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Plant dehydrins and stress tolerance
Versatile proteins for complex mechanisms

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Dehydrins (DHNs), or group 2 LEA (Late Embryogenesis Abundant) proteins, play a fundamental role in plant response and adaptation to abiotic stresses. They accumulate typically in maturing seeds or are induced in vegetative tissues following salinity, dehydration, cold and freezing stress. The generally accepted classification of dehydrins is based on their structural features, such as the presence of conserved sequences, designated as Y, S and K segments. The K segment representing a highly conserved 15 amino acid motif forming amphiphilic α-helix is especially important since it has been found in all dehydrins. Since more than 20 y, they are thought to play an important protective role during cellular dehydration but their precise function remains unclear. This review outlines the current status of the progress made toward the structural, physico-chemical and functional characterization of plant dehydrins and how these features could be exploited in improving stress tolerance in plants.

Introduction

To face various abiotic stress factors, such as drought, heat, freezing or salinity, plants as sessile organisms, have evolved special mechanisms, to prevent loss of intracellular water, i.e., dehydration. The high accumulation of late embryogenesis abundant (LEA) proteins was described as the most common mechanism developed against water stress. The LEA proteins discovered in cotton more than two decades ago,[1] were originally described to be expressed at high levels during the later stages of embryo development comprising up to 4% of cellular proteins.[2] Dehydrins (DHNs) constitute a distinct biochemical group of LEA proteins, which is known as group 2 LEA (or LEA II) proteins[3,4] or LEA-D11 proteins.[5] These proteins are known to accumulate during late embryogenesis or can be induced in vegetative tissues by various stress factors that cause cell dehydration (i.e., drought, salinity, cold, heat etc.). Because the expression of many DHNs increases by the phytohormone abscisic acid (ABA), they are also referred as RAB proteins (Responsive to ABA). Like all LEA proteins, DHNs are ubiquitous among various plant species belonging to angiosperms and gymnosperms (reviewed in refs 6 and 7) and perhaps other photosynthetic organisms, including ferns, mosses, algae and cyanobacteria.[8] They are believed to play an important protective role in plant cell during dehydration. This review will focus on the most relevant advances in understanding the structure-function relationship within DHNs and on their potential use in improving stress tolerance in plants.

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Dehydrins: Undefined Structure for Multiple Functions

DHNs are characterized by wide range of molecular masses from 9–200 kD.[9,10] As their sequence characteristics became available, DHNs were redefined on the basis of their motifs and newly defined as proteins possessing at least one copy of a conserved sequence known as K-segment in their molecules.[6,8] The K-segment is a lysine-rich amino acid (aa) sequence (EKK GIM E/DKI KEK LPG) present in 1–11 copies near the C terminus of dehydrin molecules. DHNs can also possess other conserved motifs: the tyrosine-rich Y-segment [consensus (V/T)D(E/Q) YGNP] near the N-terminus and the serine-rich S-segment formed by a stretch of 4–10 serine residues, which are a part of a conserved sequence LHRSGS4–10(E/D)3. The S-segment can undergo phosphorylation by the casein kinase 2 (CK2).[7,11] According to the presence of the K-, S- and Y-segments, DHNs can be divided into five structural subgroups: Kn, SKn, KnS, YnKn and YnSKn.[6,8,12]

In aqueous solutions, DHN molecules are present in the conformation of random coil, i.e., they form a maximum of hydrogen bonds with neighboring water molecules (intermolecular hydrogen bonds) and a minimum of hydrogen bonds between different aa residues (intramolecular hydrogen bonds). Due to low proportion of intramolecular hydrogen bonds, DHNs appear unstructured and share indeed many features with other types of intrinsically disordered/unstructured proteins (IDPs/IUPs).[13,14] Accordingly they contain high proportions of hydrophilic aa and change their conformation according to the changes in their ambient microenvironment. Based on several experimental studies, it was confirmed that the decrease in dehydrin hydration status (loss of water molecules in their ambient microenvironment) or addition of high amounts of compatible solutes (i.e., glycerol), detergents (i.e., SDS) or salts (i.e., NaCl) into a dehydrin aqueous solution, leads to conformational changes which can be monitored by the technique of far-UV circular dichroism.[15-20] Under reduced hydration, the K-segments adopt α-helical

conformation similar to class A2 amphipathic α-helices found in apolipoproteins and α-synucleins.[21] When α-helix is formed within a K-segment, negatively charged aa (with acidic pI, e.g., D and E) lie on one side of the helix, hydrophobic aa (nonpolar, e.g., I and L) lie on the opposite side of the helix, and positively charged aa (with basic pI, e.g., K and R) lie on the polar-non polar interface.[7,22]

