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Genome wide identification of SCL transcription factors in potato (*Solanum tuberosum* L.) and expression analyses under phytohormones, drought and salt stress

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Abstract

Background Abiotic stresses, such as drought and salinity, severely limit the yield and quality of potato. As a pivotal subfamily of GRAS transcription factors, SCARECROW-like (SCL) proteins play essential roles in phytohormone signaling and stress adaptation; however, their systematic characterization and functional mechanisms in potato remain largely unexplored.

Results In this study, we conducted a genome-wide identification of the SCL family, identifying 71 StSCL genes in potato. Phylogenetic analysis revealed that potato StSCL proteins exhibit the closest evolutionary relationship with their counterparts in tomato. Structural and promoter analyses demonstrated that StSCL members possess conserved GRAS and DELLA domains and are enriched with *cis*-acting elements responsive to ABA, SA, JA, GA, and various abiotic stresses. Protein-protein interaction (PPI) network analysis identified *StSCL13*, *StSCL24*, and *StSCL66* as potential hub genes. Expression profiling revealed tissue-specific patterns and broad responsiveness to hormonal and stress treatments. The *StSCL3* was localized to the nucleus and was significantly induced by ABA, GA, SA, JA, and drought stress, but suppressed under salt stress. This divergent regulation suggests that StSCL3 functions as a complex signal "integrator" that integrates multiple hormonal pathways to modulate stress adaptation and growth.

Conclusions Collectively, this study elucidates the evolutionary characteristics and regulatory

mechanisms of the potato SCL family, highlighting the pivotal role of *StSCL3* in hormone signaling and abiotic stress responses, and providing a theoretical foundation for improving stress resistance in potato.

Keywords *Solanum tuberosum* L.; SCL gene family; Abiotic stress; Phytohormone signaling, Drought and salt stress

Background

As a globally important dual-purpose crop for both staple food and vegetable purposes, potato (*Solanum tuberosum*) faces significant challenges from various biotic and abiotic stresses that compromise its stable yield and quality [1]. Among these, abiotic stresses such as drought and salinity, along with related adverse factors, have become critical bottlenecks constraining its efficient production [2, 3]. Therefore, elucidating the molecular regulatory networks underlying stress tolerance in potato and identifying key functional genes are of urgent practical significance for breeding new stress-resistant varieties and ensuring food security.

Transcription factors act as central regulators in orchestrating the intricate stress response networks of plants. SCARECROW-like (SCL) transcription factors belong to the GRAS family, which is named after the initial letters of its founding members GIBBERELLIN-ACID INSENSITIVE (*GAI*), REPRESSOR of GA1 (*RGA*), and SCARECROW (*SCR*) [4]. The first member of this family, *SCR*, was isolated from *Arabidopsis* through screening mutants with defective root development, and its mutation was found to disrupt root radial patterning and exert transcriptional regulatory functions [5]. SCL proteins are characterized by a highly conserved GRAS domain at the C-terminus and a variable region containing nuclear localization signals (NLSs) at the N-terminus [6]. The variable N-terminal region is identified as an intrinsically disordered region (IDR), which endows SCL proteins with functional polymorphism and makes them the first class of intrinsically disordered proteins discovered in plants [7, 8]. To date, GRAS/SCL genes have been identified in more than 60 plant species, with the number of family members varying

significantly from 33 in *Arabidopsis* to 188 in wheat (*Triticum aestivum*) [9, 10]. Recent studies have further confirmed that the bacterial origin of GRAS genes contributes to the establishment of genetic regulatory networks in early land plants, highlighting their evolutionary significance [11].

SCL transcription factors play crucial roles in regulating multiple plant biological processes, including growth and development as well as stress responses [9, 10]. In terms of developmental regulation, SCL members are involved in meristem maintenance, root radial patterning, somatic embryogenesis, leaf development, and secondary metabolite biosynthesis [12-15]. For instance, *AtSCL23* coordinates shoot apical meristem development through auxin-dependent lateral organ initiation, while tomato *SlSCL3* regulates the biosynthesis of volatile terpenes [16]. In stress responses, SCL proteins participate in the adaptation to both abiotic and biotic stresses: *ThSCL32* from *Salix matsudana* enhances salt tolerance by upregulating *ThPHD3* expression, and cotton *GhSCL13-2A* positively regulates resistance to Verticillium wilt by mediating jasmonic acid (JA) and salicylic acid (SA) signaling pathways [17]. Notably, microRNA (*miRNA*)-SCL modules and transcription factor complexes are two major regulatory modes of SCL gene expression, which are essential for their functional realization [18, 19]. Recent advances have revealed that SCL proteins can modulate reactive oxygen species (ROS) homeostasis by interacting with peroxidases or redox-related proteins, providing new insights into their regulatory mechanisms [14].

Despite extensive studies on SCL genes in various plant species, research gaps still exist in economically important crops, especially potato (*Solanum tuberosum*). As a vital dual-purpose crop for food and vegetables, potato is severely threatened by multiple biotic and abiotic stresses, among which bacterial wilt caused by *Ralstonia solanacearum* inflicts substantial yield losses [20-22]. However, the genome-wide identification and functional characterization of the SCL gene family (*StSCL*) in potato remain largely unexplored. Additionally, the expression patterns of potato SCL genes under hormone treatments and major abiotic stresses have not been systematically analyzed, which hinders the understanding of their roles in potato growth and stress adaptation. Recent studies on other crops have

demonstrated that genome-wide identification combined with expression profiling is an effective strategy to unravel the functional diversity of gene families [23-25], providing a feasible approach for potato SCL research.

In this study, we performed genome-wide identification of the StSCLs, a diploid potato variety with a well-annotated genome. The structural characteristics, phylogenetic relationships, and chromosomal distributions of StSCL genes were systematically analyzed. Furthermore, the expression patterns of StSCL genes under GA, auxin, drought, and salt stress conditions were investigated using quantitative real-time PCR (qRT-PCR). This study aims to establish a foundation for understanding the molecular mechanisms underlying StSCL-mediated regulation of potato growth and stress responses, and to provide potential candidate genes for potato genetic improvement.

Results and analysis

Identification and physicochemical properties of StSCLs

First, the initially identified candidate SCL genes in potato were validated by sequence alignment using the BLASTp program on the NCBI database (<https://www.ncbi.nlm.nih.gov/>) to confirm their membership in the SCL gene family. Ultimately, 71 StSCL genes were identified and designated *StSCL01* to *StSCL71* based on their ascending ID numbers.

Physicochemical property analysis of the encoded proteins (Table S1) showed that the protein encoded by *StSCL67* was the shortest (61 aa), while that encoded by *StSCL36* was the longest (826 aa). The molecular weights of these StSCL proteins ranged from 15.23 kDa (encoded by *StSCL28*) to 90.15 kDa (encoded by *StSCL36*), and their isoelectric points (pI) varied between 4.49 (encoded by *StSCL38*) and 8.42 (encoded by *StSCL51*). With the exception of proteins encoded by *StSCL1*, *StSCL21*, *StSCL28*, *StSCL29*, *StSCL39*, *StSCL48*, *StSCL57*, and *StSCL67*, the instability indices of most other StSCL proteins exceeded 40, indicating relatively low structural stability. Subcellular localization prediction revealed that: *StSCL17*, *StSCL18*, *StSCL57*, and *StSCL64* were localized in the chloroplast; *StSCL20*, *StSCL29*, *StSCL34*, *StSCL39*, *StSCL43*, *StSCL66*, and *StSCL67* to the cytoplasm; *StSCL28* to

the mitochondrion; *StSCL54* to the peroxisome; *StSCL69* to the endoplasmic reticulum; and all other *StSCL* proteins to the nucleus. The prediction results of the 3D structures of the *StSCL* gene family (Fig. S1) demonstrated high structural homology among multiple family members, specifically including *StSCL2* and *StSCL13*, *StSCL17* and *StSCL18*, *StSCL12* and *StSCL3*, *StSCL32* and *StSCL33*, *StSCL26* and *StSCL27*, *StSCL49* and *StSCL50*, *StSCL55* and *StSCL56*, *StSCL61* and *StSCL62*, *StSCL23* and *StSCL51*, *StSCL41* and *StSCL42*, and *StSCL45* and *StSCL46*.

Chromosomal distribution of *StSCLs*

To clarify the distribution pattern of potato *SCL* genes on chromosomes, we generated a physical localization map of *StSCLs* (Fig. 1). The 71 *StSCLs* were unevenly distributed across the 12 chromosomes of potato: Chromosomes 06 and 07 contained the largest number of *StSCLs* (9 each), while chromosome 3 had the fewest (only 3). The remaining chromosomes (ch01, ch02, ch04, ch05, ch08, ch09, ch10, ch11, ch12) harbored 4 to 8 *StSCLs* each.

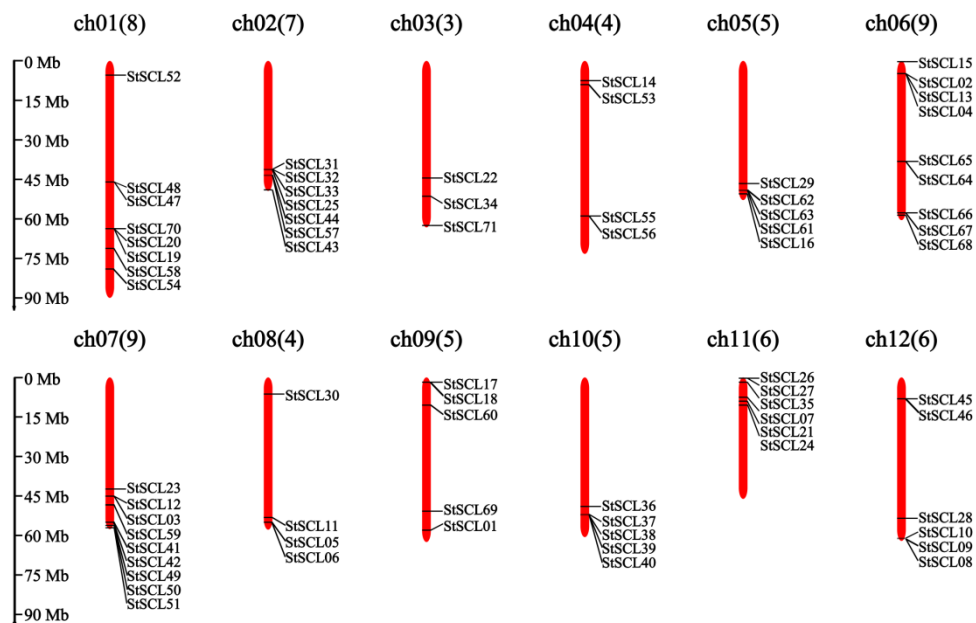


Fig. 1 Distribution of 71 *StSCLs* on 12 chromosomes. The gene IDs on the right side of each chromosome corresponds to the approximate location of each *StSCL* gene, and the

chromosome number is shown at the top of each chromosome. The scale on the left is megabits (Mb).

