Research Article

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Cloning and expression analysis of SERK1 gene in Diospyros lotus

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Abstract: Somatic embryogenesis receptor-like kinases (SERKs), a subfamily of receptor-like kinases, play important roles in response to abiotic stresses in addition to apomictic reproductive development in numerous plant species. The purpose of the present work was to determine if an ortholog of the SERK gene is present in the Diospyros lotus genome, isolate it and analyze its expression during embryogeny and abiotic stress. An ortholog of the SERK gene was isolated from the D. lotus genome, and designated as DlSERK1. The physical and chemical properties, protein structure, and evolutionary relationship of the DlSERK1 protein were analyzed by bioinformatics methods, and the expression of DlSERK1 gene during embryonic development and under low-temperature, salt, and drought stresses was examined through real-time quantitative PCR analysis. DlSERK1 contained 1,881 bp open reading frame encoding 626 amino acids, with a molecular mass of 69.18 kDa and pI of 5.34. DlSERK1 had strong hydrophilic property, signal peptide cleavage sites, and two transmembrane regions, indicating that DlSERK1 is a secretory protein. The secondary structure of DlSERK1 was consistent with the tertiary structure, both of which were dominated by random curls and alpha-helices. DlSERK1 had the typical structure of SERK proteins, and harbored multiple phosphorylation and glycosylation sites. Quantitative analysis showed that DlSERK1 was expressed during the embryonic development period, and the highest expression level was at 10 days post-flowering. The DISERK1 expression level was down-regulated under low-temperature stress and up-regulated under drought and salt stresses. Our study showed that DlSERK1 was expressed in embryo development and could respond to low-temperature, drought, and salt stresses, which lays a foundation for further research on the function of SERK1 in the apomixis growth and development of environmental adaptation in D. lotus.

Keywords: Diospyros lotus, SERK, bioinformatics analysis, gene expression

1 Introduction

Apomictic reproduction in plants is a kind of asexual reproduction in which the megaspore mother cell or nucellar cell directly develops into embryos and then forms seeds without the meiosis and double fertilization of sexual reproduction [1]. Apomictic progenies produced by apomixis inherit the same traits as their female parent without character separation, which speeds up the fixation of heterosis [2,3]. Apomictic reproduction could also be applied to cultivate high-quality rootstocks and establish a stable and efficient somatic embryo regeneration and genetic transformation system in vitro [4,5]. Therefore, studying apomictic reproductive characteristics in fruit breeding and production is of great practical value. Apomixis is not only affected by environmental conditions but regulated by multiple genes. At present, many genes related to apomictic reproductive development have been identified, such as SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK), BABY BOOM (BBM), LEAFY COTYLEDON 2 (LEC2), AGAMOUS-LIKE 15 (AGL15), WUSCHEL (WUS), and FUSCA3. SERK belongs to the leucine-rich repeat sequence receptor-like kinase (LRR-RLK) family [6], widely distributed in plants. Schmidt et al. [7] discovered the first SERK gene (DsSERK) in the hypocotyl of carrot. Subsequently, SERK genes were isolated from Arabidopsis thaliana [8], rice (Oryza sativa) [9], maize (Zea mays) [10], barley (Hordeum vulgare) [11], wheat (Triticum aestivum) [12], grape (Vitis vinifera) [13], and apple
(Malus hupehensis) [14], and were shown to play an important role in somatic embryogenesis. In general, the SERK gene consists of 11 exons and 10 introns, and the exons almost correspond to the functional domain of the coding protein, including the leucine-zipper (ZIP), leucine-rich repeat sequence (LRR), proline-rich (SPP), transmembrane (TM), and kinase domains, as well as the N- and C-terminal regions [15,16], which participate in important biological functions [17].

