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Review

Review

Annexins: central regulators of plant growth and stress signaling

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Abstract

Annexins are a family of multifunctional calcium-dependent and phospholipid-binding proteins that are widely distributed in the plant kingdom. They have a highly conserved evolutionary history that dates back to single-celled protists. Plant annexins, as soluble proteins, can flexibly bind to endomembranes and plasma membranes, exhibiting unique calcium-dependent and calcium-independent characteristics. Members of the annexin family have diverse functions, including binding to F-actin, participating in ATP and GTP hydrolysis, and even serving as peroxidases or cation channels. Annexins play pivotal roles in plant growth and stress signaling. They can respond sensitively to environmental, metabolic, and developmental signals, thereby affecting cytoskeleton remodeling and exocytosis mechanisms. Plant annexin gene families have been successfully identified in multiple species, and their expression and intracellular localization are precisely regulated by developmental processes and environmental factors. Although research on plant annexins has aroused great interest, their depth and breadth still need further expansion compared with those of animal annexins. This article provides a comprehensive and in-depth review of the characteristics and functions of plant annexin families, revealing their core roles in plant growth and adaptation, and yielding valuable references and insights for future research.

Key words annexins, abiotic stress, biotic stress, plant growth

Introduction

Annexins are a multifunctional and evolutionarily conserved multigene family of Ca^{2+}-dependent and phospholipid-binding proteins [1–3]. They are a group of primitive proteins that date back to approximately 1 to 1.5 billion years ago in the unicellular protist *Giardia lamblia* [4]. These proteins are widely distributed in plants, animals, and microorganisms [2,5,6]. Plant annexins were first discovered in tomatoes by Boustead *et al.* [7] in 1989, but research interest in plant annexins has increased. In contrast, animal annexins have been extensively studied. Animal annexins are involved in signal transduction, free cytosolic Ca^{2+} homeostasis, exocytosis and endocytosis, membrane organization, cytoskeletal dynamics, cell cycle control, and water permeability [8,9]. Plants can sense and respond to a range of environmental, metabolic, and developmental signals. The operation and control of interacting signal transduction pathways involve cells, endomembranes, and a range of integrated, peripheral, and soluble proteins. The downstream responses may require remodeling of the cytoskeleton and changes in the exocytosis mechanism and cell wall [1]. Plant annexins appear to function at all of these levels [8]. Research has shown that plant annexins, as ubiquitous soluble proteins, can bind to endomembranes and the plasma membrane with both calcium-dependent and calcium-independent properties. Some members of this multigene family can bind to F-actin, hydrolyze GTP and ATP, and act as peroxidases or cation channels [10–15]. Plant annexins seem to be at the center of signal transduction and adaptation, playing a role as core regulators or effectors in plant growth and stress signaling. Plant annexins can not only regulate cell activities such as cell division and tissue and organ development but also regulate abiotic stress (such as salt and drought stresses) and biotic stress processes caused by pathogens [3,16–18].

In addition, the identification of gene families in plants is gradually progressing. Early studies in plants revealed that these genes belong to a relatively simple gene family in both maize and tobacco [19,20]. Owing to the availability of whole-genome sequences, annexin genes were subsequently identified in Arabidopsis and rice [21,22]. In recent years, an increasing number of annexes, such as poplar and rye, have been identified in various

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plant species [23–25].

This review will introduce the basic characteristics of the plant annexin family that have been identified to date. Additionally, the specific roles and regulatory mechanisms of plant annexins in regulating plant growth and development, as well as their involvement in abiotic and biotic stress regulation, are discussed.

Characteristics of the Annexins in the Plant Kingdom

The identification of the annexin family in plants has occurred primarily within the last decade, resulting in the identification of annexin families in nearly 30 plant species. Extensive research has been conducted on the annexin family in certain plant species, including studies of expression patterns, exploration of *cis*-elements upstream of genes, and speculation regarding their functions in response to stress and plant growth. However, for some plant species, only basic information about annexin genes, such as gene quantity, accession numbers, and protein sequences, is available. The annexin protein families of Arabidopsis (*Arabidopsis thaliana*) and Indian mustard (*Brassica juncea*) were identified earlier. The *A. thaliana* annexin family, comprising AnnAt1 to AnnAt7, was the first to be identified. Comparisons and analyses of these seven annexin gene sequences were conducted by Clark *et al.* [21], who presented expression patterns for *AnnAt1* and *AnnAt2* during different developmental stages. The sequences AnnAt1 to AnnAt7 contain four characteristic structural repeats that may exhibit functional differences within these repeat regions. AnnAt1 is expressed in all examined tissues and is most abundant in stems, whereas AnnAt2 is expressed primarily in root tissue and to a lesser extent in stems and flowers. These expression patterns suggest tissue-specific and cell-specific regulation for developmental purposes, which are both distinct and overlapping [21]. *B. juncea* contains five annexin proteins, namely, AnnBj1, AnnBj2, AnnBj3, AnnBj6, and AnnBj7, which have high sequence similarity to each other and to annexins from higher plants. AnnBjs are differentially expressed in various tissues and are regulated in response to different stress conditions, such as exposure to signaling molecules, salinity, oxidative stress, and wounding. Moreover, AnnBjs contain various *cis*-elements that respond to different stress conditions, suggesting their potential importance in adapting plants to various environmental stresses [26]. In 2012, Jami *et al.* [2] analyzed the annexin gene family from unicellular green algae to multicellular plants, including sorghum, soybeans, and maize, and identified a total of 149 genes. A phylogenetic study classified these putative annexin proteins into nine arbitrary groups. However, further analysis and research on the functions and expression characteristics of annexins in each species have not yet been conducted [2]. Ten members of the rice (*Oryza sativa*) annexin gene family, encoding proteins that share 16%–64% identity, have been identified. Phylogenetic analysis classified the *O. sativa* annexins into five homologous groups, each with similar motif patterns. The expression of OsAnns is not only temporally and spatially differentially regulated but also influenced by various abiotic stresses, including salinity, drought, heat, and cold, particularly during the seedling stage. In addition, in silico analysis of the putative upstream sequences of OsAnns revealed *cis*-elements associated with stress responses [22]. Tomato (*Solanum lycopersicum*) contains nine annexin proteins, all of which feature highly conserved annexin domains. The promoters of AnnSls contain various *cis*-elements that respond to different stresses and plant hormones. Most AnnSls are induced by salt, drought, injury, and temperature stress, whereas some are induced by plant hormones, such as abscisic acid (*AnnSl3*, *AnnSl6*, *AnnSl8*, and *AnnSl9*) and gibberellin (*AnnSl1.1*, *AnnSl1.2*, *AnnSl4*, and *AnnSl7*). Tissue expression patterns demonstrate that some AnnSls are expressed in most organs (such as AnnSl1.1 and AnnSl2), whereas others are expressed in specific organs, such as the stigma, ovary, stamen, and fruit pericarp [27]. Twelve annexin proteins were identified in maize (*Zea mays*) and classified into five orthologous groups. ZmAnns are responsive to heavy metals (Ni, Zn, and Cd), Ustilago maydis, or jasmonic acid and exhibit different organ-specific expression patterns during maize development, potentially playing crucial roles in adapting to various environmental stresses [28]. Szalonek *et al.* [29] and Tang *et al.* [30] identified 14 and 9 annexin genes in the diploids *Gossypium raimondii* and *Solanum tuberosum*, respectively. Moreover, a total of 8 AnnAh genes were identified in peanuts (*Arachis hypogaea*); these genes are differentially expressed in various organs and are regulated by drought, salinity, heavy metal stress, low temperature, and hormones. AnnAh1, AnnAh2, AnnAh3, AnnAh5, AnnAh6, and AnnAh7 share amino acid sequence similarities ranging from 35.76% to 66.35% [31]. Xu *et al.* [32] analyzed the size and composition of the annexin gene family in wheat and barley, investigated the differential tissue-specific and stress-responsive expression profiles of the gene family in wheat, and discovered twenty-five, eleven, eight, and eleven putative annexin genes in *Triticum aestivum*, *Hordeum vulgare*, *Triticum urartu* and *Aegilops tauschii*, respectively. Gene expression patterns revealed differential temporal and spatial regulation of annexin genes in wheat under various abiotic stress conditions, such as salinity, drought, cold, and abscisic acid treatment [32]. Recent research has identified 13, 12, and 26 *ANN* genes in *Brassica rapa*, *Brassica oleracea*, and *Brassica napus*, respectively. These genes are highly conserved among Brassicaceae species. The different ANN group members presented varied expression patterns in different tissues and under various treatments involving abiotic stress and hormones. Conversely, members within the same group demonstrate conserved expression patterns across different tissues [33]. A total of 10 annexin genes were identified in radish (*Raphanus sativus L.*) and were clustered into five groups with similar motif patterns. Furthermore, multiple *cis*-elements involved in abiotic stress responses were identified in the promoter regions of the RsANN genes. The expression patterns of the RsANN genes displayed tissue-specific patterns across different growth stages and tissues and were induced by various abiotic stresses, such as high temperature, drought, salinity, oxidation, and ABA treatment [34]. In pepper (*Capsicum annuum*), 15 members of the *CaAnn* gene family were identified. Phylogenetic analysis revealed that CaAnn proteins can be classified into four different orthologous groups. These *CaAnn* genes exhibit tissue-specific expression patterns and are highly expressed in leaves following treatments with cold, drought, salt, and hormones such as MeJA and ABA [35]. Rye (*Secale cereale L.*), known for its remarkable tolerance to various abiotic stresses, such as cold, heat, osmotic stress, and salt stress, harbors twelve novel annexin gene families. The rye annexins have been categorized into five groups on the basis of conserved motif analyses and show significant homology with wheat annexin genes. The expression patterns of ScAnns vary under different stress conditions, including cold, heat, PEG, and NaCl treatments, suggesting that each gene plays a distinct role in stress signaling.

