



Involvement of Alfin-Like Transcription Factors in Plant Development and Stress Response

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Abstract: Alfin-like (AL) proteins are an important class of transcription factor (TF) widely distributed in eukaryotes and play vital roles in many aspects of plant growth and development. AL proteins contain an Alfin-like domain and a specific PHD-finger structure domain at the N-terminus and C-terminus, respectively. The PHD domain can bind to a specific (C/A) CAC element in the promoter region and affect plant growth and development by regulating the expression of functional genes. This review describes a variety of AL transcription factors that have been isolated and characterized in Arabidopsis thaliana, Brassica rapa, Zea mays, Brassica oleracea, Solanum lycopersicum, Populus trichocarpa, Pyrus bretschenedri, Malus domestica, and other species. These studies have focused mainly on plant growth and development, different abiotic stress responses, different hormonal stress responses, and stress responses after exposure to pathogenic bacteria. However, studies on the molecular functional mechanisms of Alfin-like transcription factors and the interactions between different signaling pathways are rare. In this review, we performed phylogenetic analysis, cluster analysis, and motif analysis based on A. thaliana sequences. We summarize the structural characteristics of AL transcription factors in different plant species and the diverse functions of AL transcription factors in plant development and stress regulation responses. The aim of this study was to provide a reference for further application of the functions and mechanisms of action of the AL protein family in plants.

Keywords: Alfin-like transcription factors; structural characteristics; growth and development; abiotic stress

1. Introduction

During the plant growth cycle, plants face various unfavorable conditions in the form of biotic and abiotic stress. Abiotic stress such as drought, salinity, and high and low temperatures are adverse environmental conditions that affect plant growth and development and ultimately reduce final crop production [1,2]. Under different stress conditions, plants modify or adapt different metabolic processes related to genetics or physiology [3,4]. Many important biological processes are associated with the regulation of gene expression. These regulatory pathways are complex and diverse and are influenced by a variety of factors, including transcription factors, which are now being studied in increasing detail for their effects on plant growth and development.

Transcription factors (TFs) are a class of regulatory proteins that bind to specific sequences upstream of the 5' end of target genes and play a critical role in the translation of stress signal perception into stress-responsive gene expression. During signal transduction, transcription factors interact with *cis*-acting elements in the promoter regions of various



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). stress-responsive target genes, thereby activating gene signaling cascades that act together and enhance plant tolerance to different environmental stresses [5]. To date, a number of stress-related transcription factors, including *DREB*, *NAC*, *MYB/MYC*, *WRKY*, *bZIP*, Alfinlike (AL) genes, and several other families, have been identified in plants [6–11]. These different families of transcription factors respond to various biotic and abiotic stresses in plants. Most plants are susceptible to biotic and abiotic stressors that affect yield and survival throughout their life cycle. Plants have developed tolerance systems that modify cellular biochemistry and physiology by altering gene expression in response to stress [12–14]. Among the TFs, stress-responsive TFs are involved in increasing plant adaptation to adverse environmental stress, such as cold, salt, and drought, as well as defense responses against invading pathogens [4,15]. The activity of these transacting factors represents a dominant and dynamic mechanism by which higher terrestrial plants can adapt to their environment [16].

AL transcription factors are a class of specific transcription factors involved in various types of stress in plants. It has been reported that the AL gene family plays crucial roles in plant growth and development, such as seed germination, root development, root hair elongation, and meristem development [17–19]. There are two main structural domains present in all AL proteins: an N-terminal conserved structural domain consisting of 140 conserved amino acid residues (the Alfin structural domain) and a C-terminal conserved PHD-finger structural domain consisting of 50 conserved amino acid residues (the Cys4-His-Cys3 type), along with a variable region, the V structural domain, located between these two structural domains [20]. Both of these particular structural domains play important roles in plants and in promoting different stress responses to different environments.

Due to the important functions and limited reports of AL transcription factors in plants, the main purpose of this review was to summarize and collate the results of other studies on AL proteins and to perform simple bioinformatics analysis of AL proteins based on the results of several years of related research. For this purpose, a phylogenetic tree of the AL gene family was constructed, and cluster analysis, motif analysis, and corresponding function analysis of AL proteins were performed. In addition, we also provide an outlook for future research in the hopes that further studies on the molecular mechanism and different signal transduction pathways of this gene family can be carried out in the future. This review will lay the foundation for revealing the uncharacterized functions of the relevant AL proteins in plants.

2. Survey Methodology

Based on previous research reports on AL proteins in A. thaliana (https://www. arabidopsis.org/, accessed on 20 September 2023), Z. mays (https://www.maizegdb.org/, accessed on 20 September 2023), B. rapa and B. oleracea (https://brassicadb.cn, accessed on 20 September 2023), Oryza sativa (https://plants.ensembl.org/index.html, accessed on 20 September 2023), S. lycopersicum (https://solgenomics.net, accessed on 20 September 2023), and P. trichocarpa (https://plants.ensembl.org/index.html, accessed on 10 January 2024), we searched for AL proteins in different species by reviewing and analyzing the contents of the relevant literature, including the earliest analyses of A. thaliana and relevant articles that have been published within the last few years. All relevant protein sequences were described in previous related studies. The different genome sequences were used to construct a phylogenetic tree using MEGA11 according to the LG + F method. Gene structures were mapped using TBtools based on relevant annotation information, and conserved structural domains were obtained using HMMER (https://www.ebi.ac.uk/ Tools/hmmer/search/hmmscan, accessed on 13 January 2024). The conserved protein motifs of the AL transcription factors from seven species were analyzed using MEME (https://meme-suite.org/meme/tools/meme, accessed on 13 January 2024). Figure quality was improved using version 2020 of Adobe Illustrator software (Adobe Illustrator 2020.lnk, accessed on 13 January 2024).