The involvement of the SERK gene in sporophyte development has been reported in A. thaliana. For example, AtSERK1 and AtSERK2 are expressed in both vegetative and reproductive tissues of A. thaliana, but their expression levels are relatively high in flowers and fruit pods, indicating that they may be involved in controlling sporophyte differentiation and thus affecting the development of male gametophytes [18]. At the same time, SERKs may function as coreceptors of GSO1/2 to transduce the TWS1 signal and ultimately regulate embryonic cuticle integrity [19]. SERKs play a critical role in regulating zygotic embryo development through controlling the division patterns of vascular precursors and ground tissue stem cells. [20] AtSERK1 is only expressed at the heart-stage during embryo development, which is similar to the expression patterns of DgSERK and DcSERK [8,21]. In addition, SERKs (SERK1 and SERK2) are also involved in microspore embryogenesis in Brassica napus L., and the expression level of BnSERK1 is significantly up-regulated within 1–5 days after microspore-derived embryogenesis, whereas the BnSERK2 expression is increased throughout microspore-derived embryogenesis [22]. It has been reported that expression of genes of the SERK family in maize are associated with embryogenesis induction [10]. Therefore, SERK genes play an important role in microspore development and reproductive development. However, SERKs also show differentiated functions, they play crucial roles in many biological processes such as brassinosteroids (BR) signaling, anther development, stomatal patterning, floral abscission, immune responses, hormone signal transduction, disease defense, and abiotic stress response [20,23–32]. For instance, AtSERK3-5 are involved in the regulation of BR signaling, affecting the growth and development of A. thaliana [29,30]. In rice, although OsSERK1 and OsSERK2 are expressed in all tissues, OsSERK1 is mainly expressed in flowers and stems, while OsSERK2 is mainly expressed in leaves. The expression of OsSERK1-2 can also be activated by pathogens, host dead cells, defense signaling molecules (such as salicylic acid), and other stress signals, modulating the immune signaling pathways [9,31]. Under salt stress, the expression of HvSERK1 and HvSERK3 in barley leaves was up-regulated at 12 h, and HvSERK1 remained at a high level until 24 h, while the HvSERK3 expression was decreased to a normal level at 24 h, suggesting that HvSERK1 and HvSERK3 may be involved in the salt tolerance pathway of barley leaves [11].

Diospyros lotus is a dioecious plant. We found that some females of D. lotus have apomixes characteristics [33], this has great application potential for D. lotus breeding research. But the type of apomixes in D. lotus is not clear enough, in particular, the regulatory mechanism of apomixes is unclear. SERK genes have been isolated and identified in many plants, but there are less reports on SERK genes in D. lotus. Lijie et al. [14] found that the SERK1 gene was highly expressed in the ovary at the bud stage of M. hupehensis var. pingyiensis Jiang, while CitSERK1 and CitSERK1-LIKE genes were highly expressed in the somatic embryo induction process of citrus [34,35]. In view of the important role of SERKs and their homologous genes in sporophyte development, reproductive development, and related resistance in higher plants, we cloned SERK homologous genes from D. lotus, and analyzed their expression patterns under somatic embryogenesis, low temperature, drought, and salt stress by reverse transcription quantitative PCR (RT-qPCR). At the same time, the results obtained from bioinformatics analysis lay a foundation for further research on the function of SERK genes and provide theoretical support for the study of the molecular mechanism of SERK genes in the growth and development process and environmental adaptation of D. lotus.

2 Materials and methods

2.1 Plant material

The samples were collected from the perennial D. lotus L. tree grown at the test base of Henan Institute of Science and Technology.

2.2 Sampling during apomictic reproductive development

When D. lotus flowers were not open, the petals changed from green to yellow, the petals surrounding the stigmas were gently removed. Polyvinyl acetate emulsion adhesive was applied evenly on the stigma to completely wrap the stigma [33]. To ensure that pollen did not enter the stigma, each stigma was treated three times at an interval of 12–24 h. The samples were collected 1 day after treatment (as the control). Afterward, samples were taken once every 3 days until white embryos were formed in
the fruit of *D. lotus*. The samples were immediately frozen in liquid nitrogen, brought back to the laboratory, and stored at −80°C.