Cis-element analysis revealed that these genes may be regulated by light, ABA, MeJA, and MYB transcription factors [25]. In the whole genome of poplar (*Populus trichocarpa*), Wei *et al.* [24] identified a total of 12 *ANN* genes distributed across 10 chromosomes. Phylogenetic analysis led to the classification of PtANNs into three subfamilies, whereby members within the same clade share similar gene structures and conserved motifs. The expression of PtANNs is induced by multiple abiotic stresses, indicating their active involvement in the response to abiotic stress [24]. Notably, extremophile plants, such as *Schrenkiella parvula* and *Eutrema salsugineum*, were found to possess ten and eight putative genes encoding annexins, respectively. These genes were grouped into six subfamilies on the basis of their phylogenetic associations. Compared with those of AtAnns, the promoters of SpAnns and EsAnns contain a greater number of *cis*-elements and transcription factor-binding sites. Given the salt tolerance of *S. parvula* and *E. salsugineum*, researchers have also investigated the response of SpAnns and EsAnns to salt stress and observed the induction of their expression in different tissues. Specifically, *SpAnn1*, *SpAnn2* and *SpAnn6* were upregulated in shoots under salt stress, whereas *EsAnn1*, *EsAnn5*, and *EsAnn7* were upregulated in roots, and *EsAnn1*, *EsAnn2*, and *EsAnn5* presented increased expression in leaves [23]. Table 1 lists the numbers, gene names, accession numbers, and references of the annexin superfamily in different plant species.

AnnAt1: the Most Extensively Studied Annexin in Plants

Among all plant annexin proteins, *A. thaliana* annexin 1 (AnnAt1) is widely regarded as the most thoroughly researched and productive. Research on AnnAt1 can be divided into three key areas. First, it plays a crucial role in the abiotic stress response process of plants, particularly in relation to osmotic (drought) stress, salt stress, and oxidative stress. Second, it regulates the biotic stress experienced by plants, particularly in response to grazing by herbivorous insects. Finally, it is involved in intracellular material transport, specifically in the transport of sugars. In a groundbreaking study, Gorecka *et al.* [36] reported that the AnnAt1 protein possesses ion channel activity and can detect changes in pH within the range of 5–7. This protein responds to these changes by adjusting ion channel conductivity, hydrophobicity, protein secondary structure, and oligomer formation [36].

Lee *et al.* [37] discovered and isolated the AnnAt1 protein via advanced techniques such as two-dimensional gel electrophoresis and matrix-assisted laser ablation/ionization time-of-flight mass spectrometry. They conducted an initial exploration of the expression pattern, subcellular localization, and mechanism of action of AnnAt1. The results revealed that AnnAt1 was highly expressed in roots but rarely expressed in flower tissues. Its expression was upregulated by ABA, mannitol, polyethylene glycol, and osmotic stress, particularly in the presence of Ca^{2+}. Subcellular localization studies revealed that AnnAt1 is present in both microsomes and cytoplasmic components of the root plasma membrane. Phenotypic experiments with the *annat1* T-DNA insertion mutant revealed hypersensitivity to osmotic stress and abscisic acid during germination and early seedling growth. These findings suggest that AnnAt1 plays a crucial role in Ca^{2+}-mediated osmotic stress and ABA signaling [37]. Additionally, Konopka-Postupolska *et al.* [38] reported that AnnAt1 expression can be upregulated by H_2O_2, SA, NaCl, and Cd^{2+}. Further research demonstrated that plants overexpressing AnnAt1 presented increased drought tolerance, whereas gene knockout plants were more susceptible to drought. Researchers have also investigated the functions of AnnAt1 at different stages of drought stress and reported that it can prevent swelling and wilting in the early stages of drought treatment. Over time, the increase in the level of the AnnAt1 protein promoted the survival of dormant buds, aiding in the recovery of plants under drought stress. Moreover, the authors reported that the accumulation of hydrogen peroxide in guard cells decreased in the overexpressing plants before and after abscisic acid treatment, whereas the knockout plants presented increased hydrogen peroxide accumulation. This finding suggested an association between AnnAt1and reactive oxygen species as well as oxidative stress [38]. Previous studies have indicated that AnnAt1 acts as a redox sensor in plant cells, limiting the excessive accumulation of reactive oxygen species and mitigating oxidative stress [12]. Its function during oxidative stress was established by expressing this *A. thaliana* membrane-associated protein in *Escherichia coli* sensitive to hydrogen peroxide. The results showed that the expression of AnnAt1 increased survival in the presence of H_2O_2 [38]. The AnnAt1 protein is sensitive to hydrogen peroxide and exhibits peroxidase activity, with the heme-binding domain playing a critical role. Mutations in this domain can lead to loss of activity [12]. The molecular mechanism underlying the antioxidant activity of AnnAt1 was elucidated by Konopka-Postupolska *et al.* [38], who reported that the reactive S3 cluster of AnnAt1 contains two Cys residues that provide a basis for the formation of disulfide bonds. These Cys residues, however, remain in a reduced state under normal conditions. The ability of AnnAt1 to form oligomers was compared between the wild-type and mutant forms, in which one or two Cys residues were replaced by Ala. Both the mutant and wild-type proteins exhibited identical patterns of dimer, trimer, and advanced oligomer formation, suggesting that dimerization was not dependent on the formation of intermolecular disulfide bonds [38]. Richards *et al.* [39] demonstrated that the ROS-regulated Ca^{2+} transporter AnnAt1 is involved in regulating the response of the root epidermis cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$) to extracellular hydrogen peroxide stress. It also affects the formation of downstream signals [39]. The expression of AnnAt1 in roots is inhibited by peroxides. In *AnnAt1*-knockout mutants, peroxide-stimulated $[Ca^{2+}]_{cyt}$ increases abnormally, accompanied by disrupted net Ca^{2+} influx and K^+ efflux. AnnAt1 is the second identified Ca^{2+} transporter protein that responds to hydrogen peroxide, in addition to the stelar K^+ outward rectifier channel [39,40]. Salt stress can induce the redistribution of the AnnAt1 protein from the cytoplasm to the membrane, leading to an increase in $[Ca^{2+}]_{cyt}$. Laohavisit *et al.* [41] investigated the role of AnnAt1 in the increase in the production of $[Ca^{2+}]_{cyt}$ induced by NaCl in roots and the root epidermis. They reported that AnnAt1 does not contribute to root Na^+ absorption but plays a crucial role as a component of the $[Ca^{2+}]_{cyt}$ signal, especially in the production of additional calcium ions and ROS by cells in saline–alkali soils. In the *annat1* mutant, there is no inflow current activated by NaCl in the root surface cortex membrane, resulting in damage to $[Ca^{2+}]_{cyt}$ signal transduction and impaired adaptive signal transduction and salt responsiveness in secondary roots [42]. Previous studies have shown that *35S:AnnAt1* plants exhibit increased drought tolerance, whereas *annat1* plants are less tolerant to drought stress than wild-type plants. However, Hu *et al.* [43] reported that AnnAt1

