

# Identification and response to abiotic stress of the alfalfa serine carboxypeptidase-like protein gene family

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## Abstract

A class of acyltransferase enzymes known as serine carboxypeptidase-like (SCPL) proteins play a pivotal role in plant growth, development, and responses to stress. While SCPL proteins have been extensively studied in various plants, their biological functions in alfalfa remain largely unexplored. In this study, 39 SCPL gene family members were identified in alfalfa and classified into three subgroups (Groups I–III). Their chromosomal structure and homology were also analyzed. Gene transcription levels were analyzed, revealing that six genes were expressed in a few specific tissues, while the remaining 33 *MsSCPL* were expressed throughout the tissues. Additionally, analysis of *cis*-acting elements revealed that the SCPL gene family contains components linked to abiotic stress. Meanwhile, transcriptome analysis revealed that 11, 16, and 14 members of *MsSCPL* gene family were found to respond to three stresses, respectively (cold, salt, and drought), in addition to *MsSCPL31/35*, which responded to all stresses at the same time. The transcriptome results were further validated by RT-qPCR. This highlights the role of *MsSCPL31/35* in abiotic stress response, suggesting their importance for plant adaptation mechanism. These results could be crucial for understanding the mechanisms behind alfalfa SCPL gene function.

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## Introduction

Known as the 'Queen of forages', alfalfa (*Medicago sativa* L.) is a vital perennial legume that provides affordable, nutritious, and easily digested fodder, with alfalfa hay containing over 20% of crude protein<sup>[1,2]</sup>. However, alfalfa yields are significantly affected by environmental stresses such as drought, alkalinity, and salinity. Enhancing the stress tolerance of alfalfa and mining its adversity-related genes are crucial steps for increasing yield. Recent advancements in sequencing technologies and declining sequencing costs have led to continuous refinement of the alfalfa genome, laying a solid foundation for genome-wide gene family research<sup>[3–5]</sup>.

The vast range of secondary metabolites produced by plants is impressive, yet many terpenes, phenols, fatty acid derivatives, and nitrogenous compounds are synthesized through a limited number of main pathways<sup>[6]</sup>. These metabolites gain their structural diversity through enzymatic modifications such as catalytic decarboxylation, oxidation/reduction, hydroxylation, glycosylation, methylation, and acylation<sup>[7]</sup>. Among these, acylation processes play a pivotal role in the formation of esters and amides, which are common modifications in secondary metabolites, by modifying oxygen- and nitrogen-containing substrates, respectively. Enzymes catalyzing these acyl activation are typically found in large protein families<sup>[8]</sup>. Acylation involves the introduction of alkyl (RCO-) or aromatic (ArCO-) acyl groups onto nitrogen, oxygen, carbon, or sulfur atoms in organic molecules<sup>[9]</sup>. Acyltransferases catalyze these reactions in organisms, influencing cellular functions and metabolism<sup>[10]</sup>. For example, SCPL genes are involved in the synthesis of galloylated catechins in tea plants<sup>[11]</sup> and chicoric acid in purple coneflower<sup>[12]</sup>. These enzymes are widespread in plants, animals, and microorganisms, transferring acyl groups from donors to acceptors<sup>[13–17]</sup>. SCPL enzymes, part of the S10 subfamily, share structural and functional similarities with

serine carboxypeptidases and possess conserved regions critical for catalysis<sup>[15–18]</sup>. SCPLs are distinct in their significant role in plant defense mechanisms<sup>[18,19]</sup>. The type I SCPL gene in tomatoes is a 'ate trauma-induced gene', meaning it is strongly induced by mechanical injury and exposure to methyl jasmonate (MeJA)<sup>[20]</sup>. Defense-related genes such as *OsPR1*, *OsPR2*, *OsPR5*, and *OsPDF1.2* were consistently up-regulated in transgenic rice plants, with those overexpressing *OsBISCL1* displaying notably higher resistance to *Pseudomonas syringae* compared to wild-type rice. Moreover, plants overexpressing *OsBISCL1* exhibited enhanced oxidative stress tolerance along with a significant increase in the expression of genes associated with oxidative stress<sup>[21]</sup>.

As an important forage crop, alfalfa is often cultivated in marginal environments, which are typically characterized by multiple abiotic stresses such as drought, salinization, and low temperatures. These stress conditions severely affect the growth and yield of alfalfa. In recent years, studies have shown that SCPL proteins play a key role in plant stress responses. For example, SCPL genes in tea plants are involved in the synthesis of galloylated catechins, enhancing the plants' tolerance to environmental stresses<sup>[11]</sup>. In purple coneflower (*Echinacea purpurea*), SCPL enzymes catalyze the production of chicoric acid, which helps plants resist pathogens and adverse conditions<sup>[12]</sup>. *Arabidopsis* plants overexpressing *TaSCPL184-6D* reportedly showed elevated proline levels and reduced malondialdehyde content, which contributed to improved tolerance to salt and drought stresses<sup>[22]</sup>. Given the unique stress challenges faced by alfalfa in marginal environments, it is hypothesized that the SCPL gene family may play an important role in alfalfa's stress adaptation. Therefore, this study aims to comprehensively analyze the SCPL gene family in alfalfa, including the number of genes, their structure, chromosomal distribution, and phylogenetic relationships. Moreover, the detailed analysis of the SCPL gene family will help

elucidate its functions in alfalfa and other closely related species. These efforts will enable researchers to gain a deeper understanding of the evolution, diversity, and physiological roles of the *SCPL* gene family.

