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Genome-wide identification of potato *Trihelix* gene family and its response to different abiotic stresses

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Abstract

The Trihelix transcription factor family, characterized by its unique triple-helix structure (helix-loop-helix-loop-helix), plays a significant role in plant growth, development, and responses to various abiotic stresses. Potato (*Solanum tuberosum* L.), as a globally important food crop, experiences significant impacts on its growth and yield due to abiotic stresses such as drought, low temperature, and salt stress. Although the functions of Trihelix transcription factors have been extensively studied in various plants, systematic analysis in potatoes remains relatively scarce. This study aims to comprehensively identify the Trihelix gene family in potatoes through bioinformatics methods and analyze their expression patterns under abiotic stresses to reveal the potential functions of this gene family in potato growth, development, and stress responses. Through genome database searches and BLAST comparisons, 35 StTrihelix genes were identified in potatoes, and phylogenetic, gene structure, functional motif, and cis-acting element analyses were conducted. The expression patterns of these genes in different tissues and under low-temperature and drought stresses were analyzed using qRT-PCR technology. Additionally, the nuclear localization of StTrihelix30 was verified through subcellular localization experiments. The results indicate that the 35 StTrihelix genes are unevenly distributed across 12 chromosomes and can be classified into five subfamilies: GT-1, GT-2, GTγ, SH4, and SIP1. Gene structure and functional motif analyses revealed high conservation within the same subfamily. Cis-acting element analysis showed that these genes are closely related to hormone responses, stress responses, and growth and development processes. Tissue expression analysis showed that *StTrihelix4* is highly expressed in stamens, while *StTrihelix13* is highly expressed in roots. qRT-PCR results indicated that most StTrihelix genes are significantly upregulated under low-temperature and drought stresses. This study systematically identified the Trihelix gene family in potatoes and revealed its important role in abiotic stress responses. It provides new insights into the functions of the Trihelix transcription factor family in potato growth, development, and stress adaptation, offering theoretical references for stress-resistant potato breeding.

Keywords Potato, Trihelix, Gene family, Abiotic stress, qRT-PCR

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Introduction

Potato (*Solanum tuberosum* L.) is a globally important crop known for its drought tolerance, adaptability, and stable yields [1], and holds an important position in the diets of many countries owing to its high productivity, versatility, affordability, and nutritional value [2]. Potato is a shallow-rooted crop that is highly sensitive to drought stress, low-temperature stress, and salinity stress, which cause a responsive regulation of several genes at the level of transcriptional regulation in plants [3–5]. Regarding the physiological phenotype of potato, drought stress can directly cause plant wilting and restrain normal plant growth, thus affecting yield [6]. Under different low-temperature stress conditions, cold stress will affect the photosynthesis and tuber formation of potato, and freezing stress will lead to wilting or even death of potato stems and leaves [7]. While under saline stress conditions, it will cause the leaves of potato plants to have chlorosis, and the root system will be damaged or even die [8]. Several transcription factors are known to be involved in the response to abiotic stresses. *GhWRKY68* responds to drought and salt stress by regulating abscisic acid (ABA) signaling and cellular reactive oxygen species [9]. In Arabidopsis, HD-ZIPIII promotes the expression of ARF and alters the content of IAA and ABA, thereby enhancing drought tolerance [10]. Overexpression of *GhGT-2* (Gh_A05G2067) in *Arabidopsis thaliana* led to a reduction in MDA and H_2O_2 levels in plants, with increased activity of active oxygen-scavenging enzymes, ultimately boosting drought tolerance. Conversely, silencing *GhGT-2* in cotton plants results in increased oxidative damage and reduced drought resistance in cotton seedlings [11].

When plants detect stress signals, cells signal the nucleus to activate specific transcriptional regulators. Transcription factors are proteins encoded by genes that contain a DNA-binding domain, oligomerization site, transcriptional regulatory domain, and a nuclear localization signal [12]. They play a crucial role in the growth and development of higher plants, as well as in responding to external environmental conditions [13]. Transcription factors play key roles in regulating gene expression and influence various biological processes in plants, such as signal transduction, stress responses, defense mechanisms, and carbohydrate metabolism [14–16]. Members of the trihelix transcription factor family are known to play crucial roles in stress responses.

The conserved DNA domain of the trihelix transcription factor comprises three tandem α-helix structures, helix-loop-helix-loop-helix [17]. This domain binds specifically to the light-responsive GT element in the DNA sequence, thereby regulating gene expression. Consequently, the trihelix transcription factor family is commonly referred to as the GT family [18]. The trihelix transcription factor family is categorized into five subfamilies, including GT-1, GT-2, SH4, GTγ, and SIPI, based on the functional domains of amino acids [19]. Various studies have demonstrated the significant role of members of this family in regulating the light response and responding to both biotic and abiotic stresses [20–23]. Additionally, members of this family were involved in regulating the growth and development of plant trichomes [24], seeds [25], stomata, and flowers. For instance, *OsGTγ-2* in rice served as a crucial positive regulator of salt stress resistance [26]. Furthermore, *ShCIGT* has been found to enhance cold and drought resistance in cultivated tomatoes [27], and *GhGT26* has been shown to improve salt tolerance in transgenic *Arabidopsis* plants [28]. Notably, *AtGTL1* bound to the GT3 box in the *AtSDD1* promoter and negatively regulated *AtSDD1* to decrease stomatal density and number, thereby improving drought tolerance and water-use efficiency in *Arabidopsis* [29, 30]. In tomatoes, the gene expression of most *SlThrelix* gene members was up-regulated by heat stress while the gene expression was down-regulated by oxidative stress (MV). There were also partial members such as *SlGT-4* and *SlGT-27* showed significant up-regulation of expression at various periods of NaCl, 4 °C, and drought stress treatments [23]. Lan et al. [31] treated eggplant cold-tolerant variety "E7134" and eggplant cold-sensitive variety "E7145" with low-temperature stress, ABA, and SA treatments and revealed that *SmTrihelix9* was found to be involved in the regulation of cold stress, while *SmGT13* and *SmGT12* were found to be involved in the cross-regulation of ABA and SA, respectively [31]. In soybeans, the overexpression of soybean GmGT2A and GmGT2B in Arabidopsis significantly enhanced resistance to salt, low temperature, and drought stress [32]. Additionally, the wheat TaGT2L1D factor suppressed the expression of AtSDD1 in Arabidopsis, leading to the negative regulation of drought resistance, as well as influencing stomatal density and the growth and development of floral organs [33].

Trihelix transcription factors have been identified in various plant species such as *Arabidopsis thaliana* [34], *Melilotus albus* [35], *Sesamum indicum* [36], *Panax ginseng* [37], *Salix matsudana* Koidz [38], Platycodon grandiflorum [39], and Solanaceae, such as *Solanum melongena* L [31]. and *Solanum lycopersicum* [23], and their functions in relation to abiotic stress have also been studied. However, trihelix transcription factors in potatoes are not well studied in relation to abiotic stress. Therefore The present study used bioinformatics to characterize the trihelix family in potatoes and investigated their expression under abiotic stress conditions. These findings offer insights into the adaptability of potatoes to abiotic stress, potential quality enhancement, and the sustainable development of the potato industry.

Materials and methods

Test materials and treatments

The experiment was conducted in 2023 at the Laboratory of Physiology and Biotechnology at the College of Horticulture, Gansu Agricultural University, China. The experimental material was Atlantic potato from the Potato Research Institute of Dingxi City, Gansu Province, China, and the initial medium consisted of MS media (4.43 $g{\cdot}L^{-1}$ MS + 1.0 $mg{\cdot}L^{-1}$ NAA + 0.2 $mg{\cdot}L^{-1}$ 6-BA + 30 $g{\cdot}L^{-1}$ sucrose + 6.5 $g{\cdot}L^{-1}$ agar). Cultivation took place in an artificial climate chamber set at 25 °C, with a photoperiod of 16 h of light, followed by 8 h of darkness. After 30 d of incubation, the well-grown potato cultures were treated with 10% osmotic (PEG), 200 mmol/L salt (NaCl) and 4 °C low temperature for 24 h, respectively. Three biological replicates were used for each treatment group. Subsequently, the leaves of the treated potato seedlings were chopped, covered in tin foil, frozen in liquid nitrogen, and stored at -80℃.