### 2.3 Abiotic stress treatment

Sufficient seeds with full grain and uniform size were sown in a nutrient bowl. The treatment was performed on seedlings with three or four leaves. Low-temperature treatment: *D. lotus* seedlings with the same growth status were placed in an incubator at 4°C, under continuous light supply and normal watering. Drought treatment: root irrigation was applied to robust *D. lotus* seedlings with same sizes with 20% PEG 6000 until saturation. The third leaf from bottom to top was collected at 0 h (CK), 6 h, 12 h, 1, 3, 5, and 7 days after abiotic treatment, frozen immediately in liquid nitrogen, and stored at −80°C until further use. Three biological replicates were conducted for each treatment. The relative electrical conductivity (REC) of the leaf at each time point was measured by a DDS-307 electrical conductivity meter, and the electrode constant was 0.990. According to the method of Bolat with some modifications [36], the conductivity of sample solution was \( R_4 \), and that of deionized water partake solution was \( R_0 \) before boiling. After boiling and cooling, the conductivity of sample solution was \( R_2 \), and that of deionized water counterpart solution was \( R_0 \). Then, \( \text{REC} \% = \frac{(R_1 - R_0)}{(R_2 - R_0)} \times 100\% \). Three biological replicates were selected at each time point of each treatment.

Total RNA was extracted using the OminiPlant RNA Kit (DNasel, CW25985; Kangwei Century Biotechnology Co., Ltd) following the manufacturer’s instructions. cDNA was synthesized by the RevertAid First Strand cDNA Synthesis Kit (#K1622; Thermo Scientific) and stored at −20°C for later use.

### 2.4 Screening and cloning of SERK in *D. lotus*

The SERK gene sequence was screened from the genome database of *Diospyros oleifera* “Youshi,” and specific primers to amplify the full length of the SERK gene were designed (upstream primer: 5′-ATGGAAAGATTGTGGTGTG GTGA-3′; downstream primer: 5′-TACCTGGGCCTGTAT AACT-3′). PCR amplification was carried out using cDNA from the leaves of the explants as the template by the homologous cloning method. The PCR reaction system was 20 μL, containing 1 μL of cDNA, 0.5 μL of each primer (10 μM), 10 μL of 2× Es TaqMster Mix (Dye), and 8 μL of ddH2O. The PCR reaction conditions were pre-denaturation at 94°C for 4 min; 94°C for 30 s, 56°C for 30 s, 72°C for 90 s, 35 cycles; extension at 72°C for 5 min. The target fragment was separated by 1.0% agarose gel electrophoresis and recovered by the Agarose gel DNA Recovery Kit. After the pMD18-T vector (TaKaRa) was connected, the plasmid was transformed into *Escherichia coli* DH5α strain by heat shock (laboratory preservation), and positive clones were identified with sequencing conducted by Sangong Biotech Co., Ltd (Shanghai, China). According to the sequencing results, the recovered fragment was confirmed as the SERK gene based on multiple characteristics of protein and gene structure and was named *DISERK1*.

### 2.5 Bioinformatics analysis of *DISERK1*

The online software BLASTP was used for gene sequence search and analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The full-length open reading frame (ORF) was predicted by the online tool ORF-finder (http://www.ncbi.nlm.nih.gov/orf/ORF.html). The physicochemical properties and hydrophilicity of the encoded *DISERK1* protein were predicted by ProtParam (http://web.expasy.org/protparam/) and ExPaSy (http://web.expasy.org/protscale/). SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) and SWISS-MODEL online tools were used to analyze the secondary structure and three-dimensional structure composition of the *DISERK1* protein. TM helices were analyzed using the TMHMM server (http://www.cbs.dtu.dk/services/TMHMM/). The subcellular localization of *DISERK* was predicted using PlantmPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/) and PredictProtein (https://predictprotein.org/). Signal peptides were predicted by SignalP v5.0. The Netphos v3.1 server, NetOGlyc v4.0, and NetNGlyc v1.0 software were used to predict phosphorylation and glycosylation sites in *DISERK*. The MotifScan program (http://myhits.isb-sib.ch/cgi-bin/motif_scan) was used to analyze the conserved motifs. Sequence alignment was performed using DNAMAN v9.0. Phylogenetic trees were prepared with MEGA v5.0 software using the neighbor-joining (NJ) method. The 2,000-bp sequence upstream of *DISERK1* was regarded as the promoter region and extracted from the *D. lotus* genome database (http://persimmon.kazusa.or.jp/blast.html) by TBtools and submitted to the Plant CARE database for identifying the cis-acting elements.
2.6 RT-qPCR