Table 1. Characteristics of the plant annexin families

Organism	Lineage	Total	Accession No.	Annxins
Brassica juncea	Dicot	5	ABB59550, ABD47519, ABD47520, ABM87934, ABD47518	AnnBj1, AnnBj2, AnnBj3, AnnBj6, AnnBj7
Arachis hypogaea	Dicot	8	KM267643, KM276779, KM276780, KM276781, KM276782, KM276783, KM276784, KM276785	AnnAh1 to AnnAh8
Arabidopsis thaliana	Dicot	8	AT1G35720, AT5G65020, AT2G38760, AT2G38750, AT1G68090, AT5G10220, AT5G10230, AT5G12380	AnnAt1 to AnnAt8
Eutrema salsugineum	Dicot	8	Thhalv10001831m.g, Thhalv10004625m.g, Thhalv10016949m.g, Thhalv10016965m.g, Thhalv10019463m.g, Thhalv10014136m.g, Thhalv10014138m.g, Thhalv10014146m.g	EsAnn1 to EsAnn8
Solanum tuberosum	Dicot	9	DMG400017714, DMG400021817, DMG402019427, DMG401019427, DMG400040554, DMG400019446, DMG400007966, DMG400007482, DMG400001879	STANN1, STANN2, STANN3.1, STANN3.2, STANN3.3, STANN4, STANN5, STANN8, STANN9
Medicago truncatula	Dicot	10	Mt5g063670, Mt3g018790, Mt8g038180, Mt3g018920, Mt8g038220, Mt8g038210, Mt3g018780, Mt8g038170, Mt8g038150, Mt8g107640	AnnMS2, MtAnn1, AnnMt1
Raphanus sativus	Dicot	10	Rs435840, Rs252780, Rs271840, Rs309650, Rs203830, Rs203830, Rs203840, Rs203840, Rs394170, Rs395890	RsANN1a, RsANN1b, RsANN2a, RsANN2b, RsANN3a, RsANN3b, RsANN4a, RsANN4b, RsANN7, RsANN8
Schrenkiella parvula	Dicot	10	Sp1g30430, Sp2g28100, Sp4g21120, Sp4g21140, Sp4g21110, Sp4g21130, Sp5g23090, Sp6g33180, Sp6g31260, SpUN1172_0010	SpAnn1, SpAnn2, SpAnn3-1, SpAnn3-2, SpAnn4-1, SpAnn4-2, SpAnn5, SpAnn6, SpAnn7, SpAnn8
Cucumis sativus	Dicot	11	Cs138380, Cs217870, Cs234800, Cs234810, Cs273000, Cs307970, Cs307980, Cs308080, Cs308090, Cs308100, Cs340270	ND
Populus alba	Dicot	12	Potri.001G024800, Potri.001G024900, Potri.001G277500, Potri.002G095600, Potri.003G200700, Potri.005G075900, Potri.007G092500, Potri.008G139500, Potri.010G101800, Potri.012G041900, Potri.013G055000, Potri.015G031800	PtANN1 to PtANN12
Brassica oleracea	Dicot	12	Bo6g043900, Bo2g166530, Bo4g187790, Bo3g032770, Bo3g032760, Bo4g187780, Bo2g057430, Bo9g172340, Bo9g172330, Bo1g039570, Bo8g025760, Bo5g083900	BoAnn1-1, BoAnn1-2, BoAnn1-3, BoAnn2-1, BoAnn3-1, BoAnn3-2, BoAnn4-1, BoAnn4-2, BoAnn5, BoAnn6, BoAnn7, BoAnn8-1
Solanum lycopersicum	Dicot	12	Solyc05g051980.2.1, Solyc01g081530.1.1, Solyc01g097510.2.1, Solyc01g097520.2.1, Solyc01g097540.2.1, Solyc01g097550.2.1, Solyc04g008270.2.1, Solyc04g055170.2.1, Solyc04g073990.2.1, Solyc04g078820.2.1, Solyc10g044690.1.1, Solyc10g047240.1.1	p34, p35, AnnSl1.1, AnnSl2 to AnnSl9
Brassica rapa	Dicot	13	Bra039578, Bra036764, Bra034402, Bra031890, Bra024346, Bra017102, Bra000091, Bra000090, Bra017103, Bra033961, Bra009049, Bra009048, Bra008892	BrAnn1-1, BrAnn1-2, BrAnn1-3, BrAnn2-1, BrAnn2-2, BrAnn3-1, BrAnn3-2, BrAnn4-1, BrAnn4-2, BrAnn5, BrAnn6, BrAnn7, BrAnn8-2
Gossypium hirsutum	Dicot	14	JX897059, JX897060, JX897061, JX897062, AAB67994, AAR13288, AAC33305, FJ415173, KC316004, KC316005, KC316006, KC316007, KC316008, KC316009	AnnGh1, AnnGh2, AnnGh3, AnnGh4, AnnGh5, AnnGh6, GhFAnX, AnxGhFx
Gossypium barbadense	Dicot	6	KC316004, KC316005, KC316006, KC316007, KC316008, KC316009	AnxGb1, AnxGb2, AnxGb3, AnxGb4, AnxGb5, AnxGb6
Vitis vinifera	Dicot	14	Vv08g00710, Vv01g05380, Vv00g25040, Vv00g00750, Vv00g00660, Vv00g00710, Vv00g00800, Vv03g02080, Vv00g00650, Vv00g00720, Vv00g00760, Vv00g25070, Vv06g10680, Vv18g03470	VvAnn1
Capsicum annuum	Dicot	15	Capana01g003267, Capana01g003268, Capana04g000557, Capana04g001093, Capana05g000872, Capana07g001263, Capana07g002415, Capana08g001265, Capana08g001266, Capana08g001267, Capana08g001268, Capana08g001269, Capana11g000652, Capana12g001722, Capana00g004266	CaAnn1 to CaAnn15

(*Continued*)

