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CONOCIMIENTOS MOLECULARES SOBRE LA EMBRIOGÉNESIS SOMÁTICA EN *Agave angustifolia*: CARACTERIZACIÓN DEL GEN AaSERK

Reyes-Díaz, Jesús Ignacio y **Amaury Martín Arzate-Fernández**

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Conocimientos moleculares sobre la embriogénesis somática en *Agave angustifolia*: caracterización del gen *AaSERK*

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ABSTRACT: The *Agave* genus, with 265 species, including 153 natives to Mexico, is of paramount significance in ethnobotanical, ecological, and economic contexts. However, over-exploitation and uncontrolled harvesting of these plants endanger their survival due to the prevention of sexual reproduction. In response, *in vitro* techniques, such as somatic embryogenesis, have been developed for vegetative propagation and species conservation. Somatic embryogenesis transitions cells to totipotency, driven by specific gene expression, endogenous hormones, and responses to external regulators. This work explores the first molecular insights into somatic embryogenesis in the *Agave* genus through the isolation and characterization of the *AaSERK* gene. Receptor-Like Kinases (RLKs) with Leucine-Rich Repeats (LRRs) have crucial roles in cellular signaling across various aspects of plant development, including embryogenesis. The presence of a Serine-Proline-Proline motif (SPP) distinguishes SERK from other RLKs, and its expression signifies embryogenic competence. The results reveal that *AaSERK* encodes a typical SERK protein with conserved domains, indicating its role in plant development. Phylogenetic analysis suggests that *AaSERK* shares evolutionary ancestry with SERKs of closely related plant species. These findings shed light on somatic embryogenesis in *Agave angustifolia* and may enhance the regeneration and transformation processes for the conservation of these valuable plants. Understanding the genetic control of totipotency and the molecular regulation of somatic embryogenesis is vital for advancing plant biotechnology and plant physiology.
Key words: Somatic embryogenesis; Somatic Embryogenesis Receptor-like Kinase gene; *Agave angustifolia.*

RESUMEN: El género *Agave*, con 265 especies, incluidas 153 nativas de México, es de suma importancia en contextos etnobotánicos, ecológicos y económicos. Sin embargo, la sobreexplotación y la recolección incontrolada de estas plantas ponen en peligro su supervivencia debido a la problemática de su reproducción sexual. En respuesta, se han desarrollado técnicas *in vitro*, como la embriogénesis somática, para la propagación vegetativa y la conservación de especies. La embriogénesis somática hace que las células manifiesten su carácter totipotente, impulsado por la expresión genética específica, hormonas endógenas y respuestas a reguladores externos. Este trabajo explora los primeros conocimientos moleculares sobre la embriogénesis somática en el género *Agave* a través del aislamiento y

caracterización del gen *AaSERK*. Las quinasas similares a receptores (RLK) con repeticiones ricas en leucina (LRR) desempeñan funciones cruciales en la señalización celular en diversos aspectos del desarrollo de las plantas, incluida la embriogénesis. La presencia de un motivo Serina-Prolina-Prolina (SPP) distingue a las *SERK* de otras RLK y su expresión significa la adquisición de la competencia embriogénica. Los resultados revelan que *AaSERK* codifica una proteína *SERK* típica con dominios conservados, lo que sugiere su papel en el desarrollo de las plantas. El análisis filogenético muestra que *AaSERK* comparte ascendencia evolutiva con *SERK* de especies de plantas estrechamente relacionadas. Estos hallazgos arrojan información importante sobre la embriogénesis somática en *Agave angustifolia* y pueden mejorar los procesos de regeneración y transformación para la conservación de esta valiosa planta. Comprender el control genético de la totipotencia y la regulación molecular de la embriogénesis somática es vital para avanzar en la biotecnología y la fisiología de las plantas.
Palabras clave: Embriogénesis somática; Gen de quinasa similar al receptor de embriogénesis somática; *Agave angustifolia*.

INTRODUCTION

The *Agave* genus, situated within the Agavoideae subfamily (Asparagaceae, Asparagales), comprises a diverse collection of 265 species. Remarkably, 153 of these species are native to Mexico, with 150 of them being exclusive to this region (Vázquez-García *et al.*, 2022). This geographical concentration establishes Mexico as the epicenter of both the origin and diversification of these plants (García-Mendoza & Galván-V., 1995). This genus holds immense significance in ethnobotanical, ecological, and economic contexts, as these plants fulfill various roles such as serving as living fences, sources of food, materials for fibers, construction, medicines, cosmetics, fodder, and the production of alcoholic beverages like bacanora, pulque, tequila, mescal, and others (Bermúdez-Bazán *et al.*, 2021; Vázquez-Delfin *et al.*, 2022).