## Materials and methods

### Methods for identifying and describing *MsSCPL* genes in alfalfa

The reference genome sequence and protein datasets for alfalfa (Zhongmu No. 1) were sourced from Figshare (<https://doi.org/10.6084/m9.figshare.12623960.v1>)<sup>[3]</sup>, while *SCPL* protein sequence data for 54 *Arabidopsis thaliana* entries was retrieved from the TAIR database ([www.arabidopsis.org](http://www.arabidopsis.org)). The genes in the alfalfa genome were subsequently co-identified using BLAST with an E-value threshold of  $> 1e-10$ . The HMM profile (PF00450) of the *SCPL* structural domain was obtained from the InterPro database ([www.ebi.ac.uk/interpro](http://www.ebi.ac.uk/interpro)) and analyzed using the Hidden Markov Model (HMM) method. Sequences were then entered into the NCBI Conserved Structural Domain Database ([www.ncbi.nlm.nih.gov/cdd](http://www.ncbi.nlm.nih.gov/cdd)) to confirm the presence of functional domains.

### Gene information and phylogenetic analysis

TBtools was used to examine and depict the chromosomal distribution of the identified *SCPL* family genes. The nomenclature of *MsSCPLs* was determined according to their chromosomal positions. The Expasy website ([https://web.expasy.org/compute\\_pi/](https://web.expasy.org/compute_pi/)) was utilized to evaluate the physicochemical properties of the identified genes. Subcellular localization predictions were performed using the WolfPSort online tool (<https://wolfsort.hgc.jp/>). Phylogenetic trees were constructed using MEGA software<sup>[23]</sup>. Multiple amino acid sequence alignments were conducted using ClusterX2.0 software with default settings. The neighbour-joining (NJ) method was applied to create a phylogenetic tree, and 1,000 bootstrap replicates were performed for validation.

### Gene structure, motif identification, and conserved domains

The MEME Sequence Analysis Tool (<https://meme-suite.org/meme>) was employed to predict conserved motifs in the *SCPLs*, with a limit of 24 motifs specified. Additionally, the gene structures of the genes were analyzed using the advanced gene structure view function in TBtools, and the results were visualized through TBtools.

### Gene duplication and synteny analysis

MCScanX was run to locate duplication points within genes and to identify regions of collinearity with *A. thaliana* and *M. truncatula*<sup>[24]</sup>. The results were subsequently visualized using TBtools.

### Promoter analysis

A 2,000 bp sequence upstream of the start codon for each identified gene was extracted and analyzed using the PlantCARE (<https://bioinformatics.psb.ugent.be/webtools/plantcare/html>) database to predict *cis*-acting elements<sup>[25]</sup>.

### Transcriptomic data analysis

The NCBI database (PRJNA276155, SRP055547) provided transcriptomic data for six alfalfa tissues: leaves, nodules, elongated stems, flowers, pre-elongated stems and roots<sup>[26]</sup>. Additionally, transcriptomic information for genes under drought, salt, and cold stress conditions was retrieved from the NCBI database (PRJNA454564, SRR7091780–SRR7091794 and PRJNA450305, SRR7160313–SRR7160357)<sup>[27]</sup>. The alfalfa reference genome (cv. Zhongmu No.1) was aligned using HISAT2<sup>[28]</sup>, resulting in the generation of SAM

files. Subsequently, the count values of the transcriptome data that corresponded to the genomic data were determined via the feature-Counts software (v2.0.1)<sup>[29]</sup>. The gene expression levels were then estimated based on the FPKM values derived from the R package DESeq2 (v1.40)<sup>[30]</sup>.

### RT-qPCR analysis of genes

Total RNA was isolated using TRIzol Reagent (Vazyme, Nanjing, China) and single-stranded cDNA was synthesised using a First Strand cDNA Synthesis Kit (Vazyme, Nanjing, China). RT-qPCR was conducted using a SYBR Premix Ex Taq (Takara, Japan) and a 7,500 real-time fluorescence quantitative PCR system. Primers for three *MsSCPL* genes were designed using NCBI's Primer-BLAST (Supplementary Table S1), and *Actin* from alfalfa was used as an internal reference gene. Gene expression levels were calculated using the  $2^{-\Delta\Delta Ct}$  method<sup>[31]</sup>. Each experiment was conducted with three technical replicates. Analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test for post hoc multiple comparisons, and different lowercase letters indicate significance at  $p < 0.05$ .

### Plant growth and treatments

Zhongmu No.1 alfalfa seeds were cold-primed at 4 °C for three days and then hydroponically grown in a greenhouse under a 16/8 h light/dark photoperiod at 24/20 °C day/night temperatures and 70%–80% relative humidity for two weeks. During cold treatment, leaves were maintained at 4 °C and sampled at 0, 2, 6, 12, and 24 h. To simulate salt stress, NaCl was applied at a concentration of 250 mM, and root tips were collected at six time points: 0, 1, 3, 6, 12, and 24 h post-treatment. For drought stress, mannitol (400 mM) was used to treat the root tips, with samples collected at six intervals: 0, 1, 3, 6, 12, and 24 h after treatment.