Organism	Lineage	Total	Accession No.	Annxins
Glycine max	Dicot	22	Gm08g14460, Gm05g31250, Gm13g01870, Gm13g27000, Gm11g21460, Gm04g27100, Gm13g26990, Gm15g38040, Gm15g38010, Gm13g26960, Gm11g21480, Gm15g14350, Gm15g38060, Gm13g27010, Gm13g27020, Gm15g38070, Gm09g30190, Gm07g12030, Gm08g06100, Gm20g01460, Gm07g28080, Gm13g26040	ND
Brassica napus	Dicot	26	BnaA10g22020D, BnaC09g46410D, BnaA10g22010D, BnaC09g46400D, BnaAnng37420D, BnaC02g43450D, BnaA06g23960D, BnaC03g49290D, BnaC06g08410D, BnaA08g06260D, BnaC08g06690D, BnaAnng04520D, BnaC05g27530D, BnaC01g16910D, BnaA10g20320D, BnaC09g44350D, BnaA04g22190D, BnaC04g45920D, BnaA03g18080D, BnaC03g21600D, BnaA02g13560D, BnaC02g45910D, BnaA03g18070D, BnaC03g21590D, BnaA04g22180D, BnaC04g45910D	BnaAnn1A-1, BnaAnn1A-2, BnaAnn1C-1, BnaAnn1C-2, BnaAnn1C-3, BnaAnn2A-1, BnaAnn2A-2, BnaAnn2C-1, BnaAnn2C-2, BnaAnn3A-1, BnaAnn3A-2, BnaAnn3C-1, BnaAnn3C-2, BnaAnn4A-1, BnaAnn4A-2, BnaAnn4C-1, BnaAnn4C-2, BnaAnn5A, BnaAnn5C, BnaAnn6A, BnaAnn6C, BnaAnn7A, BnaAnn7C, BnaAnn8A-2, BnaAnn8C-1, BnaAnn8C-2
Nicotiana tabacum	Dicot	ND	Y14972, Y14773, X93308, AAX78199.	VCaB42, Ntp32.1, Ntp32.2, Cap32.1, NtAnn12
Triticum urartu	Monocots	8	TRIUR3_22191, TRIUR3_11010, TRIUR3_11009, TRIUR3_18622, TRIUR3_04012, TRIUR3_16577, TRIUR3_03306, TRIUR3_32496	TuAnn1, TuAnn2, TuAnn3, TuAnn6, TuAnn9, TuAnn10, TuAnn11, TuAnn12
Oryza sativa	Monocots	10	Os01g31270, Os02g51750, Os05g31750, Os05g31760, Os06g11800, Os07g46550, Os08g32970, Os09g20330, Os09g27990, Os09g23160	OsAnn1, OsAnn3, OsAnn4, OsAnn9, OsAnn10
Sorghum bicolor	Monocots	10	Sb04g027590, Sb10g007760, Sb03g004990, Sb01g035050, Sb09g018980, Sb01g035040, Sb02g041850, Sb02g024090, Sb07g020760, Sb02g026390	ND
Brachypodium distachyon	Monocots	11	Bd1g18990, Bd1g45487, Bd1g62120, Bd1g62130, Bd2g13620, Bd2g26760, Bd2g26770, Bd3g36240, Bd3g58830, Bd4g29680, Bd4g31920	ND
Hordeum vulgare	Monocots	11	MLOC_15770.2, MLOC_54933.3, MLOC_54932.1, MLOC_15543.3, MLOC_55134.1, MLOC_54650.4, MLOC_51591.2, MLOC_3056.1, AK370408, AK248515, AK249691	HvAnn1 to HvAnn12 (except HvAnn4)
Aegilops tauschii	Monocots	11	AEGTA07604, AEGTA29052, AEGTA21287, AEGTA42363, AEGTA16509, AEGTA11977, AEGTA10144, AEGTA28110, AEGTA31729, AEGTA27331, AEGTA15612	AeAnn1 to AeAnn12 (except AeAnn8)
Zea mays	Monocots	12	Zm05g40790, Zm06g16450, Zm08g03950, Zm03g04200, Zm06g23270, Zm01g15800, Zm08g13570, Zm06g23280, Zm07g13390, Zm02g30240, Zm04g13650, Zm02g31380	ZmAnn33, ZmAnn35
Secale cereale	Monocots	12	SECCE1Rv1G0011310, SECCE1Rv1G0035230, SECCE1Rv1G0035220, SECCE2Rv1G0112000, SECCE2Rv1G0076590, SECCE6Rv1G0429840, SECCE7Rv1G0468070, SECCE7Rv1G0468080, SECCE5Rv1G0326380, SECCE5Rv1G0330660, SECCE6Rv1G0410290, SECCE4Rv1G0252830	ScANN1 to ScANN12
Triticum aestivum	Monocots	25	Traes_1AS_3264B32DD1.1, Traes_1DS_DD0F4640E.1, Traes_1AL_A5FB19E3C.1, Traes_1BL_E0E972134.1, Traes_1BL_04EBF6A19.1, Traes_2AL_788CA6B69.1, Traes_2DL_227971FCE.1, Traes_2BS_879851C74.1, Traes_3AL_A966AD43D.2, Traes_3B_93504D484.1, Traes_4BL_8BD1B62C8.2, Traes_4DL_D0B60B7B5.2, Traes_4AS_63C8858E9.1, Traes_4DL_87FDF914B.1, Traes_5BL_56B9E776F.1, Traes_5DL_E1D06F29F.1, Traes_5BL_23101F4FA.2, Traes_5DL_7A3A1FDF9.2, Traes_6AL_CFE60316E.1, Traes_6DL_6F7AB41471.1, Traes_7AS_FFB7CAFC3.1, Traes_7DS_3F6DCEAA8.1	TaAnn1-A, TaAnn1-D, TaAnn2-A, TaAnn2-B, TaAnn3-A, TaAnn3-B, TaAnn4-A, TaAnn4-D, TaAnn5-B, TaAnn6-A, TaAnn6-B, TaAnn7-B, TaAnn7-D, TaAnn8-A, TaAnn8-D, TaAnn9-B, TaAnn9-D, TaAnn10-A, TaAnn10-B, TaAnn11-A, TaAnn10-D, TaAnn11-B, TaAnn11-D, TaAnn12-A, TaAnn12-D
Picea sitchensis	Gymnosperm	3	PsABK22223, PsABK21977, PsACN40166	ND

ND, not determined.

negatively regulates plant responses to drought and salt stress. The *35S:AnnAt1* plants presented the greatest sensitivity to drought and salt stress, whereas the *annat1* mutant presented lower sensitivity and greater survival rates than the wild-type plants. This apparent discrepancy was later explained by the influence of different lighting conditions. AnnAt1 was confirmed to regulate plant salt and drought stress in a light-dependent manner, with positive and negative effects under short- and long-day conditions, respectively. However, AnnAt1 positively regulates stress responses under biotic stress conditions [43]. Clark *et al.* [41] demonstrated that the level of AnnAt1 mRNA is regulated by heavy metal therapy or injury. Microarray and proteomic analyses of the abundance and expression patterns of AnnAt1 in various cell types, tissues, organelles, and physiological responses suggested that AnnAt1 has multiple functions and may play important roles independent of abiotic stress signals [41]. Recent research has shown that AnnAt1 acts as a positive defense regulatory factor, playing a role in both local and systemic defenses against herbivorous grassland noctuid larvae. Loss of function in the *annat1* mutant led to an increase in cytosolic free calcium and a reduction in defense-related responses, which are normally induced by herbicides and injury. Consequently, larvae that fed on the *annat1* mutant gained significantly more weight than those that fed on the wild type did, whereas larvae that fed on the AnnAt1-overexpressing lines gained less weight [44]. Finally, AnnAt1 is involved in the transport of sugars from the phloem to the root tip, indirectly affecting the photosynthetic rate of cotyledons. Under sugar-free conditions, the roots of the *annat1* mutant plants presented a sugar deficiency phenomenon, leading to decreased primary root growth and changes in columnar cells within the root cap. Additionally, the expressions of photosynthesis genes and chlorophyll accumulation were significantly upregulated in the cotyledons. Moreover, postphloem sugar transport in the *annat1* mutant root tip was impaired, restricting the transport of intercellular polysaccharides to the root tip due to increased ROS and callose levels [45]. The subcellular localization of AnnAt1 has been extensively studied. Initially, it was believed to be located in microsomes and cytoplasmic components of roots [37]. However, Hu *et al.* [43] demonstrated that AnnAt1 is located mainly in the endoplasmic reticulum. Recent research provides a detailed description of the distribution and subcellular localization of AnnAt1-GFP in different plant cells. AnnAt1-GFP accumulated in the root cap and epidermal cells but was depleted in dividing cells within the root meristem tissue. During root hair development, AnnAt1-GFP accumulates at the tips of germinating and growing root hairs. In aboveground plants, AnnAt1-GFP is located mainly in the cortex of trichomes and epidermal cells of hypocotyls, cotyledons, true leaves, and petioles. At the subcellular level, AnnAt1-GFP is enriched in the plasma membrane and vesicles of nondividing cells, as well as in the mitosis and microtubules of dividing cells. AnnAt1-GFP also accumulates around the nuclear membrane of elongated hair droplet cells. These observations demonstrate the complex developmental and subcellular localization patterns of AnnAt1 within living *A. thaliana* plants [46].

Annexins Regulate Plant Growth and Development

Plant annexins play crucial roles in regulating various cellular processes, including cell division, cell elongation, and exocytosis. They also have a significant effect on the growth and development of multiple tissues and organs, such as pollen development, fiber formation, and seed germination.