However, the over-exploitation of specific *Agave* species, coupled with uncontrolled plant extraction, presents a substantial danger to their survival (Aguirre-Dugua & Eguiarte, 2013). This risk is intensified because these plants are harvested before reaching the flowering stage, thereby preventing sexual reproduction (Monja-Mio *et al*., 2021). In response to this pressing need for a substantial number of individuals from selected genotypes, *in vitro* culture techniques, particularly somatic embryogenesis has been developed as a promising vegetative propagation process, offering an alternative for the conservation of *Agave angustifolia* (Reyes-Díaz *et al*., 2017). Also, preservation methods such as cryopreservation and encapsulation have been utilized to conserve somatic embryos long-term (Arzate-Fernández *et al*., 2016).

Somatic embryogenesis (SE) plays a vital role in the mass propagation and genetic transformation of various plant species (Ramírez-Mosqueda, 2022). In this process, the transition to developmental totipotency in cells is initiated by temporarily exposing them to genes responsible for maintaining the meristematic state regulated by endogenous hormone levels like response to exogenous plant growth regulators (PGR) and controlling different stages of plant morphogenesis during SE (Loyola-Vargas & Ochoa-Alejo, 2016). A widely accepted model suggests that the conditions inducing somatic embryogenesis led to the dedifferentiation of somatic plant cells, followed by the acquisition of developmental totipotency. At this totipotent stage, cells can respond to specific developmental signals guiding them toward embryogenesis. Understanding the genetic control of totipotency is pivotal for advancing plant biotechnology and enhancing our knowledge of plant physiology (Schmidt *et al*., 1997).

Thus, the identification and characterization of genes associated with somatic embryogenesis offer the potential to assess the embryogenic capacity of somatic cells at early developmental stages and provide insights into the molecular regulation of this process *(De Oliveira Santos et al., 2005; Liu et al.*, 2018; Ramasamy *et al*., 2022).

Receptor-Like Kinases (RLKs) with Leucine-Rich Repeats (LRRs) within the family have pivotal roles in various aspects of cellular signaling in plant development. This includes their involvement in disease resistance, microsporogenesis, vascular tissue differentiation, embryo pattern formation, control of cellular death, and self-incompatibility (Fisher & Turner, 2007; Hu *et al.*, 2005). RLKs function by facilitating the transmission of external signals and information from neighboring cells, thereby triggering specific responses. In case of somatic embryogenesis process, the responses of cultured cells are contingent on the cell type and the composition of the culture medium (Baudino *et al*., 2001; Ma *et al*., 2012; Nodine *et al*., 2007; Ramasamy *et al*., 2022; D.-Z. Zhao *et al*., 2002).

The expression of flavin monooxygenase-encoding genes is stimulated during the establishment of cell or tissue polarity and embryo development in the presence of synthetic auxins like 2,4-dichlorophenoxyacetic acid (2,4-D) in the culture medium (Ramírez-Mosqueda, 2022). These enzymes play a crucial role in auxin biosynthesis, resulting in an increase in endogenous indoleacetic acid (IAA) levels, a key step in meristem formation and, by extension, embryo development (Wójcik *et al*., 2020). It has been observed that cells originating from provascular cells promote most somatic embryos, with their embryogenic commitment confirmed through the expression of a Receptor-Like Kinase known as Somatic Embryogenesis Receptor-Like Kinase (*SERK*). This gene expression serves as a recognized marker of embryogenic competence (Koehler *et al*., 2020; Maulidiya *et al*., 2020; D.-Z. Zhao *et al*., 2002).

What distinguishes *SERK* from other RLKs is the presence of a Serine-Proline-Proline motif (SPP) situated between five LRRs and the transmembrane (TM) domain (Cueva *et al*., 2012; De Oliveira Santos *et al*., 2005; Hecht *et al*., 2001; Santos *et al*., 2009).

SERK genes are well-known for their pivotal role in signal transduction during embryogenic cell development, with their expression mainly occurring in the early stages of embryogenesis *(Hecht et al*., 2001). This expression gradually diminishes as development progresses. In *Daucus carota* and *Arabidopsis thaliana*, for instance, *SERK* genes are only expressed in embryogenic structures, not in non-embryogenic cultures (Hecht *et al*., 2001; Salaj *et al.*, 2008; Schmidt *et al*., 1997).

While the role of *SERK* genes in many plant species has been extensively studied, the molecular aspects of somatic embryogenesis in the *Agave* genus remain unexplored, and genes associated with this process have not been yet identified. This study represents the first characterization of a *SERK* gene from *A. angustifolia*, referred to as *AaSERK.* The results of this study may offer insights to enhance the regeneration rate of somatic embryogenesis and improve the genetic transformation process.