Extraction of RNA in potato

RNA extraction was performed using the plant extraction kit RNAplant-RTR2303 (Real-Times Biotechnology Co. Ltd., Beijing, China) in accordance with the provided operating instructions. The quality and quantity of RNA were assessed using a Pultton P200 Micro Volume Spectrophotometer (Pultton Technology Ltd., USA). Following confirmation of suitability, the extracts were stored at -80 °C icebox [40].

Identification of trihelix family genes in potatoes

Trihelix family protein sequences were retrieved from the *Arabidopsis* genome databaseTAIR (https://www.arabidopsis.org/), the rice genome database RGAP (http://rice.plantbiology.msu.edu/index.shtml), tomato and pepper Phytozome v13 database (https://plants.ensembl.org/index.html). These sequences were compared to those in the potato genome database (http://solgenomics.net/) using the BLAST tool. Subsequently,, according to the Pfam (http://pfam.sanger.ac.uk/) found in trihelix transcription factor family Pfam number (PF13837) of the family of transcription factors, using SMART (http://smart.embl.de/) out the conservative sequence of potato, The conserved domain was identified. The trihelix gene family in the potato genome was named based on its chromosomal location.

Physicochemical properties, secondary structure, and predicted subcellular localization of the trihelix family of protein in potato

Basic information such as the theoretical isoelectric point (pI), molecular weight, and instability index of potato trihelix family proteins was assessed using the ExPASy (https://web.expasy.org/protparam/). Additionally, the alpha helices, beta bridges, extended strands, and random coils of these proteins were predicted using the online secondary structure tool SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa%20_sopma.html).

Chromosomal localization, collinearity analysis of the trihelix family genes in potato

The chromosomal positions of StTrihelix family members were determined by extracting annotated information from the potato genome using the TBtools v1.09876 software for visualization. Collinearity genes within the potato genome and across potato, Arabidopsis, tomatoes and pepper and rice genomes were analyzed and visualized using TBtools. Phylogenetic analysis of trihelix protein sequences from potato, Arabidopsis, and rice was conducted with MEGA 11 software using the neighbor-joining method with 1,000 bootstrap repeats and default parameters.

Conservation of motifs, gene structure, and cis-acting element analysis

The functional motifs in potato triple helix protein were analyzed using MEME (http://meme-suite.org/tools/meme). Gene structure analysis and visualization were performed using the online software GSDS 2.0 (http://gsds.cbi.pku.edu.cn/). Additionally, cis-acting elements located 2 kb upstream of the promoter of StTrihelix family members were analyzed using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

Tissue expression profiling

Download tissue expression data for Flower, Sepal, Leaf, Stem, Petiole, Stolon, Tuble, Root in potato (RH89-039-16) from Spud DB (http://solgenomics.net/). Subsequently, the data were sorted using Excel 2023, and a heat map illustrating the expression of StTrihelix genes was generated using TBtools v1.09876.

qRT-PCR analysis of trihelix family genes in potato

Primers for qRT-PCR were designed and synthesized by Bioengineering Co. (Shanghai, China) (Supplementary Table S2) Synthesis of cDNA was performed using the Primer Script RT Reagent Kit (TaKaRa). The expression analysis of this gene family under PEG, low temperature (4℃), and NaCl(200 mmol/L) treatments was conducted using a real-time fluorescence quantitative PCR instrument (LightCycler® 96 Real-Time PCR System, Roche, Switzerland) along with a SYBR Green I kit (TaKaRa). Three biological replicates were used for the quantitative analysis, with potato Ef1a was used as the internal reference gene. The PCR amplification reaction volume was 20 μL, which included 2 μL cDNA, 1 μL of each upstream and downstream primer, 10 μL SYBR, and 6 μL ddH_2O. The reaction protocol included pre-denaturation at 95℃

for 30 s, followed by 40 cycles of denaturation at 95℃ for 5 s, annealing at 60℃ for 30 s, and extension at 72℃ for 30 s. The relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method in Excel and visualized using TBtools software [41] .

Subcellular localization of StTrihelix30

Primers *StTrihelix30*-F (5′-TTTGGAGAGGACACGCT CGAGATGCTGGAAAGTTCTGTTTTGTTGTTG-3′) and *StTrihelix30*-R (5′- GCCCTTGCTCACCATGAAT TCACATTCTGTTCTTTCTGATGCTTGTT-3′) were used to clone the *StTrihelix30* fragment into the pCAMBIA1300-35 S-EGFP vector, resulting in the creation of the pCAMBIA1300:*StTrihelix30*-GFP fusion expression plasmid. An empty vector was used as the positive control (35 S::GFP). The fusion construct was transferred into DH5α through thermal excitation and then into *Agrobacterium tumefaciens* strain GV3101 via freeze-thawing after successful sequencing at Sangyo Bioengineering Co. (Shanghai, China). The bacterial solution was cultured in LB medium supplemented with kanamycin (50 μg mL^{-1}) and rifampicin (50 μg mL^{-1}) and incubated overnight at 28 °C with 200 rpm shaking until the OD_{600} reached 0.6 as described by Gou et al. [42]. For Agrobacterium-mediated infiltration of tobacco leaves, the resuspended bacterial solution was injected onto the abaxial surface of *Nicotiana benthamiana* leaves, which were then incubated for 2 d before observation using a laser confocal microscope.

Results

Analysis of sttrihelix physicochemical properties and chromosome localisation

A total of 35 potato trihelix genes, named *StTrihelix1* to *StTrihelix35*, were identified according to their position on the chromosomes. The 35 StTrihelix genes were unevenly distributed across 12 chromosomes (Fig. 1-A). Chr01 contained the highest number of genes, with six, followed by Chr09 and Chr12 with five. Chr08 harbored four genes, and Chr02 and Chr03 included three. Chr04, Chr06, and Chr11 contained two genes. Only one StTrihelix gene was distributed on Chr05, Chr07, and Chr10.

Analysis revealed that proteins from the subfamily GT-2 had a higher average amino acid size, molecular weight/KD, aliphatic index, and instability index than those of the other subfamilies (Fig. 1-B). The amino acid lengths ranged from 106 to 852, with *StTrihelix10* being the longest and StTrihelix27 the shortest. The molecular weight varied from 94365.2 to 111497.17 Da, and the isoelectric point ranged from 4.6 (*StTrihelix22*) to 9.87 (*StTrihelix27*) (Supplementary Table S1). Notably, most StTrihelix proteins were alkaline. All StTrihelix proteins were hydrophilic. Subcellular localization predictions indicated that StTrihelix proteins were primarily located in the nucleus, except for *StTrihelix4*, *StTrihelix10*, *StTrihelix19*, and *StTrihelix35* in the chloroplasts, *StTrihelix2* in the peroxisomes, and *StTrihelix20* in the mitochondria.