Annexins play important roles in many cellular processes

One example is VCaB42, which is a vacuole-associated annexin. Its expression is closely associated with the expansion of tobacco cells and may be involved in the vacuolation process of expanding cells. Research has shown that VCaB42 levels correlate with age-associated and hormonally induced changes in cell volume in tobacco (*Nicotiana tabacum*) BY-2 suspension cultures. This correlation suggests a potential role for VCaB42 in vacuole biogenesis [47]. In *N. tabacum*, Proust *et al.* [48] identified two annexins, Ntp32.1 and Ntp32.2, which are homologous to bell pepper Cap32.1 and were named Sp32 annexin. The expression of Sp32 is regulated by developmental processes in plants, with the highest expression level observed in growing and dividing tissues. In cultured BY-2 cells, Sp32 is expressed only in exponentially growing cells. Moreover, during the cell cycle, Sp32 is expressed at the G2/M and G1/S transitions and peaks at mitosis termination. Sp32 is also present in intercellular junctions and forms a ring structure under the plasma membrane, indicating the potential involvement of annexins in cell wall maturation [48]. In *Zea mays*, annexin p35 regulates exocytosis from maize root cap protoplasts in the presence of Ca^{2+} and GTP. Maize annexin p35 is expressed in root cap cells, differentiating vascular tissues, and elongating cells. Protoplasts dialyzed against the maize p35 protein show enhanced exocytosis at physiological Ca^{2+} concentrations, which can be blocked by dialysis with antibodies specific for maize annexins. Overall, annexin p35 can increase exocytosis when Ca^{2+} level increases [49]. In *A. thaliana*, AnnAt1 and AnnAt2 are involved in secretion during early growth and development in seedlings. These annexins are detected in the cytoplasm and periphery of cells with high secretory activity. Annexins have also been localized in the cytoplasm and at the periphery of highly secretory cell types in plants, particularly in developing sieve tubes, outer root cap cells, and root hairs. They can also be found near the apical meristem, in some leaf cells, and in phloem-associated cells at different developmental stages. The localization pattern of these annexins in the same cell at the same developmental stage is closely related to the active secretion process of cell wall materials [50].

Annexins regulate tissue and organ development

As early as 1996, researchers analyzed the expression characteristics of annexins during bell pepper fruit development. In this study, they isolated annexin cDNA and examined the differential expression patterns of the corresponding transcripts during the early stages of development and ripening. Furthermore, researchers discovered approximately 30 kDa annexin peptides at each stage of bell pepper fruit development and successfully purified them *in vitro* [48]. In the case of mimosa plants, the potential contribution of annexin to nyctinastic movement in the pulvinus has been explored. Researchers have reported that the expression of annexins in various tissues of mimosa plants is regulated throughout their development. Specifically, they reported that the amount of annexins in the pulvinus increases at night and that annexins are primarily distributed in the cytoplasm. During the day, the localization of annexins shifts, and annexins become predominantly distributed in the cell periphery. Additionally, researchers have shown that mimosa annexins are sensitive to abscisic acid but

do not respond to cold or mechanical stimuli [15]. Arabidopsis Annexin5 plays a crucial role in pollen development, germination, and pollen tube growth by facilitating calcium-modulated endomembrane trafficking. Researchers have used Ann5-RNAi lines to observe severely sterile pollen grains. Furthermore, both brefeldin A and lactrunculin can inhibit pollen germination and pollen tube growth. In contrast, plants overexpressing Ann5 presented increased resistance to BFA, leading to increased pollen germination and pollen tube growth. This effect seems to be regulated by calcium. Additionally, overexpression of Ann5 promoted Ca^{2+}-dependent cytoplasmic streaming in pollen tubes in response to BFA. However, compared with the wild-type, overexpression of Ann5 did not affect pollen germination or pollen tube growth in response to lactrunculin [51]. ZmANN33 and ZmANN35 encode proteins involved in plasma membrane recovery during seed germination. Compared with that under normal-temperature conditions, the expressions of both genes were upregulated during seed germination but repressed by chilling. *A. thaliana* seedlings expressing ZmANN33 or ZmANN35 presented longer seedling lengths than wild-type plants during the recovery period following chilling stress. Furthermore, these transgenic plants presented lower levels of lipid peroxidation and greater peroxidase activity than the wild-type plants. Moreover, the root cells of these transgenic plants presented greater numbers of intact plasma membranes after chilling stress. These findings highlight the positive roles played by these two gene products in plant recovery from chilling injury and their contribution to maintaining plasma membrane integrity. Additionally, both ZmANN33 and ZmANN35 were found to localize to the cytosol near the plasma membrane [52].

Annexins participate in regulating fiber elongation

Multiple annexins that are involved in regulating fiber elongation were identified in the cotton fiber cDNA library. As early as 1999, Shin *et al.* [14] identified a novel cotton fiber annexin. Through Northern blot analysis, they demonstrated that the annexin gene is highly expressed during the elongation stages of cotton fiber differentiation, suggesting its role in cell elongation [14]. Several annexin proteins, including GhFAnnx, GhAnx1, AnGb5/6, AnnGh3, and GhAnn2, were subsequently shown to be involved in fiber formation. The annexin GhFAnnx exhibits preferential expression in different developmental fibers, particularly at the edge and inner side of the apex of the *Gossypium hirsutum* fiber tips. Conversely, expressions in roots, stems, and leaves are low. GhFAnnx is associated with the polar expansion of *G. hirsutum* fibers during elongation [53]. Like GhFAnnx, GhAnx1 from *G. hirsutum* is highly expressed in fibers, especially during the elongation stage. Analysis of its protein sequence revealed high similarity and evolutionary associations with annexins from higher plants. In addition to its function in fiber elongation, GhAnx1 also plays potential roles in antioxidant activity, as *E. coli* cells expressing GhAnx1 are protected from tert-butyl hydroperoxide stress [54]. The annexin AnxGb6 is believed to act as an adaptor that associates the fiber cell membrane with actin assembly, thus participating in the regulation of *Gossypium barbadense* fiber elongation. Six *G. barbadense* annexin genes (AnxGb) are highly expressed in elongating cotton fibers, but only AnxGb6 exhibits fiber-specific expression. AnxGb6 has strong Ca^{2+}- and lipid-binding abilities and is capable of interacting with AnxGb5 to generate protein macrafts in the cell membrane, which may serve as a stabilized scaffold for Actin1 organization. Moreover, the expression of the *AnxGb6* gene affects the direction and density of actin assembly, subsequently impacting *G. barbadense* fiber length and elongation rate. Both AnxGb5 and the fiber-specific actin gene GbAct1 can interact with AnxGb6. Overexpression of AnxGb6 in *A. thaliana* results in increased F-actin accumulation in the basal part of the root cell elongation zone, thereby increasing root elongation without affecting the number of root cells [55,56]. Furthermore, Li *et al.* [57] isolated and identified the cotton annexin AnnGh3 and investigated its expression pattern during fiber development. AnnGh3 is initially expressed in initiating fiber cells and reaches its highest expression level during rapid fiber elongation. As fiber cells further develop, its expression dramatically decreases to a relatively low level. Additionally, its expression in fibers is regulated by phytohormones and Ca^{2+}. The annGh3 protein is localized in the cytoplasm. The overexpression of AnnGh3 in *A. thaliana* leads to a significant increase in trichome density and length on the leaves of the transgenic plants, suggesting its involvement in fiber cell initiation and elongation in *G. hirsutum* [57]. GhAnn2, derived from the tetraploid species D genome, is highly expressed in elongated fibers and is induced by plant hormones, such as IAA and GA3, which play critical roles in fiber elongation. Silencing of the *GhAnn2* gene inhibits fiber elongation and secondary cell wall synthesis in the transgenic plants, resulting in shorter and thinner mature fibers, as well as a lower rate of Ca^{2+} influx from the extracellular to the intracellular environment, than in the wild-type [30]. Moreover, Yang *et al.* [58] conducted comparative proteomic analysis to explore the mechanism of cell elongation in developing cotton fibers. The results demonstrated a strong correlation between development-dependent proteins and fiber biochemical processes, as well as morphogenesis. This research provides a comprehensive overview of development-dependent protein changes in cotton fibers, serving as a framework for further functional research on target proteins associated with fiber development [58]. Table 2 shows the characteristics of the annexins that regulate growth and development.