The fruits which were capped by white latex for 1, 4, 7, 13, 16, 19, and 22 days and leaves treated with low temperature, drought, and salt stress were used as test materials. RNA was extracted, reverse transcribed to synthesize cDNA, and real-time quantitative PCR was performed. The RT-qPCR was performed on a CFX96 instrument (Bio-Rad), and the PCR reaction was prepared according to the instruction of the SYBR Green qPCR Kit (TaKaRa). Primer sequences were: SERKrt1F, TGCCATCTGAACCACC ACTC and SERKrt1R, ACGGCTGAATGACGTGTGT. The 10-μL PCR reaction system contained 5.0 μL of SYBR Premix Ex Taq II enzyme, 0.7 μL of cDNA, 0.4 μL of each primer (10 μM), and 3.5 μL of ddH2O. The amplification parameters were as follows: 95°C pre-denaturation for 30 s, 1 cycle; 95°C denaturation for 5 s, 56°C renaturation for 30 s, 40 cycles. The comparative threshold 2−ΔΔCT method was applied to quantify the relative expression of target genes [37]. Four independent assays were carried out.

3 Results

3.1 Cloning of the SERK1 gene in D. lotus

cDNA of D. lotus leaves was used as the template for PCR amplification. After cloning and sequencing, the ORF length of DISERK1 sequence was 1,881 bp (Figure 1). Comparison of nucleotide sequence length showed that the DISERK1 gene was 72 bp longer than SERK of D. oleifera, and these two sequences length shared 96.17% similarity (Figure 2). Moreover, compared with the homologous SERK sequences in other plants, this fragment showed 100% similarity with tetraploid hybrid offspring of Malus, 100% similarity with PpSERK2, and 98.90% similarity with SERK1 of D. officinale, indicating that the full-length cDNA sequence of DISERK1 was cloned.

3.2 Bioinformatics analysis of DISERK1

3.2.1 Homology of DISERK1 protein

Through BLASTP searches against the NCBI database, the protein sequence encoded by DISERK1 showed high similarity with SERKs of other species. The similarities between the DISERK1 protein with SERKs of Sesamum indicum (XP_011074139.1), Populus euphratica (XP_011002514.1), V. vinifera (XP_002270847.1), Carica papaya (ABS32233.1), and Solanum lycopersicum (NP_001233866.1) were 93.25, 92.27, 91.94, 91.56, and 89.94%, respectively, indicating that the cloned DISERK1 gene was homologous with SERKs.

3.2.2 Physicochemical properties of DISERK1

The ORF of DISERK1 was 1,881 bp in length and encoded a protein of 626 amino acids that had a calculated molecular mass of 69.18 kDa and a predicted pl of 5.34. The molecular formula of the DISERK1 protein was C_{3109}H_{4893}N_{839}O_{907}S_{20}, its lipid solubility index was 101.37, and its instability coefficient was less than 40 (39.52), belonging to the stable protein. Among the 20 amino acid residues in DISERK1, leucine was the most (15.5%), followed by glycine (7.8%), while cysteine (1.3%) was the least. The total number of negatively charged residues (Asp + Glu) was 74 and that of positively charged residues (Arg + Lys) was 56. The total average hydrophilic coefficient of DISERK1 was −0.098, suggesting that DISERK1 is a hydrophilic protein.