MATERIALS AND METHODS

Establishment of embryogenic culture

Aseptic, fully developed zygotic embryos (Figure 1a) were meticulously dissected from seeds of *Agave angustifolia* and served as the initial explants for inducing callus formation, as shown in Figure 1a. The cultures of embryogenic callus (Figure 1b) were initiated on a medium containing one-quarter strength of Murashige and Skoog (MS) nutrients (Murashige & Skoog, 1962). This medium was supplemented with 3.0 mg L^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1.0 mg L^{-1} of 6-benzyladenine (BA) as plant growth regulators (Reyes-Díaz *et al*., 2017). The cultures were then incubated under conditions featuring 16 hours of light and 8 hours of darkness, utilizing cool white light at an intensity of 60 µmol m^{-2} s^{-1}, at a temperature of 25 ± 2 °C. Remarkably, mature somatic embryos (Figure 1d) were observed after a duration of 120 days from the initiation of the culture.

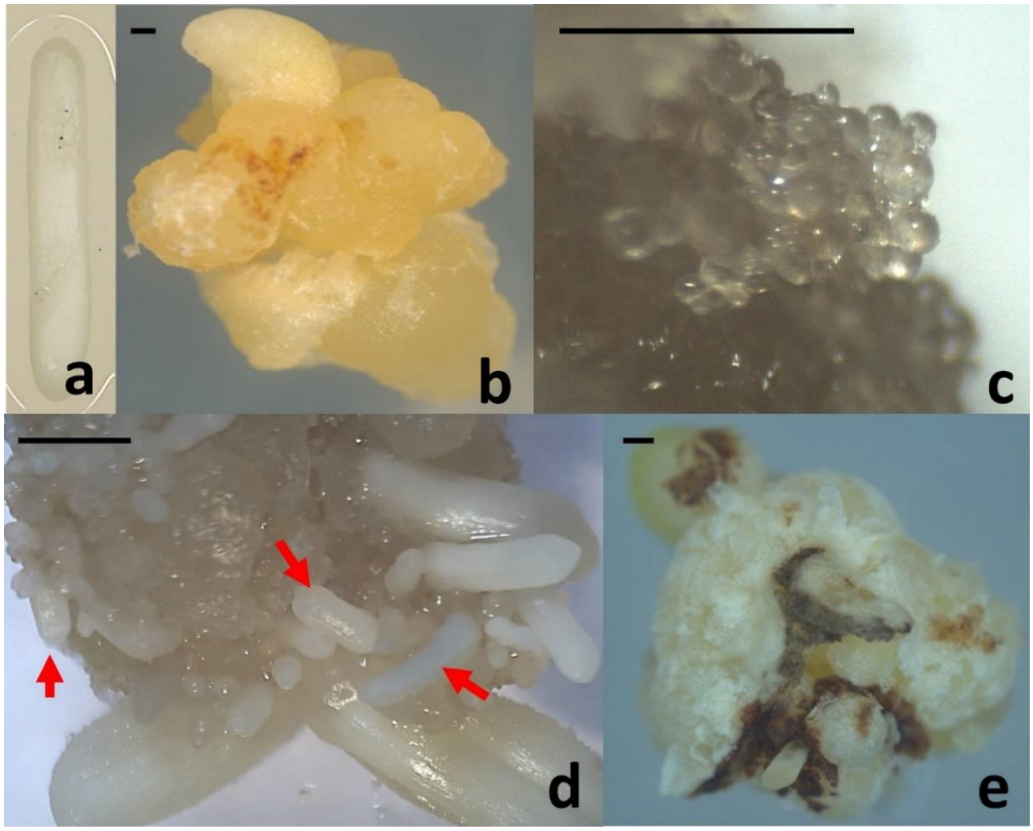

Figure 1. *Agave angustifolia* somatic embryogenesis process: (a) Initial explant. (b) Embryogenic callus. (c) Embryogenic callus with globular somatic embryos (60 days after the induction) used for *SERK* gene isolation. (d) Mature somatic embryos (arrows) (120 days after the induction). (e) Non-embryogenic callus. Bar = 5mm
Figura 1. Proceso de embriogénesis somática de *Agave angustifolia*: (a) Explante inicial.b) Callo embriogénico. c) Callo embriogénico con embriones somáticos globulares (60 días después de la inducción) utilizados para el aislamiento del gen *SERK*. d) Embriones somáticos maduros (flechas) (120 días después de la inducción). e) Callo no embriogénico. Barra = 5 mm.