3. Structural Characteristics and Classification of the Alfin-Like Transcription Factors

Alfin-like transcription factors are small and unique transcription factors in plants that play different roles at different stages of growth and development. Alfin-like transcription factors were first identified in *Medicago sativa (alfalfa)*. In that study, Alfin was revealed to be the first storage protein synthesized during the development of somatic cells and syncytial embryos. In somatic embryos, the predominance of Alfin 7S storage proteins is associated with an increase in their mRNA accumulation. Mature (14 d) somatic embryos were closest to stage VI syncytial embryos in terms of protein and mRNA accumulation. Developmental comparisons also indicate that the synthesis patterns of individual storage proteins are regulated independently of each other during *alfalfa* embryogenesis [21]. AL proteins also showed 50–66% sequence identity to alfalfa Alfin1 [20]. Previous studies reported that AL proteins are mainly localized in the nucleus and play crucial roles in plant transcriptional regulation [11,20,22,23]. AL transcription factors are two-domain proteins that can bind to highly methylated forms of histones and function in plant abiotic stress [23,24]. AL proteins have two conserved domains located at the N-terminus and C-terminus, namely, the DUF3594 domain (or Alfin domain) and the PHD-finger domain. In addition to shaping the stress response, reactive oxygen species (ROS) act as signaling mediators to maintain redox homeostasis. Recent studies have shown that reactive oxygen species production and redox metabolism are closely related to endoplasmic reticulum stress. The reactive oxygen species (ROS)-related transcription factor *GmAlfin09* and the peroxidase *GmPRDX6* have been identified in soybeans, and experimental results confirm that GmAlfin09 promotes the up-regulation of *GmPRDX6*, which reduces ROS levels and promotes endoplasmic reticulum stability [25]. Although the DUF3594 domain has been less well studied, the highly conserved nature of the DUF3594 domain across species suggests that AL proteins may have a fundamental biological function in plants. The AL family is a plant-specific subfamily of plant homeodomain (PHD) finger proteins [22]. Similarly, PHD-finger proteins exhibit root-specific salt responses after binding to conserved GNGGTG or GTGGNG sites in the promoter regions of target genes [26]. PHD-finger proteins play crucial roles in different physiological processes in plants. For example, it mediates epigenetic silencing and vernalization-induced flower regulation in Arabidopsis [23,27-29]; it also binds to histone H3K4me3/2 [23] and to OBERON1 (OBE1) and OBERON2 (OBE2) to maintain root tip meristematic tissue [30]. In soybean, the histone methylation and acetylation of different lysine residues provide a platform for *GmPHD* to bind to stress-related genes and regulate their expression under salt stress [31]. The N-terminal DUF3594 structural domain (also known as the PAL structural domain) [32] consists of 140 highly conserved amino acids that functionally mark the transcriptional start site of all genes and recognize trimethylation of Lys-4 of histone H3 (H3K4me3) [18,33,34]. This structural domain contains five α -helix structures and two β -structures and exists as a conserved homodimer of AL family proteins [32]. However, no AL proteins containing this structural domain have been reported in animals, fungi, or prokaryotes [35].

Previously, several AL transcription factors were identified in different crop species, such as *A. thaliana, B. rapa, B. oleracea, Z. mays, S. lycopersicum, O. sativa, P. trichocarpa, P. bretschenedri,* and *M. domestica,* which contained 7, 15, 12, 18, 11, 9, 9, 15 and 11 AL members, respectively [11,14,36–41]. To better understand the evolutionary relationships of AL TFs, based on the similarity of protein sequences, an unrooted phylogenetic tree was constructed using the LG + F method. According to the phylogenetic tree, the AL protein family members of seven species were divided into four branches (Figure 1), which is consistent with the previously reported *A. thaliana* AL protein classification [36]. Interestingly, the distribution of the AL genes varied among the groups based on the species; for example, almost all the maize AL genes were clustered into groups one and two, with the third group containing the fewest members and no members from maize. Nine *PtAL* genes were distributed across all groups except for the third group, and they exhibited different levels of stress response to salt, drought, cold, and heat [39]. This difference might be due to the high sequence similarity between the different maize AL proteins. The various

AL genes clustered in different groups perform specific functions. *AtAL5*, in the third group, regulates various signaling pathways in plants, and overexpression of *AtAL5* repressed the transcription of downstream negative regulatory genes, including SHMT7, TAC1, OFE, FAO, and CAX1, to improve salt, drought, and cold stress tolerance in transgenic seedlings [20]. BoAL8 is responsive to different abiotic stresses [38]. It has also been suggested that the novel Cys4-His-Cys3 potential zinc finger structure is distributed within group one of alfalfa proteins; Alfin1 might play a role in transcriptional regulation; and Alfin1 might bind to G-rich DNA elements in the promoter of *MsPRP2*, a salt-inducible and root-specific gene [26]. AL6 in A. thaliana responds differently to phosphorus-stimulated elongation of root hairs and AL7 to salt stress [17,36]. BoAL12 and SIAL3, in the fourth group, also exhibit some stress responses to different abiotic stresses. In addition, AL proteins can bind to PRC1 to form complexes and thus participate in many complex biological processes. For example, the SUMO protease FUG1 physically interacts with AL3, and interference with its potential SUMOylation site disrupts its nuclear localization. AL3 interacts with LHP1 of the PRC1 complex, and the FUG1-AL3-LHP1 module is essential for conferring repeat amplification-associated epigenetic silencing of the SUMO protease FUG1, histone reader AL3, and PRC1 complex, which are integral to repeat expansion-induced epigenetic silencing in A. thaliana [42].

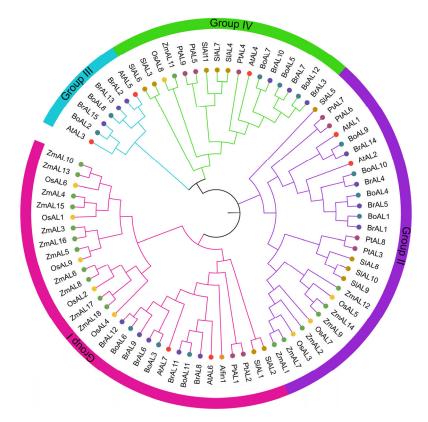


Figure 1. Phylogenetic analysis of *Arabidopsis thaliana, Brassica rapa, Zea mays, Brassica oleracea, Solanum lycopersicum, Populus trichocarpa* and *Oryza sativa* AL transcription factors. The graph was constructed in MEGA11 using the LG + F approach.