Phylogenetic tree of StSCLs, AtSCLs, CaSCLs, SlSCLs, and NtSCLs

To elucidate the evolutionary relationships among SCL family members across different species, a phylogenetic tree was constructed using 71 StSCL, 34 AtSCL, 52 SlSCL, 65 CaSCL, and 56 NtSCL protein sequences (Fig. 2). The analysis revealed that the 278 SCL proteins were clustered into four major groups: I, II, III, and IV. Among these, Group IV was the largest, accounting for 33.2% of the members, followed by Group II (31.65%), Group III (17.98%), and Group I (17.26%). Further evolutionary analysis indicated that potato StSCL proteins exhibited the closest phylogenetic relationship with tomato SlSCL proteins. Most members showed an interspersed clustering pattern in the phylogenetic tree, whereas their relationships with *Arabidopsis* (34 AtSCL), pepper (65 CaSCL), and tobacco (56 NtSCL) were comparatively distant.

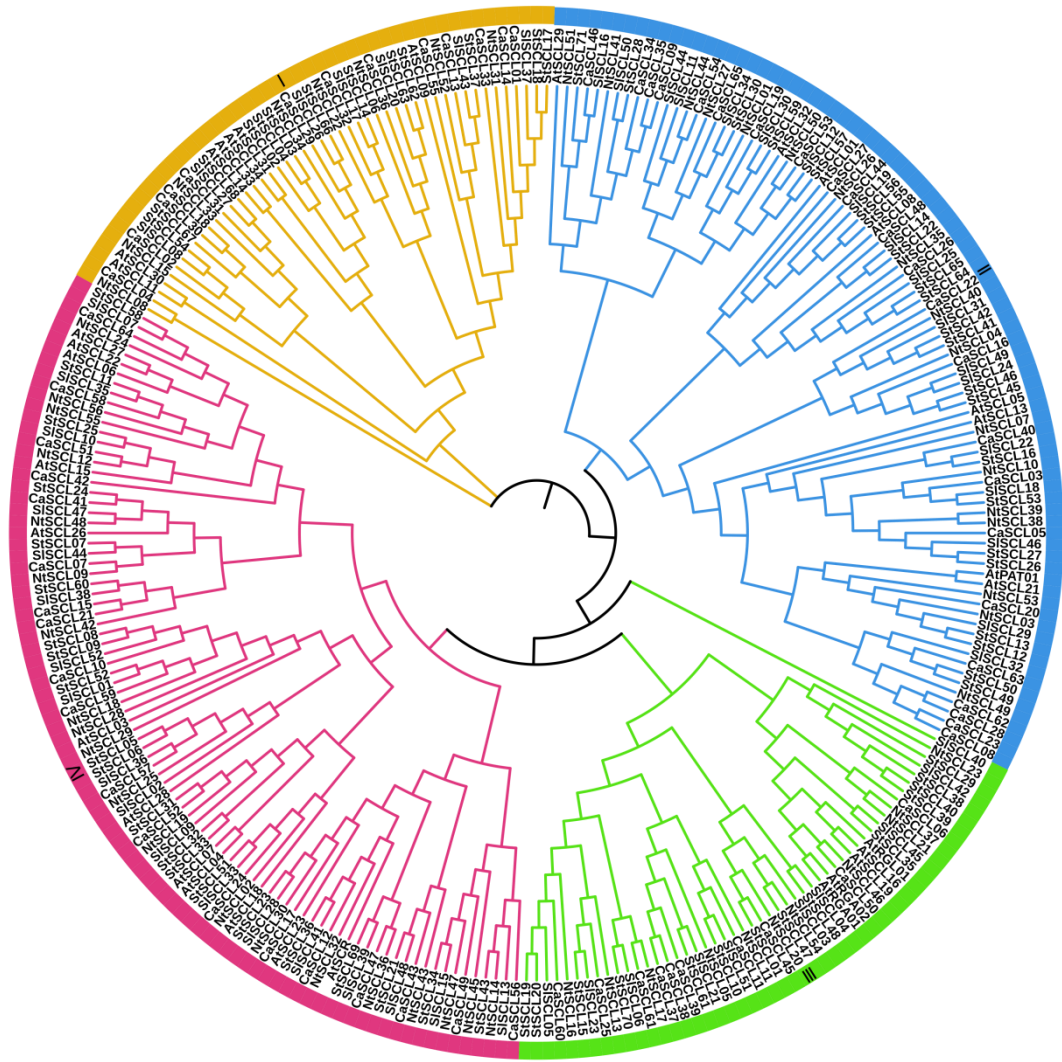


Fig. 2 Phylogenetic tree of SCL proteins from potato, *Arabidopsis*, tomato, pepper, and tobacco. A neighbor-joining (NJ) phylogenetic tree was constructed based on a ClustalW multiple sequence alignment (default parameters) of the full-length amino acid sequences of 71 potato, 52 tomato, 56 tobacco, 65 pepper, and 34 *Arabidopsis* SCL proteins. The tree was generated using MEGA software (version 7.0.14) with 1,000 bootstrap replicates. The SCL proteins were divided into four groups (I, II, III, and IV), which are distinguished by orange, blue, green, and purple lines, respectively.

Collinearity relationships of StSCLs and AtSCLs, CaSCLs, SiSCLs, as well as NtSCLs

To further explore the potential evolutionary mechanisms of the StSCLs, we evaluated the synteny relationships of SCL genes among the five species based on sequence homology

and positional information (Table S2, Fig. 3). The results showed that there were 37, 22, 14, and 5 SCL members participating in the potato-pepper, potato-*Arabidopsis*, potato-tomato, and potato-tobacco synteny relations, respectively. Among the above collinear gene pairs, *StSCL70* with *CaSCL*, *CaSCL*, *SlSCL*, and *SlSCL*, respectively. *StSCL4*, *StSCL33*, *StSCL57*, each corresponded to two 2 *AtSCL* members (*AtSCL31/AtSCL33*, *AtSCL04/AtSCL07*, *AtSCL16/AtSCL32*, *AtSCL31/AtSCL33*), respectively. in addition, *StSCL17* and *StSCL58* each corresponded to 3 *AtSCL* members (*AtSCL09/AtSCL14/AtSCL33*, *AtSCL06/AtSCL22/AtSCL27*), respectively, indicating simultaneous collinear relations.

Intraspecific synteny analysis revealed a large number of highly homologous syntenic gene pairs within the *StSCL* gene family, and these gene pairs exhibited significant regional clustered distributions on all 12 chromosomes. For instance, both *StSCL47* and *StSCL48* are located on chromosome 1, *StSCL32* and *StSCL33* on chromosome 2, *StSCL55* and *StSCL56* on chromosome 4, *StSCL62* and *StSCL63* on chromosome 5, *StSCL66* and *StSCL67* on chromosome 6, *StSCL41* and *StSCL42* on chromosome 7, *StSCL05* and *StSCL06* on chromosome 8, *StSCL17* and *StSCL18* on chromosome 9, *StSCL36* and *StSCL37* on chromosome 10, *StSCL26* and *StSCL27* on chromosome 11, and *StSCL08* and *StSCL09* on chromosome 12 (Fig. 3, Fig. 1).

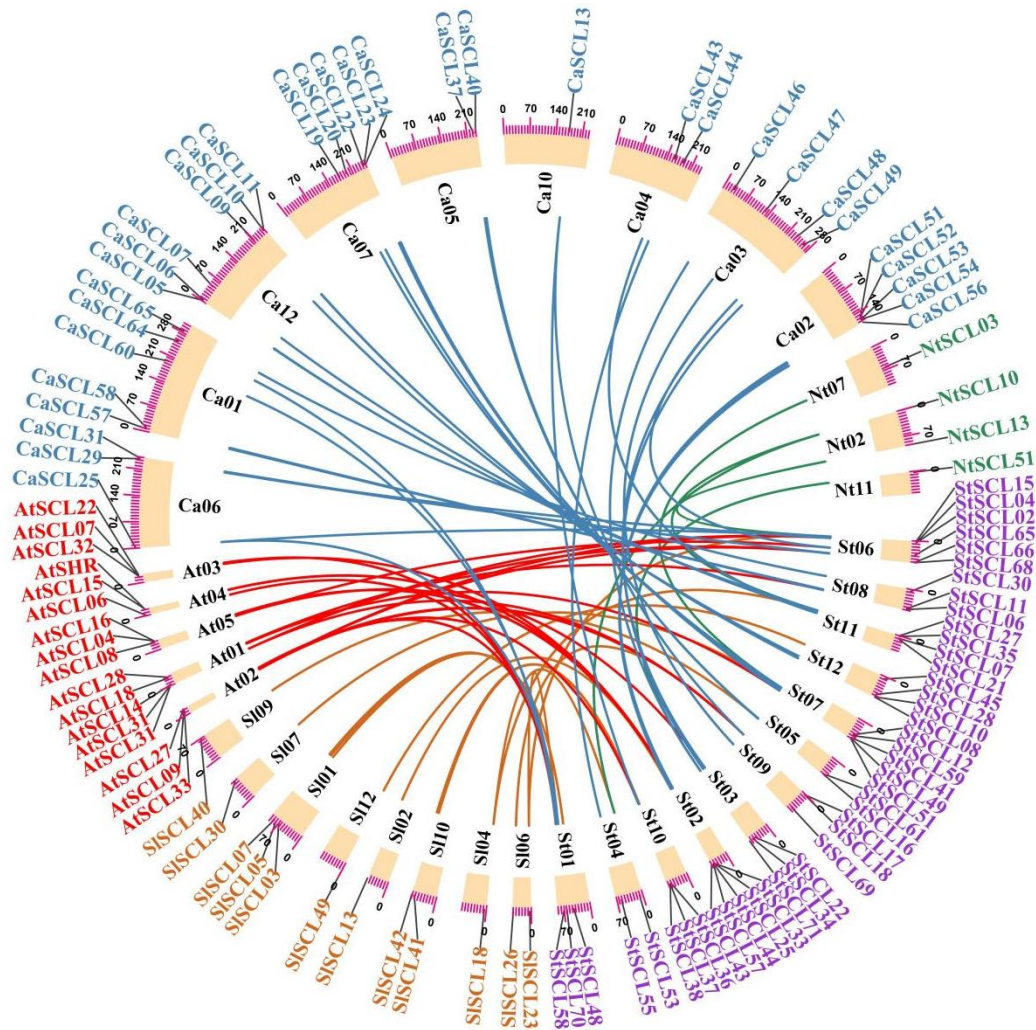


Fig. 3 Collinearity of SCL family in potato, *Arabidopsis*, tomato, tobacco, and pepper. The red line indicates the collinearity of SCL gene pairs between potato and *Arabidopsis*; the blue line represents the collinearity of SCL gene pairs between potato and pepper; the green line shows the collinearity of SCL gene pairs between potato and tobacco; and the orange line shows the collinearity of SCL gene pairs between potato and tomato.