Cloning and sequencing of *Agave angustifolia SERK* (*AaSERK*) gene

Genomic DNA was extracted from 60-day-old embryogenic callus with globular somatic embryos (Figure 1c) and from callus without embryogenic response (Figure 1e) using the modified CTAB method (Aboul-Maaty & Oraby, 2019). The DNA was subsequently dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.0). The quality and concentration of the extracted DNA were assessed through agarose gel electrophoresis and bio photometry (Eppendorf AG, Germany), respectively.

To amplify a fragment of *Agave angustifolia* SERK (*AaSERK*), degenerate primers were designed based on consensus SERK sequences from *Zea mayz*, *Oryza sativa*, *Daucus carota*, *Arabidopsis thaliana*, and *Cyrtochilum loxense* stored in the NCBI database (www.ncbi.nlm.nih.gov/). These primers were crafted from conserved regions and possessed the following sequences: forward primer 5′-NTGGTGAGGTGGCGGAGG-3′ and reverse primer 5′-TGTHACRTGGGTRTNCTTCTARTCCAT-3′. Polymerase Chain Reaction (PCR) was conducted using these primers. The PCR reaction was prepared with a final volume of 10 µl, consisting of 10 ng of genomic DNA, 6.3 µl of Taq DNA polymerase buffer, 5 mM dNTPs, 15 mM $MgCl_2$, 1 U of MyTaq DNA Polymerase (Bioline, USA), and 0.4 µM of each forward and reverse primer (Sigma, USA). The thermal amplification parameters for the PCR reaction included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of amplification (95 °C for 30 s, 47.5 °C for 30 s, 72 °C for 2 min), and a final extension step at 72 °C for 10 min. The amplifications were conducted in a thermal cycler (Multigene Optimax, Labnet, USA). Subsequently, the PCR products were subjected to 1% (w/v)

agarose gel electrophoresis and visualized using a gel documentation system (UVP Transilluminator 95-0403-01, UK).

The *AaSERK* fragment, amplified from genomic DNA, underwent purification using the GenElute™ PCR Clean-up Kit (Sigma, USA) following the manufacturer's instructions. The purified fragment was then sequenced twice, once from each direction using the forward and reverse primers, at both the Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA) and the Universidad Nacional Autónoma de México (UNAM). BLASTn and BLASTx tools were employed to compare the nucleotide and translated sequences, respectively. Sequences exhibiting the most significant homology in both nucleotide and amino acid sequences were selected for multiple sequence alignments using Mega 6.0, then conserved domains were identified.

For the analysis of the relationship between the *AaSERK* protein and known *SERK* protein sequences from six phylogenetically related species (selected based on identity percentage), a consensus and unrooted phylogenetic tree was constructed using the UPGMA method within the Mega program (version 6.06).

RESULTS

In this investigation, a fragment of the *Agave angustifolia* Somatic Embryogenesis Receptor-Like Kinase (*AaSERK*) gene was successfully amplified from DNA obtained from embryogenic calli. It should be noted that its amplification was not possible in non-embryogenic callus (Figure 2).

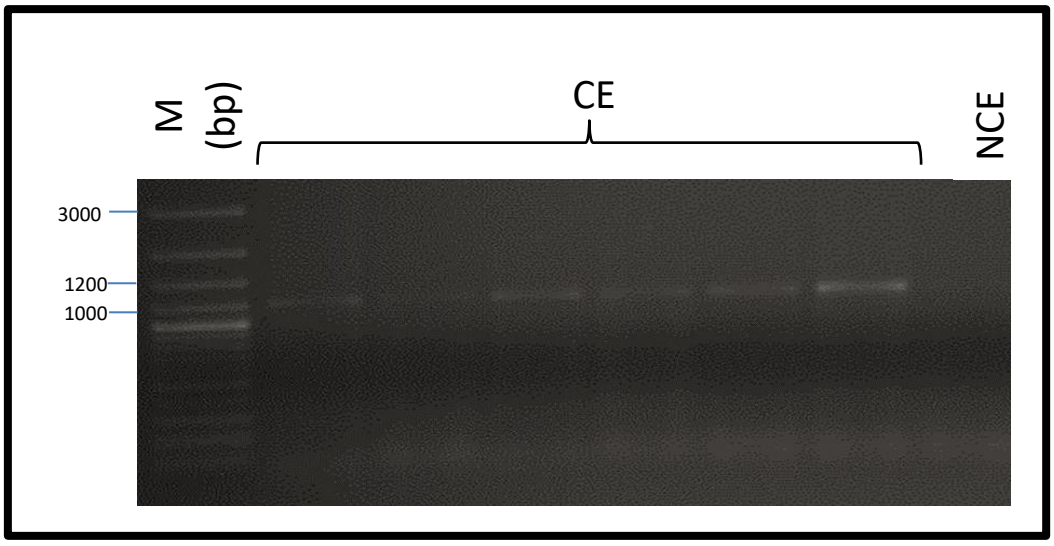

Figure 2. Amplification of *Agave angustifolia* Somatic Embryogenesis Receptor-Like Kinase gene from DNA of embryogenic (CE) and non-embryogenic calli (NCE): molecular weight marker (M), base pairs (bp).
Figura 2. Amplificación del gen Receptor similar a la Quinasa de la Embriogénesis Somática de *Agave angustifolia* a partir de ADN de callos embriogénicos (CE) y no embriogénicos (NCE): marcador de peso molecular (M), pares de bases (pb).