Similarly, cluster and conserved motif analyses revealed that the AL family proteins contained two conserved domains, the Alfin domain and the PHD domain (Figure 2B). The PHD-finger and Alfin domain distribution sites indicated that these two domains had coevolutionary connections with one another. To gain insight into the diversity of the gene structures, we performed a simple structural analysis of the full-length cDNA of AL genes (Figure 2C). Structural analyses revealed that most of the genes with similar numbers and lengths of introns/exons were typically clustered into the same group. The AL proteins distributed in different groups are essentially composed of 5–6 CDSs, and only individual

CDSs differ in number. The lengths of the introns are different across all AL members but somewhat similar within the same group. In general, similarities in the gene structure are closely mirrored in the phylogenetic tree. Motif analysis revealed that almost all the AL proteins in the different species contained the two essential structural domain motifs. Among them, motifs 2 and 6 encode the PHD-finger structural domain, and motifs 1, 3, and 5 encode the DUF3594 structural domain (Figure 3A). Motifs 4 and 7 are absent only in *ZmAL17* and *ZmAL9*, while both motif structures are present in all the other proteins. Motif 8 is present in most AL proteins, and a small number of AL members do not have this structure. We speculate that this difference may be related to later evolution. None of the 27 AL proteins clustered in the second group had motif 9, which was present in members of the other three groups. We speculate that this pattern is related to the proximity of evolutionary relationships between species. The fact that all the AL family members with similar exon/intron numbers and motif patterns clustered in the same group implies that they have similar functional roles in plants. However, there is little information about the function of AL transcription factors in plants, and further investigation is required to determine the complete regulatory mechanism involved.

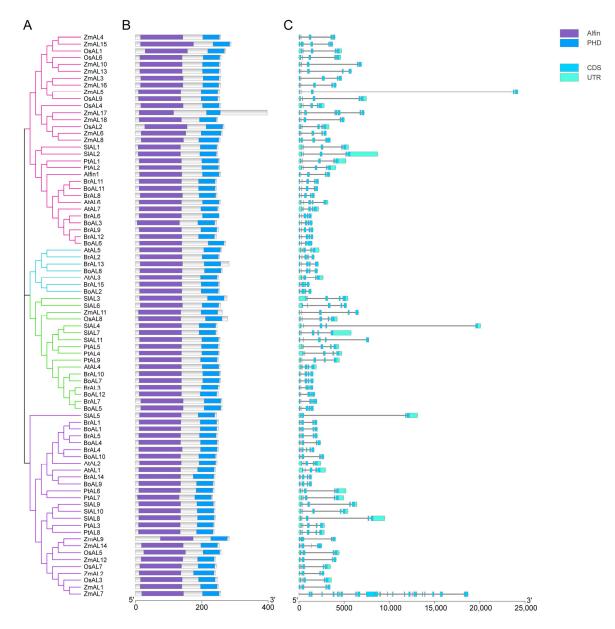


Figure 2. Cluster analysis of A. thaliana, Z. mays, B. oleracea, B. rapa, S. lycopersicum, O. sativa, and

P. trichocarpa AL transcription factors. (**A**): Phylogenetic tree of 82 AL transcription factor proteins; (**B**): Conserved structural domains. Purple for Alfin structure, blue for PHD structure; (**C**): Gene structure. CDS and UTR are shown in different coolers, and introns are represented by a black line.

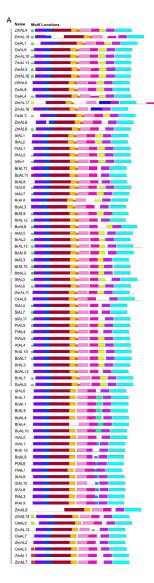




Figure 3. Motif analysis of *A. thaliana, Z. mays, B. oleracea, B. rapa, S. lycopersicum, O. sativa,* and *P. trichocarpa* AL transcription factors (**A**): Structure of 82 AL transcription factor protein conserved motifs. Different color boxes represent different motifs; (**B**): 20 motifs. Protein conserved motifs were analyzed using MEME (Introduction-MEME Suite) (https://meme-suite.org/meme/tools/meme, accessed on 13 January 2024) for AL transcription factors in seven species.

4. Role of Alfin-Like Transcription Factors in Plant Growth and Development

4.1. Alfin-Like Transcription Factors Regulate Seed Shape and Seed Germination

Rice is one of the most important food crops in the world. Recently, grain-related AL genes have been identified in rice. The *OsAL* genes were analyzed and found to be localized in the nucleus and not to have transcriptional self-activating activity. Most of the natural variants of *OsALs* were significantly associated with yield traits, grain size differences, and drought tolerance. It was observed that the *OsAL7.1* and *OsAL11* transgenic plants exhibited significantly regulated seed shape under drought stress and were more sensitive to ABA and mannitol at the germination stage [43]. The PAL domain at the N-terminus of AL proteins can bind to the PRC ring finger and participate in the seed germination process,

and the function of AL PHD-PRC1 complexes is to convert the H3K4me3 active state to the H3K4me3 repressive state of seed developmental genes during seed germination. T-DNA insertion mutant analysis revealed that simultaneous loss of *AtAL6/AtAL7* and *AtBMI1a/AtBMI1b* retards seed germination due to loss of function of the AL PHD-PRC1 complex [19].

4.2. Alfin-Like Transcription Factors Regulate Plant Root Development

Phosphorus deficiency is a limiting factor for plant growth, and a limited amount of plant-available soil phosphorous leads to severe yield losses [44]. Phosphorus deficiency causes a decrease in the number of primary roots and an increase in lateral root density and length [45–48]. Alfin-like transcription factor AL6 pure mutants produced shorter root hairs under phosphorus deficiency conditions, probably caused by altered expression of its downstream target genes. In addition to affecting root hair length, the per2 mutant also exhibited changes in primary root elongation, lateral root numbers, anthocyanin accumulation, and phosphorous ion concentrations. These results suggest that AL6 acts as a novel upstream regulator of root hair formation during phosphorus deficiency in A. thaliana [17]. Overall, we speculate that there are likely to be growth- and development-related functions of AL transcription factors, but confirmation of this speculation requires additional work in the future.