Evolutionary analysis of trihelix family members

Phylogenetic analysis was conducted using trihelix protein sequences from *Oryza sativa*, *Arabidopsis thaliana*, *Solanum lycopersicum* L., *Capsicum annuum* L. and *Solanum tuberosum* (Fig. 2). The results indicated that the trihelix members could be classified into five distinct subfamilies, GT-1, GT-2, GTγ, SH4, and SIP1. The largest subfamily among them was SIP1, which consisted of 57 members, followed by the GT-2, SH4, and GT-1 subfamilies with 29, 332, and 24 members, respectively. The smallest subfamily, GTγ, had 19 members. Potato trihelix members were predominantly found in the SIPI subfamily (12 members), followed by the SH4 subfamily (9 members). The GT-2 subfamily consisted of 6 members, whereasThe subfamilies are GT-1 and GTγ, each with five and three members, respectively. The distribution of potato trihelix family members across different subfamilies mirrored their overall distribution (Fig. 2).

Note In the figure, GT-1 represents subfamily 1, GT-2 represents subfamily 2, GT-γ represents subfamily 3, SH4 represents subfamily 4, SIP1 represents subfamily 5, the same below.

Collinearity analysis of the potato trihelix transcription factor family

Collinearity analysis revealed that five gene pairs exhibited segmental duplications, suggesting that fragment duplications may contribute to the expansion of trihelix-containing genes in potatoes (Fig. 3-A). Comparative analysis of homologous genes in potato, Arabidopsis, and rice indicated close collinear relationships between trihelix-containing genes in potato and Arabidopsis, with 15 and 6 pairs of homologous genes identified, respectively. It is noteworthy that the number of homologous *threlix* gene pairs of potato was higher with tomato and pepper, with 17 and 26 homologous gene pairs, respectively, possibly since they all are members of the *Solanaceae* family, indicating that the *StThrelix* genes are more closely related to *Solanaceae* plants in the evolutionary process (Fig. 3-B).

Secondary structure analysis of trihelix-containing proteins in potatoes

The prediction of the secondary structure indicated that 35 StTrihelix proteins primarily consisted of random coils ranging from 39.19% (*StTrihelix27*) to 58.05% (*StTrihelix4*), followed by alpha-helix structures ranging from 29.08% (*StTrihelix24*) to 53.02% (*StTrihelix26*)

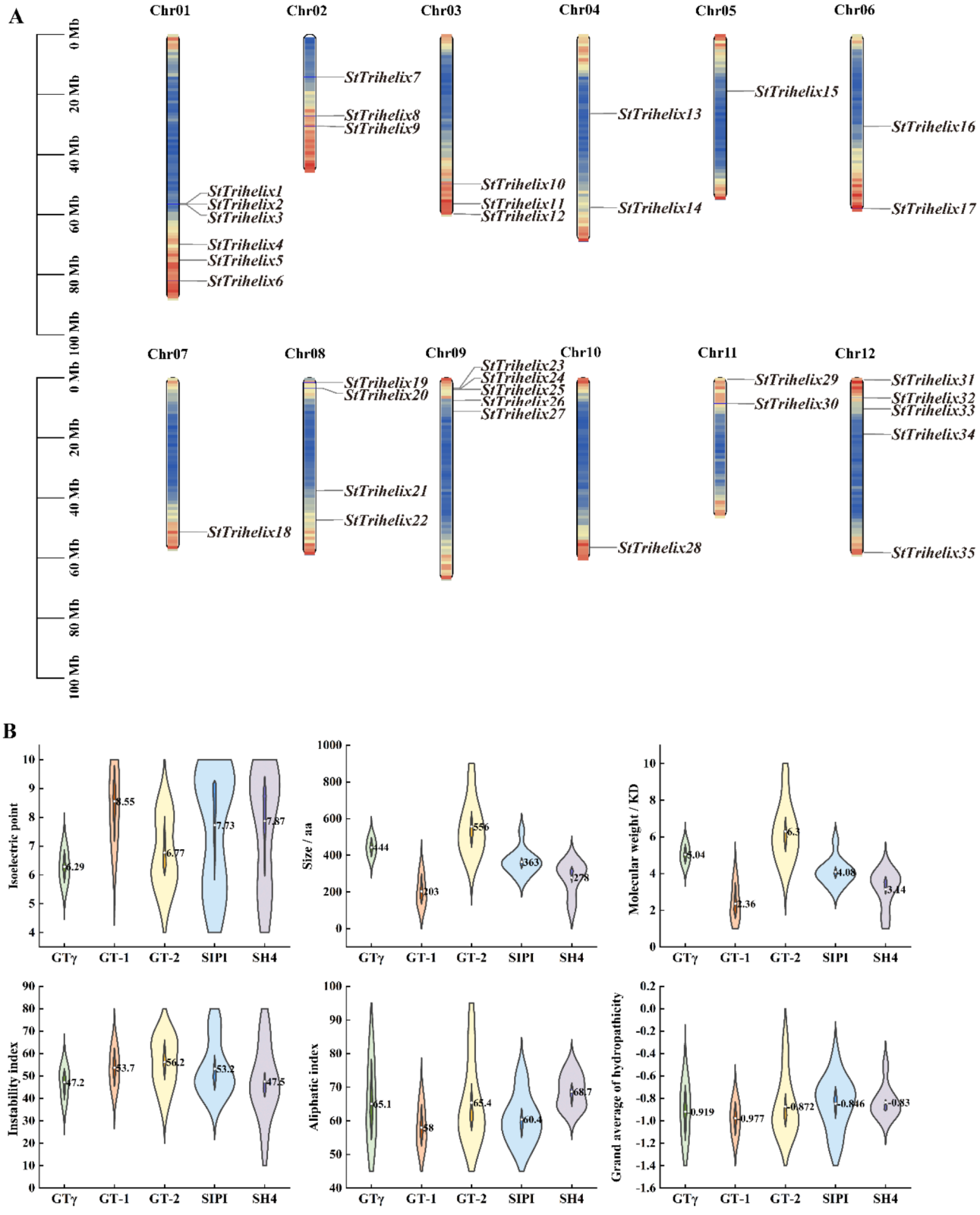

Fig. 1 The distribution of the StTrihelix gene on different chromosomes of potato and the physicochemical properties of StTrihelix protein. A Chromosome length (Mb) is represented on the left scale, with trihelix gene markers located on the right side of each chromosome. Chromosomes are color-coded to indicate gene density, with red representing the highest density and blue representing the lowest density. B The physical and chemical properties of StTrihelix protein were drawn by Violin box plots. White dots represent the average