Phylogeny of StSCLs and gene structure analysis

The phylogenetic tree of the StSCL gene family reveals that the 71 StSCL genes can be categorized into three distinct groups (Fig. 4A). Analysis of the conserved motifs within the StSCL proteins shows the following composition: *StSCL67* contains only motif6; *StSCL28* comprises motifs 3 and 4; *StSCL2*, *StSCL5*, *StSCL39*, and *StSCL71* each possess five motifs;

StSCL60 contains six motifs; *StSCL7*, *StSCL23*, *StSCL25*, *StSCL38*, *StSCL58*, and *StSCL69* each have seven motifs; *StSCL11*, *StSCL21*, *StSCL30*, and *StSCL36* contain eight motifs; 33 *StSCL* genes contain nine motifs; and the remaining 21 *StSCL* genes harbor the complete set of motifs (Fig. 4B).

Analysis of the conserved domains in the *StSCL* gene family (Fig. 4C) indicates that 48 members contain the conserved GRAS domain, while 23 members harbor the GRAS superfamily domain, supporting the classification of *SCL* genes within the GRAS transcription factor family. Notably, *StSCL35* additionally contains a DELLA domain, *StSCL58* carries an Amelogenin superfamily domain, and *StSCL29* possesses a calmodulin-binding superfamily domain.

Examination of the intron-exon structures in the *StSCL* gene family (Fig. 4D) shows that *StSCL3*, *StSCL4*, *StSCL20*, *StSCL34*, and *StSCL70* contain two exons, while the remaining 66 *StSCL* genes possess a single exon. In terms of intron numbers, 10 *StSCLs* contain three introns, 38 *StSCLs* contain two introns, 6 *StSCLs* contain one intron, and 18 *StSCLs* lack introns entirely.

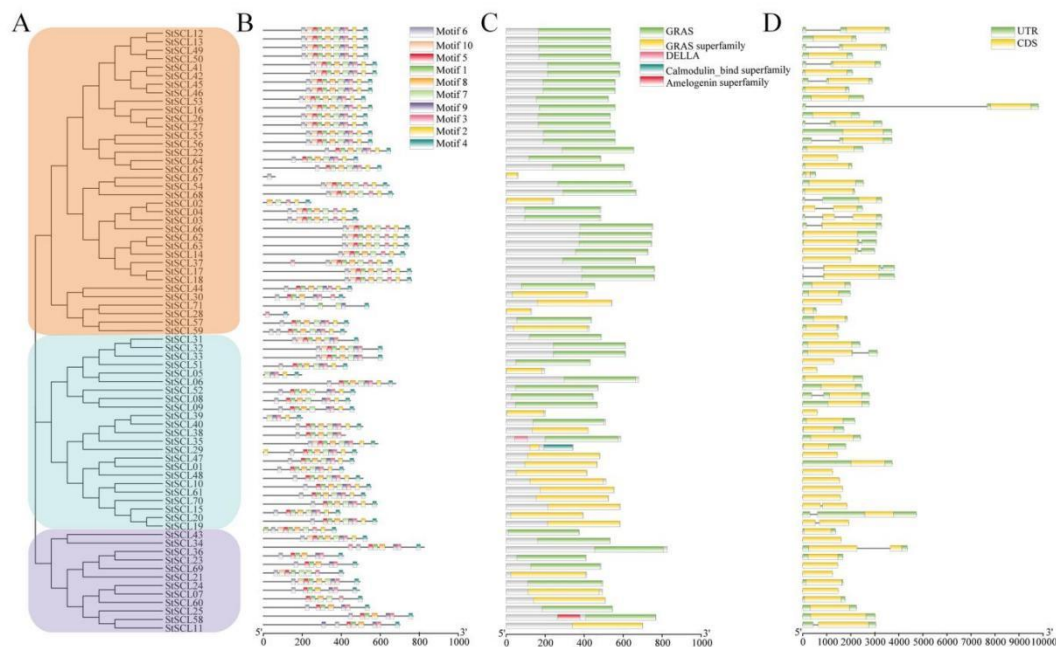


Fig. 4 Phylogenetic relationships and gene structure of *StSCLs*. **A** Phylogenetic tree of 71 *StSCL* proteins. Different subgroups are represented by different background colors. **B**

Distribution of conserved motifs in StSCL proteins. The length of the black line represents the size of the protein, which can be estimated by the the scale at the bottom. Motifs 1~10 are distinguished by different colored rectangular boxes. **C** Domain analysis of StSCL proteins. Different colors represent different domains. **D** Intron-exon structure of the StSCL gene. Coding sequences (CDS) and introns are represented by yellow rectangular boxes and black lines, respectively.

Analysis of *cis*-acting elements in the StSCL promoters

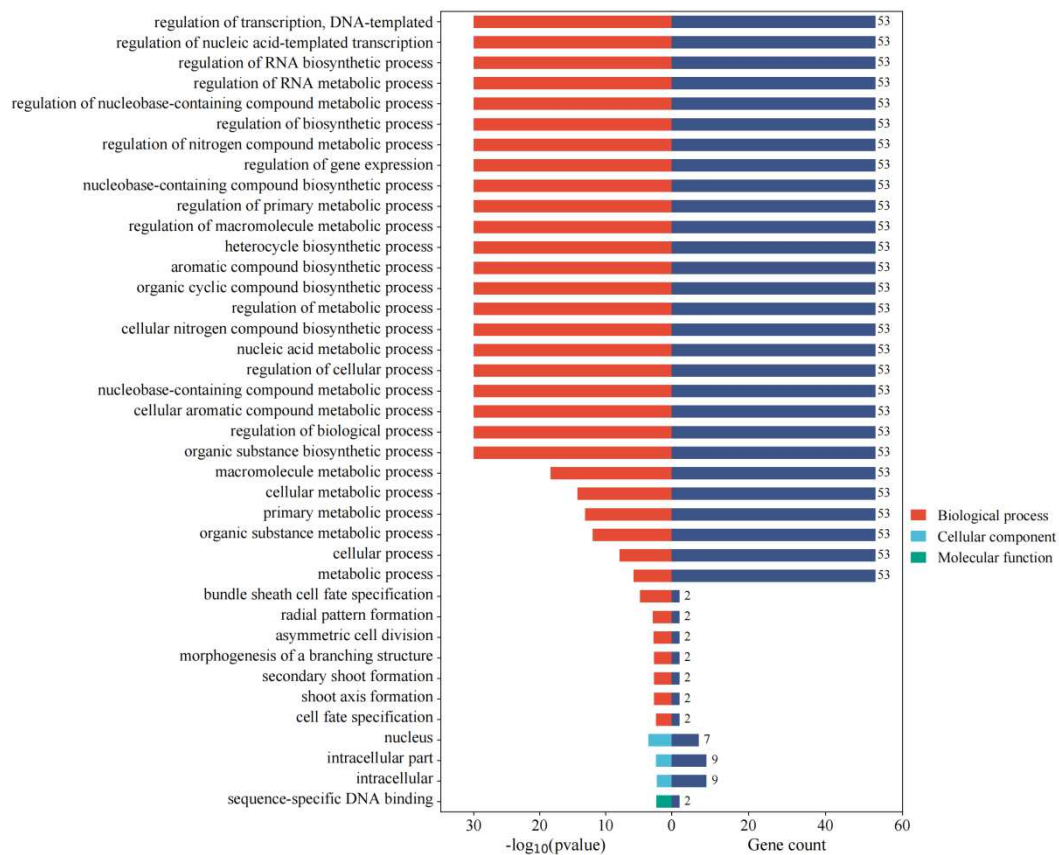
Cis-acting elements in promoter regions serve as core molecular switches for transcriptional regulation. Analysis of the promoters revealed that the 2000 bp upstream sequences of *StSCL* genes contain CAAT-box, TATA-box, stress-response elements, hormone-response elements, light-response elements, and development-related elements (Fig. S2). Most StSCL genes harbor CAAT-box and TATA-box, and the number of TATA-boxes generally exceeds that of CAAT-boxes (Fig. 5A), reflecting a high density and regulatory activity of transcription start sites in the promoter regions.

Stress-response elements encompass 15 types, including ARE, TC-rich repeats, DRE1, MYB, DRE, SARE, MBSI, as-1, WRE3, STRE, WUN-motif, LTR, MBS, GC-motif, and H-box. Among these, ARE (anaerobic response element) and STRE (stress response element) are present at relatively high frequencies (Fig. 5B).

Hormone-response elements comprise five categories: abscisic acid-response elements (ABRE), methyl jasmonate-response elements (CGTCA-motif), salicylic acid-response elements (TCA-element), gibberellin-response elements (TATC-box, GARE-motif, P-box), and auxin-response elements (TGA-element, TGACG-motif, ERE) (Fig. 5B). Notably, 54% of StSCL genes contain ABRE, and 51% possess auxin-response elements, which aligns with the finding that 48 StSCL members harbor the conserved GRAS domain and 23 members contain the GRAS superfamily domain (Fig. 4C).