5. Role of Alfin-Like Transcription Factors in Plant Biotic and Abiotic Stress

5.1. Role of Alfin-Like Transcription Factors in Biotic Stress

Biotic stress, such as those applied by insects, pathogens, and weeds, affects plant morphological, physiological, and metabolic processes as well as defense responses [49–51]. Maize anthracnose, caused by fungal infiltration, severely compromises maize yield. In maize leaves infected by Gramineae anthracnose, ZmAL5a expression continuously increased with time. It has been shown that inoculation of plant leaves with B. graminids activates the resistance of distal uninoculated plant tissues to fungal pathogens and that this signaling involves the accumulation of β -1,3-glucanase in SA and abscisic acid (ABA) signaling. Gene expression analysis of ZmAL5a in the C_4 crop maize further showed that the ZmAL5a, β -1, and 3-GA2 genes not only respond to abiotic stress (salt and drought) but also that ZmAL5a is involved in the signaling pathway for β -1,3-GA2 gene expression [52]. B. oleracea contains 12 ALs transcription factors, and it was observed that various abiotic and biotic stress significantly enhanced the transcript levels of these ALs, particularly the expression of BoAL8 and BoAL12, after inoculation with Pectobacterium carotovorum subsp. carotovorum [38]. The Vitis quinquangularis AL transcription factor VqAL4 binds directly to the G-rich element (CACCTC) promoter of VqNSTS4 to activate its expression. In addition, overexpression of VqAL4 positively regulates powdery mildew resistance in grapes by inducing stilbene accumulation and SA signaling [53]. Recent studies have shown that in tobacco mosaic virus (TMV)-infected cells, the NLR protein induces phosphorylation of the transcription factor AL7 through activation of the mitogen-activated protein kinase (MAPK) cascade. AL7 suppressed downstream ROS-scavenging genes and enhanced ROS accumulation in the infected cells. However, under normal conditions, NLR proteins inhibit AL7 phosphorylation to prevent the down-regulation of ROS-scavenging genes and excess ROS accumulation [54].

5.2. Role of Alfin-Like Transcription Factors in Abiotic Stress

To date, studies on AL transcription factors are few. In 1992, the *Alfin1* gene was first identified in *alfalfa*, and its overexpression was induced by salt stress [21]. A total of 7 *AL* genes were identified in *A. thaliana* [55], and *Thellungiella halophila ALs* have a high degree of sequence identity with those of *A. thaliana*. In plants, *AL* genes regulate multiple signaling pathways related to environmental factors and abiotic stress tolerance [26,36]. In addition, four *AhAL* genes were isolated in *Atriplex hortensis*, all of which bind to *cis*-acting and transinhibitory nuclear localization proteins. Previously, *AhAL1*-expressing transgenic plants

were shown to have greater survival under salt and drought stress conditions, with lower MDA contents and less water loss than wild-type plants. *AhAL1* can bind to the promoter regions of GRF7, DREB1C, and multiple groups of PP2C genes to inhibit their expression, resulting in abscisic acid (ABA)-mediated stomatal closure, seed germination inhibition, and primary root elongation enhancement in transgenic plants. However, the expression levels of the stress-related positive regulatory genes DREB1A, DREB2A, and ABFs were increased in AhAL1 overexpressing plants [22]. With the continuous development of bioinformatics, an increasing number of AL transcription factors have been identified in plants. Fifteen BrALs were identified in kale, and all of these BrALs were induced by cold, salt, and drought stress. Similarly, 18 ZmAL genes were identified in maize, and various abiotic stresses increased the transcript levels of many AL genes in maize. The AL family comprises nonspecific transcription factors involved in abiotic stress response in dicotyledonous plants, and early studies have shown that the expression of OsALs is regulated by different environmental stimuli and phytohormones, with overexpression of OsAL7.1 and OsAL11 in rice impairing drought tolerance at the maturity stage [43]. Both ING proteins and AL proteins are involved in chromatin regulation of nuclear proteins through binding to H3K4me3/2. The Alfin proteins are also considered H3K4me3/2 readers [19,23] and perform functions related to AtRING1 and AtBMI1 interactions [56]. Six members of the AL gene family were identified in grapevine, and analysis of the upstream promoter region revealed a large number of cis-acting elements related to phytohormones and abiotic stress responses. Expression pattern analysis revealed that these AL transcription factors are closely related to the phytohormones ABA, drought, salt, and low-temperature stress. In particular, members of this family strongly respond to salt stress [57].

In recent years, an increasing number of researchers have begun to study AL transcription factors. The Daucus carota DcPSY2 gene promoter contains Alfin response elements that bind to DcAL4 and DcAL7 to regulate various developmental and stress-related regulatory mechanisms. Similarly, the expression of both the DcAL4 and DcAL7 genes was induced by ABA treatment and salt stress conditions. Furthermore, the ecotypic expression of DcAL4 and *DcAL7* enhanced the survival of *A. thaliana* seedlings during salt stress conditions [58]. In apples, all 11 MdAL genes exhibited differential expression patterns after exposure to various abiotic stress conditions. Similarly, compared with the wild type, the overexpression of MdAL4 positively regulates A. thaliana seedling tolerance to drought stress by reducing drought stress damage such as from ROS, malondialdehyde (MDA), and electrolyte leakage [41]. Recently, genome-wide identification and analysis of the AL protein family in cultivated tomato (S. lycopersicum) and three wild relatives (S. pennellii, S. pimpinellifolium, and S. lycopersicoides) were performed to evaluate the AL response to different abiotic stresses, and it was revealed that nuclear localized SIAL3 gene expression was induced by both drought and salt stress [11]. Fifteen PbAL genes have been studied and reported in P. bretschenedri, and analysis of the promoter regions revealed a number of stress-related cis-acting elements associated with hormonal and environmental stress responses. The differential expression of *PbAL* genes under indoleacetic acid, gibberellin, melatonin, and abscisic acid treatments was subsequently analyzed via qRT-PCR, which revealed that 15 *PbAL* genes responded to both abiotic stress and fruit growth and development [40]. Nine AL transcription factors were identified in poplar, and analysis of these nine proteins revealed that most of the *PtAL* genes were highly expressed at low temperature (4 $^{\circ}$ C), at high temperature (39 °C), under 100 mM NaCl treatment, and under drought treatment. PtAL4 and PtAL6 are sensitive to different types of stress, and PtAL5 and PtAL7 exhibit resistance to different types of stress. PtAL genes play important roles in plant growth and development and in response to abiotic stress, providing valuable insights into plant stress tolerance [39].