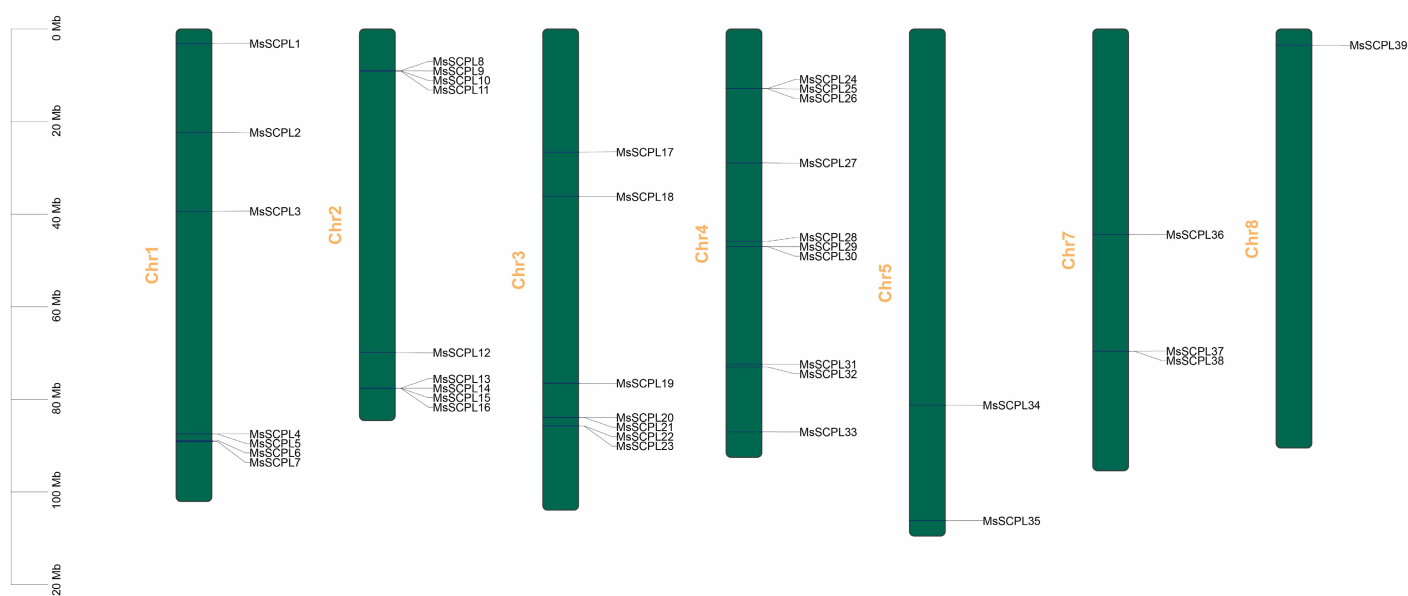
## Results

### Identification and description of *MsSCPL* genes in alfalfa

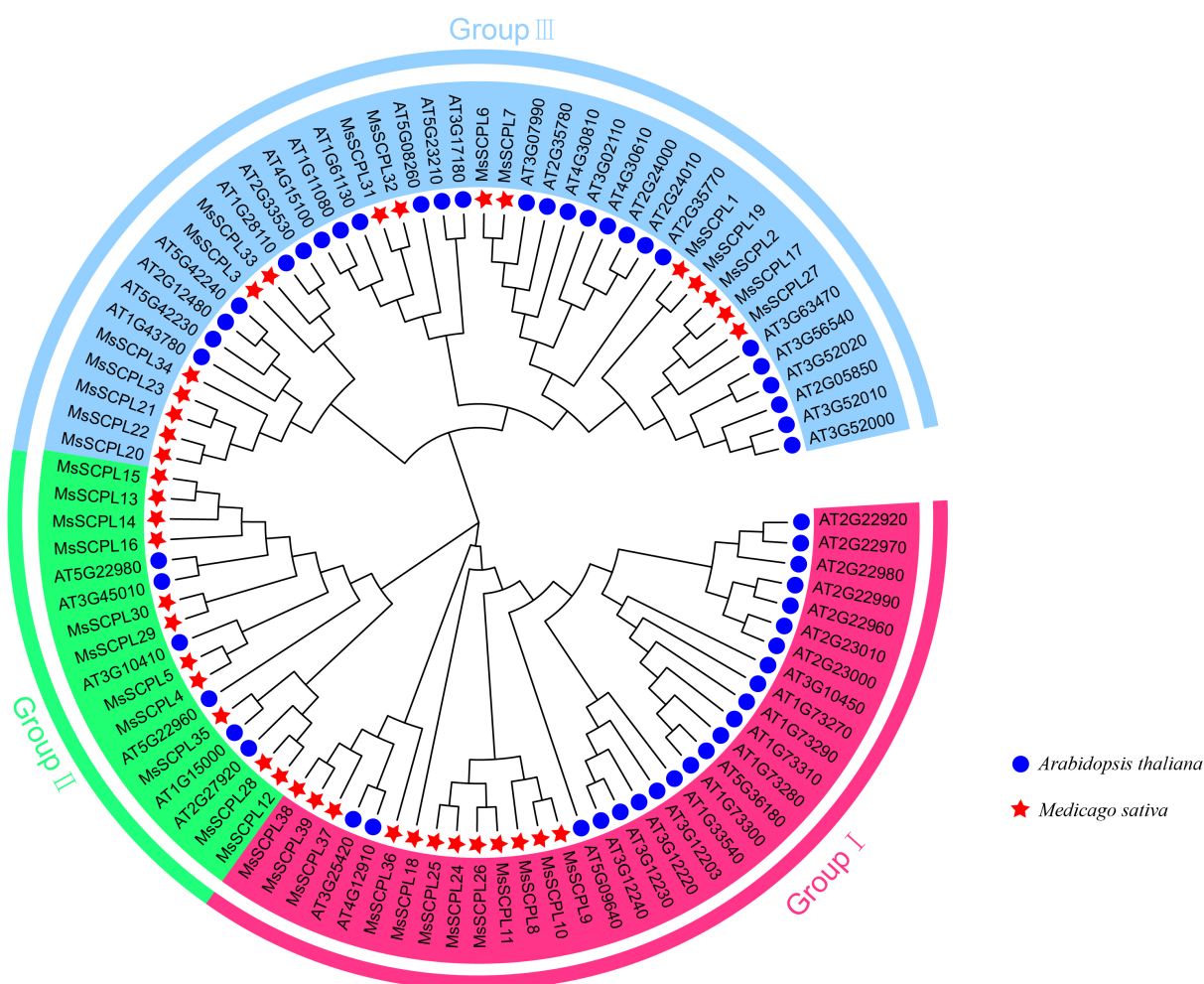
The 39 *MsSCPL* genes identified in the alfalfa genome are catalogued with their gene IDs, genomic positions, CDS lengths, protein sizes, molecular weights, isoelectric points, and predicted subcellular localizations (Supplementary Table S2). Among these 39 *MsSCPL* members, *MsSCPL26* has the shortest CDS sequence at 636 bp, while *MsSCPL19* has the longest, measuring 3,432 bp. The molecular weights of the proteins for these *MsSCPL* members vary from 15.24 kDa (*MsSCPL27*) to 128.54 kDa (*MsSCPL19*), and the pI values range from 4.74 (*MsSCPL11*) to 9.18 (*MsSCPL3*). According to the subcellular localization results, six members are found in the extracellular space, 13 in the chloroplast, seven in the vacuole, six in the cytoplasm, five in the nucleus, and two in the mitochondria.