3.2.3 Hydrophilic of DISERK1 protein

Analysis by ExPASy software showed that there were several strong hydrophilic regions in the N-terminal, C-terminal, and intermediate regions of DISERK1 (Figure 3). The site with the strongest hydrophilicity was found at amino acid residue 266 (Lys), with a value of −2.733. The most hydrophobic site was found to be residue 14 (Leu), with a value of 2.867.
3.2.4 Secondary and tertiary structures of DlSERK1 protein

The secondary structure prediction of DlSERK1 (Figure 4) revealed that it was mainly composed of 39.30% alpha-helices (blue line), 4.31% beta-turns (green line), 42.65% random coils (purple line), and 13.74% extended strands (red line). Through homology modeling, it was found that the tertiary structure of DlSERK1 (Figure 5) had the most similarity with the 3tl8.2.A model, and the tertiary structure was dominated by random coils and alpha-helices, which is consistent with the prediction results of secondary structure.

3.2.5 Subcellular localization, TM structure, and signal peptide prediction of DlSERK1

DlSERK1 was predicted to be localized to the cell membrane considering the results from both plant-MPLoc and Predict Protein. TM prediction results (Figure 6) showed that DlSERK1 contained two TM domains, i.e., TM helices at amino acid residues 4–26 and 240–262, respectively. Therefore, DlSERK1 was presumed to be a TM protein. The DlSERK1 protein may contain a signal peptide (Sec/SPI), and the cleavage site was between residues 26 and 27, with a probability of 0.98 (CS > 0.5), indicating that the protein belongs to the secretory protein.

3.2.6 Prediction of phosphorylation and glycosylation sites of DlSERK1 protein

The phosphorylation sites of DlSERK1 were predicted by the NetPhos v3.1 server (Figure 7). The results showed...
that there were 43 phosphorylation sites in *DlSERK1*, including 27 serine (Ser) sites, 12 threonine (Thr) sites, and four tyrosine (Tyr) sites. NetOGlyc v4.0 and NetOGlyc v1.0 were used to predict the O-terminal and N-terminal glycosylation sites in *DlSERK1*. Three O-terminal glycosylation sites were located at residues 394 and 607, respectively, and five N-terminal glycosylation sites were located at residues 115, 150, 163, 184, and 381.

### 3.2.7 Conserved motif prediction of *DlSERK1* protein

Analysis based on MotifScan showed that *DlSERK1* contained several typical conserved domains of SERK proteins, including an N-terminal region LRRNT_2 (between residues 26 and 66), a leucine zipper (between residues 33 and 54), four leucine repeats LRR_1 (between residues 94 and 116, 118 and 140, 142 and 164, and 166 and 189), a proline-rich domain with the Ser-Pro-Pro (SPP) motif, a TM domain (between residues 204 and 231), a TM domain (between residues 240 and 262), and a protein kinase active domain comprising 11 sub-domains (between residues 302 and 589). Additionally, a protein kinase ATP-binding site (between residues 308 and 330) and a serine/threonine kinase activation site (between residues 425 and 437) were observed.

### 3.2.8 Multiple alignment of amino acid sequences and construction of phylogenetic tree

The protein sequences of SERKs from other plant species were downloaded from NCBI, including *D. oleifera* Cheng (AKN89445.1), *S. indicum* (XP_011074139.1), *P. euphratica* (XP_011002514.1), *V. vinifera* (XP_002270847.1), *C. papaya* (ABS32233.1), *Prunus persica* (XP_007201734.1), *Theobroma caca* (XP_007020220.1), *Prunus mume* (XP_008245841.1),...
Figure 7: Predicted phosphorylation sites of DISERK1.