Sequencing of the fragment obtained indicates that *AaSERK* encompassed 968 nucleotides, which encodes a protein of 327 amino acids. This nucleotide sequence has been formally submitted to GenBank under the accession number KX247683.1 (available at https://www.ncbi.nlm.nih.gov/nuccore/1134518460). Furthermore, the amino acid sequence of *AaSERK* obtained in our study, with the accession number APX18295.1 (accessible at www.ncbi.nlm.gov/protein/1134518461), exhibited a noteworthy degree of identity with other *SERK* proteins, exceeding 80%. The highest similarity was observed with *ClSERK* from *Cyrtochilum loxense* (97% similarity, 619 amino acids, CBV98085.1), *DcSERK* from *Dendrobium catenatum* (97% similarity, 633 amino acids, AKN89445.1), *CmSERK* from

Cattleya maxima (89% similarity, 357 amino acids, CCD32850.1), *AcSERK* from *Ananas comosus* (85% similarity, 629 amino acids, AEC46975.1), *CnSERK* from *Cocos nucifera* (82% similarity, 629 amino acids, AAV58833.2), and *PdSERK* from *Phoenix dactylifera* (82% similarity, 621 amino acids, XP_008780820.1).

The confirmation of *AaSERK* identity was corroborated by the presence of characteristic domains commonly observed in *SERK* proteins from other species (as depicted in Figure 3a). Multiple reports suggest that the length of *SERK* proteins typically is considered within the range of 250 to 1650 amino acids. What unites these proteins is the presence of a distinctive domain known as SPP, which is notably absent in other Leucine-Rich Repeat Receptor-Like Kinases (LRR-RLKs) (De Oliveira Santos *et al*., 2005; Hecht *et al*., 2001; Koehler *et al*., 2020; Salaj *et al*., 2008; Santos *et al*., 2009; Schmidt *et al*., 1997). In this research, the SPP domain was successfully identified, which exhibited a high degree of identity in comparison to other *SERK* proteins found in phylogenetically related species to *Agave angustifolia* (as seen in Fig. 3b). Lastly, the transmembrane and kinase domains were found to be highly conserved due to structural constraints imposed by their catalytic requirements (Schwessinger & Rathjen, 2015).