The 39 genes are distributed across eight chromosomes, primarily concentrated on chromosomes 1, 2, 3, and 4 (Fig. 1). Chromosome 4 had the most *MsSCPL* genes, totaling 10. Interestingly, only one gene, *MsSCPL39*, was discovered on chromosome 8. A phylogenetic tree was built using sequences from 39 alfalfa proteins and 54 *Arabidopsis thaliana* *SCPL* gene family proteins to improve understanding of the taxonomic and genetic relationships between members of the alfalfa gene family (Fig. 2). The *SCPL* gene family can be categorized into three subgroups: Group I, II, and III. Group I contains 12 members, Group III has 11 members, and Group IIII, has the largest number, with 16 members. It is possible to predict the functions of proteins that occur in the same group because they generally have similar characteristics.

## Identification of the *SCPL* gene family in alfalfa



**Fig. 1** Chromosomal positions of members of the *SCPL* gene family in alfalfa.

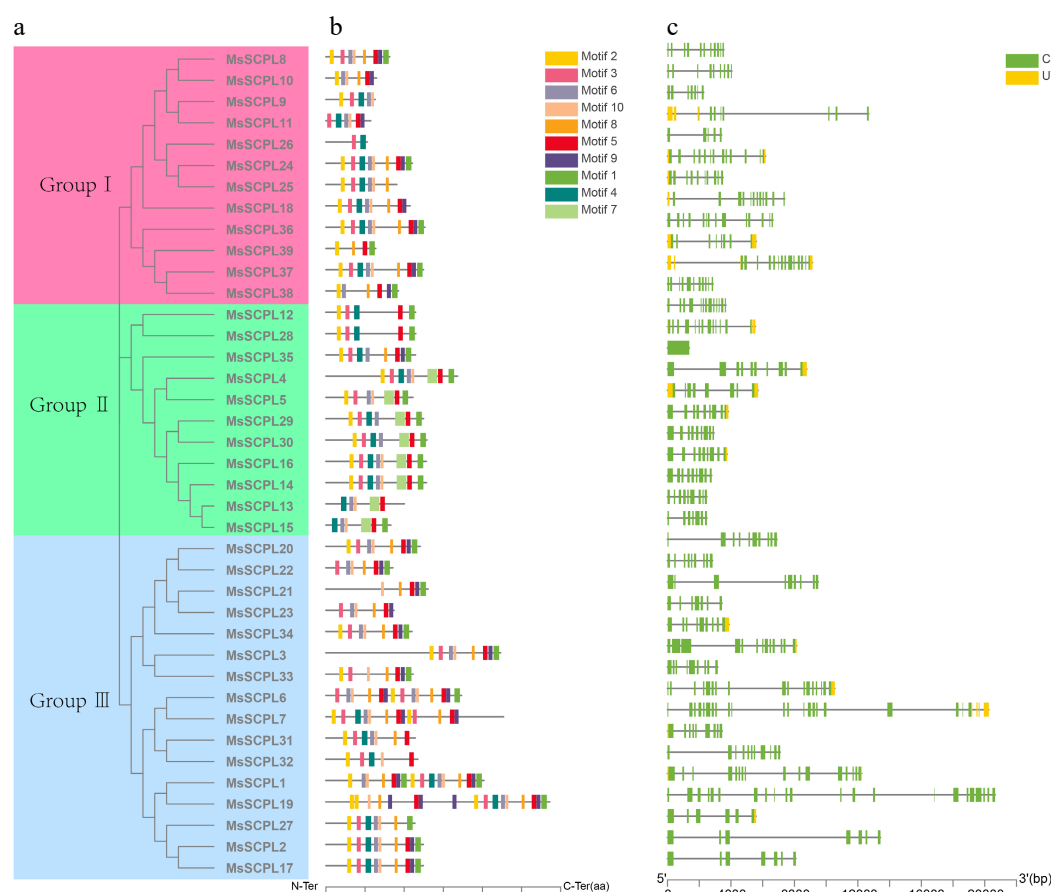


**Fig. 2** Phylogenetic tree of the *SCPL* gene family in *A. thaliana* and *M. sativa*.

## *MsSCPL* gene structure and conserved motif analysis

The conserved motifs of *SCPL* proteins were analyzed using the MEME online software to enhance understanding of the range and overlap of motifs within the *MsSCPL* gene family. Among the 39 *MsSCPL* members, 10 conserved motifs (motif1–motif10) were

identified (Fig. 3). Notably, motifs 3 and 4 were found exclusively in *MsSCPL*26 of Group I, while the majority of members contained 7 to 8 motifs. The motifs of *SCPL* proteins within the same subgroup are identical or similar. For instance, motif 5 is widespread in the *SCPL* proteins of Group II, whereas motifs 1 and 4 are predominantly



**Fig. 3** Structural characterization of SCPL in alfalfa. (a) Phylogenetic analysis of proteins. (b) Predicted motifs of proteins. (c) Exon-intron structure of the gene.

present in the majority of Group II members. Although only one protein in Group III lacks motif 8, all Group III proteins possess the conserved motif 10.

### Analysis of gene synteny and gene duplication events in *MsSCPL* genes

In alfalfa, tandem and segmental duplication events of *MsSCPL* were investigated to clarify the evolutionary processes that contributed to the expansion of the *MsSCPL* gene family (Fig. 4). Seven pairs of tandem duplication events were identified in the *MsSCPL* genes of alfalfa, and the data on these duplication events shed light on the diversity and complexity of the alfalfa genome, indicating a significant number of duplication events within the *MsSCPL* family. A covariance map was created to illustrate potential evolutionary events influencing the *MsSCPL* gene family across *M. sativa*, *A. thaliana*, and *M. truncatula* (Fig. 5). A total of 28 co-lineage pairs of SCPL genes were identified between alfalfa and *M. truncatula*, while seven co-lineage pairs were found between alfalfa and *A. thaliana*. Notably, the number of co-lineage pairs of SCPL genes between *M. truncatula* and alfalfa was four times greater than that between *A. thaliana* and alfalfa.