Figure 8: Multiple sequence alignment of amino acid sequences of DISERK1 and SERKs in other plants. Conserved sequence characteristics of SERK are indicated with colored underlines (red, signal peptide; orange, leucine zipper; yellow, leucine repeat sequence; green, serine-proline-rich domain; blue, TM domain; and purple, kinase domain).
Nicotiana tabacum (XP_016455663.1), and A. thaliana (ACN59271.1 and CAF33246.1). Multiple sequence alignment was performed by DNAMAN. The results showed that the similarity among these sequences was 90.81%. Among them, DlSERK1 had the highest similarity with D. oleifera SERK2 (94.47%), followed by SISERK2 (93.25%). Analysis results showed that these sequences had typical structural and functional domains of SERK proteins (Figure 8). This confirms that DlSERK1 is a member of the SERK family and belongs to SERK/LRR-RLK genes.

To explore the evolutionary relationship between DlSERK1 and SERKs from other species, a phylogenetic tree was constructed by MEGA v5.0 based on the NJ method (Figure 9). It was found that DlSERK1 was closest to DolSERK2; the two were clustered in a small clade with VvSERK1 first, and then clustered with SISERK2, NtSERK, SISERK1, and SERKs of other dicotyledons. SERK sequences were clustered in different clades of monocotyledonous plants including Brachypodium distachyon and rice, and were relatively distant from each other. The results of the phylogenetic tree were consistent with the homology comparison results. Among dicotyledons, SERKs of peach, plum, and apple of Rosaceae belonged to one group, and those of tobacco, tomato, and potato of Solanaceae belonged to another group. While AtSERK1 and AtSERK2 were clustered to one branch, AtSERK3, AtSERK4, and AtSERK5 were in separate clades, indicating that different SERK proteins in the same plant species can also be divergent.

3.2.9 Analysis of regulatory elements in the DlSERK1 promoter

The 2,000 bp nucleotide sequence upstream of the DlSERK1 gene was analyzed by Plant CARE to obtain the regulatory elements (Table 1). The prediction results showed that this
sequence contained not only a large number of the core sequence (TATA-box) in the promoter, the CAAT-box in the promoter and enhancer regions, and other basic promoter elements across eukaryotes, but also included nine light-responsive elements (G-box, GATA-motif, TCT-motif, and AE-box), 12 hormone response-related cis-elements (TGACG-motif, CGTCA-motif, ABRE, and P-box), two stress response-related cis-elements (ARE and MBS), and two responsive elements involved in the regulation of corn protein metabolism (O2-site).

### 3.3 Changes of REC of leaves under abiotic stress

In order to calibrate the degree of cell damage under low temperature, drought, and salt stress at different times, and to determine the rationality of the sampling time of gene expression in the later period, we measured the REC. With the extension of the treatment time, the REC of leaves increased in varying degrees compared with CK (0 h), indicating that the cell membrane was damaged to varying degrees (Figure 10). The REC of leaves increased linearly within 12 h under 4°C and peaked (11.76%) at 12 h, which was increased by 4.96% compared with CK. Although a downward trend was observed after 12 h, the REC was always higher than that of CK. The REC showed a gradual downward trend within 12 h of drought treatment with 20% PEG 6000. At 12 h, the REC of leaves was the lowest (5.74%); it then rose until the maximum value appeared at 5 days (12.32%), which was increased by 4.17% compared to that of CK and 2.15 times relative to that at 12 h. Within 7 days of 250 mM NaCl treatment, the REC first showed an upward trend within 6 h, then decreased to the CK level at 12 h, and then gradually increased. At 5 days, the REC reached a maximum value (9.74%), which was increased by 2.08% compared to CK. The results showed that low-temperature, drought, and salt treatment increased the REC of seedling leaves, and the membrane was damaged under abiotic stress. The damage intensity of the cell membrane under different stresses was in an order of 4°C > 20% PEG 6000 > 250 mM NaCl.