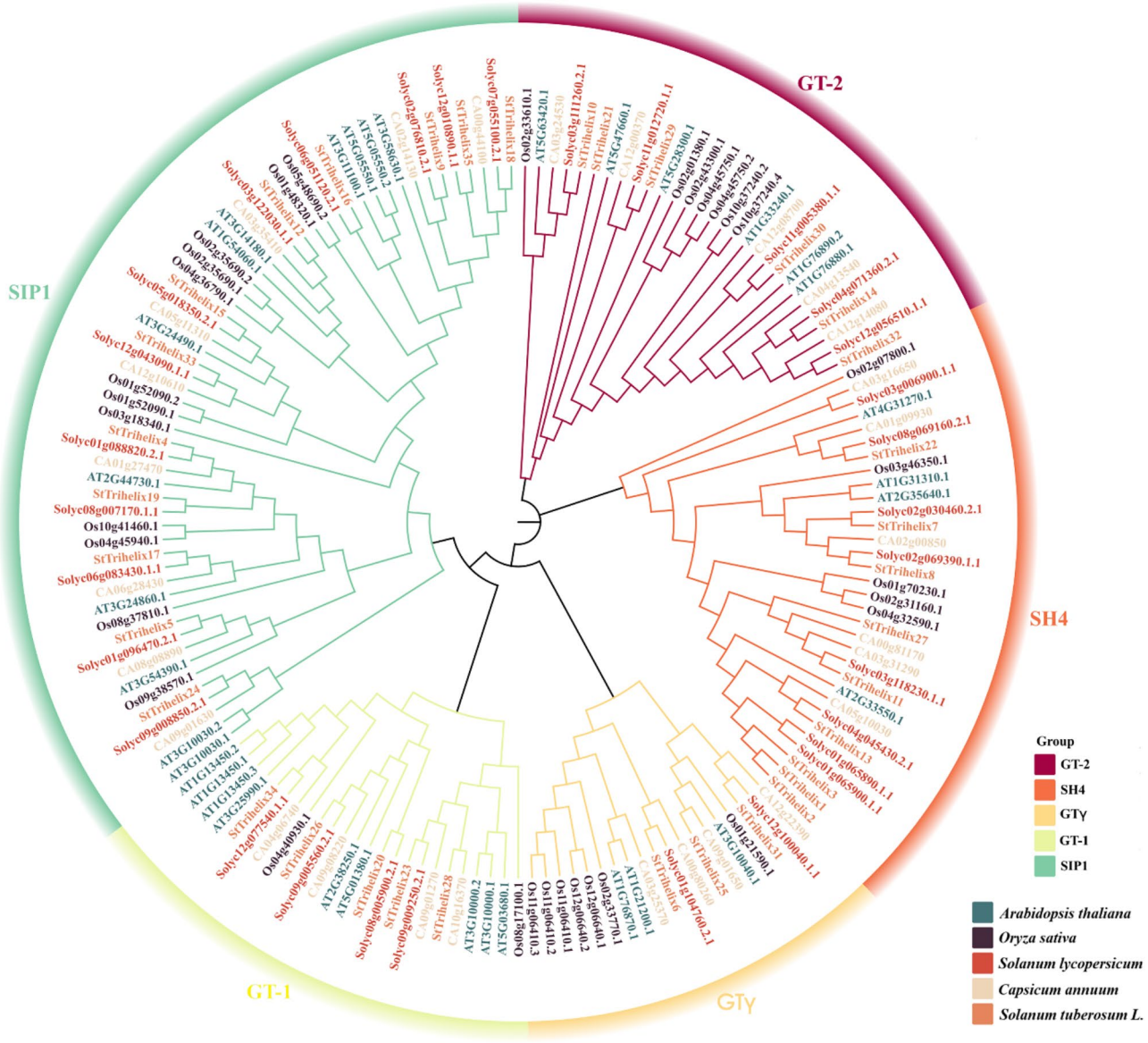

Fig. 2 Secondary structure analysis of the trihelix protein sequence in potatoes

(Fig. 4). Additionally, extended strands were observed, varying from 2.53% (*StTrihelix13*) to 16.7% (*StTrihelix10*) (Fig. 4). The final prevalent structure was the beta turn, with percentages ranging from 0.61% (*StTrihelix4*) to 10.38% (*StTrihelix27*) (Fig. 4).

Gene structure and motif analysis of sttrihelix

The majority of genes within the same subfamily exhibited consistent exon numbers and lengths (Fig. 5). Specifically, StTrihelix genes typically ranged from 1 to 3 exons. Notably, *StTrihelix1*, *StTrihelix3*, and *StTrihelix4* consisted of a single exon, whereas *StTrihelix10* was characterized by 18 exons and 17 introns. Furthermore, motif analysis revealed that the number of conserved motifs in the StTrihelix gene varied from one to seven (Fig. 5). Nonetheless, members of the same subfamily displayed comparable compositions and distributions of conserved motifs. It is noteworthy that *StTrihelix7* exclusively contained a single motif, Motif 1(Fig. 5).

Analysis of cis-acting elements of the trihelix-containing gene in potatoes

The analysis of cis-acting elements revealed that these genes encompassed elements associated with light responsiveness, hormone responsiveness, anaerobic induction, growth and development, and abiotic stresses (Fig. 6). Notably, all StTrihelix genes contained light-responsive elements, suggesting a significant role for trihelix in the regulation of light signaling in potatoes. The hormone response elements include TGACG

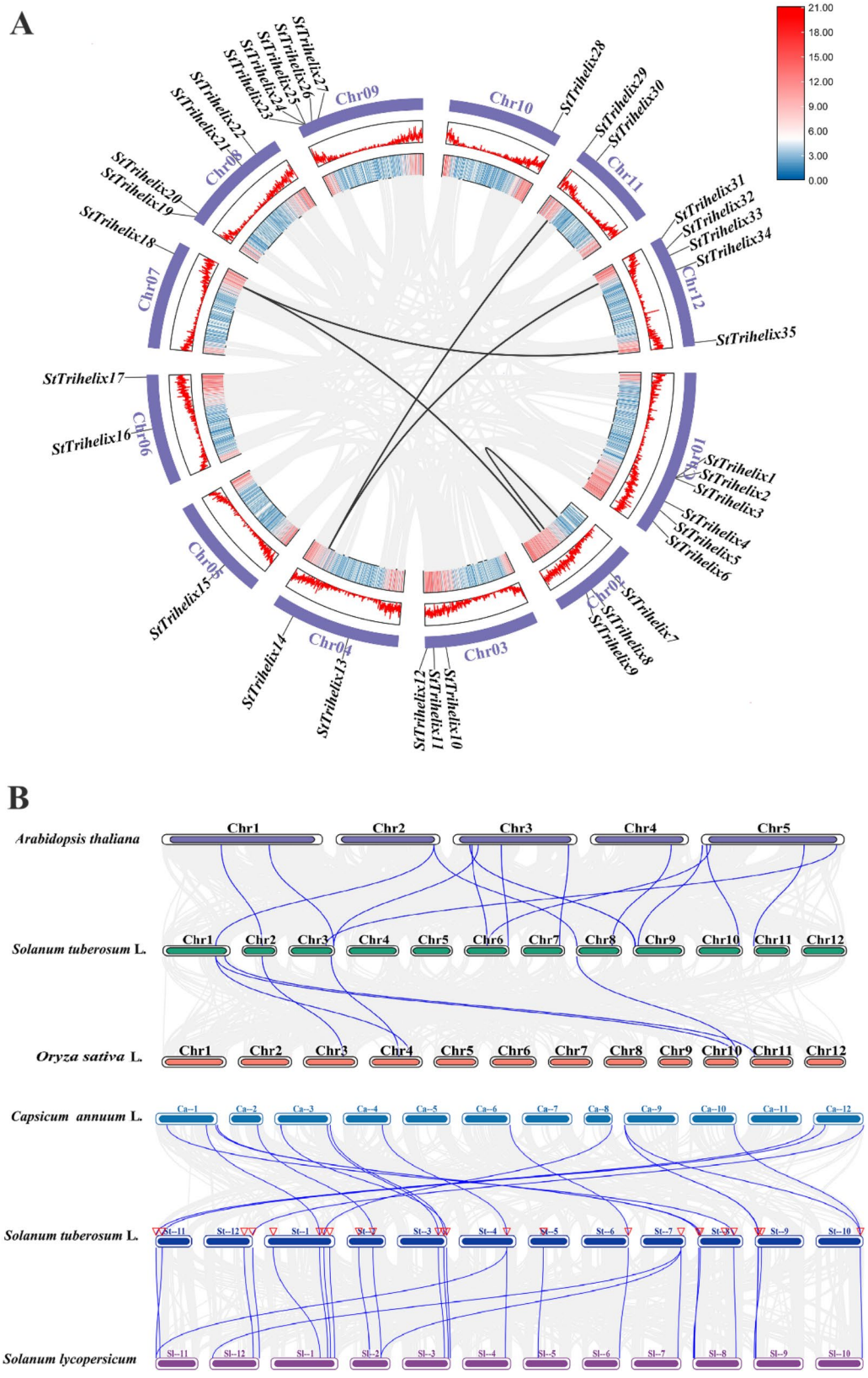

Fig. 3 Colinearity between (**A**) StTrihelix family members in potato and (**B**) trihelix genome in *Arabidopsis thaliana*, *Oryza sativa* L., *Capsicum annuum* L. *Solanum lycopersicum* and *Solanum tuberosum* L. The blue lines represent colinear blocks among genes containing trihelix motifs, and grey lines indicate colinear blocks within and between genomes