Annexins Regulate Plant Abiotic Stress

In recent decades, the plant annexin family has been shown to respond to various abiotic stresses in multiple species, such as Arabidopsis, mustard, maize, and poplar. The expression patterns of the genes AnnBj1, AnnBj2, AnnBj3, AnnBj6, and AnnBj7, which are members of *B. juncea* annexin, are regulated by various stress conditions, including exposure to signaling molecules, salinity, and oxidative stress. The promoters of these genes contain different *cis*-elements that respond to various stress conditions [26]. *B. juncea annexin* (*AnnBj1*) is regulated by abscisic acid, ethephon, salicylic acid, methyl jasmonate, osmotic stress-inducing chemicals (mannitol), heavy metal stress ($CdCl_2$), and oxidative stress. Transgenic tobacco plants with ectopic expression of AnnBj1 were generated under the control of the 35S promoter and exhibited significant tolerance to drought stress, salt stress, heavy metal stress, and oxidative stress at the seedling stage [59]. These transgenic plants also retained relatively high chlorophyll levels in response to the aforementioned stresses. In addition to transgenic *A. thaliana* plants, transgenic cotton plants expressing AnnBj1 also presented increased tolerance to different abiotic stresses, such as sodium chloride, mannitol, polyethylene glycol, and hydrogen peroxide. Compared with the wild-type plants, the transgenic plants presented greater retention of total chlorophyll, reduced accumula-

Table 2. Characteristics of annexins regulating growth and development

Annexins	Accession No.	Species	Function	Regulation	Ref.
AnnAt1	AT1G35720	*Arabidopsis thaliana*	Secretion process	Positive	[50]
AtAnn5	AT1G68090	*Arabidopsis thaliana*	Pollen development	Positive	[51]
AnxGb6	KC316009	*Gossypium barbadense*	Fiber elongation	Positive	[55]
AnxGb5	KC316008	*Gossypium barbadense*	Fiber elongation	Positive	[55]
GhFAnnx	ACJ11719	*Gossypium hirsutum*	Fiber elongation	Positive	[53]
GhAnx1	AY351650	*Gossypium hirsutum*	Fiber elongation	Positive	[54]
GhAnn2	AAB67994	*Gossypium hirsutum*	Fiber elongation	Positive	[30]
AnnGh3	JX897059	*Gossypium hirsutum*	Fiber elongation	Positive	[57]
VCaB42	ND	*Nicotiana tabacum*	Vacuole biogenesis	Positive	[47]
Sp32	ND	*Capsicum annuum*	Cell wall maturation	Positive	[48]
p35	X98245.1*	*Zea mays*	Exocytosis	Positive	[49]
ZmANN33	CAA66900	*Zea mays*	Seed germination	Positive	[52]
ZmANN35	X98245.1*	*Zea mays*	Seed germination	Positive	[52]

*These accession numbers are mRNA numbers.

tion of hydrogen peroxide, increased fresh weight and dry weight, and greater relative water content after stress treatment. These responses are associated with increased total peroxidase activity and increased levels of osmoprotectants, specifically proline and sucrose [60]. The promoter of the maize annexin gene contains different stress-responsive *cis*-elements, suggesting that these genes play important roles in maize adaptation to various environmental stresses [28]. The expressions of annexin genes in *P. trichocarpa* can be induced by multiple abiotic stresses, indicating their widespread involvement in the response to abiotic stresses [24]. The annexin genes of Brassicaceae species respond to different abiotic stresses and hormone treatments. *BnaANN* genes play important roles in jasmonate signaling and multiple stress responses in *B. napus* [33].

Drought and osmotic stress

AnnAt1 and AnnAt4 are involved in osmotic stress and abscisic acid signaling in *A. thaliana*. These proteins bind to each other in a Ca^{2+}-dependent manner and function cooperatively under drought stress, regulating plant stress responses in a light-dependent manner. The *annat1* and *annat4* single mutants exhibit drought tolerance, and this tolerance is further enhanced in the *annat1annat4* double mutant. However, transgenic plants overexpressing *AnnAt4* (*35S:AnnAt4*) are more sensitive to stress treatment under long-day conditions. After osmotic stress, the expressions of key genes encoding enzymes involved in ABA and proline synthesis strongly increased in the *annat1annat4* plants but slightly increased in the *annat1* and *annat4* plants but not in the *35S:AnnAt4* plants. Additionally, *annat4* plants are more drought-tolerant under short days than under long days [43]. Liu *et al.* [61] reported that AtANN2 and AtANN3 confer drought tolerance under short-day and long-day conditions, respectively. Under their respective functional photoperiods, *AtANN2* and *AtANN3* are upregulated in mannitol-treated roots. Their encoded proteins are rapidly targeted to the plasma membrane, where they mediate significant Ca^{2+} flow across the membrane. The functions of AtANN2 and AtANN3 are repressed by AtCRY2, which affects the plasma membrane localization of AtANN2 and AtANN3, thereby inhibiting transmembrane Ca^{2+} flow [61]. In *A. thaliana*, AnnAt8 is upregulated by dehydration stress and positively regulates abiotic stress at different stages of plant growth and development. Compared with wild-type plants, transgenic plants overexpressing AnnAt8 presented greater seed germination rates, greater plant growth, and greater chlorophyll retention under dehydration stress treatment. Furthermore, these transgenic plants presented greater levels of proline and lower levels of malondialdehyde than did the WT plants [62]. In wild tomato (*Solanum pennellii*), AnnSp2 positively regulates drought resistance by modulating ABA synthesis and scavenging ROS. *AnnSp2* is highly expressed in leaves, flowers, and fruits, and its expression is induced by plant hormones and abiotic stress. Tomato plants overexpressing AnnSp2 presented increased tolerance to drought stress, decreased accumulation of ROS, and increased peroxidase activity. Under drought stress, the ABA content of the AnnSp2-overexpressing plants significantly increased, resulting in stomatal closure and reduced water loss. Additionally, AnnSp2-transgenic plants are less sensitive to ABA during the seed germination and seedling stages [17]. In addition, the annexin AnnSp2 can interact with SpUSP, which plays a crucial role in drought tolerance by influencing ABA-induced stomatal movement and alleviating oxidative stress. Under drought stress, the ABA content significantly increases in SpUSP-overexpressing plants, inducing stomatal closure and reducing water loss, ultimately increasing drought tolerance [63]. The potato annexin gene *STANN1* is upregulated under drought stress and positively regulates the drought resistance of *S. tuberosum* plants by maintaining effective photosynthesis. Under drought conditions, transgenic potato plants ectopically expressing *STANN1* are more tolerant to water deficit in their roots, retain more water in green tissues, and maintain chloroplast functions. Moreover, drought-induced reductions in the maximum efficiency and electron transport rate of photosystem II, as well as the quantum yield of photosynthesis, were less severe in the transgenic plants than in the wild-type plants [29]. In cotton, the overexpression of GhAnn1 significantly enhanced ABA biosynthesis. Under both normal and stress conditions, the ABA content in the root tissues of overexpression lines (OEs) seedlings remained at a higher level compared to that of the wild type. GhAnn1 may be associated with drought and oxidative stress responses through ABA signaling [64].

In *Vitis vinifera L.*, the annexin gene *VvANN1* plays a positive role in the drought stress response by regulating ROS homeostasis. *VvANN1* is significantly induced by osmotic stress, and its ectopic expression in *A. thaliana* enhances drought and osmotic tolerance by modulating the levels of MDA, H_2O_2, and O^{2-}. Furthermore, in response to drought stress, VvbZIP45 can regulate *VvANN1* expression by directly binding to the promoter region of *VvANN1* [65]. In *O. sativa*, OsANN3 is a positive regulator of ABA-dependent stress tolerance. It is induced by PEG and ABA treatments. Overexpression of OsANN3 in *O. sativa* significantly increases survival rates and results in better growth, such as longer root length and more pronounced stomatal closure, under drought stress. On the other hand, knocking down *OsANN3* results in sensitivity to drought. Additionally, overexpression of OsANN3 enhances sensitivity to exogenous ABA, reducing water loss by regulating ABA-dependent stress response pathways [66]. In contrast to OsANN3, OsANN10 negatively regulates plant responses to osmotic stress. Knocking down *OsANN10* significantly decreases the content of H_2O_2 by increasing peroxidase and catalase activities, thereby reducing oxidative damage in *O. sativa* leaves [16]. Certain *O. sativa* annexins play critical roles in drought-related ABA responses by modulating ROS production, mediating Ca^{2+} influx, or phosphorylating other proteins. Compared with those of the wild-type plants, the plants in which *OsANN4* was knocked down via RNA interference presented some significant phenotypic changes, such as a lower rooting rate and shorter shoot and root lengths. Moreover, the activities of superoxide dismutase and catalase are significantly reduced, and net Ca^{2+} influx is inhibited in the RNAi strain. Additionally, OsCDPK24 interacts with and phosphorylates OsANN4, potentially causing conformational changes that affect its binding to Ca^{2+} [67]. Like OsANN4, OsANN9 may also function as a positive regulator in response to drought stress by regulating antioxidant accumulation. After exposure to drought stress, both the *OsANN9*-RNAi lines and the *osann9* mutants were hypersensitive to drought and severe wilting, whereas the *OsANN9*-OE lines grew better than the WT plants did. In addition, POD, SOD and CAT activities were significantly increased in *OsANN9*-OE plants but decreased in *OsANN9*-RNAi and *osann9* plants after drought treatment [68].

