Preliminary molecular detection of the somatic embryogenesis receptor-like kinase (*VpSERK*) and knotted-like homeobox (*VpKNOX1*) genes during in vitro morphogenesis of *Vanilla planifolia* Jacks

Marco A. Ramírez-Mosqueda¹ · Lourdes G. Iglesias-Andreu¹ · Luis Sáenz² · Iván Córdova²

Received: 30 September 2017 / Accepted: 11 January 2018 / Published online: 24 January 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

This work aimed to evaluate the embryogenic competence of different tissues from different stages (friable callus, bud-regenerating callus, and whole buds) of *Vanilla planifolia*, through the molecular detection of the somatic embryogenesis receptor-like kinase (*VpSERK*) and knotted-like homeobox (*VpKNOX1*) genes. RNA was extracted with Trizol®, cDNA was obtained, and the studied transcripts were amplified. Using non-specific primers, *VpSERK* and *VpSTM* gene expression was detected in the three stages evaluated. This study might contribute to providing an explanation for the recalcitrance of this *Vanilla* species to somatic embryogenesis.

Keywords *Vanilla planifolia* · *VpSERK* · *VpKNOX1* · Somatic embryogenesis

Introduction

There are currently no somatic embryogenesis (SE) protocols available for vanilla (*Vanilla planifolia* Jacks.), nor has the embryogenic competence of its tissues been evaluated. Moreover, no molecular studies have assessed the cause of recalcitrance to somatic embryogenesis in *V. planifolia*. However, the early expression of the somatic embryogenesis receptor kinase 1 (*SERK1*) gene is closely related to the formation of embryogenic cells in several plant species (Perez-Núñez et al. 2009; Cueva et al. 2012; Huang et al. 2014; Ahmadi et al. 2016). This suggests that the level of *SERK1* expression can indicate embryogenic competence in *V. planifolia* in vitro cultures. The expression of a class

I KNOX gene in *Arabidopsis*, Shoot Meristemless (*STM*), is associated with the organogenic pathway (including SE) (Zhao et al. 2002). Expression of the *STM* gene may occur after the induction of organogenesis, specifically during bud differentiation (Bao et al. 2009).

Friable callus (FC), callus with buds (CB), and regenerated buds (RB) produced through indirect organogenesis as described by Ramírez-Mosqueda and Iglesias-Andreu (Ramírez 2015) were used in this study (Fig. 1a). To extract RNA, Trizol® (Invitrogen) was used (Montero-Córtes et al. 2010). Omniscript (Quiagen) was used to synthesize cDNA following the manufacturer's instructions. To isolate the *VpSERK* transcript, primers specific for coconut palm (*Cocos nucifera* L.) with the following sequences were used: *CnSERK*-Fw: 5'-TAT CTG GTA CAT TGG TCC CTC-3'; *CnSERK*-Rv: 5'-CTC TTC AGC AGG CAC ATC-3' (Perez-Núñez et al. 2009). DNA amplification was performed in an AXIGEN® (Applied Biosystems, Thermo Fisher Scientific Corp., CA, USA) thermocycler. Amplifications were carried out in 35 cycles using the following program: initial denaturation at 94 °C for 2 min, subsequent denaturation at 94 °C for 2 min, annealing at 60 °C for 40 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 10 min. The *SERK* plasmid was extracted with the QIAprep Spin Miniprep kit (Qiagen, Hilden, North Rhine-Westphalia, Germany) and was used as the positive control (Perez-Núñez et al. 2009), and a reaction mixture with no DNA

✉ Marco A. Ramírez-Mosqueda
marcoa.rm.07@gmail.com

✉ Lourdes G. Iglesias-Andreu
liglesias@uv.mx

¹ Instituto de Biotecnología y Ecología Aplicada (INBIOTECA), Universidad Veracruzana, Av. de las Culturas Veracruzanas No. 101, Campus para la Cultura, las Artes y el Deporte, Col. Emiliano Zapata, 91090 Xalapa, Veracruz, Mexico

² Centro de Investigación Científica de Yucatán, Calle 43 No. 130, Colonia Chuburná de Hidalgo, Mérida, Yucatán, Mexico

Fig. 1 Plant material and molecular profiles in different phases of indirect organogenesis in *Vanilla planifolia*. **a** Friable callus (FC), bud-regenerating callus (CB) and regenerated buds (RB). **b** Analysis of *VpSERK* expression; **c** analysis of *VpKNOX1* expression. *M* = 1 kb molecular weight marker (Invitrogen). B has only five lanes, since we did not have the plasmid generated from *Cocos nucifera*

template was used as the negative control. To isolate the *VpKNOX1* transcript, primers specific to coconut palm were used to amplify a 416-bp fragment and had the following sequences: *CnKNOX1*-FW 5'-CCC CAA ACC CAG AGC TAA AA-3' and RV 5'-TTG CCT TGA TGA TGC T GT CA-3' (Montero-Cortés et al. 2010). *VpKNOX1* amplification by PCR was performed as indicated above over 36 cycles in an AXIGEN® thermocycler using the following program: initial denaturation at 94 °C for 2 min, subsequent denaturation at 94 °C for 50 s, annealing at 50 °C for 50 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products were analyzed by 1% agarose gel electrophoresis and purified using the Nucleo Spin Extract Kit (Macherey-Nagel, Düren, Zúrich, Germany) according to the directions of the manufacturer. Gene amplification of both genes was performed in duplicate using a different sample from each tissue.