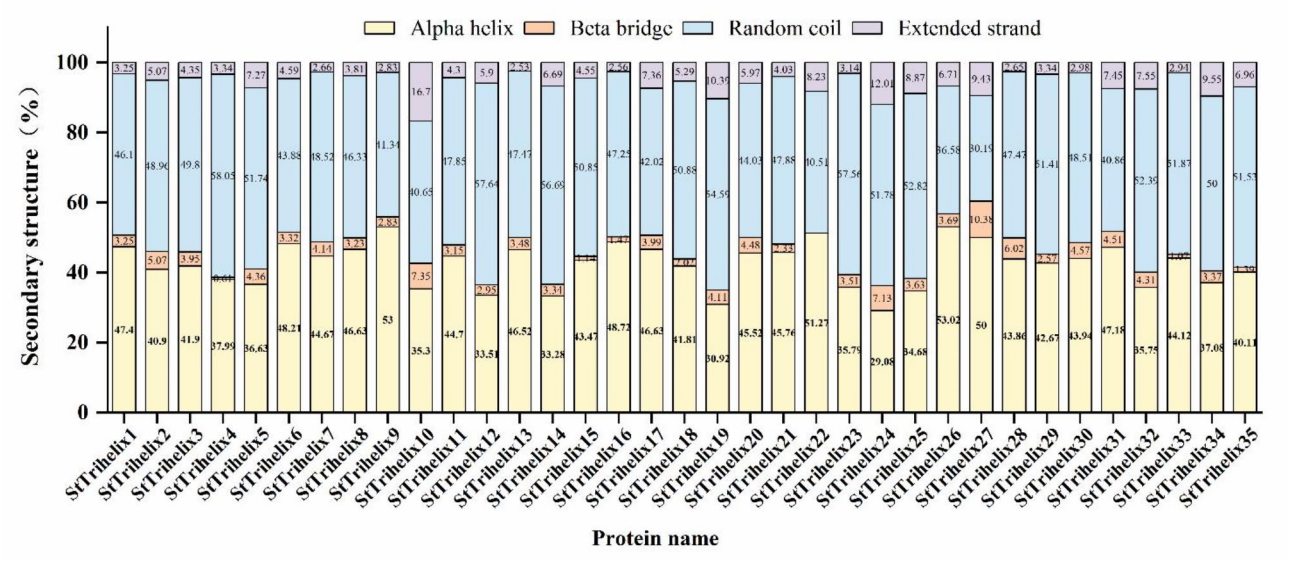

Fig. 4 Secondary structure of the potato trihelix protein

motifs associated with the response of methyl jasmonate (MeJA), TCA elements associated with the response of salicylic acid (SA), and P-box and GARE motifs associated with the response of gibberellin (GA) (Fig. 6). Additionally, various elements associated with adverse responses or defense mechanisms were identified, including MYB-binding sites (MBS), low-temperature response elements (LTR), mechanical damage signaling elements (WUN motifs), and defense and stress response elements (TC-rich repeats). The presence of G-box, GT1-motif, MBS, and ABRE elements in a relatively high proportion suggests the crucial involvement of this gene family in ABA, drought, and salt stress responses.

Expression analysis of the potato trihelix-containing genes in different tissues

Results of differential expression analysis of the potato trihelix family genes in different tissues are shown in Fig. 7. The findings revealed that *StTrihelix4* is highly expressed in the stamen. Additionally, *StTrihelix5* and *StTrihelix13* displayed higher expression levels in roots than in other tissues. Furthermore, *StTrihelix32* showed elevated expression levels in flowers, petioles, and stems, which surpassed those in roots, stolons, stamens, and leaves. Conversely, *StTrihelix1*, *2*, *3*, *6*, *21*, *27*, and *28* generally exhibited low expression levels in different tissues (Fig. 7). Overall, most StTrihelix gene members were expressed in various potato tissues.

Expression analysis of trihelix-containing genes in response to abiotic stress in potato

The relative expression levels of the 35 StTrihelix genes were analyzed using qRT-PCR under low-temperature, PEG, and NaCl treatments. The results indicated that *StTrihelix5* was not expressed (Fig. 8). After 24 h of NaCl treatment, 15 StTrihelix genes were upregulated. Notably, *StTrihelix31* peaked at 55.88 folds after 24 h under PEG treatment. Conversely, *StTrihelix26*, *StTrihelix27*, and *StTrihelix34* were downregulated under PEG treatment compared to the control, whereas other StTrihelix genes were upregulated. *StTrihelix4* was downregulated under low-temperature treatment. The relative expression levels of *StTrihelix3*, *9*, *15*, *22*, *30*, *32*, *33*, and *35* were higher than those of the control, with *StTrihelix30* exhibiting the highest fold change at 54.6 times that of the control. These findings suggest a potential association between the trihelix-containing genes in potatoes and their responses to low temperature, drought, and salt stress (Fig. 8).

Subcellular localization of StTrihelix30

The subcellular localization of *StTrihelix30* was investigated. The findings indicated that the positive control was present in the cytoplasm, cell membrane, and nucleus, whereas *StTrihelix30* was specifically localized in the nucleus (Fig. 9). This is consistent with the results of subcellular localization prediction. These results provide additional evidence supporting the involvement of the trihelix gene family in nuclear function (Fig. 9).

Discussion

Transcriptional regulation of genes is crucial for growth and responses to environmental stress. Different transcription factors play key roles in this process by interacting with the cis-acting elements or other transcription factors involved in gene expression [43, 44]. Trihelix transcription factors are a plant-specific class of GT factors because of their specific binding to GT elements