Salt stress

The plant annexin genes that are involved in regulating salt tolerance are involved primarily in a few plant species, namely, Arabidopsis, tomato, and cotton. These genes often play a role in salt tolerance, which is closely associated with drought tolerance. Furthermore, salt stress sensors in plant roots play crucial roles in detecting and responding to early signals during salt stress episodes. Currently, various proteins, including annexins, are considered potential candidates for salt stress sensors [69].

AnnAt1 and *AnnAt4* are not only responsible for regulating osmotic stress but also play a role in regulating plant salt stress. Single-mutant *annAt1* and *annAt4* plants demonstrated tolerance to salt stress, a trait that is even more pronounced in double-mutant *annAt1annAt4* plants. However, it should be noted that *AnnAt4*-overexpressing transgenic plants (*35S:AnnAt4*) exhibit increased sensitivity to stress treatments under long-day conditions [43]. The expression of the *AnnAt8* gene is significantly induced by NaCl, up to several hundred times. Transgenic plants overexpressing *AnnAt8* presented increased rates of seed germination, improved plant growth, and increased chlorophyll retention under NaCl treatment. These plants also presented elevated level of proline and reduced level of malondialdehyde, which may be attributed to alterations in the expressions of stress regulatory genes in transgenic tobacco [62]. Under the treatments of the plant hormone ABA and salt stress, the expression of GhAnn1 is significantly upregulated. The overexpression of GhAnn1 enhances cotton's tolerance to salt stress. Specifically, GhAnn1 regulates the biosynthesis pathways of ABA and phenylpropanoid, and boosts the expression of genes related to Na^+ and K^+ transporters. GhAnn1 improves cotton's salt tolerance by regulating ABA accumulation, coordinating ion homeostasis, and involving in the phenylpropane biosynthesis pathway [64]. The expression of the annexin gene *AnnBj2* is tissue specific, with relatively high expression observed in roots and under NaCl treatments. The plants overexpressing AnnBj2 are insensitive to NaCl [70]. Similarly, the *AnnSp2* gene was isolated from drought-resistant wild tomato (*S. pennellii*). Overexpressing *AnnSp2* in tomato plants increases their tolerance to salt stress. The over-expressing plants also presented reduced levels of reactive oxygen species, elevated total chlorophyll content, decreased lipid peroxidation levels, increased peroxidase activity, and increased level of proline [17]. Additionally, annexin genes in *C. annuum* are tissue-specific and are widely expressed in pepper leaves after exposure to cold, salt, drought, or exogenously applied MeJA and ABA. Among the *CaAnn* genes, *CaAnn9*-silenced pepper seedlings appear to be more vulnerable to salt stress, as evidenced by chlorophyll degradation, increased accumulation of reactive oxygen species, and a decrease in antioxidant defense capacity [35].

Other stresses

Annexins play crucial roles in regulating various abiotic stresses, such as temperature stress and heavy metal stress, in plants. However, the specific mechanisms by which annexins mediate these stress responses require further investigation. In the case of cold-acclimated wheat (*T. aestivum*), two annexins with molecular masses of 39 kDa and 22.5 kDa potentially participate in the low-temperature signal transduction pathway. These annexins, known as p39 and p22.5 proteins, are associated with the microsomal fraction. Their levels are induced by low temperatures, gradually increasing after exposure for one day and reaching a maximum level. The p39 protein is localized to the plasma membrane, but the function of the p22.5 protein remains unknown [71]. In *O. sativa*, the annexin OsANN1 enhances heat stress tolerance by modulating the production of H_2O_2. Plants with reduced OsANN1 expression are more sensitive to heat stress, whereas those with increased OsANN1 expression exhibit improved growth under abiotic stress conditions. Under high-temperature stress, the OsANN1 protein significantly accumulates in the cytoplasm, thereby promoting the activities of superoxide dismutase and catalase. This regulation of H_2O_2 content and redox homeostasis helps protect cells from heat stress-induced damage. Furthermore, OsANN1 can interact with OsCDPK24, signaling a potential additional regulatory layer in abiotic stress responses [72]. Another example is *B. juncea* annexin-3, which has been found to mitigate oxidative stress in plants. Continuous expression of *AnnBj3* in *A. thaliana* effectively alleviates the oxidative stress caused by methyl viologen. AnnBj3 plays a role in detoxifying hydrogen peroxide and exhibits antioxidative properties. Transgenic plants expressing AnnBj3 also exhibit increased peroxidase activity, providing comprehensive

cellular defense against methyl viologen-induced cytotoxicity [73]. *S. tuberosum* annexin STANN1 not only promotes drought resistance in transgenic plants but also protects the reaction centers of plants from photooxidative damage under drought conditions. Compared with wild-type plants, transgenic potato plants expressing STANN1 retain more water in their green tissues, maintain chloroplast function, and accumulate greater levels of chlorophyll b and xanthophylls. Moreover, the negative effects of drought on the maximum efficiency and electron transport rate of photosystem II, as well as the decrease in the quantum yield of photosynthesis, are less pronounced in transgenic plants [29]. Finally, certain annexin genes in maize and cotton have been found to respond to heavy metals, including Ni^{2+}, Zn^{2+}, and Cd^{2+}. However, the specific mechanisms by which these annexins contribute to heavy metal tolerance have not yet been studied in detail [28,74].

Annexins Regulate Plant Biotic Stress

In *N. tabacum* BY2 cell suspension cultures, the *Ntann12* gene is induced by pathogenic bacteria, such as *Rhodococcus fascians* and *Pseudomonas syringae*. The protein encoded by this gene is located in the cytoplasm. In the *pNtann12-GUS* transgenic plants, GUS staining specific to *R. fascians*-infected plants was observed on the leaf surface. Additionally, GUS blue spots appeared in the emerging adventitious meristems but were not observed in the noninfected transgenic plants [75]. Jami *et al.* [59] generated transgenic tobacco plants that ectopically overexpressed AnnBj1. They demonstrated that overexpression of AnnBj1 provides tolerance to various abiotic and biotic stresses. The transgenic plants presented increased resistance to the oomycete pathogen *Phytophthora parasitica* var. *nicotianae*, as well as increased expressions of several pathogenesis-related proteins [59]. Resistance to Powdery Mildew 8.1 (*RPW8.1*) is one of the few broad-spectrum resistance genes. ANNEXIN 8 can negatively regulate RPW8.1-mediated cell death and disease resistance in *A. thaliana*. Loss-of-function or RNA silencing of *AtANN8* resulted in increased expression of RPW8.1- and RPW8.1-dependent necrotic lesions in leaves and increased defense against powdery mildew. Conversely, overexpression of AtANN8 compromised RPW8.1-mediated disease resistance and cell death [76]. OsANN1 is induced by *Magnaporthe oryzae* infection and negatively regulates blast disease resistance in *O. sativa*. OsANN1 interacts with the cytochrome P450 monooxygenase HAN1, which is also a negative regulator of *O. sativa* resistance, by acting as an upstream molecule and stabilizing HAN1. The OsANN1-HAN1 module impairs blast disease resistance by inactivating biologically active JA-Ile and JA signaling in *O. sativa* [3]. AtANN1 serves as a positive defense regulatory factor in the local and systemic defenses of plant attacks by the larvae of the herbivorous *Spodoptera littoralis*. *S. littoralis* that fed on ann1 gained significantly more weight than larvae that fed on the wild-type did, whereas those that fed on ANN1-overexpressing lines gained less weight [44]. Table 3 shows the characteristics of the annexins that regulate stress.