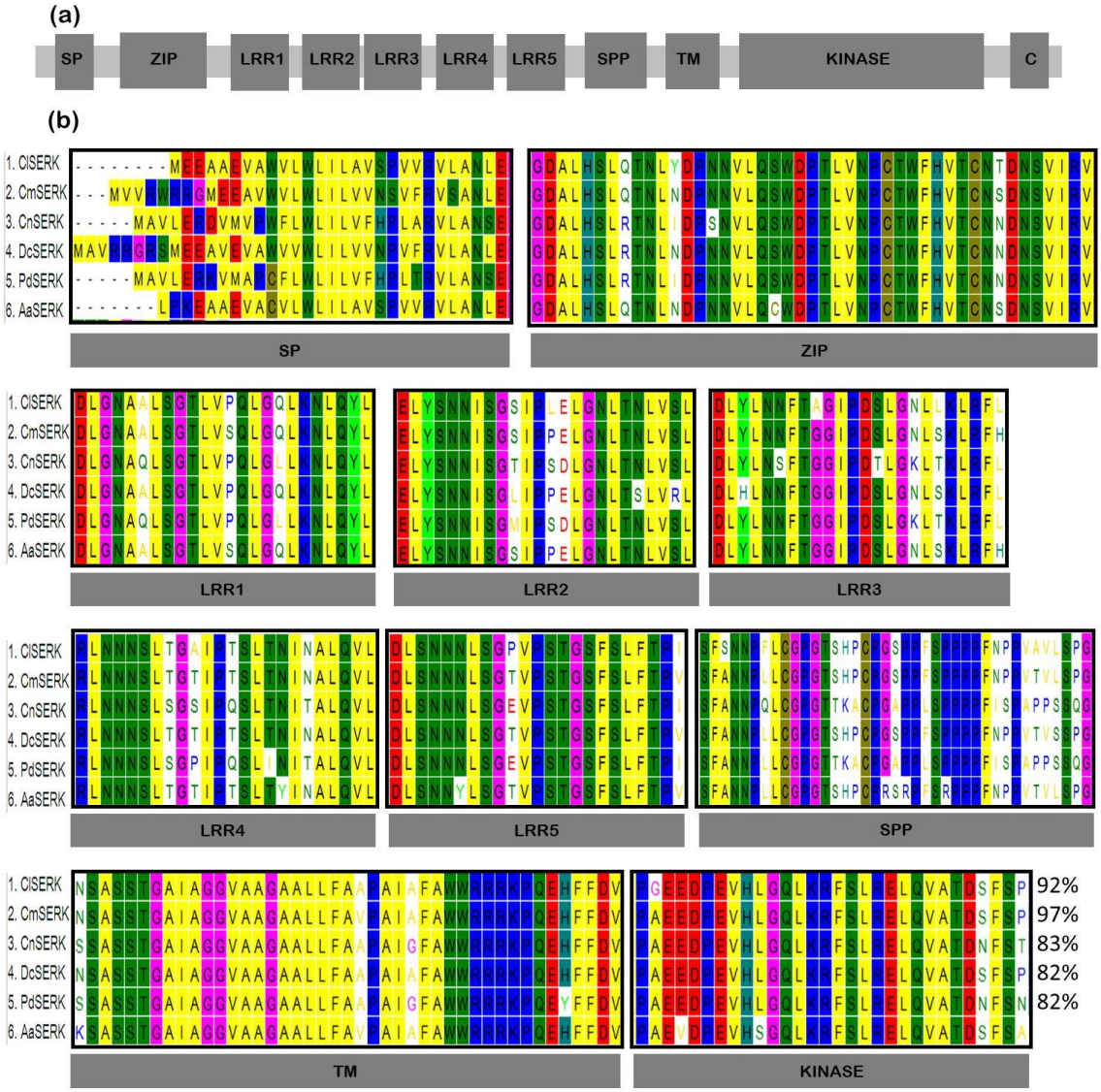

Figure 3. (a). Dibujo esquemático de los dominios típicos en un gen Receptor Similar a la Quinasa de la Embriogénesis Somática (SERK). SP Péptido señal, ZIP cremallera de leucina, LRR1 – LRR5 repeticiones ricas en leucina, SPP motivo serina-prolina-prolina, TM motivo transmembrana, C región C-terminal. (b). Estructura de la proteína SERK identificada de Agave angustifolia (AaSERK) y alineación de la secuencia de aminoácidos

predicha que indica el grado de similitud (%) con algunos miembros de las proteínas quinasas de la familia SERK: (1. *Cyrtochilum loxense* SERK, ClSERK, 97%; 2. Cattleya maxima SERK, CmSERK, 89%; 3. Cocos nucifera SERK, CnSERK, 82%; 4. Dendrobium catenatum SERK, DcSERK, 97%; 5. Phoenix dactylifera SERK, PdSERK, 82%; 6. AaSERK). Se destacan residuos de aminoácidos idénticos en todas las proteínas enumeradas.

Figura 3. (a). Dibujo esquemático de los dominios típicos en un gen de la Embriogénesis Somática Receptor Similar a la Quinasa (SERK). Péptido señal SP, cremallera de leucina ZIP, repeticiones ricas en leucina LRR1 – LRR5, motivo SPP serina-prolina-prolina, motivo transmembrana TM, región C C-terminal. (b). Estructura de la SERK identificada de Agave angustifolia (AaSERK) y alineación de la secuencia de aminoácidos predicha que indica el grado de similitud (%) con algunos miembros de las proteínas quinasas de la familia SERK: (1. *Cyrtochilum loxense* SERK, ClSERK, 97%; 2. Cattleya maxima SERK, CmSERK, 89%; 3. Cocos nucifera SERK CnSERK, 82%; 4. Dendrobium catenatum SERK, DcSERK, 97%; 5. Phoenix dactylifera SERK, PdSERK, 82%; 6. AaSERK). Se destacan residuos de aminoácidos idénticos en todas las proteínas enumeradas.

Our phylogenetic analysis revealed a clear grouping of *SERK* proteins in accordance with two taxonomic categories. The first cluster included monocot *SERK* proteins (*DcSERK*, *CmSERK*, *ClSERK*, *AaSERK*, *CnSERK* and *PdSERK*). In contrast, the second cluster included dicot *SERK* proteins (*AtSERK*) (as shown in Figure 4). Additionally, we observed the highest similarity of *AaSERK* with the predicted *DcSERK*, *CmSERK*, and *ClSERK*.