The above studies showed that the *AL* gene family is widely involved in plant growth and development and in response to various biotic and abiotic stresses, as shown in Figure 4 and Table 1.

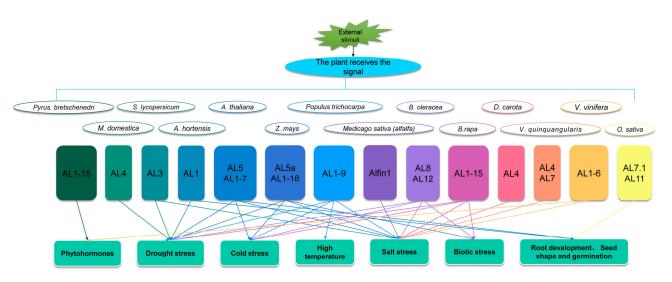


Figure 4. Research progresses on AL transcription factors.

Table 1. Research progresses or	AL transcription factors.
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Species	Gene	Function	References
Alfalfa	Alfin1	salt stress	[18,26,33]
A.thaliana	AL5 AL7	salt, drought, and cold stress salt stress	[20] [36]
	AL6	control root hair elongation under phosphate deficient conditions	[17]
A. hortensis	AL1	drought stress, mediated ABA pathway, consistent seed germination, reduced initial rooting	[22]
O.sativa	AL7.1, AL11	Regulation of seed shape, responds to ABA and mannite	[43]
Z.mays	AL5a AL1-18	salt, drought stress, and fungal pathogens salt, drought, and cold stress	[52] [14]
B.oleracea	AL8, AL12	salt, drought, cold stress, and fungal stress	[38]
B.rapa	AL1-15	salt, drought, cold stress, and fungal stress	[37]
M.domestica	AL4	drought stress	[41]
S.lycopersicum	AL3	salt, drought stress	[11]
D.carota	AL4, AL7	salt stress	[58]
V.quinquangularis	AL4	Promotion of stilbene accumulation, activation of SA signaling, and enhancement of resistance to powdery mildew	[53]
V.vinifera	AL1-6	salt stress, phytohormones	[57]
P. bretschenedri	AL1-15	response to indole acetic acid, gibberellin, melatonin, and abscisic acid	[40]
P. trichocarpa	AL1-9	salt, drought, cold, and high temperature stress	[39]

6. Conclusions and Perspectives

Alfin-like proteins play an important role in many aspects of plant growth and development and in response to various stressors. In recent years, researchers have successively cloned and identified members of the *AL* gene family in different plants and performed biological functional analysis. This review is based on genomic data from different species, and this comprehensive analysis as well as the review of previous functions identified by bioinformatics methods can not only help us better understand the functions that these genes are already known to have but also provide more research directions for elucidating the functions of these genes in the future. The involvement of AL genes in salt, drought, and cold stress conditions in plants has been extensively reported, and these studies have led to a deeper understanding of the members of the AL gene family for future studies. Our analyses revealed that the evolutionary relationships between different species are relatively similar and that proteins grouped in the same phylogenetic group have similar gene structures and functions. Therefore, we hypothesized that most of the genes with unknown functions may also respond to abiotic stress in plants. Alfin-like transcription factors are commonly found in higher plants, and with the development of transcriptome sequencing technology and the updating of whole genome annotations, an increasing number of AL genes will be identified or reannotated in different plant species. However, further research is still needed to determine the exact functions of the AL protein. At present, many studies have reported the functional role of the plant AL gene family in determining the effects of plant shape, seed germination, root hairs, and abiotic stress. These studies are limited to the identification and initial characterization of the gene family, and the complete molecular mechanisms and signal transduction pathways of ALs in plant-specific biological functions have not been thoroughly studied. Recent studies have also revealed that some ALs can regulate different hormonal signals. Therefore, we speculate that these genes not only play a role in one signaling pathway but can also interact with multiple signaling pathways. However, studies on the molecular mechanisms of AL genes are still rare.

In conclusion, an in-depth study of the biological functions of AL proteins and the construction of molecular regulatory networks will enrich and improve our understanding of the roles of AL proteins in various biological events. Furthermore, such research will also provide a valuable scientific basis for the study of new AL proteins. Therefore, identifying additional *AL* gene family members involved in various types of stress and analyzing their mechanisms of action in plants are highly important and valuable. These studies will be highly important for plant stress resistance breeding and genetic characterization.