### Examination of *cis*-elements in *MsSCPL* gene promoter regions

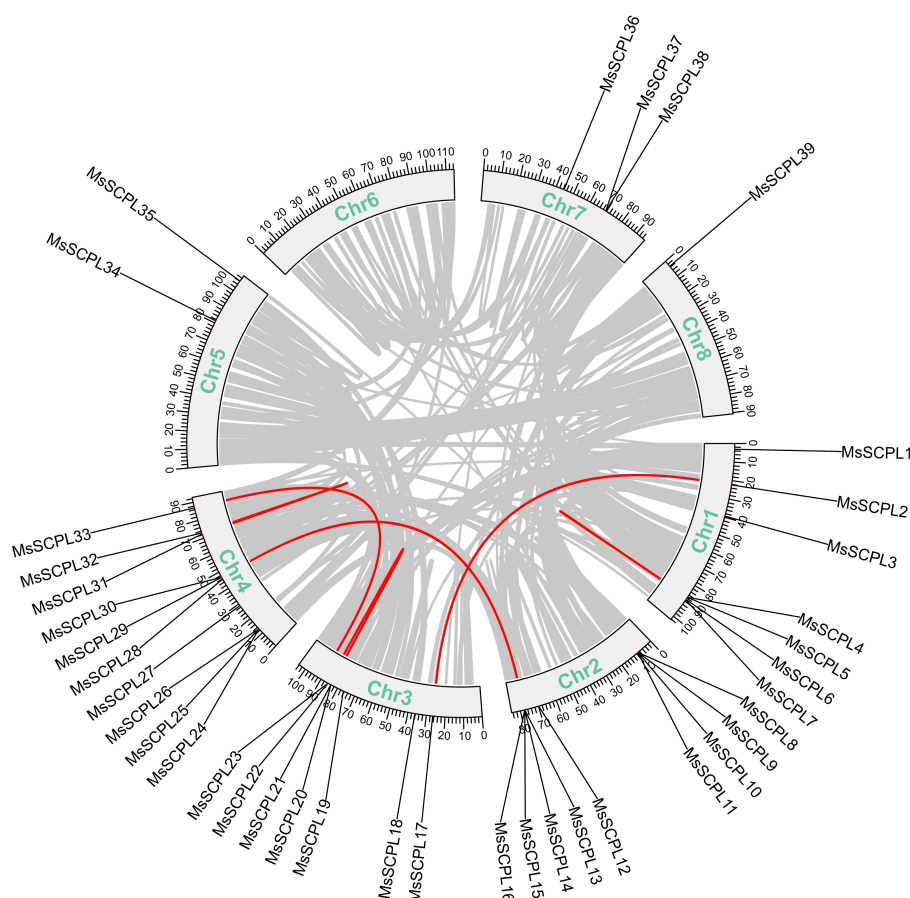
To better understand the biological roles of *MsSCPL* genes in alfalfa, an analysis was conducted of *cis*-elements in the promoter sequences of the genes, located around 2 kb upstream of the start codon (ATG). Thirteen distinct *cis*-elements related to hormone-responsive, plant development regulatory, and stress were identified in promoter regions (Fig. 6). Among these, hormone-responsive

elements are the most prevalent, comprising approximately 57%, while stress-related elements account for 30%, and development-related elements make up the remaining 13%. Within the hormone-related elements, those associated with jasmonic acid and abscisic acid responses (such as CGTCA-motif and ABRE) are more numerous, and some genes also contain auxin and gibberellin response elements (e.g., TGA-box, P-box, etc.). Stress-related elements primarily include anaerobic ARE elements, drought-related MBS elements, and low temperature-related LTR elements. It is noteworthy that most of the *MsSCPL* genes have ARE, ABRE, CGTCA, and TGACG elements, with ARE being present in all members of Group I.

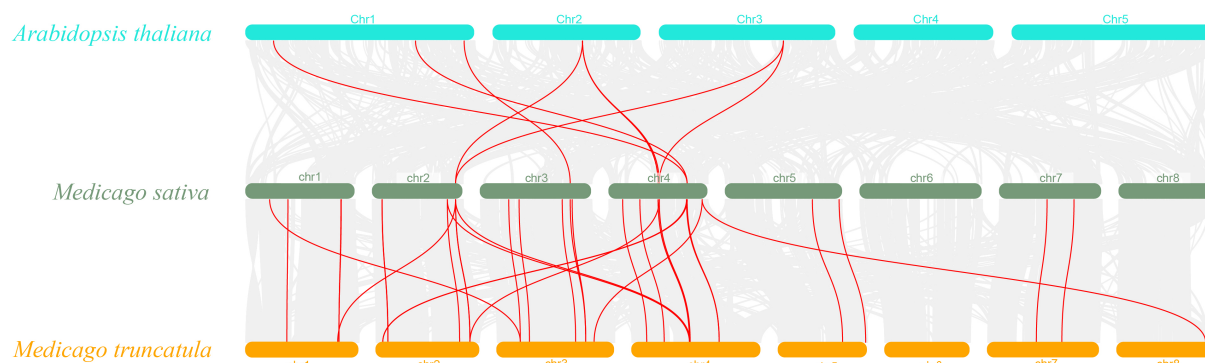
### *MsSCPL* gene expression trends in alfalfa tissues

Transcriptome data for six distinct alfalfa tissues were sourced from a freely available database to analyze the expression patterns of the *MsSCPL* genes in these various tissues. Thirty-three *MsSCPL* genes were expressed in six tissues, and the remaining six genes were partially expressed in six tissues (Supplementary Table S3). Specifically, only *MsSCPL36*, *MsSCPL20*, and *MsSCPL22* are expressed in flowers and nodules, while *MsSCPL12* is not expressed in post-elongated stems and roots. Additionally, *MsSCPL1* and *MsSCPL30* are each absent in only one tissue: *MsSCPL1* is not expressed in roots, and *MsSCPL30* is not expressed in post-elongated stems. In addition, the expression abundance of these *MsSCPL* genes differed significantly between tissues. For instance, *MsSCPL20/22/36* was found to be co-expressed in flowers and roots, but its expression level was significantly higher in flowers than in roots. Although 33 genes were expressed in all six tissues, *MsSCPL6/7/9/18/24* were predominantly expressed in leaves while *MsSCPL2/17/19/25/26/29/35/37/38* were highly expressed in roots.