### 3.4 Expression analysis of DISERK1

The expression of DISERK1 during the ovaries of different flower development stages in D. lotus was analyzed by RT-qPCR (Figure 11). The results showed that DISERK1 was expressed during the embryonic development period. At 1–3 days post-treatment (DPT), some flowers bloomed, roughly in bud stage. At 4–7 DPT, most flowers bloomed, which was the full flowering stage. About a week after flowering, the stigma turned brown, and the petals turned yellow.

**Table 1: Cis-acting elements of the DISERK1 gene**

| Type of cis-acting element        | Associated element | Number | Function of response                      |
|----------------------------------|--------------------|--------|------------------------------------------|
| Light response-related element   | G-box              | Five   | Light-responsive element                 |
|                                  | GATA-motif         | Two    | Light-responsive element                 |
|                                  | TCT-motif          | One    | Light-responsive element                 |
|                                  | AE-box             | One    | Light-responsive element                 |
| Hormone response-related cis-element | TGACG-motif       | Four   | MeJA responsiveness                      |
|                                  | CGTCA-motif        | Four   | MeJA responsiveness                      |
|                                  | ABRE               | Three  | Abscisic acid responsiveness             |
|                                  | P-box              | One    | Gibberellin-responsive element           |
| Stress response-related cis-element | ARE                | One    | Anaerobic-induction element              |
|                                  | MBS                | One    | MYB binding site involved in drought inducibility |
| Growth-related cis-element       | O2-site            | Two    | Zein metabolism regulation               |

![Figure 10](image-url) Relationship between treatment time and REC under different stress treatments. Different lowercase letters indicate significant differences at \( P < 0.05 \).
gradually withered, which was the late flowering stage. The highest DISERK1 expression in ovary was observed at 10 days of flowering, which was 1.63 times higher than that at Day 1 of flowering. It was speculated that DISERK1 may promote the development of somatic embryos.

The expression patterns of DISERK1 under various abiotic stresses (4°C, PEG, and NaCl) were studied. DISERK1 was expressed in leaves at low temperature for 7 days, and the expression was almost unchanged after 6 h of treatment. The DISERK1 expression decreased rapidly after 12 h of treatment, which was less than half of the CK, and remained at a low level until 7 DPT. Under PEG treatment, the expression of DISERK1 was up-regulated in leaves, and the peak expression appeared at 5 DPT, which was about 2.73 times that of the CK. Under the condition of high-salt treatment, the expression of DISERK1 in leaves increased first and then decreased. The expression increased significantly within 3 days of treatment, and peaked at 3 DPT, which was about 1.9 times that of CK. However, at 5 DPT, the DISERK1 expression decreased to the lowest level, and then returned to the normal level at 7 DPT.

4 Discussion

In this study, the full-length cDNA of DISERK1 (1,881 bp), encoding 626 amino acids, was cloned from the D. lotus leaf. Bioinformatics analysis showed that the DISERK protein encoded by this gene had high similarities with SERK proteins of other plants. DISERK also contained common conserved structural domains of SERK proteins, including a signal peptide, leucine zipper structure, proline-rich structure, TM structure, leucine-repeat sequence, and intracellular protein kinase activity domain, which were the typical structural characteristics of the SERK protein family. Therefore, it was speculated that DISERK was a member of the SERK protein family and was thus named DISERK1. DISERK1 had high similarities to other plant SERK proteins in amino acid composition, molecular weight, and pl. For example, DISERK1, AtSERK1, PpSERK2, MtSERK1, OsSERK1, and CitSERK1 proteins were composed of 626, 625, 626, 627, 628, and 621 amino acids, respectively; their molecular weights were 69.18, 69, 68.99, 69.125, 69.5, and 68.4 kDa, respectively; and their pls were 5.34, 5.25, 5.38, 5.56, 5.98, and 5.48, respectively [9,18,34,38].