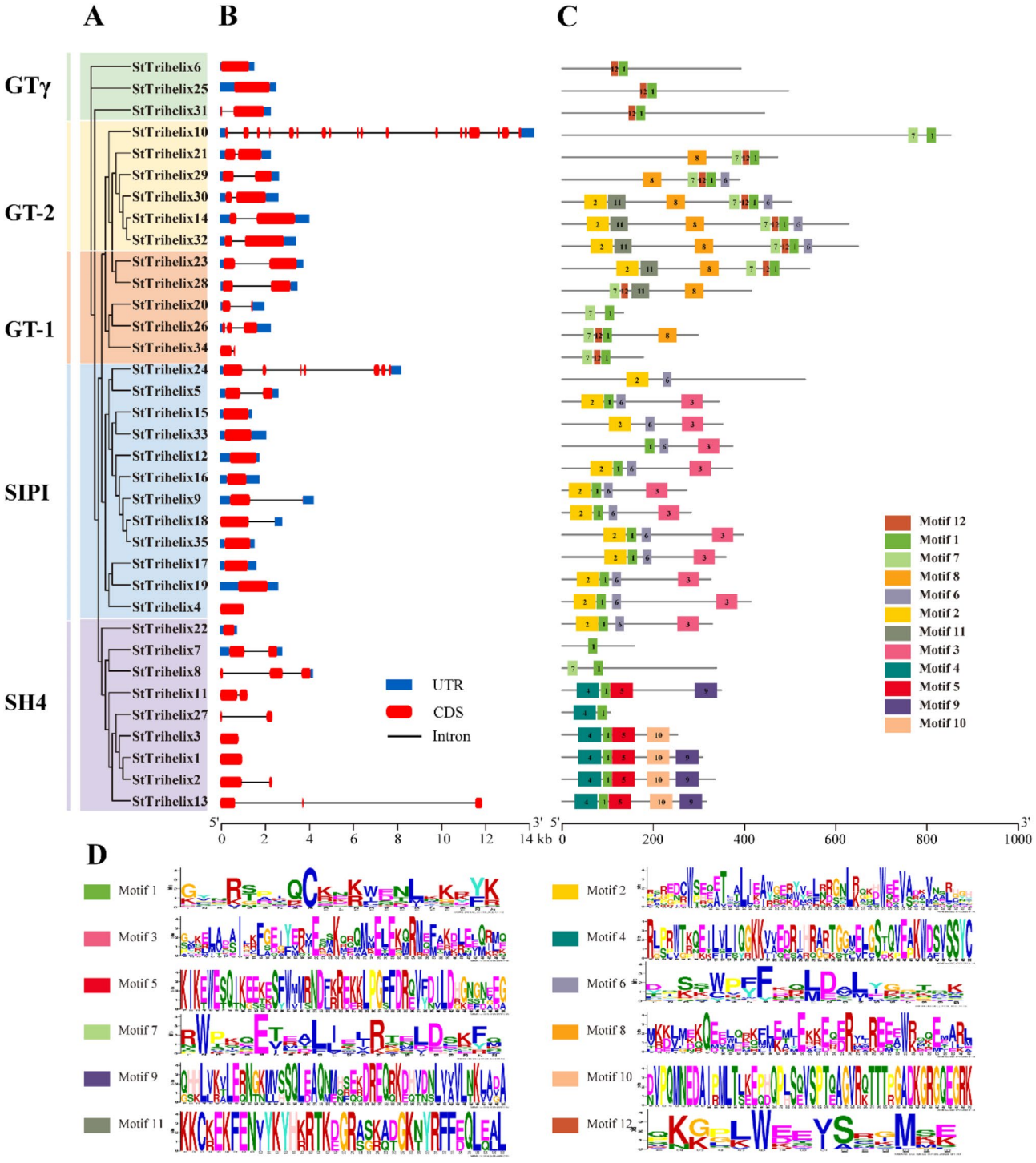

Fig. 5 Analysis of gene structure and protein-conserved motifs of the trihelix gene family. **A**, phylogenetic structure; **B**, gene structure map; **C**, conserved motif diagram; **D**, conserved motif logo diagram

[45–47]. Genome duplication during evolution provides plants with ample genetic material and a wealth of genetic variation, enabling them to better adapt to a range of environments, including drought, high salinity(200 mmol/L NaCl), and extreme temperatures [28, 48]. Trihelix proteins are directly or indirectly involved in various physiological processes, such as stress responses, development of perianth organs, trichomes, stomata, seed abscission layers, and regulation of late embryogenesis [32, 49, 50].

In the present study, 35 StTrihelix genes were identified in the StTrihelix family, similar to the 36 and 34 genes

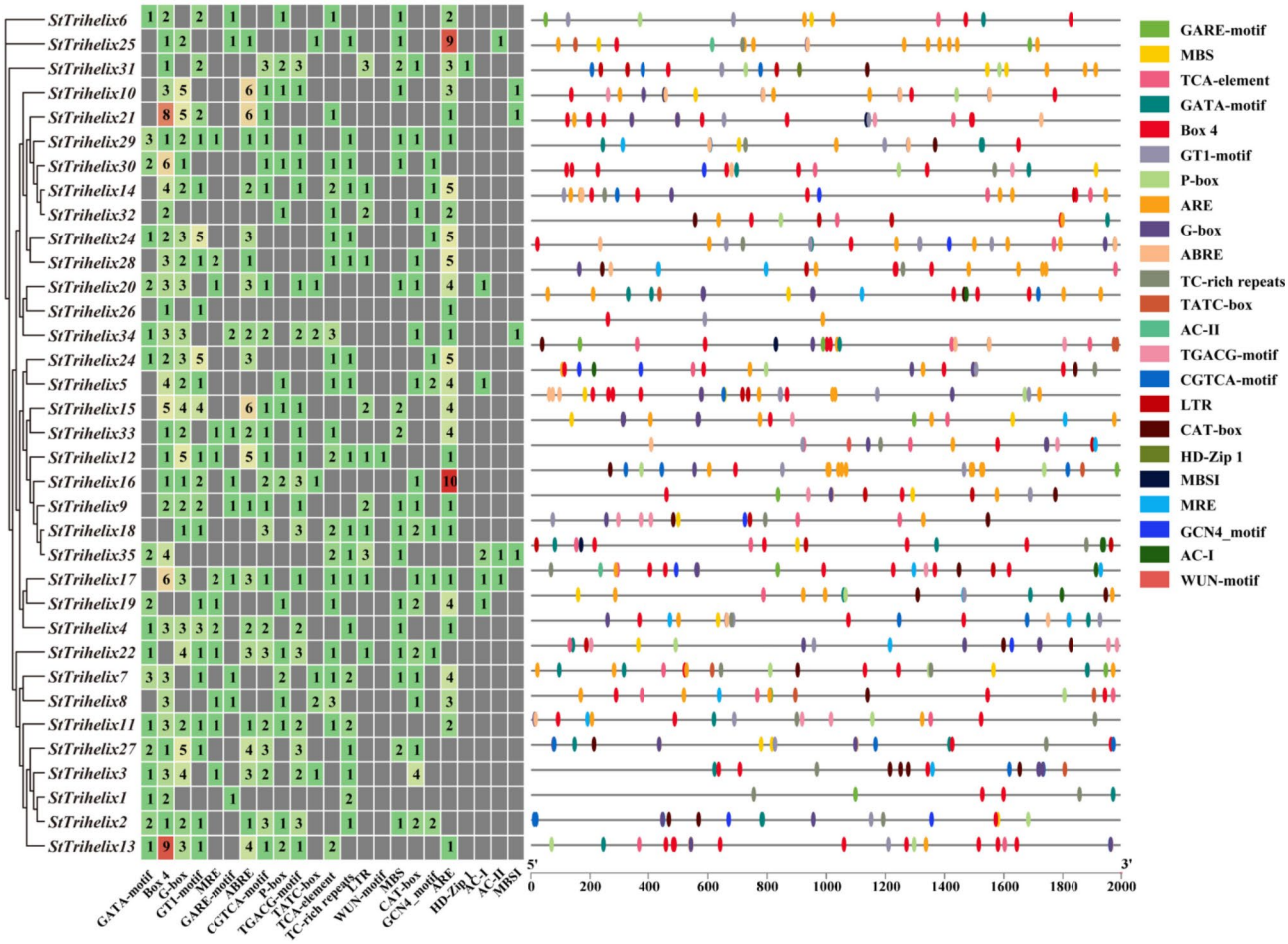

Fig. 6 Analysis of the *cis*-acting elements of the trihelix family genes in potato

identified in tomato and *Melilotus albus*, respectively [23, 35]. However, in comparison to *Populus trichocarpa* [41], *Sorghum bicolor* [48], *Chenopodium quinoa* [51], *Oryza sativa* [49], and *Zea mays* [52], the number of trihelix genes varied [51–55].

The trihelix gene family was initially classified into three subfamilies: GTα, GTβ, and GTγ [56]. Subsequently, based on the functional domains of amino acids, it was further categorized into five subfamilies: GT-1, GT-2, SH4, GTγ, and SIPI [19]. In the present study, through phylogenetic analysis, StTrihelix genes were also classified into five subfamilies: GT1, GT2, SH4, SIP1, and GTγ. The distribution of trihelix members was found to be the lowest in the GT-1 and GTγ subfamilies, whereas the SIP1 subfamily had the highest number of members. This distribution pattern was consistent with the observation of the largest number of SIP1 subfamily members in the trihelix genes of *Platycodon grandiflorum* [39]. In *chrysanthemum*, the SIP1 subfamily had the highest distribution of trihelix members, whereas the GT-1 subfamily had the lowest distribution. Noteworthily, unlike in potato, the SH4 subfamily in *chrysanthemum* does not contain trihelix members [57]. The present study aimed to predict the subcellular localization of the potato trihelix transcription factor family and revealed that the majority of the genes in this family are expressed in the nucleus. The findings suggest a potentially significant role for the transcription factor family in signal transduction within the potato nucleus.