Light-response elements include nine types: ACE, AE-box, box S, chs.CMA1a, I-box, MRE, G-box, CAT-box, and CCGTCC-box. Among these, 59% of StSCL genes contain the

251 molecular functions (MFs), only two genes are enriched in sequence-specific DNA binding.



253 **Fig. 6** GO analysis of StSCL. Gene Ontology (GO) terms of molecular function (MF),
 254 biological process (BP), and cellular component (CC) categories for potato StSCL were
 255 enriched using the g:Profilerr Analyzer tool. The number on the right shows the gene counts
 256 associated with the GO terms.

257 Protein-protein interaction analysis of StSCLs

258 Based on the STRING database, the protein – protein interaction (PPI) network among
 259 members of the potato StSCL family was further analyzed (Fig. 7 and Table S3). The results
 260 revealed that 51 StSCL members formed a complex interaction network through 610
 261 interaction edges. Among them, *StSCL13*, *StSCL24*, and *StSCL66* had the highest number of
 262 interacting partners (35 each), followed by *StSCL01* and *StSCL54* (each interacting with 34
 263 proteins), suggesting that these five genes may serve as central hubs within the StSCL family
 264 interaction network. In contrast, *StSCL52* exhibited the fewest interactions (only 8 partners:

StSCL21, *StSCL23*, *StSCL29*, *StSCL40*, *StSCL48*, *StSCL59*, *StSCL61*, and *StSCL68*).

Although *StSCL52* lacks a high-homology 3D structure (Fig. S1) and contains relatively few types and numbers of response elements, its core interacting partners (such as *StSCL13*, *StSCL24*, and *StSCL66*) are enriched with elements like ABRE and STRE, which are associated with hormone response and stress adaptation.

Strong interaction relationships ($|r| > 0.95$) further indicated that *StSCL13* strongly interacts with *StSCL01*, *StSCL06*, *StSCL07*, *StSCL16*, *StSCL17*, *StSCL24*, *StSCL27*, *StSCL28*, *StSCL30*, *StSCL32*, *StSCL43*, *StSCL46*, *StSCL54*, and *StSCL55*; *StSCL24* strongly interacts with *StSCL01*, *StSCL06*, *StSCL07*, *StSCL13*, *StSCL16*, *StSCL17*, *StSCL27*, *StSCL28*, *StSCL30*, *StSCL32*, *StSCL43*, *StSCL46*, *StSCL54*, *StSCL55*, *StSCL58*, *StSCL65*, *StSCL66*, and *StSCL70*; and *StSCL66* strongly interacts with *StSCL01*, *StSCL06*, *StSCL13*, *StSCL15*, *StSCL24*, *StSCL27*, *StSCL28*, *StSCL30*, *StSCL46*, *StSCL50*, *StSCL54*, *StSCL65*, and *StSCL67*. These strong interaction clusters may play key regulatory roles in biological processes mediated by the StSCL family. Additionally, 20 StSCL members (*StSCL02*, *StSCL03*, *StSCL04*, *StSCL05*, *StSCL08*, *StSCL12*, *StSCL18*, *StSCL26*, *StSCL31*, *StSCL33*, *StSCL34*, *StSCL39*, *StSCL41*, *StSCL45*, *StSCL49*, *StSCL51*, *StSCL56*, *StSCL62*, *StSCL64*, and *StSCL69*) showed no detectable interactions, possibly due to sequence divergence or structural differences that preclude effective protein binding.

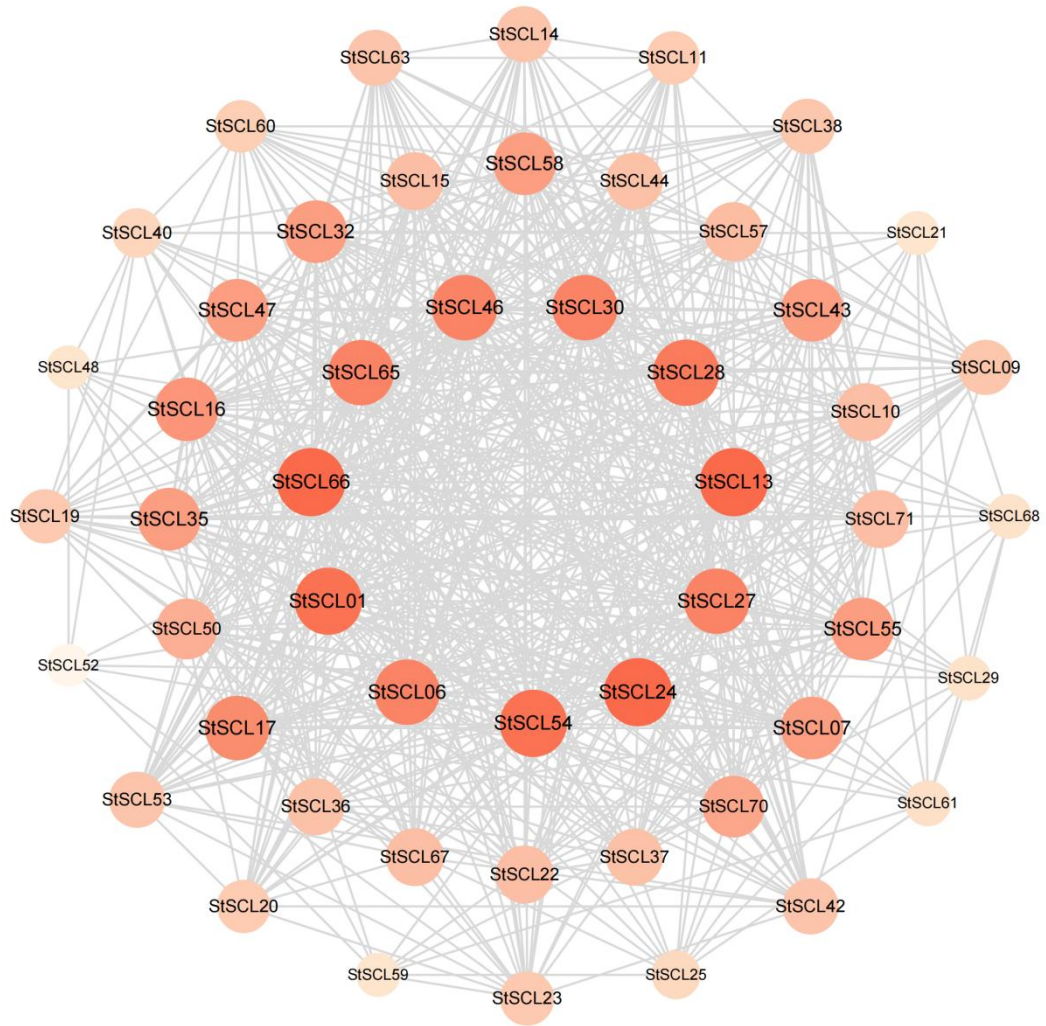


Fig. 7 Protein-protein interaction (PPI) network of the StSCLs. The nodes in the network represent StSCL family members, and the grey lines indicate the protein-protein interaction relationships between different StSCLs. The orange nodes highlight the core hub members in the PPI network (including *StSCL01*, *StSCL06*, *StSCL13*, *StSCL24*, *StSCL27*, *StSCL28*, *StSCL30*, *StSCL46*, *StSCL54*, *StSCL65* and *StSCL66*), which exhibit more extensive interaction connections (more grey lines) compared to other members, suggesting their key regulatory roles in the StSCL family interaction network.

Expression profile analysis of StSCLs

The SCL gene family plays a central role in maintaining meristems, regulating root development, orchestrating secondary metabolism, and mediating diverse stress responses.

However, functional information on SCLs in potato remains limited. To investigate their roles, we analyzed transcriptome data of the StSCL gene family across different tissues and under various treatments, visualizing the expression patterns of 55 differentially expressed StSCLs (Fig. 8A, Table S4).

Tissue development heatmap analysis (Fig. 8A) showed an overall marked transcriptional downregulation of StSCLs. Specifically, 22 StSCLs were downregulated across all six tissues examined, with *StSCL14*, *StSCL17*, *StSCL32*, and *StSCL35* showing pronounced downregulation—especially *StSCL32* and *StSCL35*. In leaves, whole mature flowers, mature whole fruit, tubers, roots, and stolons, only 2, 5, 5, 9, 10, and 16 StSCLs were upregulated, respectively. These findings align with the observation that the StSCL family contains only four types of development-related *cis*-acting elements (A-box, O2-site, circadian, and GCN4_motif), each present in low copy numbers per gene (Fig. 5B).

The tuber development heatmap (Fig. 8B) revealed a relatively balanced up- and downregulation of StSCLs across seven tuber developmental stages. Fourteen StSCLs (*StSCL6*, *StSCL7*, *StSCL15*, *StSCL19*, *StSCL21*, *StSCL34*, *StSCL47*, *StSCL48*, *StSCL51*, *StSCL59*, *StSCL61*, *StSCL68*, *StSCL69*, and *StSCL70*) were upregulated at all stages, with *StSCL6* and *StSCL59* showing the most pronounced upregulation. Thirteen StSCLs (*StSCL14*, *StSCL16*, *StSCL22*, *StSCL25*, *StSCL29*, *StSCL32*, *StSCL42*, *StSCL50*, *StSCL54*, *StSCL55*, and *StSCL57*) were downregulated across all stages, with *StSCL54* and *StSCL55* being the most significantly suppressed. *StSCL35* was notably upregulated in young tubers but significantly downregulated in the other six stages, which correlates with its DELLA domain—a region often involved in stage-specific regulation of plant growth and development (Fig. 4C). This pattern suggests that *StSCL35* may function during early tuber differentiation but becomes inactive during tuber maturation.