The use of non-specific primers showed the presence of a distinctive 180-bp fragment in all the samples examined (Fig. 1b). *VpSERK* expression was observed in all tissue types evaluated. However, in callus with buds, a decrease in the intensity of this fragment was observed. Fragments of the *VpSERK* transcript have been detected in different plant species, showing sizes ranging from 194 to 281 bp (Cueva et al. 2012; Ahmadi et al. 2016). The lower intensity of the fragments observed in *V. planifolia* friable callus tissue may be due to lower embryogenic competence.

A 380-bp fragment was also observed in all samples evaluated (Fig. 1c). Unlike the expression of the *VpSERK* gene, the expression of *VpKNOX1* increased in friable callus and callus with buds, and decreased slightly in the regenerated buds. The *VpKNOX1* gene was expressed in all three tissues. Elhiti and Stasolla (2012) determined that expression of the *VpKNOX1* gene was involved in bud formation during indirect organogenesis in turnip (*Brassica napus* L.).

Expression of the *VpSERK* gene was detected in the three stages evaluated, but there was a decrease in expression during the bud-induction phase in callus, most likely because this gene participates in somatic embryogenesis. Besides, the expression of the *VpKNOX1* gene increased as organogenesis progressed. These preliminary results bring us closer to understanding the recalcitrance of this spice toward somatic embryogenesis through a molecular study of two genes (*VpSERK* and *VpKNOX1*) that are involved in the morphogenesis of plants grown in vitro. However, studies allowing the purification, linkage, cloning, and sequencing of these amplification products are required to clearly identify the presence of these genes in this species.

Acknowledgements The authors thank the “Programa para el Desarrollo Profesional Docente (PRODEP)” for financial support provided for the project “Biotechnological Basis for the Genetic Improvement of *Vanilla planifolia*” within the “Conservation, Management and Plant Breeding network”. MARM thanks the Consejo Nacional de Ciencia y Tecnología (CONACyT) for a grant scholarship No. 275736 which allowed the realization of this work. We thank Jaime A. Teixeira da Silva for revising the manuscript, improving the English and offering critical analysis of the scientific content.

Compliance with ethical standards

Conflict of interest The author(s) declare that they have no conflicting interests.

References

- Ahmadi B, Masoomi-Aladizgeh F, Shariatpanahi ME, Azadi P, Keshavarz-Alizadeh M (2016) Molecular characterization and expression analysis of *SERK1* and *SERK2* in *Brassica napus* L.: implication for microspore embryogenesis and plant regeneration. Plant Cell Rep 35:185–193

- Bao Y, Dharmawardhana P, Arias R, Allen MB, Ma C, Strauss SH (2009) *WUS* and *STM*-based reporter genes for studying meristem development in poplar. *Plant Cell Rep* 28:947–962
- Cueva A, Concia L, Cella R (2012) Molecular characterization of a *Cyrtocilum loxense* somatic embryogenesis receptor-like kinase (*SERK*) gene expressed during somatic embryogenesis. *Plant Cell Rep* 31:1129–1139
- Elhiti M, Stasolla C (2012) *In vitro* shoot organogenesis and hormone response are affected by the altered levels of *Brassica napus* meristem genes. *Plant Sci* 190:40–51
- Huang YW, Tsai YJ, Chen FC (2014) Characterization and expression analysis of somatic embryogenesis receptor-like kinase genes from *Phalaenopsis*. *Genet Mol Res* 13:10690–10703
- Montero-Córtes M, Sáenz L, Córdova I, Quiroz A, Verdeil JL, Oropeza C (2010) GA3 stimulates the formation and germination of somatic embryos and the expression of a *KNOTTED*-like homeobox gene of *Cocos nucifera* (L.). *Plant Cell Rep* 29:1049–1059
- Pérez-Núñez MT, Souza R, Saénz L, Chan JL, Zuniga-Aguilar JJ, Oropeza C (2009) Detection of a *SERK*-like gene in coconut and analysis of its expression during the formation of embryogenic callus and somatic embryos. *Plant Cell Rep* 28:11–19
- Ramírez-Mosqueda MA, Iglesias-Andreu LG (2015) Indirect organogenesis and assessment of somaclonal variation in plantlets of *Vanilla planifolia* Jacks. *Plant Cell Tiss Organ Cult* 123:657–664
- Zhao Q-H, Fisher R, Auer C (2002) Developmental phases and *STM* expression during *Arabidopsis* shoot organogenesis. *Plant Growth Regul* 37:223–231