The results showed that SERK proteins of different plants had similar primary structure and physicochemical properties, thus it was speculated that they play similar roles in the process of plant growth and development. The secondary and tertiary structures were consistent with the prediction results, which revealed mainly random coils and alpha-helices. There was a cleavage site of signal peptide between residues 26 and 27. It was speculated that DISERK1 is a secretory protein with a TM structure located on the cell membrane, and harbors multiple phosphorylation and glycosylation sites, which is consistent with the function of

Figure 11: DISERK1 gene expression analysis during embryogenesis and abiotic stress.
SERK proteins in signal transduction. DISERK1 had the highest similarity with DoSERK2, was close to SERKs of dicotyledonous plants such as sesame, tobacco, and tomato, and far from SERKs of monocotyledonous plants such as rice and B. distachyon.

The expression level of DISERK1 was the highest at 10 days after flowering, which was the late flowering stage of D. lotus. The fruit began to expand about half a month after flowering, and the DISERK1 expression returned to the normal level. According to the DISERK1 expression at different stages of ovary development, it can be inferred that DISERK1 may play a certain regulatory role in embryogenesis in D. lotus. This is similar to the results of D. officinale [39], citrus [34], and apple [14]. DoSERK2 was widely expressed at all stages of protocorm development, and the expression was the highest at the stage of seed embryo activation. The expression of DoSERK2 was low and showed little difference from protocorm formation to the seedling stage. The expression signal of CitSERK1 could be detected in flowers and fruits at 30 and 60 days after flowering, but not in fruit at 180 days after flowering. The expression levels of MhsERK1 and MhdSERK1 were the highest in the ovary of M. hupehensis and in the ovary of the hybrid progeny, respectively. The expression of SERK1 was the highest in the ovary of M. hupehensis at the bud stage, while the expression was the highest in the ovary of hybrid progeny at the flowering stage, which may be the main reason for the decline in apomixis ability of hybrid progeny.

A large number of studies have shown that SERK proteins play an important role in the regulation of abiotic stress. Ma et al. found that SERK1 could be induced by low-temperature treatment at 4°C for 24 h in non-embryogenic callus of pineapple [40]. Xue et al. found that low-temperature accumulation could induce the PsSERK2 expression in Paeonia suffruticosa “Luhehong,” and PsSERK2 plays a positive regulatory role in sleep release [41]. In this study, we found that the expression level of DISERK1 decreased significantly with the increase of low-temperature treatment time (low temperature for 12 h) by RT-qPCR technique. DISERK1 plays a negative regulatory role in low temperature stress. Many gene families play different roles in different plants, such as the MYB gene family has positive and negative regulatory functions [42,43]. Most of the MYBs involved in the control of flavonoid biosynthesis are positive regulators that enhance the expression of the structural flavonoid pathway genes [2]. However, repressors have also been characterized, such as FaMYB1 in strawberry (Fragaria x ananassa Duch.) and VvMYB4 in the berries of grapevine [44,45]. The expression level of DISERK1 was up-regulated at 5 DPT during drought stress, which was similar to that of BnaA07g23390D and BnaA07g29610D in B. napus under drought treatment, with the BnaA07g23390D expression being up-regulated at 1 h of treatment and the BnaA07g29610D expression being up-regulated at 0.5 h [46]. The expression of DISERK1 was up-regulated after salt treatment, similar to GmSERK1, HvSERK1, HvSERK3, MdSERK4, MdSERK10, BnaC01g43240D, and BnaC01g07810D [32,47–49]. The results indicate that DISERK1 can respond to low-temperature, drought, and salt stress signals. Plant CARE analysis showed that DISERK1 may be involved in the light-response process, response to the signal regulation of hormones such as MeJA and abscisic acid, and protein metabolism. This indicates that the regulation of gene expression is extremely complex, and DISERK1 may have multiple functions and participate in various biological processes.

5 Conclusion

In summary, the cloning of SERK has laid a foundation for studying the role of this gene in the development of embryogenesis in D. lotus, and it is of great significance for the in-depth study of the molecular mechanism of embryonic development and the function of environmental adaptability in D. lotus.

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