Table 3. Characteristics of annexins regulating stress

Annexins	Accession No.	Species	Stresses	Regulation	Ref.
AnnAt1	AT1G35720	*Arabidopsis thaliana*	Drought stress, osmotic stress, salt stress	Negative	[43]
AnnAt4	AT2G38750	*Arabidopsis thaliana*	Drought stress, osmotic stress, salt stress	Negative	[43]
AtANN2	AT5G65020	*Arabidopsis thaliana*	Drought stress	Positive	[61]
AtANN3	AT2G38760	*Arabidopsis thaliana*	Drought stress	Positive	[61]
AnnAt8	AT5G12380	*Arabidopsis thaliana*	Dehydration stress, salt stress	Positive	[62]
AtANN8	AT5G12380	*Arabidopsis thaliana*	Powdery mildew	Negative	[76]
AtANN1	AT1G35720	*Arabidopsis thaliana*	Herbivorous animals attack	Positive	[44]
AnnBj1	DQ191753	*Brassica juncea*	Drought stress, salt stress, heavy metal, oxidative stress, oomycete pathogen	Positive	[59]
AnnBj2	DQ388683	*Brassica juncea*	Salt stress	Positive	[70]
AnnBj3	DQ388684	*Brassica juncea*	Oxidative stress	Positive	[73]
CaAnn9	Capana08g001266	*Capsicum annuum*	Salt stress	Positive	[35]
GhAnn1	AAB67993	*Gossypium hirsutum*	Salt stress	Positive	[64]
Ntann12	AAX78199	*Nicotiana tabacum*	Pathogenic bacterium	Positive	[75]
OsANN3	Os07g0659600	*Oryza sativa*	ABA-dependent stress	Positive	[66]
OsANN10	Os09g0368850	*Oryza sativa*	Osmotic stress	Negative	[16]
OsANN4	LOC_Os05g31750	*Oryza sativa*	Drought stress	Positive	[67]
OsANN9	LOC_Os05g31760	*Oryza sativa*	Drought stress	Positive	[68]
OsANN1	LOC_Os02g51750	*Oryza sativa*	Heat stress	Positive	[72]
OsANN1	LOC_Os02g51750	*Oryza sativa*	Blast disease	Negative	[3]
AnnSp2	LOC107018370	*Solanum pennellii*	Drought stress, salt stress	Positive	[17,63]
STANN1	PGSC0003DMG400017714	*Solanum tuberosum*	Drought stress, photooxidative damage	Positive	[29]
p39, p22.5	ND	*Triticum aestivum*	Low-temperature stress	Positive	[71]
VvANN1	Vv18g03470	*Vitis vinifera*	Drought stress, osmotic stress	Positive	[65]

ND, not determined.

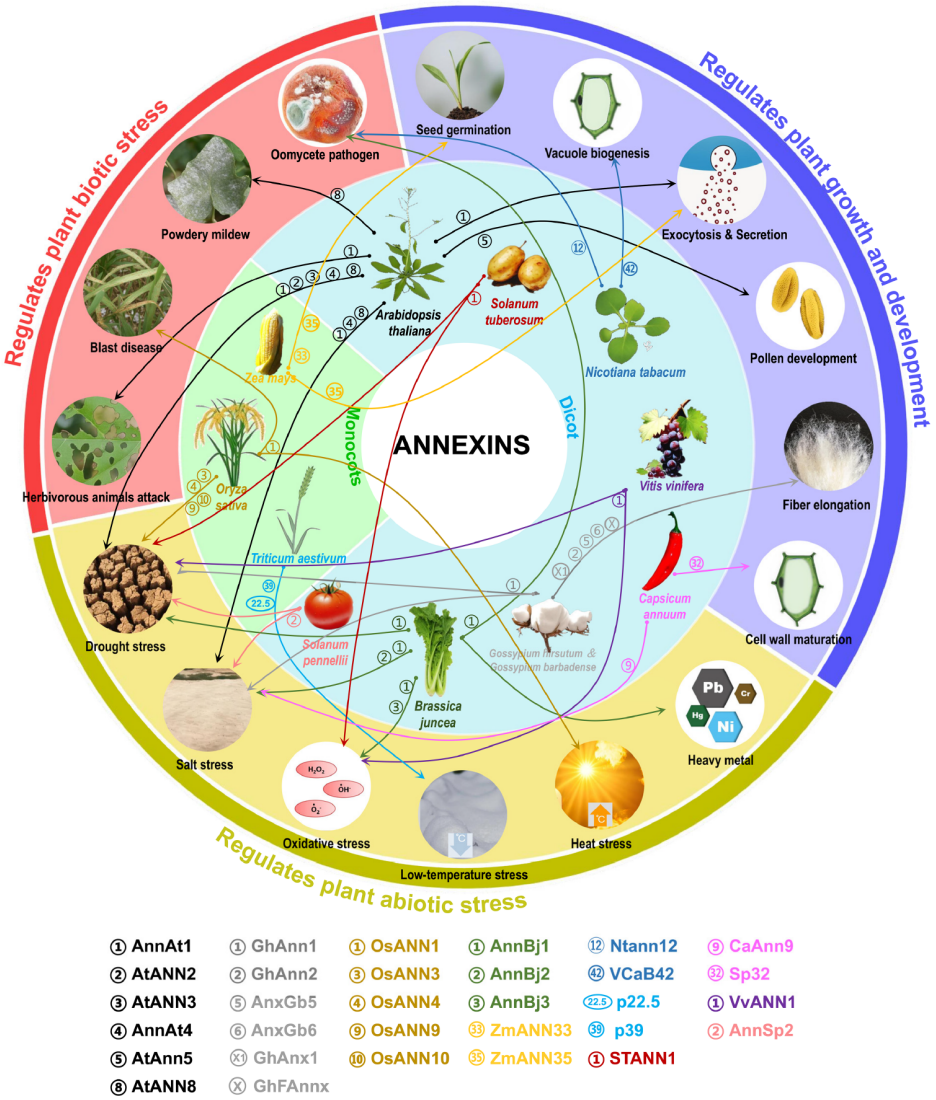


Figure 1. Working model of plant annexins Different annexins in plants regulate plant growth and development and stress tolerance in different ways.

Conclusions and Prospects

Owing to the availability of genome sequences from different species, including various model organisms and crops, annexin families across the plant kingdom are being rapidly identified. Plant annexins are potentially multifunctional proteins *in vivo* that participate in actin modeling, membrane dynamics, and the regulation of $[Ca^{2+}]_{cyt}$ and ROS. Annexins play important roles in plant development, stress responses, and defense mechanisms, and their regulatory patterns in these processes exhibit acute and long-term responses. Examining annexin genes holds great value for elucidating the role of annexin signaling in abiotic and biotic stress tolerance, which are areas that remain largely unexplored. The continuous enrichment of our understanding of signaling processes might help assign specific roles to different plant annexins. In this article, we review various plant annexins that regulate stress responses, some of which are involved in regulating at least two stress responses, including salt and drought stresses (Figure 1). Whether plant annexins serve to integrate these processes warrants further investigation. Although some mechanisms through which annexins regulate physiological processes have been preliminarily analyzed, many topics remain worthy of exploration, such as the downstream proteins that interact with annexins, how they affect annexin function, and the relationship between annexins and lipids. Future studies should focus primarily on the following aspects. First, which annexin activities are subject to group modifications, such as glutathionylation? Second, genetic engineering utilizing annexin genes might offer an excellent platform for developing crops with increased abiotic or biotic tolerance. Biological processes involving annexin have been identified through gene knockout and imaging techniques, while the expression patterns of annexin genes have been explored via microarray and RNA interference assays, implicating annexin in certain cellular processes or pathways. Future experiments elucidating the role of plant annexins through electrophysiology should focus on *in vivo* membranes. Finally, annexin biologists will be anticipated to answer the following questions, and most importantly to explore the underlying

mechanisms in the years to come. Do annexins have different functions in different cell types? How are annexins secreted? Can annexins be classified into functionally integrated groups, or are they functionally independent?

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Conflict of Interest

The authors declare that they have no conflict of interest.

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