Heatmap analysis under abiotic stresses and hormone treatments (Fig. 8C) indicated that, except under BAP (6-Benzylaminopurine, a synthetic plant growth regulator) treatment, StSCLs were overall markedly upregulated under the other seven treatments—particularly

under mannitol (27 StSCLs) and salt stress (25 StSCLs). Eleven StSCLs were commonly upregulated under both water stress and mannitol-induced drought, suggesting that these genes represent core family members in potato drought response. In contrast, the responsive genes under heat and NaCl stress were relatively distinct, reflecting stress-specific regulation within the StSCL family. Under BAP treatment, only *StSCL46* was upregulated, while the remaining StSCLs were downregulated or unchanged, consistent with the tissue development heatmap results (Fig. 8A). The StSCL family exhibited clear functional divergence in response to three plant hormones: 12 genes (*StSCL1*, *StSCL4*, *StSCL22*, *StSCL25*, *StSCL27*, *StSCL40*, *StSCL42*, *StSCL46*, *StSCL54*, *StSCL60*, *StSCL65*, and *StSCL66*) were upregulated, whereas seven (*StSCL6*, *StSCL9*, *StSCL11*, *StSCL14*, *StSCL17*, *StSCL36*, and *StSCL58*) were downregulated, likely to maintain hormonal homeostasis and avoid physiological damage from overresponse. *StSCL46* was significantly upregulated, indicating that it may be recognized and bound by transcription factors from all three hormone pathways to activate transcription and mediate cross-talk among them—consistent with the enrichment of corresponding hormone-response *cis*-elements in its promoter region (Fig. 5B) and the predominance of stress-responsive genes among its strong interaction partners (Fig. 7). *StSCL6* was significantly downregulated under all eight treatments, which correlates with the limited number of stress-response elements in its promoter (Fig. 5), suggesting that it primarily functions in tuber development (Fig. 8B) and shows low sensitivity to stress and exogenous hormones.

Heatmap analysis under biotic stresses (Fig. 8D) revealed that after inoculation with *Phytophthora infestans*, StSCLs showed differential up-/downregulation, indicating potential involvement in basal or induced immune pathways against pathogens. Notably, *StSCL23* was significantly upregulated, while *StSCL54* was significantly downregulated. Given that both can interact via *StSCL6* or *StSCL20* (Fig. 7), we speculate that *StSCL23* acts as a positive regulator of late blight resistance by activating defense responses, whereas *StSCL54* serves as a negative regulator that relieves suppression of resistance pathways. They may form protein

complexes with *StSCL23* or *StSCL54* to finely tune the immune response to *P. infestans*. Under treatment with BABA and BTH (both disease-signal inducers), 29 StSCLs were upregulated under both conditions, suggesting that they may represent conserved core responders in disease resistance regulation. Moreover, 21 StSCLs were synchronously upregulated under all three biotic stresses, aligning with the enrichment of TC-rich repeats (a defense-related element) in their promoter regions (Fig. 5).

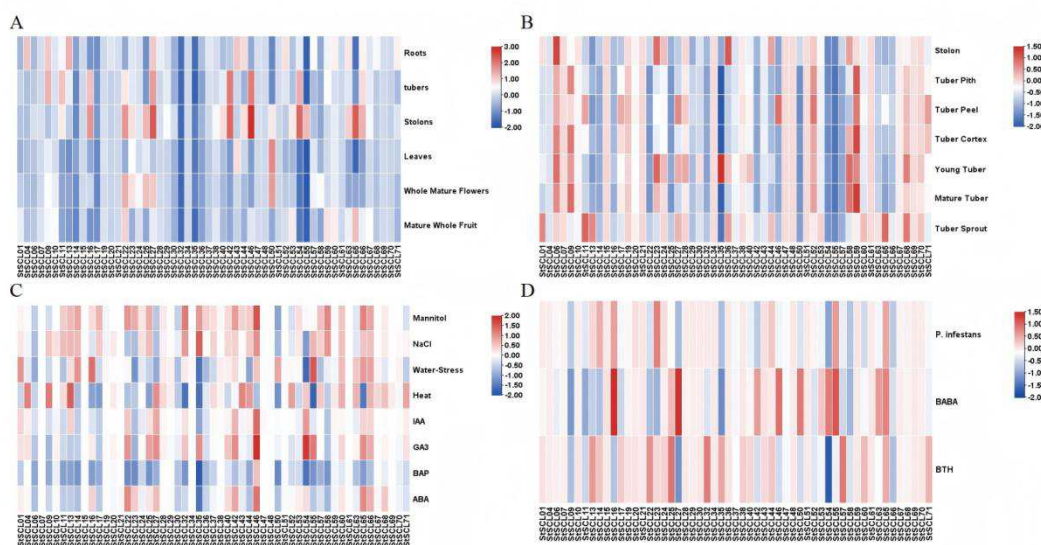


Fig. 8 Relative expression profiles of StSCLs. **A** Expression profiles of StSCLs in different tissues or developmental stages; **B** Expression profiles of StSCLs during tuber development; **C** Expression profiles of StSCLs under abiotic stress and plant hormone treatments; **D** Expression profiles of StSCLs under biotic stress. The color scale below the heat map represents the expression levels: red indicates high transcript abundance, and blue indicates low transcript abundance.

Expression analysis of *StSCL3* under hormone, drought and salt stress

Among the 16 StSCL members that showed no significant transcriptional differences in the previous heatmap analysis (Fig. 8), *StSCL3* contains a complete conserved GRAS domain (Fig. 4). Since GRAS family proteins have been widely confirmed to participate in phytohormone signaling and stress response regulation, the structural integrity of *StSCL3*

supports the execution of its disease-related functions. Furthermore, *cis*-element analysis revealed that the promoter region of *StSCL3* is enriched with multiple hormone-responsive elements, such as abscisic acid (ABRE), salicylic acid (TCA-element), and methyl jasmonate (CGTCA-motif) (Fig. 5). Consequently, *StSCL3* was selected for RT-qPCR analysis under four phytohormone treatments (ABA, GA, SA, MeJA) as well as salt and drought stress (Fig. 9, Tables S5). The results showed that *StSCL3* responded most rapidly to GA treatment, peaking on day 1 after treatment (Fig. 9B). Under ABA and SA treatments, the expression peaks of *StSCL3* appeared on day 3 (Fig. 9A, C). For SA, the delay may be attributed to processes such as cytoplasmic-nuclear translocation of the NPR1 protein and assembly of transcriptional complexes [26]. Both pathways involve additional molecular steps, leading to longer response times. In contrast, the response to MeJA was slowest, peaking on day 4 (Fig. 9D). Among the treatments, ABA induced the most pronounced upregulation of *StSCL3*, followed by MeJA, GA, and SA. These findings suggest that *StSCL3* is involved in multiple plant hormone related signaling pathways.

Under drought stress, *StSCL3* was significantly upregulated on day 2 (Fig. 9E). Combined with the presence of stress-response elements in the *StSCL3* promoter (Fig. 5B, which are more inclined to respond to osmotic stress and hypoxia signals caused by drought) and the GO enrichment results (Fig. 6, indicating that the StSCL family is primarily involved in hormone-mediated stress responses), we speculate that drought stress activates *StSCL3* expression via ABRE elements, enabling it to play an important role in potato drought adaptation.

In contrast, *StSCL3* showed consistent downregulation under salt stress (Fig. 9F). This downregulation may involve two mechanisms: first, salt stress may suppress hormone pathways that normally upregulate *StSCL3*; second, the plant may downregulate genes unrelated to salt-tolerance mechanisms to better adapt to the saline environment.

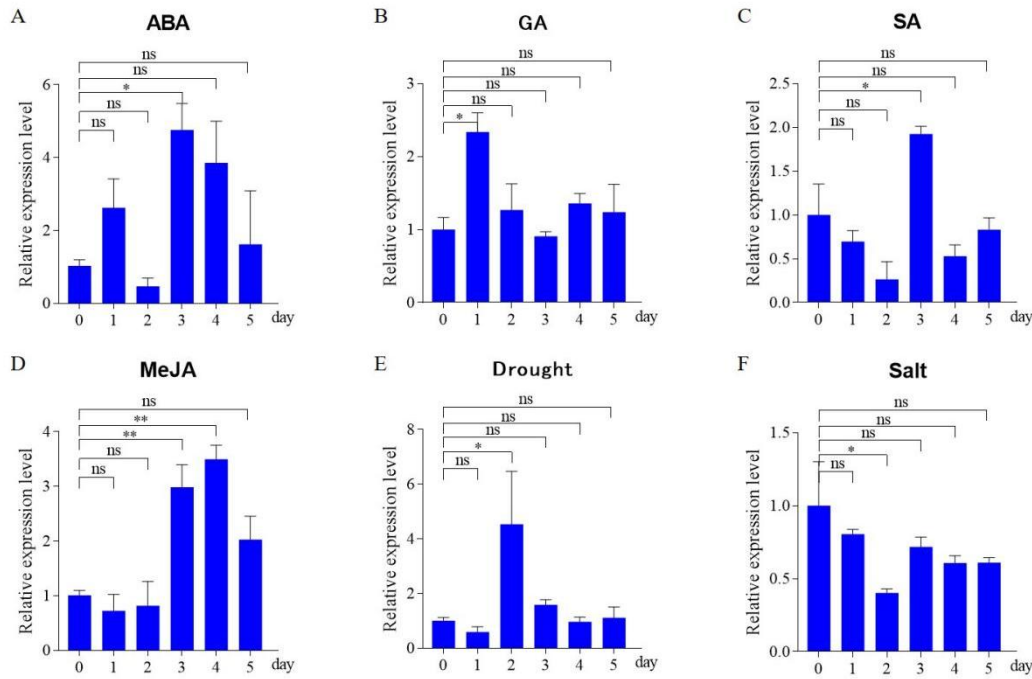


Fig. 9 Expression of *StSCL3* under hormone, salt, and drought stress. Expression analysis of *StSCL3* under ABA, GA, SA, MeJA, drought, and salt treatment by RT-qPCR. According to the One-way ANOVA analysis and multiple comparison tests, expression of *StActin* was used as a reference gene, and expression of *StSCL3* at 0 time-point was set as 1. The x-axis represents the time points after treatment. Error bars represent the standard deviations from three biological replicates. Asterisks indicate that the expression levels of treatment groups show a significant difference in transcript abundance compared with the control. (ns: no significant difference, $p < 0.05$, $p < 0.01$).