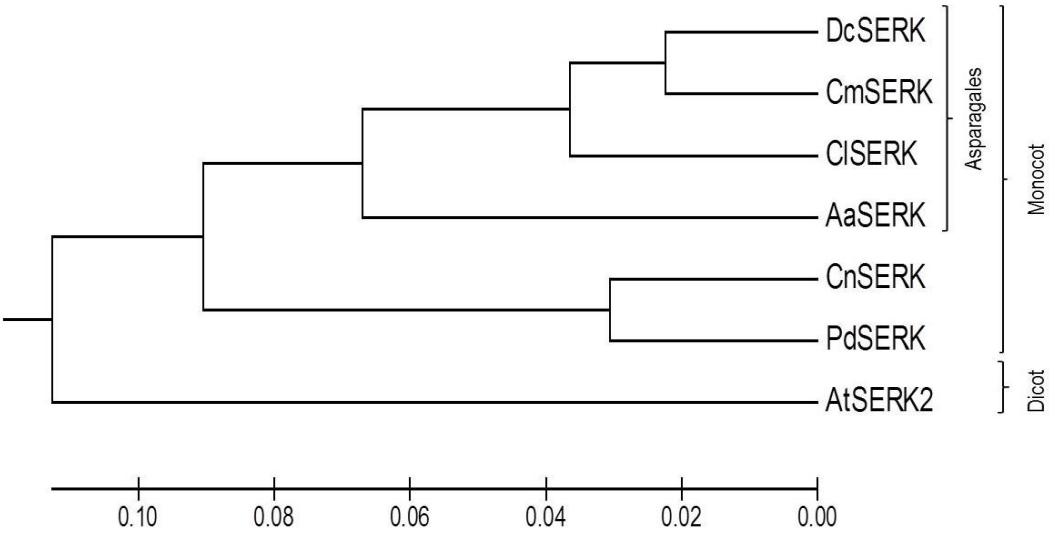

Figure 4. Phylogenetic tree of *AaSERK* with other reported *SERK* sequences depicting the interrelationship of different *SERK*: *Dendrobium catenatum SERK* (*DcSERK*), *Cattleya maxima SERK* (*CmSERK*), *Cyrtochilum loxense SERK* (*ClSERK*), *Agave angustifolia* (*AaSERK*), *Cocos nucifera SERK* (*CnSERK*), *Phoenix dactylifera SERK* (*PdSERK*) and *Arabidopsis thaliana SERK* (*AtSERK*).

Figura 4. Árbol filogenético de AaSERK con otras secuencias de SERK reportadas que representan la interrelación de diferentes SERK: *Dendrobium catenatum* SERK (DcSERK), *Cattleya maxima* SERK (CmSERK), *Cyrtochilum loxense* SERK (ClSERK), *Agave angustifolia* (AaSERK), *Cocos nucifera* SERK (CnSERK), *Phoenix dactylifera* SERK (PdSERK) y *Arabidopsis thaliana* SERK (AtSERK).

DISCUSSION

Somatic embryogenesis serves as the cornerstone of cellular totipotency in higher plants. In the controlled environment of *in vitro* conditions, a single or a few somatic cells within an explant must possess the competency to receive a developmental signal, whether endogenous or exogenous, that triggers the transformation toward the embryogenic pathway (Mikuła *et al*., 2022; Von Arnold *et al*., 2002). In the case of the *Agave* genus, somatic embryogenesis protocols have been established for various species, including *A. angustifolia* (Reyes-Díaz *et al*., 2017). Likewise, epigenetic mechanisms, such as DNA methylation and histone modifications in the micropropagation of *A. angustifolia* (De-la-Peña *et al*., 2012; Duarte-

Aké *et al.*, 2016) has been studied, but the absence of reports on the molecular mechanisms such those regulate the somatic embryogenesis process is still unclear.