**Fig. 4** Schematic representation of collinear connections of the gene in alfalfa. Segmental duplication events in the alfalfa genome are indicated in red, while collinear blocks are represented by grey lines.



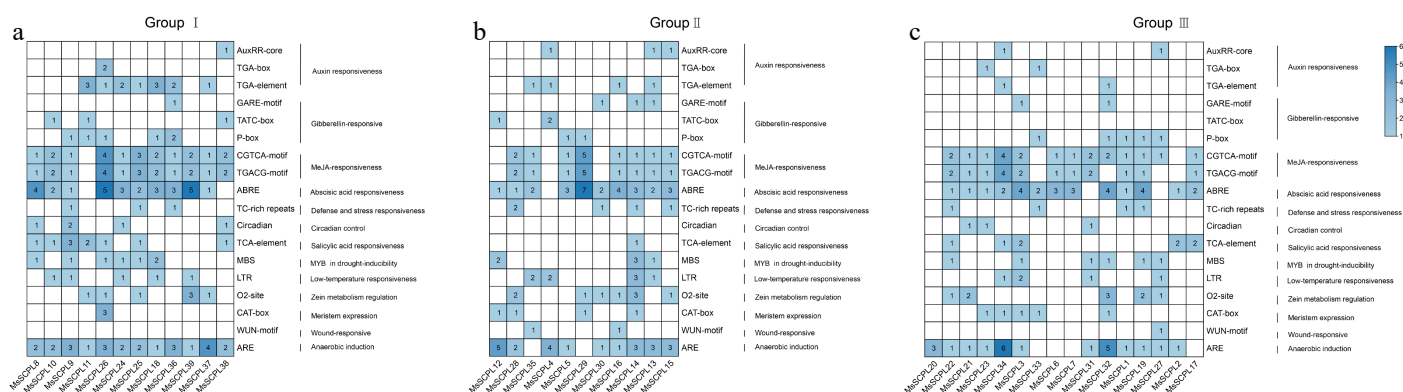
**Fig. 5** Synteny analysis of the *SCPL* gene between *M. sativa* and two representative plant species. Red lines highlight co-linear *SCPL* gene pairs, while grey lines in the background illustrate co-linear blocks between *M. sativa* and the selected plant species.

### Analysis of *MsSCPL* gene expression in abiotic stresses

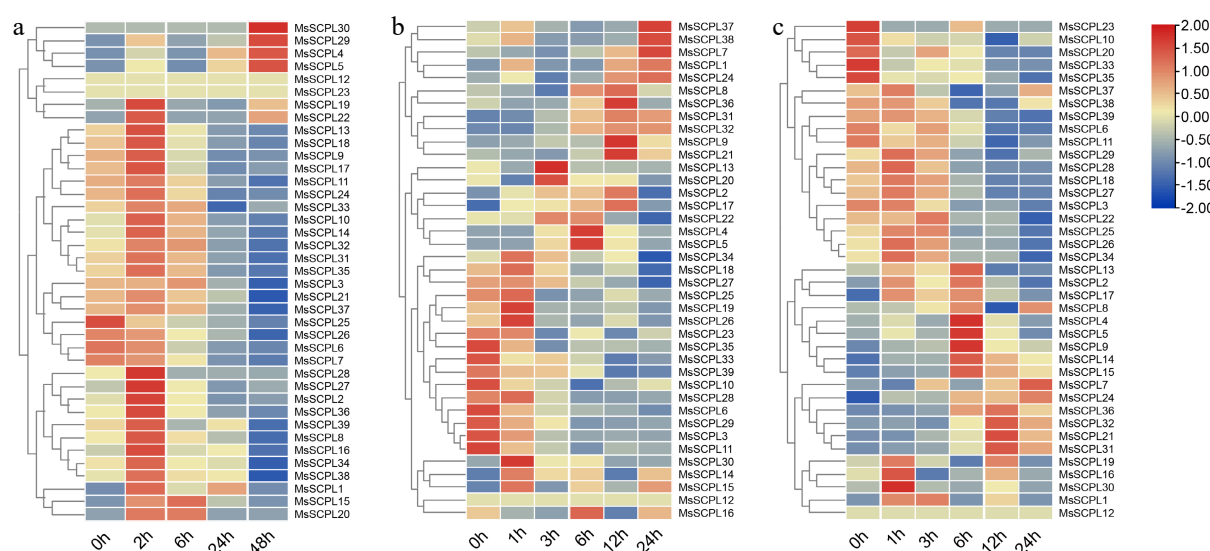
A public database was utilized to collect and analyze transcriptome data of alfalfa seedlings subjected to three stresses, aiming to elucidate the diverse expression levels of the *MsSCPL* genes under these three abiotic stresses. Expression profiling showed that 11, 16, and 14 *MsSCPL* genes were responsive to drought, salt and cold stresses, respectively (Supplementary Tables S4–S6; Fig. 7). Notably, *MsSCPL*6/7/29 responded to both salt and cold stress, while nine genes—*MsSCPL*17/19/27/28/32/33/34/37—were responsive to both salt and drought stress. Remarkably, *MsSCPL*31/35 showed responses to all three stresses; therefore, these two genes were selected for RT-qPCR validation analysis.