The *StSCL3* protein localizes in the nucleus

Based on bioinformatics online prediction, the *StSCL3* protein is mainly localized in the nucleus. To further verify this prediction, the full-length coding sequence of the target gene *StSCL3* (1608 bp, with 3 bp as the stop codon) was amplified from potato cDNA by PCR, and the primers are listed in Supplementary Table 6. The *StSCL3* gene was digested with restriction enzymes *Sal* I and *Kpn* I (Fig. S3). Subsequently, this fragment was cloned into the pCAMBIAsuper1300 empty vector digested with the same two enzymes (Fig. 10). The recombinant plasmid was transformed into *Escherichia coli* competent cells TOP10. After

sequence verification, the recombinant plasmid was introduced into *Agrobacterium tumefaciens* strain GV3101. *Agrobacterium*-mediated infiltration was used for transient expression of the recombinant plasmid in tobacco epidermal cells to perform subcellular localization. The p1300-35S-H2B-mCherry vector was used as a nuclear marker (red fluorescence) to detect the GFP fluorescence signal. Fluorescence signal of the 35S:*StSCL3*-GFP fusion vector (Fig. S3) was detected in the nucleus, indicating that the *StSCL3* protein is localized in the nucleus (Fig. 10). This result is consistent with previous bioinformatics predictions and functional analysis conclusions, confirming that *StSCL3* can regulate the transcription of genes related to hormone signal transduction and stress response by binding to *cis*-acting elements in the promoter region of target genes in the nucleus.

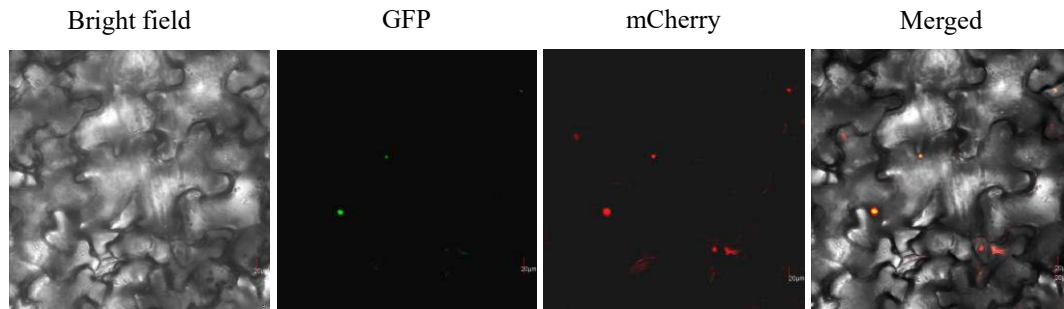


Fig. 10 Subcellular localization analysis of 35S: *StSCL3*-GFP. The 35S:*StSCL3*-GFP fusion protein were delivered into cells via *A. tumefaciens* and transiently expressed in tobacco leaves, then visualized after 18 h of transformation using confocal laser scanning microscopy. p1300-35S-H2B-mCherry (nuclear marker) red fluorescence was used as control. Under the green fluorescence excitation channel, *StSCL3*-GFP clearly localized to the nucleus (green) , which appears yellow in the merged image. Scale bars = 20 μ m.

Discussion

Soil salinization, drought stress, and pathogen infection represent major abiotic and biotic constraints limiting the growth, development, and yield formation of potato [27-29]. The SCL gene family, a pivotal subfamily of plant-specific GRAS transcription factors, is known to play critical and conserved roles in phytohormone signaling, stress responses, and

developmental regulation, including root patterning and gibberellin signaling [4, 16, 30]. Although SCL genes have been extensively studied in species such as *Arabidopsis* [7], cucumber (*Cucumis sativus*) [31], pear (*Pyrus bretschneideri*) [32], pine (*Pinus taeda*) [33], and wheat (*Triticum aestivum*) [34], however, systematic research on the StSCL family remains scarce. Our preliminary analysis previously showed that a member of the SCL family in potatoes (named *Stsl-1*) is elicited by the exogenous phytohormones such as MeJA, ABA, and SA. To gain a more comprehensive understanding of the characteristics of the StSCL gene family, this study systematically identified their evolutionary and functional diversity, and characterized the expression profiles of StSCLs at the genomic level in response to exogenous phytohormones, drought and salt stress.

Characteristics and evolution of the StSCLs

In this study, a total of 71 StSCL genes were identified (Table S1). These genes were found to be unevenly distributed across the 12 chromosomes of potato (Fig. 1). This pattern, which is consistent with the non-uniform distribution of tomato SlSCL genes [35], is common among plant multi-gene families and is often associated with genomic evolutionary events such as chromosomal segment duplications and tandem repeats [36]. Intraspecific synteny analysis revealed a large number of highly homologous syntenic gene pairs within the StSCL gene family, and these gene pairs exhibited significant regional clustered distribution characteristics on all 12 chromosomes (Fig. 3, Fig. 1). This phenomenon indicates that tandem duplication may be one of the key evolutionary driving forces contributing to the expansion of the StSCL gene family. Meanwhile, the regional clustered distribution pattern on chromosomes also suggests that these homologous genes may originate from a common ancestral gene, which have been retained through gene duplication events during evolution. They are likely to maintain similar functions or participate in coordinated regulatory pathways, providing an evolutionary basis for the stability and diversity of the functions of the StSCL gene family.

To further explore the potential evolutionary mechanisms of the StSCLs, we evaluated

the synteny relationships of SCL genes among the five species based on sequence homology and positional information (Table S2, Fig. 3). The results showed that there were 37, 22, 14, and 5 SCL members participating in the potato-pepper, potato-*Arabidopsis*, potato-tomato, and potato-tobacco synteny relations, respectively. Among the above collinear gene pairs, *StSCL70* corresponded to CaSCL, CaSCL, SlSCL, and SlSCL, respectively; *StSCL4*, *StSCL33*, *StSCL57*, and *StSCL66* each corresponded to 2 AtSCL members), respectively; *StSCL17* and *StSCL58* each corresponded to 3 AtSCL members, respectively, and simultaneously had collinear relations. The difference in the number of such interspecific syntenic gene pairs indicates that SCLs are relatively conserved during plant evolution, and also represents one of the origin characteristics of homologous genes [30]. This is consistent with the phenomenon that multiple syntenic SCL gene pairs exist between the genomes of wheat and *Arabidopsis thaliana*, as well as *Oryza sativa*, further confirming that the conservation of SCL genes is cross-species universal [37]. Collectively, this suggests that these genes may play important roles during plant evolution and indicates that whole-genome or segmental duplications have been major drivers for the expansion of the StSCL gene family in solanaceous species [38].

Subsequent analysis of the 3D structures (Fig. S1), physicochemical properties, conserved domains, and gene structures (Fig. 4) provided insights into the structural basis of functional diversification within the potato StSCL family. Although all StSCL genes encode proteins containing the GRAS or GRAS superfamily domain confirming their classification as typical GRAS transcription factors, their members vary in sequence length, isoelectric point, and instability index. Notably, the majority of StSCLs were predicted to reside in the nucleus, consistent with their core function as transcriptional regulators [33, 39]. The high homology observed among the 3D structures of multiple family members often reflects functional conservation, consistent with findings in foxtail millet where conserved motifs contribute to functional diversification[40]. For instance, tomato *SlSCL3* shares high sequence and 3D structural homology with *AtSCL3*, both containing conserved GRAS domains (LRI, VHIIID, LRII) that support conserved metabolic regulatory roles [41, 42]. Similarly, the homology of *ThSCL32* in *Tamarix hispida* with *AtSCL32* allows its conserved domains to mediate

regulatory functions under salt stress [43]. Therefore, the structural conservation observed in potato StSCLs suggests potential functional redundancy or cooperative interactions, providing a solid structural basis for further investigating their specific roles in stress responses.

Phylogenetic analysis showed that the StSCLs can be divided into four subgroups (Fig. 2). Notably, StSCLs exhibited the highest homology with tomato SCL proteins, which is consistent with the close phylogenetic relationship between potato and tomato within the family Solanaceae, indicating that the evolution of SCLs tracks the phylogenetic positions of the species [37]. This conservation is evident not only at the sequence level but also in gene function and regulatory mechanisms. Specifically, the phylogenetic tree constructed solely for StSCLs grouped them into three major clades (Fig. 4A), whereas the cross-species tree (Fig. 2) clustered them with SCLs from *Arabidopsis*, tomato, and other species into four larger groups. These two levels of evolutionary analysis corroborate each other, indicating that the StSCLs underwent early divergence during evolution, giving rise to distinct phylogenetic branches. The clear division into four subgroups is not a result of random clustering but is closely related to the functional conservation of SCL proteins. Previous studies have confirmed that the SCLs, as an important branch of the GRAS transcription factor superfamily, plays key roles in regulating plant growth and development (e.g., plant architecture and floral organ development), hormone signaling (e.g., gibberellin and abscisic acid pathways), and stress responses (e.g., drought and salinity) [44]. Given that Group IV accounts for the highest proportion in this analysis, it is hypothesized that this group may contain core functional members of the SCL family, whose conserved structures and functional domains have been maintained over long-term evolution. In contrast, the lower proportions of Groups I and III may represent functionally specialized members that have diverged during species-specific evolutionary processes.

Gene structure and promoter analysis of the StSCLs

Gene structure analysis indicated that most StSCLs encode proteins containing either the GRAS domain or the GRAS superfamily domain (Fig. 4C), which aligns with the GO

enrichment results showing significant terms such as "transcription regulation" and "regulation of biological processes" (Fig. 6). Notably, the majority of StSCLs were predicted to reside in the nucleus (Fig. 4C), consistent with their core function as transcriptional regulators [7, 36, 42]. The widespread enrichment of abscisic acid response elements (ABRE) and auxin response elements in the promoters of most StSCLs (Fig. 5) corresponds with their gene structure. Given that GRAS proteins are widely recognized as key mediators in phytohormone signaling, particularly for auxin and gibberellin pathways [45], the alignment between conserved domains and regulatory elements implies that hormone signals may serve as upstream switches that activate or repress the transcription of most StSCL genes.

Although the GRAS domain is conserved, notable structural diversification was also observed regarding specific domains and intron-exon structures. *StSCL35* additionally contains a DELLA domain, *StSCL58* carries an Amelogenin superfamily domain, and *StSCL29* possesses a calmodulin-binding superfamily domain (Fig. 4C). This domain specificity likely results from structural fusion during evolution, leading to functional specialization, a pattern consistent with the evolutionary acquisition of pleiotropic regulatory functions through domain fusion, as observed in the *Ghd7* of rice (*Oryza sativa* L.) [46]. The presence of the DELLA domain in *StSCL35* suggests its potential involvement in gibberellin signaling pathways, aligning with the typical functions of the DELLA subfamily within the GRAS family[31]. Furthermore, examination of the intron-exon structures (Fig. 4D) revealed that *StSCL3*, *StSCL4*, *StSCL20*, *StSCL34*, and *StSCL70* contain two exons, while the remaining 66 StSCL genes possess a single exon. In terms of intron numbers, 10 StSCLs contain three introns, 38 StSCLs contain two introns, six StSCLs contain one intron, and 18 StSCLs lack introns entirely. This variation may reflect the gain or loss of introns during evolution, resembling the structural pattern observed in *Arabidopsis* GRAS genes, which are predominantly single-exonic or contain few introns [47]. Such structural diversity is further exemplified by *StSCL3*, which contains two introns, whereas some genes, such as *StSCL67*, encode only 61 amino acids (Fig. 4D). The presence of introns may enable alternative splicing, thereby increasing proteomic complexity, while the occurrence of ultra-short genes hints at

highly specialized functions or potential roles as regulatory non-coding RNAs [48].