Understanding the regulation of somatic embryogenesis during tissue and organ culture could significantly enhance the development of more efficient regeneration and transformation protocols. Schmidt et al. (1997) reported a correlation between the expression of the *SERK* gene and the potential of cells to exhibit embryogenic characteristics, especially in the presence of 2,4-D, a known promoter of totipotency. The genetic expression, such as key transcription factor, is crucial for triggering and regulating the processes involved in embryo formation from somatic cells (Kikuchi *et al.*, 2006). However, not all the samples express those genes at sufficient levels to initiate and complete the process of somatic embryogenesis (Méndez-Hernández *et al.*, 2019; Schwessinger & Rathjen, 2015), as it was observed in our assay in embryogenic and none embryogenic calli, respectively. From a genetic variability perspective, differences in the expression of these specific genes may be due to the presence of allelic variants, epigenetic modifications, or differences in transcriptional regulation (Pikaard & Mittelsten Scheid, 2014; Sivanesan *et al.*, 2022). Some cells may have the necessary genetic combination to activate these genes and trigger somatic embryo formation, while other cells may lack this combination or be subject to transcriptional repression (Fambrini *et al*., 2022). Additionally, the physiological mechanisms (endogenous production of acid abscisic) that regulate the cellular ability to form somatic embryos are closely related to the genetic activity (Kikuchi *et al.*, 2006). From this point, cells must be in a receptive physiological state, characterized by a high rate of cell division and plasticity, to respond to the activation of *SERK* genes related to somatic embryogenesis (Meira *et al*., 2024). Also, external factors such as nutrient availability, the presence of auxins in the culture medium, and environmental signals can also modulate the expression of these genes and thus influence in the cellular ability to initiate the process of somatic embryo formation (Long *et al*., 2022). *SERK* proteins play a pivotal role in regulating various facets of plant growth and development, encompassing the differentiation of somatic embryos. While the *SERK* gene family has been extensively scrutinized in well-established model plants like arabidopsis, rice, and maize, it remains comparatively less explored in non-model plant species (Baudino *et al*., 2001; Hecht *et al*., 2001; Singla *et al*., 2009). In the present study, a Somatic Embryogenesis Receptor-Like Kinase gene from *Agave angustifolia* (*AaSERK*) present only in embryogenic callus was isolated, characterized, and reported for the first time, similar expression was reported in carrot (Guzzo *et al*., 1994; Schmidt *et al*., 1997) and arabidopsis (Salaj *et al*., 2008). This pattern of *SERK* gene expression in cultured cells indicates its involvement in a signaling pathway responsible for orchestrating developmental modifications in response to specific culture conditions. These alterations include the activation of cell division and the reprogramming of cellular properties (Hu *et al*., 2005). It is believed that the level of expression of the *SERK* gene plays a fundamental role in determining cell fate, which can influence processes such as organogenesis or in this case somatic embryogenesis (Singla *et al*., 2009), thus underlining its importance in cell differentiation (Santos *et al*., 2009), as well as it is regarded as an indicator of cells with the ability to generate somatic embryos (Porras-Murillo *et al*., 2018).

This *AaSERK* gene encodes a Leucine-Repeat Receptor protein kinase and exhibits similarities to other *SERK* (Baudino *et al*., 2001; Cueva *et al*., 2012), suggesting that it functions as a Leucine-Rich Repeat Receptor-Like Kinase (LRR-RLK). In the model for RLK function, plant RLKs typically exist as monomers until the binding of an extracellular signal molecule induces receptor dimerization (Becraft, 2002). This brings the intracellular kinase domains of individual monomers into proximity, allowing for transphosphorylation, activation of kinase domains, and the regulation of cellular responses (Becraft, 1998; Maulidiya *et al*., 2020; L. Zhao *et al*., 2020).

LRR-RLKs are essential in regulating various physiological processes, including embryo pattern formation, microsporogenesis, vascular tissue differentiation, maintenance of meristematic cells, organ morphology, inflorescence structure, brassinosteroid (BR) signaling, and cellular death control (Cueva *et al*., 2012; De Oliveira Santos *et al*., 2005; Hecht *et al*., 2001; Koehler *et al*., 2020; Santos *et al*., 2009).

Structural and sequence analyses (as shown in Figure 3b) reveal that *AaSERK* encodes a typical *SERK* protein with conserved roles in plant development, displaying a high degree of sequence and protein domain homology with other *SERK* proteins. The characteristic distribution of *SERK* protein domains, including a signal peptide; a ZIP domain known to play a role in protein oligomerization (Shah *et al*., 2001); five LRR units that are exposed at the cell membrane, and these are presumed to be responsible for interactions with other signaling molecules, such as brassinosteroid receptors, as well as for the perception of external signals (auto- and transphosphorylation events between respective intracellular kinase domains), and signal transduction during somatic embryogenesis induction (Kobe & Deisenhofer, 1994; Schwessinger & Rathjen, 2015); a Pro-rich domain containing the SPP motif, a transmembrane domain, and a Serine - Threonine protein kinase, is also evident in *AaSERK*.

Phylogenetic analysis (as seen in Figure 4) indicates that *AaSERK* shares a close relationship with *SERK* sequences from other plant species, particularly with monocot SERKs belonging to the order Asparagales, such as *DcSERK*, *CmSERK*, and *ClSERK*. This could suggest a common evolutionary origin of *SERK* among these plants.

CONCLUSIONS

It can be inferred that *AaSERK* plays a crucial role in mediating somatic embryogenesis, where cells with embryogenic competence respond to biochemical signals triggered by the presence of auxins in a culture medium and transform into somatic embryos. The expression of *SERK* genes has been shown to be associated with various stages of embryogenesis in other plant species, further supporting this hypothesis.

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