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References

- 1. Ashraf, M.Y. Heavy Metals-Environmental Pollutants or Potential Stress Ameliorants in Plants. View project Development of Nutrient Enrich Manure Phosphate (NEM-Phos) as efficient fertilizer for crop production on calcareous soils View project. *Pak. J. Bot.* **2009**, *41*, 647–654.
- Xu, Y.; Huang, B. Exogenous Ascorbic Acid Mediated Abiotic Stress Tolerance in Plants. In Ascorbic Acid in Plant Growth, Development and Stress Tolerance; Springer International Publishing: Berlin/Heidelberg, Germany, 2018; pp. 233–253. ISBN 9783319740577.
- 3. Zhu, J.-K. Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. 2002, 53, 247–273. [CrossRef] [PubMed]
- 4. Yamaguchi-Shinozaki, K.; Shinozaki, K. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* **2006**, *57*, 781–803. [CrossRef] [PubMed]
- 5. Akhtar, M.; Jaiswal, A.; Taj, G.; Jaiswal, J.P.; Qureshi, M.I.; Singh, N.K. DREB1/CBF Transcription Factors: Their Structure, Function and Role in Abiotic Stress Tolerance in Plants. *J. Genet.* **2012**, *91*, 385–395. [CrossRef] [PubMed]

- 6. Su, J.; Song, S.; Wang, Y.; Zeng, Y.; Dong, T.; Ge, X.; Duan, H. Genome-Wide Identification and Expression Analysis of DREB Family Genes in Cotton. *BMC Plant Biol.* **2023**, *23*, 169. [CrossRef] [PubMed]
- Kurowska, M.; Daszkowska-Golec, A. Molecular Mechanisms of SNAC1 (Stress-Responsive NAC1) in Conferring the Abiotic Stress Tolerance. *Plant Sci.* 2023, 337, 111894. [CrossRef] [PubMed]
- 8. Peng, Y.; Tang, N.; Zou, J.; Ran, J.; Chen, X. Rice MYB Transcription Factor OsMYB1R1 Negatively Regulates Drought Resistance. *Plant Growth Regul.* 2023, *99*, 515–525. [CrossRef]
- 9. Mahiwal, S.; Pahuja, S.; Pandey, G.K. Review: Structural-Functional Relationship of WRKY Transcription Factors: Unfolding the Role of WRKY in Plants. *Int. J. Biol. Macromol.* **2024**, 257, 128769. [CrossRef]
- 10. Zhang, B.; Feng, C.; Chen, L.; Li, B.; Zhang, X.; Yang, X. Identification and Functional Analysis of bZIP Genes in Cotton Response to Drought Stress. *Int. J. Mol. Sci.* 2022, 23, 14894. [CrossRef]
- 11. Jin, R.; Wang, J.; Guo, B.; Yang, T.; Hu, J.; Wang, B.; Yu, Q. Identification and Expression Analysis of the Alfin-like Gene Family in Tomato and the Role of SIAL3 in Salt and Drought Stresses. *Plants* **2023**, *12*, 2829. [CrossRef]
- Zaynab, M.; Kanwal, S.; Furqan, M.; Islam, W.; Noman, A.; Ali, G.M.; Rehman, N.; Zafar, S.; Sughra, K.; Jahanzab, M. Proteomic Approach to Address Low Seed Germination in Cyclobalnopsis Gilva. *Biotechnol. Lett.* 2017, 39, 1441–1451. [CrossRef] [PubMed]
- 13. Zaynab, M.; Sharif, Y.; Fatima, M.; Afzal, M.Z.; Aslam, M.M.; Raza, M.F.; Anwar, M.; Raza, M.A.; Sajjad, N.; Yang, X.; et al. CRISPR/Cas9 to Generate Plant Immunity against Pathogen. *Microb. Pathog.* **2020**, *141*, 103996. [CrossRef] [PubMed]
- 14. Zhou, W.; Wu, J.; Zheng, Q.; Jiang, Y.; Zhang, M.; Zhu, S. Genome-Wide Identification and Comparative Analysis of Alfin-like Transcription Factors in Maize. *Genes Genom.* **2017**, *39*, 261–275. [CrossRef]
- 15. Golldack, D.; Li, C.; Mohan, H.; Probst, N. Tolerance to Drought and Salt Stress in Plants: Unraveling the Signaling Networks. *Front. Plant Sci.* **2014**, *5*, 151. [CrossRef]
- 16. Carroll, S.B. Evolution at Two Levels: On Genes and Form. PLoS Biol. 2005, 3, e245. [CrossRef] [PubMed]
- 17. Chandrika, N.; Sundaravelpandian, K.; Yu, S.M.; Schmidt, W. ALFIN-LIKE 6 Is Involved in Root Hair Elongation during Phosphate Deficiency in Arabidopsis. *New Phytol.* **2013**, *198*, 709–720. [CrossRef] [PubMed]
- Winicov, I. Alfin1 Transcription Factor Overexpression Enhances Plant Root Growth under Normal and Saline Conditions and Improves Salt Tolerance in Alfalfa. *Planta* 2000, 210, 416–422. [CrossRef]