To verify the transcriptome data findings, RT-qPCR tests were conducted on two selected genes (*MsSCPL*31/35). Under the three

stress conditions, the expression patterns of *MsSCPL*31/35 varied over time (Fig. 8). Interestingly, under drought stress, the expression of *MsSCPL*31 showed an upward and then downward trend, and the expression was considerably increased compared to the control at 12 h. The expression of *MsSCPL*31 increased to a peak at 3 h of salt stress treatment, followed by a downward trend. Under cold stress, the expression of *MsSCPL*31 was up-regulated at 2 h of the cold stress treatment, and then showed a downward trend. Under drought stress, the expression of *MsSCPL*35 was first up-regulated at 0–1 h, then down-regulated at 3 h, then up-regulated to the maximum at 6 h, and finally showed a continuous down-regulation trend. After just 1 h of salt stress treatment, the level of expression of *MsSCPL*35 was considerably above that observed in the control, and then declined as a whole with the prolongation of the time of salt



**Fig. 6** Analysis of *cis*-acting elements in alfalfa *SCPLs*. (a) Panel represents the *cis*-acting elements of gene family members from Group I. (b) Panel represents the *cis*-acting elements of gene family members from Group II. (c) Panel represents the *cis*-acting elements of gene family members from Group III.



**Fig. 7** Expression of genes in response to cold, salt, and drought conditions. (a) Expression of genes under cold stress. (b) Expression of genes under salt stress. (c) Expression of genes under drought stress.

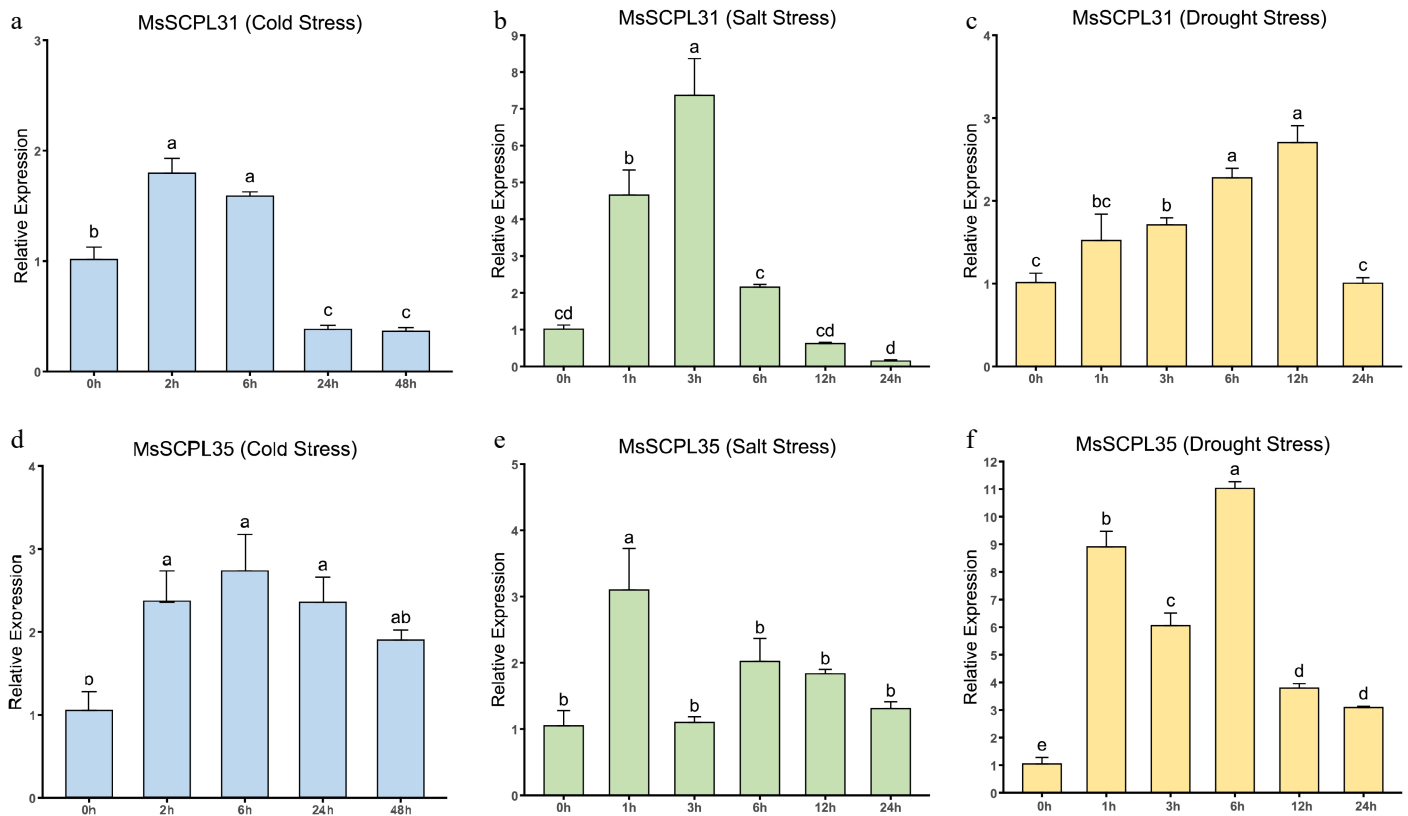
stress; and the expression of *MsSCPL35* first rose and then fell in response to cold stress. These genes should be further investigated for their roles under abiotic stress.

## Discussion

The *SCPL* gene, originating from an ancestral serine carboxypeptidase family, is significant in plant growth, maturation, and stress adaptation [21,32,33]. Genome-wide analyses of the *SCPL* gene family have been conducted in several plants, identifying 54, 210, 71, 41, and 96 *SCPL* genes in *Arabidopsis* [34], wheat [22], rice [35], soybean [36], and cotton [37], respectively. Comparing the number of *SCPL* genes across species shows that the *MsSCPL* count in alfalfa is relatively low. For instance, rice has 71 *SCPL* genes, soybean has 73, and wheat contains as many as 210. This high number in wheat is attributed to its complex heterozygous hexaploid nature, which underwent two whole-genome duplication events. In contrast, the alfalfa genome analyzed in this study is based on the haploid genome of 'Zhongmu No.1', leading to the identification of only 39 *SCPL* genes. Additionally, variations in threshold settings and screening strategies may also contribute to these differences in gene identification.