Analysis of *cis*-acting elements associated with stress, phytohormones, light, and plant growth and development identified various motifs in the promoter regions of StSCL genes (Fig. 5), suggesting their potential roles in regulating various plant responses. These elements can help predict transcription factor binding sites in gene promoters, providing valuable clues for functional studies [49]. For example, *AtSCL3* has been shown to positively regulate the gibberellin (GA) pathway and act as a DELLA repressor to maintain GA homeostasis during root development [44]. Most StSCL genes harbor CAAT-box and TATA-box, consistent with their conserved roles as essential elements for transcriptional initiation in eukaryotes [50]. StSCLs carrying these *cis*-acting elements also contain the conserved GRAS domain (Fig. 4C), indicating functional specialization of the *cis*-acting elements and their co-evolution with gene family diversification [51]. Light-response elements include nine types: ACE, AE-box, box S, chs.CMA1a, I-box, MRE, G-box, CAT-box, and CCGTCC-box. Among these, 59% of StSCL genes contain the G-box, a core element for light-signal transduction that can bind transcription factors such as HY5 to mediate photomorphogenesis and the expression of photosynthesis-related genes [52]. In summary, the StSCLs appears to be primarily involved in hormone-mediated stress responses and light signaling, a pattern consistent with the coordinated regulation of stress resistance and yield traits through hormone- and light-response elements observed in *TaSCLs* of wheat [53].

Finally, combining promoter features (Fig. 5) with expression profiles (Fig. 8) revealed that genes consistently upregulated during tuber development (e.g., *StSCL6*, *StSCL21*) are likely to be enriched with specific hormone-response elements related to tuber formation, such as those for cytokinins or gibberellins. These associations suggest that the combination of *cis*-acting elements in promoters can, to some extent, predict the expression preferences of genes. The widespread enrichment of hormone- and stress-responsive elements is consistent with the functional roles of the GRAS/SCL family in *Arabidopsis* and rice, reaffirming the central and conserved function of these transcription factors in plant environmental adaptation [45].

Expression patterns and functions of the StSCLs

The GRAS family is one of the major families involved in plant growth and development, and SCL constitutes a subfamily within it. Previous studies have shown that SCARECROW (SCR), a GRAS-containing transcription factor, controls asymmetric cell division in flowering plants by regulating the radial patterning of root ground tissues and cell proliferation in leaves [54]. *PpSCR1* participates in moss leaf and midvein development; its role in regulating cell division and proliferation is ancient and conserved between flowering plants and mosses [15]. The SHORTROOT (SHR) transcription factor, together with its target transcription factors Scarecrow (SCR), Scarecrow-like 23 (*SCL23*), and JACKDAW (JKD), promotes lateral organ formation and controls shoot meristem size [42]. *CsSCL1* plays a role in maintaining meristematic capacity and root radial patterning [55].

Tissue-specific expression profiling revealed that different StSCLs exhibit distinct expression patterns in various potato organs and at different tuber developmental stages (Figs. 8A, 8B). For example, *StSCL46* shows specifically high expression in stolons, suggesting its potential involvement in stolon growth regulation. Such high expression differentiation is an important indicator of gene functional specialization and also correlates to some extent with the phylogenetic grouping (Fig. 4), implying that genes within the same evolutionary clade may retain similar tissue-expression preferences. Moreover, genes responding to drought and ABA treatments are indeed enriched with ABRE elements, indicating that the expression response pattern of a gene may correspond to the type and number of elements in its promoter (Fig. 5).

Regarding stress responses, expression profiling further validated predictions from the *cis*-acting element analysis (Figs. 8C, 8D). Most StSCLs responded to at least one stress or hormone treatment. Notably, *StSCL6* was significantly downregulated under all eight abiotic-stress and phytohormone treatments, whereas *StSCL46* was specifically upregulated only under BAP-induced biotic stress. Such contrasting patterns suggest that although the entire family broadly participates in stress responses, different members may be responsible for sensing and transducing distinct types of stress signals. For instance, genes enriched with

ABA-response elements are more likely to dominate drought or osmotic-stress responses, while genes enriched with salicylic acid- or jasmonic acid-response elements may be more inclined toward biotic-stress defense [56]. This "division of labor" enables plants to mount precise responses to complex and variable environments by activating different StSCL gene modules.

Heatmap analysis under biotic stresses (Fig. 8D) revealed that four StSCLs were consistently downregulated, hinting that these StSCLs may serve as key nodes linking "actual *P. infestans* infection immunity" and "induced pre-activated defense." StSCLs responding only to a single treatment reflect functional specialization within the family during disease resistance. In summary, the StSCL family regulates potato disease resistance through the coordinated action of core conserved members and specifically responsive members, providing target directions for future dissection of its molecular mechanisms in pathogen resistance. Overall, the StSCL family coordinates potato growth, development, and stress adaptation through tissue-specific expression and differentiated responses to adversity and hormones.

PPI network analysis revealed a complex interaction network comprising 51 StSCL proteins connected by 610 interaction edges (Fig. 7). This network highlights extensive regulatory cooperation within the family. Proteins such as StSCL13, StSCL24, and StSCL66 possess the highest number of interaction partners and can be regarded as central hubs in the network. These hub genes likely play key roles in integrating upstream and downstream signals and coordinating the activity of family members. Strong interaction relationships further suggest the existence of functional modules, within which genes may cooperate in the same biological pathway [57]. Some genes that showed co-regulatory trends in expression profiles also displayed potential interactions in the PPI network, supporting the hypothesis that "co-expressed genes may encode interacting proteins" [58]. Most members that are interaction-active and broadly responsive in expression are predicted to localize to the nucleus, which aligns perfectly with their core function as transcriptional regulators. Based on the principle that similar structures may mediate similar protein–protein interactions, gene pairs

with highly homologous 3D structures may also exhibit similarities in expression patterns and interaction networks.

The roles of the StSCLs in hormone signal transduction and abiotic stress responses

The SCL family functions as a critical hub in the plant hormone-signaling network, coordinating growth, development, and stress responses [59]. Systematic promoter analysis reveals that upstream regulatory regions are enriched with *cis*-acting elements responsive to abscisic acid (ABRE), salicylic acid (TCA-element), methyl jasmonate (CGTCA-motif), and auxin (TGA-element). This co-occurrence suggests that StSCLs are cross-regulated by diverse hormonal signals, acting as downstream effectors that convert perceived signals into specific transcriptional programs [60, 61].

The candidate gene *StSCL3* serves as a prime example of this integration through distinct temporal dynamics. *StSCL3* responded most rapidly to GA treatment, peaking on day 1, likely via the nuclear degradation of DELLA repressor proteins [62]. Conversely, peaks under ABA and SA treatments appeared on day 3, reflecting the time required for signal transduction through the "PYR/PYL-SnRK2-ABF" cascade and NPR1 nuclear translocation, respectively [63, 64]. The JA treatment triggered the slowest response (day 4), indicating a late-response role [64]. This diversity, combined with the enrichment of multiple hormone-responsive elements in its promoter, positions *StSCL3* as a complex "signal processor" rather than a simple switch, operating at the node of ABA-GA crosstalk to balance stress adaptation and growth [44]. Furthermore, members with special domains, such as the DELLA-containing *StSCL35*, define specialized hormonal functions, potentially fine-tuning GA signaling during tuber development [65]. These findings align with conserved GRAS functions in model plants.

Under abiotic stress, *StSCL3* exhibits divergent regulation. Under drought, endogenous ABA accumulation activates the classical signaling cascade: PYR/PYL receptors activate SnRK2 kinases, which phosphorylate ABF/bZIP transcription factors to bind ABRE elements in the *StSCL3* promoter, thereby enhancing drought tolerance [66, 67]. In contrast, salt stress

triggers significant downregulation of *StSCL3*. Although salt stress induces ABA, specific ionic signals triggered by Na⁺ toxicity (involving Ca²⁺, ROS, and the SOS pathway) may antagonize ABA signaling [68]. Moreover, the absence of salt-specific *cis*-elements (e.g., DRE) in the *StSCL3* promoter renders it insensitive to osmotic components. This downregulation likely represents an adaptive strategy reallocating limited resources toward urgent physiological processes like ion homeostasis rather than growth-related signals incompatible with the stress [69]. These findings highlight the capacity of the StSCL family to decode complex environmental crosstalk [70].

Conclusions

This study systematically identified 71 StSCLs in potato, confirming their evolutionary conservation with tomato and expansion via tandem duplication. Structural analysis revealed conserved GRAS domains alongside diverse *cis*-elements, implying broad regulatory potential. Network analysis identified key hub genes, while expression profiling highlighted tissue specificity and stress responsiveness. *StSCL3* was validated as a nuclear integrator of GA, ABA, SA, and JA signals, acting as a complex "signal processor". Crucially, *StSCL3* enhanced drought tolerance via ABA signaling but was suppressed under salt stress, reflecting distinct adaptive mechanisms for ionic versus osmotic stress. These findings elucidate the regulatory mechanisms of the StSCLs and provide a foundation for improving stress resistance in potato.

