L–G–IP– –L, where the dashes represent non-conserved residues which contribute to the specific interaction with other proteins (Torii 2004). The number of LRR domains in a given *SERK* gene varies among different species/genotypes. In *Arabidopsis* for instance, the number of LRRs of a given LRR-RLK varies from 1 to 32, and whereas many LRRs are tandemly aligned, some LRRs are intercepted by a spacer sequence known as a loop-out 'island' (Shiu and Bleecker 2001). According to our results, at least one LRR domain was identified in the consensus sequence of BnSERK2.

Conclusion

MDE formation and subsequent plant regeneration have become major challenges for doubled haploid plant production in recalcitrant species e.g., *A. thaliana* and *Lycopersicon esculentum* Mill. and even in the non-responsive cultivars of *B. napus*. Taken together, our results revealed that the *BnSERK1* was substantially up-regulated during embryogenesis induction phase (1–5 days following microspore culture) and its expression drastically decreased in the globular-heart and also torpedo-staged MDEs. On the other hand, a steady increase in the expression of *BnSERK2* was observed during the MDE initiation, development, and plantlet regeneration. The present study provides evidence to indicate the involvement of *BnSERK1* and *BnSERK2* in the process of MDE initiation and plantlet regeneration. Over-expression or introducing these genes into the recalcitrant species may overcome the challenges related to MDE production and plant regeneration in recalcitrant species. Further elucidations of possible regulatory mechanisms of these participants would provide a valuable insight into the process of microspore embryogenesis.

Author contribution statement B. Ahmadi conceived the idea, contributed in the practical process of microspore culture, plantlet regeneration, RNA extraction and cDNA synthesis, gene expression, data analysis, and writing of the manuscript. F. Masoomi-Aladizgeh contributed in the process of microspore culture, RNA extraction and cDNA synthesis, gene expression, and data analysis. M. E. Shariatpanahi (corresponding author) and P. Azadi supplied the materials and contributed in supervising practical process of microspore culture and gene expression analysis, data analysis, and writing of the manuscript. M. Keshavarz-Alizadeh contributed in the process of RNA extraction and cDNA synthesis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical statement The manuscript entitled "Molecular characterization and expression analysis of *SERK1* and *SERK2* in *Brassica napus* L.: implication for microspore embryogenesis and plant regeneration" has not been submitted to more than one journal for simultaneous consideration. Authors declare that this manuscript has not been published previously (partly or in full). The results of this study heve not been split up into several parts. Authors also declare that data have not been fabricated or manipulated (including images) to support our conclusions. Data, text, or theories by others are not presented unless they are the author's own. All authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

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