- 19. Molitor, A.M.; Bu, Z.; Yu, Y.; Shen, W.-H. Arabidopsis AL PHD-PRC1 Complexes Promote Seed Germination through H3K4me3to-H3K27me3 Chromatin State Switch in Repression of Seed Developmental Genes. *PLoS Genet.* **2014**, *10*, e1004091. [CrossRef]
- Wei, W.; Zhang, Y.; Tao, J.; Chen, H.; Li, Q.; Zhang, W.; Ma, B.; Lin, Q.; Zhang, J.; Chen, S. The ALfin-like Homeodomain Finger Protein AL5 Suppresses Multiple Negative Factors to Confer Abiotic Stress Tolerance in Arabidopsis. *Plant J.* 2015, *81*, 871–883. [CrossRef]
- Krochko, J.E.; Pramanik, S.K.; Bewley, J.D. Contrasting Storage Protein Synthesis and Messenger RNA Accumulation during Development of Zygotic and Somatic Embryos of Alfalfa (*Medicago sativa* L.). *Plant Physiol.* 1992, 99, 46–53. [CrossRef]
- Tao, J.J.; Wei, W.; Pan, W.J.; Lu, L.; Li, Q.T.; Ma, J.B.; Zhang, W.K.; Ma, B.; Chen, S.Y.; Zhang, J.S. An Alfin-like Gene from Atriplex Hortensis Enhances Salt and Drought Tolerance and Abscisic Acid Response in Transgenic *Arabidopsis. Sci. Rep.* 2018, *8*, 2707. [CrossRef] [PubMed]
- Lee, W.Y.; Lee, D.; Chung, W.; Kwon, C.S. Arabidopsis ING and Alfin1-like Protein Families Localize to the Nucleus and Bind to H3K4me3/2 via Plant Homeodomain Fingers. *Plant J.* 2009, *58*, 511–524. [CrossRef] [PubMed]
- 24. Winicov, I.; Valliyodan, B.; Xue, L.; Hoober, J.K. The MsPRP2 Promoter Enables Strong Heterologous Gene Expression in a Root-Specific Manner and Is Enhanced by Overexpression of Alfin1. *Planta* **2004**, *219*, 925–935. [CrossRef] [PubMed]
- 25. Chen, K.; Guo, D.; Yan, J.; Zhang, H.; He, Z.; Wang, C.; Tang, W.; Zhou, Y.; Chen, J.; Xu, Z.; et al. The Alfin-like Transcription Factor GmAlfin09 Regulates 2 Endoplasmic Reticulum Stress in Soybean via a Peroxidase. *bioRxiv* 2023. [CrossRef]
- Bastola, D.R.; Pethe, V.V.; Winicov, I. Alfin1, a Novel Zinc-Finger Protein in Alfalfa Roots That Binds to Promoter Elements in the Salt-Inducible MsPRP2 Gene. *Plant Mol. Biol.* 1998, 38, 1123–1135. [CrossRef] [PubMed]
- 27. Greb, T.; Mylne, J.S.; Crevillen, P.; Geraldo, N.; An, H.; Gendall, A.R.; Dean, C. The PHD Finger Protein VRN5 Functions in the Epigenetic Silencing of Arabidopsis FLC. *Curr. Biol.* **2007**, *17*, 73–78. [CrossRef] [PubMed]
- 28. Sung, S.; Schmitz, R.J.; Amasino, R.M. A PHD Finger Protein Involved in Both the Vernalization and Photoperiod Pathways in Arabidopsis. *Genes Dev.* **2006**, *20*, 3244–3248. [CrossRef]
- 29. Sung, S.; Amasino, R.M. Vernalization in Arabidopsis Thaliana Is Mediated by the PHD Finger Protein VIN3. *Nature* 2004, 427, 159–164. [CrossRef]
- Saiga, S.; Furumizu, C.; Yokoyama, R.; Kurata, T.; Sato, S.; Kato, T.; Tabata, S.; Suzuki, M.; Komeda, Y. The Arabidopsis *OBERON1* and *OBERON2* Genes Encode Plant Homeodomain Finger Proteins and Required for Apical Meristem Maintenance. *Development* 2008, 135, 1751–1759. [CrossRef]
- Wu, T.; Pi, E.-X.; Tsai, S.-N.; Lam, H.-M.; Sun, S.-M.; Kwan, Y.W.; Ngai, S.-M. GmPHD5 Acts as an Important Regulator for Crosstalk between Histone H3K4 Di-Methylation and H3K14 Acetylation in Response to Salinity Stress in Soybean. *BMC Plant Biol.* 2011, 11, 178. [CrossRef]
- 32. Ling, P.; Wang, L.; Zhang, Y.; Dong, A.; Shen, W.H.; Ying, H. Structural Analysis of the Arabidopsis AL2-PAL and PRC1 Complex Provides Mechanistic Insight about Active-to-Repressive Chromatin State Switch. *J. Mol. Biol.* **2018**, 430, 4245–4259.
- 33. Winicov, I.; Bastola, D.R. Transgenic Overexpression of the Transcription Factor Alfin1 Enhances Expression of the Endogenous MsPRP2 Gene in Alfalfa and Improves Salinity Tolerance of the Plants. *Plant Physiol.* **1999**, *120*, 473–480. [CrossRef] [PubMed]