Materials and Methods

Plant material and treatments

The potato plants used in this study were the diploid line *Solanum phureja* DM1-3 R44, which were provided by the Institute of Vegetable and Flowers, Chinese Academy of Agricultural Sciences (CAAS). These plants were grown in a growth chamber under the following conditions: 26 °C/18 °C (day/night) temperature regime, 60-70% relative humidity,

and a 16:8 h light: dark photoperiod. When seedlings reached the 7-8 leaf stage, the following treatments were applied separately: salt stress was induced by irrigating the roots with 200 mM NaCl , while drought stress was simulated by root irrigation with 300 mM mannitol [71]. For hormone treatments, leaves were sprayed with 1 mM salicylic acid (SA), 50 μ M abscisic acid (ABA), 50 μ M gibberellic acid (GA₃), or 25 μ M jasmonate (JA) [72]. During the spraying process, both adaxial and abaxial surfaces of all leaves were uniformly moistened to form condensed water droplets without causing runoff. Immediately after spraying, the treated plants were covered with black plastic bags, and this treatment was performed only once. Subsequently, leaf samples were collected from the treated plants at 1, 2, 3, 4, and 5 days post-treatment (with day 0 set as the control group). Each set of samples was rapidly frozen in liquid nitrogen and then stored at -80°C for subsequent analysis. Each treatment was performed in three biological replicates.

Genome-wide identification of the SCL transcription factor family in *Solanum tuberosum*, *Capsicum annuum*, *Nicotiana tabacum*, and *S.lycopersicum*

The potato genome sequence was obtained from the Potato Genome Sequencing Consortium (PGSC, <http://potato.plantbiology.msu.edu/>) , while the genomes of pepper, tobacco, and tomato were retrieved from the Ensembl Plants database (<https://plants.ensembl.org/>). First, using AtSCL protein sequences (downloaded from The *Arabidopsis* Information Resource, TAIR) as queries, BLAST searches were performed against the genomes of potato, pepper, tobacco, and tomato with a cutoff E-value $<10^{-5}$ to identify potential SCL candidate genes. Subsequently, the hidden Markov model (HMM) profile of the GRAS domain (PF03514) was acquired from the Pfam database (<http://pfam.sanger.ac.uk/>). Further screening was conducted using BLASTP against SMART (<http://smart.embl-heidelberg.de/>) and HMMER 3.0 (<https://www.ebi.ac.uk/Tools/Hmmer/>) with default parameters to remove redundant sequences and eliminate candidates lacking the conserved PF03514 domain.

Chromosomal localization, subcellular localization, and physicochemical property

analysis of StSCLs

The physical positions of StSCL genes on each chromosome were retrieved from the potato genome database, and the chromosomal location map of these StSCL genes was plotted using TBtools software[73]. The subcellular localization of StSCL proteins was predicted via the Plants-mPLoc (<http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/>). Basic physicochemical properties including sequence length, molecular weight, isoelectric point, and hydrophilicity were obtained using the ProtParam tool of ExPASy (<http://web.expasy.org/Protparam/>). Three-dimensional structural models of the StSCLs were predicted by UniProt (<https://www.uniprot.org/>).

Phylogenetic tree and collinearity analysis of StSCLs with those from four other species

First, the amino acid sequences of StSCL, AtSCL, CaSCL, SlSCL, and NtSCL were aligned using ClustalW. A phylogenetic tree was then constructed from the alignment file with MEGA7 software (<http://www.megasoftware.net/>) using the neighbor-joining method, and bootstrap analysis was performed with 1,000 replicates. Finally, the tree was annotated and color-coded via the iTOL website (<https://itol.embl.de>). Additionally, homology analysis among potato, *Arabidopsis*, pepper, tobacco, and tomato was conducted using TBtools (v2.096), and synteny relationships among SCL family members across the five species were visualized with the Advanced Circos module (<http://circos.ca/>).

Analysis of phylogeny, conserved motifs, conserved domains, and gene structures of StSCLs

First, multiple sequence alignment of the StSCL gene family was performed using ClustalW. Based on the alignment results, a phylogenetic tree was constructed with the neighbor-joining (NJ) method in MEGA7 software (<http://www.megasoftware.net/>), and its topological reliability was assessed using 1,000 bootstrap replicates. Subsequently, conserved motifs of the StSCL protein family were predicted using the MEME online program (<http://meme-suite.org/tools/meme>). Sequence analysis was carried out with the EMBL-EBI Pfam 32.0 batch search tool (<http://pfam.xfam.org/search#tabview=tab1>). Combined with

results retrieved from the NCBI Conserved Domain Database (CDD, <https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>), the conserved domains of the StSCL protein family were identified and validated. Furthermore, by aligning the DNA and cDNA sequences of StSCL genes, their intron – exon structures were predicted using the online Gene Structure Display Server (GSDS, <http://gsds.cbi.pku.edu.cn/>). Finally, TBtools software was employed to integrate and visualize the results from phylogenetic tree construction, conserved motif prediction, conserved domain identification, and gene structure analysis.

Prediction of *cis*-acting elements, gene ontology (GO) enrichment analysis, and transcriptome-based expression profiling of StSCLs

First, upstream sequences (2000 bp from the start codon) of StSCL genes were extracted from the potato genome database. The *cis*-acting elements in the promoter regions of StSCLs were then predicted using PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>). Finally, the results were visualized via the OmicStudio platform (<https://www.omicstudio.cn/home>).

Gene Ontology (GO) enrichment analysis of StSCL gene sequences was performed using the online tool g:Profiler (<https://biit.cs.ut.ee/gprofiler/gost>) with a significance threshold of $p < 0.001$. The enriched terms were visualized using the Bioinformatics online platform (<http://www.bioinformatics.com.cn/>).

RNA-Seq data of the potato DM genotype were downloaded from the Spud DB database (<http://spuddb.uga.edu/PotatoGenomicTools>). The expression levels of StSCLs were analyzed across different tissues, tuber developmental stages, and under various biotic/abiotic stresses and hormone treatments. Finally, a gene expression heatmap was generated using TBtools, with expression values represented as $\log_{10}(\text{FPKM})$.

Protein-protein interaction (PPI) analysis of StSCLs

Using the online database STRING v12.0 (<https://cn.string-db.org/>), protein sequences interacting with 71 known sequences were retrieved, and a protein – protein interaction network was constructed with a confidence threshold of 0.150. The network was then

visualized graphically using Cytoscape software (version 2.8.3).

RNA isolation and RT-qPCR analysis

Leaf samples were ground to a fine powder in liquid nitrogen, and total RNA was isolated using the TransZol Up Plus RNA kit (Trans, Beijing, China) following the manufacturer's recommended protocol. Subsequently, the purified RNA was used as a template for first-strand cDNA synthesis with TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix for qPCR (Trans, Beijing, China), which concurrently eliminates genomic DNA contamination. All primer sequences employed in this study were designed via the NCBI Primer-BLAST tool 16 (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>), and their detailed information is provided in Table S6. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed on a QuantStudio 3 real-time PCR system (Thermo Fisher Scientific, Shanghai, China). The 20 µL reaction mixture consisted of 1 µL cDNA template, 0.4 µL of each specific primer (*StSCL-F* and *StSCL-R*), 10 µL SuperMix, 0.4 µL DyeII, and 7.8 µL nuclease-free water. The thermal cycling program was set as follows: initial denaturation at 94 °C for 30 s, followed by 40 cycles of denaturation at 94 °C for 5 s and annealing/extension at 60 °C for 30 s. Each sample was analyzed in triplicate, and the relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method [74]. Three biological replicates were included for each sample. Following a previously described method, statistical analysis of the RT-qPCR data was performed using GraphPad Prism 10.0, where the Student's *t*-test was adopted for evaluating the significance of observed differences at a threshold of $p \leq 0.05$. One-way analysis of variance (ANOVA) was applied to evaluate differences between groups, with significance levels denoted as $p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$. Each experiment was repeated three times.

Subcellular localization of StSCL3

To investigate the subcellular localization and expression pattern of StSCL3 in potato, the full-length coding sequence (CDS) of *StSCL3* lacking the stop codon was cloned into the pCAMBIA1300 vector. Initially, the complete CDS of *StSCL3* (1605 bp) was amplified from

potato cDNA via PCR using a pair of primers with homologous arms. The amplified fragment was then inserted into the pCAMBIA1300 vector, which had been pre-linearized with the restriction enzymes *Sal* I and *Kpn* I. Subsequently, the resulting pSCL3-GFP fusion plasmid was transformed into *E. coli* DH5 α competent cells. Positive clones were validated through bacterial liquid PCR and Sanger sequencing (Sangon Biotech, Shanghai, China). The confirmed recombinant plasmid was further introduced into *A. tumefaciens* strain GV3101, and single colonies were selected for PCR-based positive screening. Finally, the recombinant *Agrobacterium* harboring the pSCL3-GFP vector was infiltrated into tobacco leaves for transient expression assays, as described previously [75]. GFP fluorescence signals were observed and analyzed using a laser scanning confocal microscope (LSCM).

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Supplementary material

The Supplementary Material for this article can be found online at

Supplementary Material 1. Supplementary Fig. 1: Predictive 3D structures of StSCLs.

Supplementary Material 2. Supplementary Fig. 2: *Cis*-acting elements on the promoters of StSCLs.

Supplementary Material 3. Supplementary Fig. 3: Construction of pCAMBIAsuper1300 subcellular localization vector.

Supplementary Material 4. Supplementary Fig. 3: Supplementary File-YC6314F ABX7470-9

Supplementary Material 5. Supplementary Table. 1: Physiochemical properties of StSCLs.

Supplementary Material 6. Supplementary Table. 2: The information of synteny analysis among five species.

Supplementary Material 7. Supplementary Table. 3: Protein and protein interaction information of StSCLs.

Supplementary Material 8. Supplementary Table. 4: The transcriptome datas corresponding to the StSCLs heatmap analysis.

Supplementary Material 9. Supplementary Table. 5: The qRT-PCR results of *StSCL3*.

Supplementary Material 10. Supplementary Table. 6: Primers of PCR and RT-qPCR.

Declarations

Ethics approval and consent to participate

All materials used in this study complies with both local and national regulations.

Consent for publication

Not applicable.

Availability of Data and Materials

The datasets supporting the conclusions of this article are included within the article and its supplementary files. All other relevant raw data supporting the results are available from the corresponding author. Further inquiries can be directed to the corresponding author. All related resources are publicly available.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

LL conceptualized and designed the study. SL interpreted and analyzed the data, and drafted the manuscript. XZ conducted the bioinformatics analysis and mapping, designed and executed the experimental work. YZ and LW assisted in enhancing the bioinformatics content and refining the visual representations. GG and WL provided project supervision and critically revised the manuscript. All authors contributed to the development of this article and approved the submitted version.

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Supplementary Files

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- [FigS3SupplementaryFileYC6314FABX74709.pdf](#)
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