In this study, RNA-seq data from various alfalfa tissues (leaves, elongated stems, roots, pre-elongated stems, nodules, and flowers) revealed that *MsSCPL* genes exhibit high expression levels in roots

and flowers, suggesting their potential roles in plant development and formation processes. The *SCPL* acyltransferase is involved in various metabolic pathways, which are likely strongly linked to the plant's response to abiotic stress [36]. Moreover, the analysis showed that six genes are located extracellularly, while seven genes are situated in vesicles, indicating that *MsSCPL* genes may participate in diverse secondary metabolic pathways associated with abiotic stress responses. Promoter analysis of *MsSCPLs* revealed elements associated with developmental processes, resistance to environmental stress and reaction to hormones (Fig. 6). These results suggest that *MsSCPLs* may play critical roles in alfalfa's responses to different types of stress. Previous studies have shown that the *SCPL* gene family is relevant to how plants respond to salt and drought stress. For example, following three hours of ABA treatment and 68 h of salt and drought treatments, *TaSCPL1-24D* showed the highest expression levels in wheat [22]. It was reduced under cold, salt stress, and drought stress. This suggests that the *CsSCPL* gene may play a regulatory role in the response of tea tree to these stresses [11]. In potatoes, *StSCPL* genes showed significant expression under salt stress, heat stress, drought stress, and hormone treatments [38]. The RT-qPCR results of this study also demonstrated that the expression patterns of *MsSCPL31* and *MsSCPL35* under different abiotic stresses showed significant time dependence and stress specificity.



**Fig. 8** Expression of genes under drought, salt and cold stress conditions as determined by RT-qPCR. (a) Expression levels of *MsSCPL31* under cold stress. (b) Expression levels of *31* under salt stress. (c) Expression levels of *MsSCPL31* under drought stress. (d) Expression levels of *MsSCPL35* under cold stress. (e) Expression levels of *MsSCPL35* under salt stress. (f) Expression levels of *MsSCPL35* under drought stress. Different lowercase letters indicate significance at  $p < 0.05$ .

*MsSCPL31* is significantly up-regulated at 12 h under drought stress, suggesting its involvement in early stress responses; it reaches a peak at 3 h under salt stress, indicating a potential role in early defense mechanisms; and it is up-regulated at 2 h under cold stress, likely participating in rapid signal transduction. The peak expression of *MsSCPL35* at 6 h under drought stress may reflect its involvement in the early stress response rather than sustained stress acclimation; it is significantly up-regulated at 1 h under salt stress, followed by a decline, suggesting its role in early stress responses; and it displays an initial increase followed by a decrease under cold stress, likely contributing to early defense mechanisms. These distinct expression profiles are presumably closely related to their subcellular localization, protein interactions, and mechanisms of stress signal transduction. Future research could employ approaches such as gene knockout, overexpression, and protein interaction analysis to further elucidate the specific molecular mechanisms underlying these genes.

Alfalfa, as a high-quality forage crop, is rich in protein and has always held a significant position in the forage industry. However, most alfalfa cultivation areas are located on marginal lands, often facing stresses such as drought and salinity, making large-scale cultivation in these challenging environments difficult. In recent years, the rapid advancement of transgenic and gene editing technologies has facilitated significant progress in the development of stress-resistant alfalfa varieties. This research study identified 39 *SCPL* gene family members in alfalfa, and some were found to respond to drought, salt, and cold stresses. These responses can be functionally validated using molecular techniques, paving the way for the development of new resilient alfalfa varieties. Additionally, this enhances understanding of the biological functions of genes in alfalfa and

other plants, as well as the molecular mechanisms underlying abiotic stress regulation.

## Conclusions

This study comprehensively identified and analyzed the members of the alfalfa *SCPL* gene family. Phylogenetic analysis categorized the family members into Groups I, II, and III based on the distribution of the 39 gene loci across four chromosomes. Phylogenetic analysis showed that the *MsSCPL* gene family exhibited significant homology to the gene family found in *Arabidopsis*, and the expansion of the gene family in alfalfa was primarily driven by segmental duplications rather than tandem duplications. According to the gene expression data from different types of tissue, 33 *MsSCPL* genes were expressed in six tissues, while the remaining 6 genes were expressed in some tissues. *MsSCPL* genes are evidently crucial for plant growth and development. Moreover, the analysis of *cis*-acting elements has revealed that *SCPL* genes encompass several elements associated with abiotic stresses. The transcriptome data under different stresses showed that 11, 16, and 14 genes responded to cold, salt, and drought stresses, respectively, while *MsSCPL31/35* surprisingly responded to all three stresses simultaneously, and the transcriptome data were further validated by RT-qPCR. This study provides useful information about *MsSCPL* genes, but lacks further functional validation. In the future, techniques such as overexpression, gene editing, and molecular interactions may be used to validate and analyze the functions of the *MsSCPL* gene family. In summary, this study deepens the understanding of *MsSCPL* genes in response to abiotic stresses and provides candidate genes for stress-resistant alfalfa breeding.

## Author contributions

The authors confirm their contributions to the paper as follows: methodology: Zhu H, Liu H, Wu Y; investigation: Zhu H, Liu Q; software: Zhu H, Liu H, Xu Y; validation: Liu Q, Xu Y, Qu X, Chen Y; data curation & resources: Wu Y; original draft: Zhu H; funding acquisition, supervision & project administration: Yang Q, Long R; writing - review & editing: Liu Q, Qu X, Chen Y, Yang Q, Long R. All authors reviewed the results and approved the final version of the manuscript.

## Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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