Structural variations in genes play a crucial role in gene evolution because the integration and recombination of gene fragments can affect the number of exons/introns [58]. For example, *PtrGT42* and *PtrGT47* in the SIP1 subfamily of the *Populus trichocarpa* trihelix transcription factor family contain 17 exons, in contrast to the usual two or three exons found in most other genes in this subfamily [55]. Similarly, *StTrihelix10* in the GT-2 subfamily of the potato trihelix transcription factor family contained 18 exons, whereas most genes in this subfamily typically have only two exons. Furthermore, *StTrihelix24* in the SIP1 subfamily contained six exons in contrast to the usual single exons found in most other genes in this subfamily. These findings suggest that these specific genes have undergone multiple genetic evolutionary

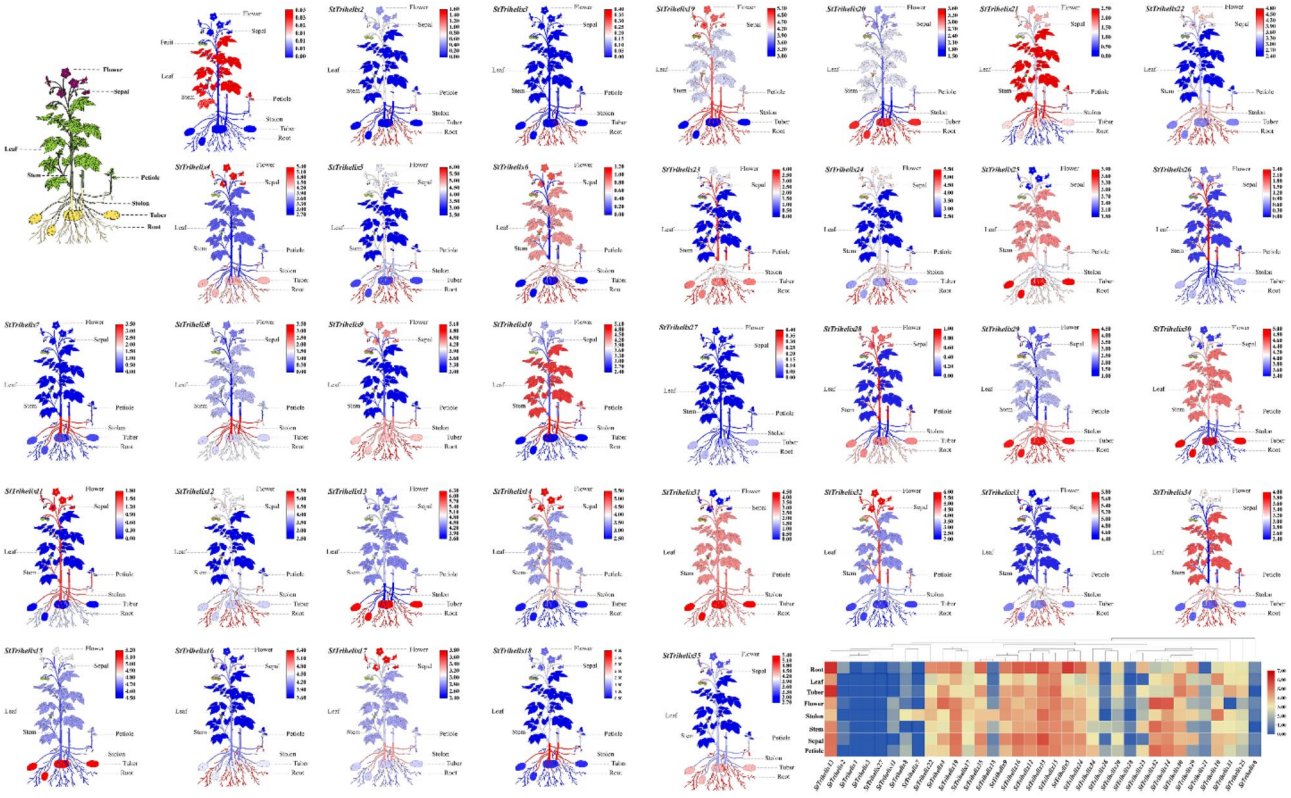

Fig. 7 Expression profile of the trihelix genes in different potato tissues and varieties

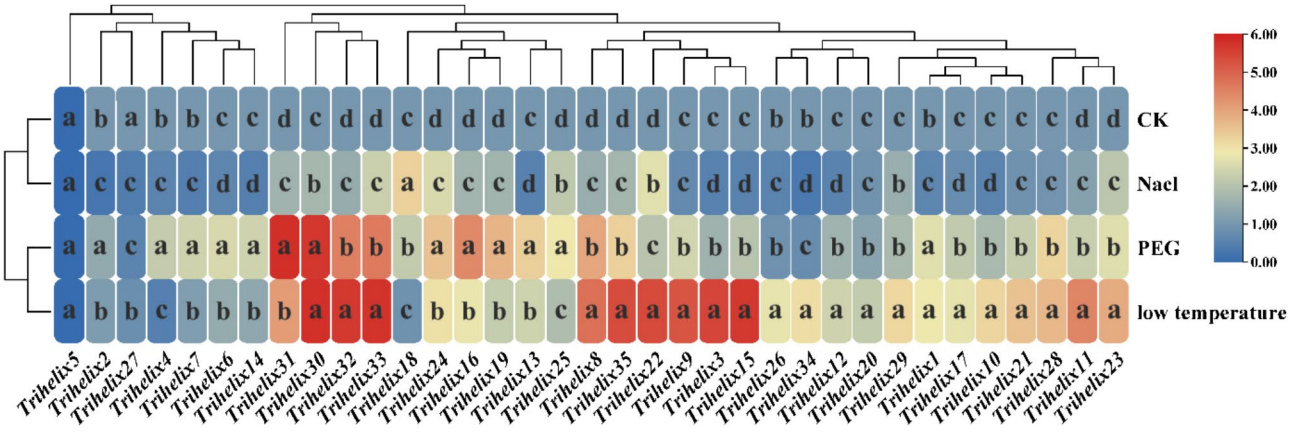

Fig. 8 Relative expression levels of potato trihelix genes under different abiotic stress treatments. The error bar represents the mean ± SE of the three biological replicates. Different letters indicate significant differences, and the same lowercase letters indicate no significant difference ($p < 0.05$)

events, resulting in an increased number of exons and potentially indicating functional divergence. Within a specific subfamily, most genes exhibit similar structures and conserved motifs, suggesting comparable functions and stable evolutionary patterns. Nonetheless, within the same subgroup, certain genes possess unique motifs that distinguish them from the others [55]. For instance, *StTrihelix7* exclusively contains Motif 1, which confers distinct functions compared with other genes.