- 34. Schindler, U.; Beckmann, H.; Cashmore, A.R. HAT3. 1, a Novel Arabidopsis Homeodomain Protein Containing a Conserved Cysteine-rich Region. *Plant J.* **1993**, *4*, 137–150. [CrossRef] [PubMed]
- 35. Bienz, M. The PHD Finger, a Nuclear Protein-Interaction Domain. Trends Biochem. Sci. 2006, 31, 35–40. [CrossRef] [PubMed]
- 36. Song, Y.; Gao, J.; Yang, F.; Kua, C.-S.; Liu, J.; Cannon, C.H. Molecular Evolutionary Analysis of the Alfin-like Protein Family in Arabidopsis Lyrata, Arabidopsis Thaliana, and Thellungiella Halophila. *PLoS ONE* **2013**, *8*, e66838. [CrossRef] [PubMed]
- 37. Kayum, M.A.; Park, J.I.; Ahmed, N.U.; Jung, H.J.; Saha, G.; Kang, J.G.; Nou, I.S. Characterization and Stress-Induced Expression Analysis of Alfin-like Transcription Factors in Brassica Rapa. *Mol. Genet. Genom.* **2015**, *290*, 1299–1311. [CrossRef] [PubMed]
- 38. Kayum, M.A.; Park, J.-I.; Ahmed, N.U.; Saha, G.; Chung, M.-Y.; Kang, J.-G.; Nou, I.-S. Alfin-like Transcription Factor Family: Characterization and Expression Profiling against Stresses in Brassica Oleracea. *Acta Physiol. Plant.* **2016**, *38*, 127. [CrossRef]
- Rehman, S.; Sabir, I.A.; Wang, P.; Li, H.; Ahmad, Z.; Manzoor, M.A.; Zhuge, Q. Genome-Wide Identification of Alfin Like (AL) Transcription Factors and Their Regulatory Role in Abiotic Stress Responses in Poplar (*Populus trichocarpa*). *Plant Stress* 2023, *8*, 100168. [CrossRef]
- Manzoor, M.A.; Li, G.; Xinya, W.; Wang, M.; Zhao, Y.; Sabir, I.A.; Shah, I.H.; Wang, H.; Abdullah, M.; Kim, G.T.; et al. The Alfin-like Transcription Factors: Identification, Characterization, and Expression Analysis in Pyrus Bretschenedri Provide Insight into Its Divergent Functions on Abiotic Response. *Sci. Hortic.* 2023, *321*, 112320. [CrossRef]
- 41. Wang, P.; Lu, S.; Li, W.; Ma, Z.; Mao, J.; Chen, B. Genome-Wide Characterization of Alfin-like (AL) Genes in Apple and Functional Identification of MdAL4 in Response to Drought Stress. *Plant Cell Rep.* **2023**, *42*, 395–408. [CrossRef]
- Sureshkumar, S.; Bandaranayake, C.; Lu, J.; Dent, C.I.; Atri, C.; York, H.M.; Tamizhselvan, P.; Shamaya, N.; Folini, G.; Bhagat, P.K.; et al. SUMO Protease FUG1, Histone Reader AL3 and the PRC1 Complex Are Integral to Repeat-Expansion Induced Epigenetic Silencing in Arabidopsis Thaliana. *bioRxiv* 2023. [CrossRef]
- 43. Yang, Y.; Ma, X.; Xia, H.; Wang, L.; Chen, S.; Xu, K.; Yang, F.; Zou, Y.; Wang, Y.; Zhu, J. Natural Variation of Alfin-like Family Affects Seed Size and Drought Tolerance in Rice. *Plant J.* **2022**, *112*, 1176–1193. [CrossRef] [PubMed]
- Chiou, T.-J.; Lin, S.-I. Signaling Network in Sensing Phosphate Availability in Plants. Annu Rev Plant Biol. 2011, 62, 185–206. [CrossRef] [PubMed]
- López-Bucio, J.; Hernández-Abreu, E.; Sánchez-Calderón, L.; Nieto-Jacobo, M.F.; Simpson, J.; Herrera-Estrella, L. Phosphate Availability Alters Architecture and Causes Changes in Hormone Sensitivity in the Arabidopsis Root System. *Plant Physiol.* 2002, 129, 244–256. [CrossRef] [PubMed]
- Lynch, J.P.; Brown, K.M. Topsoil Foraging—An Architectural Adaptation of Plants to Low Phosphorus Availability. *Plant Soil* 2001, 237, 225–237. [CrossRef]
- Williamson, L.C.; Ribrioux, S.P.C.P.; Fitter, A.H.; Leyser, H.M.O. Phosphate Availability Regulates Root System Architecture in Arabidopsis. *Plant Physiol.* 2001, 126, 875–882. [CrossRef] [PubMed]
- 48. Bates, T.R.; Lyncu, J.P. Stimulation of Root Hair Elongation in Arabidopsis Thaliana by Low Phosphorus Availability. *Plant Cell Environ.* **1996**, *19*, 529–538. [CrossRef]
- 49. Sun, L.; Xing, S.; Zhang, J.; Yang, J.; Wang, X.; Dong, Y. Function of the Transcription Factors in Plant Domestication and Stress Resistance. *Genom. Appl. Biol.* 2009, 28, 569–577.
- 50. Qu, L.-J.; Zhu, Y.-X. Transcription Factor Families in Arabidopsis: Major Progress and Outstanding Issues for Future Research. *Curr. Opin. Plant Biol.* **2006**, *9*, 544–549. [CrossRef]
- Riechmann, J.L.; Heard, J.; Martin, G.; Reuber, L.; Jiang, C.-Z.; Keddie, J.; Adam, L.; Pineda, O.; Ratcliffe, O.J.; Samaha, R.R.; et al. Arabidopsis transcription factors: Genome-wide comparative analysis among eukaryotes. *Science* 2000, 290, 2105–2110. [CrossRef]
- 52. Wang, J.J.; Sun, N.; Liu, Z. Bioinformatics and Expression Analysis of Maize Alfin-like Transcription Factor ZmAL5a. *Mol. Plant Breed.* **2019**, *17*, 4859–4864.
- 53. Yan, C.; Yang, N.; Li, R.; Wang, X.; Xu, Y.; Zhang, C.; Wang, X.; Wang, Y. Alfin-like Transcription Factor VqAL4 Regulates a Stilbene Synthase to Enhance Powdery Mildew Resistance in Grapevine. *Mol. Plant Pathol.* **2023**, *24*, 123–141. [CrossRef] [PubMed]
- Zhang, D.; Gao, Z.; Zhang, H.; Yang, Y.; Yang, X.; Zhao, X.; Guo, H.; Nagalakshmi, U.; Li, D.; Dinesh-Kumar, S.P.; et al. The MAPK-Alfin-like 7 Module Negatively Regulates ROS Scavenging Genes to Promote NLR-Mediated Immunity. *Proc. Natl. Acad. Sci. USA* 2023, 120, e2214750120. [CrossRef] [PubMed]
- 55. Liang, X.; Lei, M.; Li, F.; Yang, X.; Zhou, M.; Li, B.; Cao, Y.; Gong, S.; Liu, K.; Liu, J.; et al. Family-Wide Characterization of Histone Binding Abilities of PHD Domains of AL Proteins in Arabidopsis Thaliana. *Protein J.* **2018**, *37*, 531–538. [CrossRef]
- Huang, Y.; Jiang, L.; Liu, B.-Y.; Tan, C.-F.; Chen, D.-H.; Shen, W.-H.; Ruan, Y. Evolution and Conservation of Polycomb Repressive Complex 1 Core Components and Putative Associated Factors in the Green Lineage. *BMC Genom.* 2019, 20, 533. [CrossRef] [PubMed]

- 57. Xie, M.; Wang, P.; He, H.; Cao, X.; Mao, J.; Chen, B. Identification and Expression Analysis of Alfin-like Transcription Factor Family in Vitis vinifera. *Acta Bot. Boreali-Occident. Sin.* **2020**, *40*, 1467–1474.
- 58. Quiroz-Iturra, L.F.; Simpson, K.; Arias, D.; Silva, C.; González-Calquin, C.; Amaza, L.; Handford, M.; Stange, C. Carrot DcALFIN4 and DcALFIN7 Transcription Factors Boost Carotenoid Levels and Participate Differentially in Salt Stress Tolerance When Expressed in Arabidopsis Thaliana and Actinidia Deliciosa. *Int. J. Mol. Sci.* **2022**, *23*, 12157. [CrossRef]

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