Understanding tissue-specific expression is crucial for understanding gene function in plants [59]. The present study examined the expression patterns of trihelix-containing genes in different potato tissues, revealing that *StTrihelix4* was highly expressed in the stamen, whereas *StTrihelix5* and *StTrihelix13* showed elevated expression levels in the roots (Fig. 8). Most StTrihelix members displayed varying degrees of expression across different potato tissues, which is consistent with previous findings. Notably, the expression of strawberry FvTrihelix [60] was

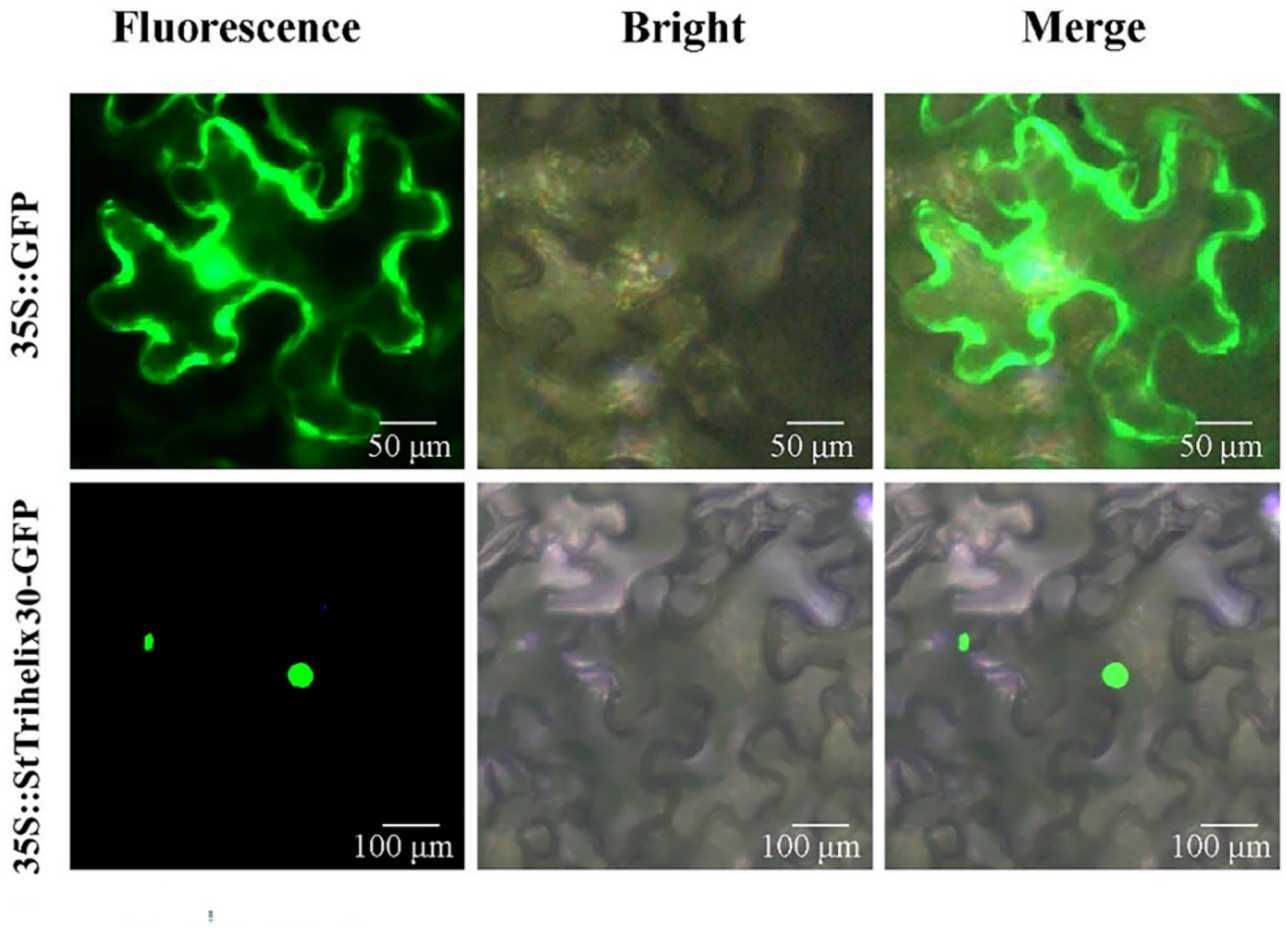

Fig. 9 Subcellular localization of *StTrihelix30*

also detected in various tissues, indicating that trihelix family genes exhibit tissue-specific expression patterns in potatoes.

Adverse environmental conditions, such as drought, high and low temperatures, and soil salinity, can significantly affect plant growth and productivity. In response to such stresses, plants may modulate the expression of stress-related genes to adapt better to challenging environments. Studies have found that in Arabidopsis, *AtGTL2* and *AtGTL1* in GT-2 can regulate the expression of cell cycle-related genes [29]; members of the GTγ subfamily are involved in the regulation of abiotic stress responses such as cold, drought, and salt [61]. Predictive analysis of cis-acting elements upstream of StTrihelix genes found that in the 2000 bp sequence upstream of the promoter region of the gene, there are response elements related to light response, hormone response, anaerobic induction, growth and development, and abiotic stress. This family has been speculated to play important roles in response to ABA, drought, and salt stress. Real-time fluorescence quantification revealed significant variations in trihelix expression in different potato varieties under various stress conditions. In tomato, six *SlGT* genes were highly up-regulated and others were slightly induced under salt stress. Notably, these six *SlGT* genes were distributed in all five subgroups of Trihelix genes (GT-1, GT-2, SH4, SIP1,and GT-γ) [23]. And in this work, StTrihelix genes exhibited both up and down regulation of gene expression in response to salt stress, the up-regulated StTrihelix genes were also distributed in all five subgroups after salt stress treatment of Potato seedlings for 24 h. It suggests that most subgroup members of Trihelix genes are responsive to salt stress.Moreover, StTrihelix genes primarily exhibited positive regulation under drought stress, similar to findings in *Sibirica alba* [35]. Additionally, StTrihelix genes predominantly showed positive regulation under low temperature stress, aligning with research conducted in apple, indicating similar outcomes [62]. In eggplant, the expression of most *SmGT* genes was up-regulated in the cold-tolerant variety "E7134" after 24 h of low-temperature stress treatment, and this finding is consistent with our results. Interestingly, while the expression of *SmGT25* and *SmGT27* was down-regulated after 1 day of low-temperature stress treatment, the *SmGT25* and *SmGT27* gene expression was also up-regulated as the days of low-temperature

treatment increased [31]. Thus, we speculated that the expression of *StTrihelix4*, *StTrihelix18*, and *StTrihelix27* in potatoes was down-regulated after 24 h of low-temperature treatment at 4 °C, probably due to the limitation of the treatment time, and that the expression of these genes might also be up-regulated with the prolongation of the low-temperature exposure time. Our experimental data demonstrated the nuclear localization of *StTrihelix30* in tobacco cells, confirming previous predictions and supporting similar findings on *P. grandiflorus PgGT1* [39]. Overall, further exploration of the interactions, functional analyses, and roles of trihelix genes in plant development and stress responses will provide a more comprehensive understanding of their regulatory mechanisms and biological functions.

Conclusion

In this study, genome-wide identification of the triple helix gene family of potato (*Solanum tuberosum* L.) was performed, revealing 35 triple helix genes distributed on 12 chromosomes. Phylogenetic analysis divided these genes into five subfamilies (GT-1, GT-2, GT-γ, SH4, and SIP1), indicating that these genes are highly structurally conserved in Arabidopsis. qRT-PCR analysis showed that 15 stritrihelix genes were significantly upregulated under salt stress, while most of the Stritrihelix genes were significantly upregulated under drought and low temperature. It is worth noting that *StTrihelix30* was the most sensitive to low temperature, and its expression was increased by 54.6 times. Subcellular localization experiments confirmed the nuclear localization of *StTrihelix30*, which was consistent with the predicted transcriptional regulation. These findings highlight the important role of stritrihelix gene in abiotic stress response and provide a theoretical basis for future research on potato stress resistance breeding.

Supplementary Information

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Supplementary Material 1

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Author contributions
All of the authors contributed to the conception and design of the study. HYY: Data curation, Writing – original draft. YW: Visualization, Writing – review & editing. WXY: Writing – review & editing. TTL: Data curation, Writing – review & editing. XJF: Writing – review & editing. BY: Writing – review & editing. GYS: Data curation, Methodology, Software, Supervision, Writing – review & editing.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare no competing interests.

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