The changes in protein conformation result also in changes in protein function. This phenomenon, which is characteristic for IDPs/IUPs, is called "moonlighting."[13,14] In the case of IDPs/IUPs, the changes in protein ambient microenvironment, such as availability of water molecules, result in protein conformational and functional changes. The amphipathic α-helices can interact with partly dehydrated surfaces of various other proteins and also with surfaces of biomembranes. It has been proposed by Ingram and Bartels[4] that several K-segments in one DHN molecule can form bundles when present in α-helical conformation thus enhancing their amphipathic character in protein-protein or protein-biomembrane interactions. The binding of DHNs to the partly dehydrated surface of other proteins enhances formation of amphipathic α-helices in a DHN molecule and protects other proteins from further loss of water envelope (which can lead to irreversible changes in the protein conformation, i.e., protein denaturation). It has been suggested that these interactions between partly dehydrated surfaces of DHN molecules and other proteins and/or biomembranes (observed by Koag et al.[23] in case of maize DHN1), present the basis of dehydrin protective functions. Kovacs et al.[19] reported the protective activities of two dehydrin proteins isolated from *Arabidopsis thaliana*, ERD10 and ERD14, against thermal aggregation of citrate synthase, firefly luciferase, inactivation of lysozyme and thermal inactivation of alcohol dehydrogenase. Cryoprotective activity has been also reported for several DHNs, such as COR85 from spinach,[24] WCS120 from common wheat,[25] and PCA60 from peach.[26] As shown by Reyes et al.[27] the presence of K-segments is essential for dehydrin cryoprotective activity. In an opposite way, DHNs might also prevent heat inactivation and recently, Brini et al.[28] showed that the wheat dehydrin DHN-5 improved the activity and/or thermostability of the fungal β-glucosidase (bglG) and glucose oxidase (GOD/POD) enzymes in vitro. It is therefore plausible to imagine that DHNs can act as chaperones on other proteins and help them to fold properly and/or prevent their aggregation under heat or freezing stress. However, classical chaperones not only prevent inappropriate protein aggregation but also form specific complexes with target proteins through interaction of hydrophobic patches.[29] It is therefore rather difficult for DHNs to establish specific interactions with other proteins especially under dry state, that's why some authors described these dehydrin protective functions based on non-specific protein-protein interactions, as "molecular shield" effect.[30]

Moreover, when cells lose water, the relative distribution among intracellular complexes may change leading to undesirable interactions and aggregation/denaturation of several protein and membrane-associated complexes. As they can accumulate to relatively large amounts in various compartments inside the cells under dehydration, it has been previously proposed that DHNs may simply act as "space-fillers," i.e., they can participate in keeping the original, non-harmful distances among intracellular complexes.[11,30] Briefly, due to their unfolded state, higher accumulation and capability to bind water, DHNs can under dehydration, help in keeping the original cell volume, thus preventing cellular collapse.

Some DHNs, which contain relatively large amounts of H, R and other reactive aa residues on their surface, exhibit also reactive oxygen species (ROS) scavenging and metal ion binding properties. Both functions are mediated by direct interactions between the aa residue and the ROS species (superoxide anion radical O_2^-; singlet oxygen $^1O_2$; hydroxyl radical HO^-; Hydrogen peroxide H_2O_2) or the metal ions (Co^{2+}; Cu^{2+}; Fe^{2+}; Fe^{3+}; Ni^{2+}; Zn^{2+}). The interactions of the aa residue with ROS lead to oxidation of the residue, whereas the interactions with metal ions lead to the formation of covalent bonds. Binding of free metal ions prevents the intracellular compounds from excessive ROS formation since free metal ions act as catalyzers of synthesis of various ROS. DHNs can thus function also as antioxidants (e.g., CuCOR15 and CuCOR19 in *Citrus unshiu*),[18,31] ion sequestrants [e.g., VCaB45 in celery (*Apium graveolens*) vacuoles where it binds Ca^{2+}],[32] or metal ion transporters in plant phloem sap [e.g., ITP protein from castor bean (*Ricinus communis*) which binds Fe^{2+} and Fe^{3+}].[33]

Induction and Modification of Dehydrins in Response to Abiotic Stresses

Like other LEA proteins, DHNs accumulate to high amounts in plant embryos in later stages of their development (embryo maturation and desiccation). In vegetative tissues, they are hardly detected and their presence is limited to young plant organs and those exhibiting rapid cell division or cell elongation, e.g., root tips, elongating stems, petioles, etc.[34,35] Whereas once plants are exposed to various stresses related to cellular dehydration (e.g., drought, osmotic stress, salinity, temperature), DHNs accumulate to high amounts in all vegetative tissues.[36,37] Stress-inducible DHN encoding genes contain ABA-responsive elements (ABRE), C-repeat/drought-responsive/low-temperature-responsive elements (CRT/DRE/LTRE), myeloblastosis (MYB) and myelocytomatosis (MYC) regulatory elements in their promoter regions. Their expression is regulated by ABA-dependent and ABA-independent signaling pathways. ABA-dependent signaling pathways include either bZIP transcription activators named ABFs or AREBs (ABRE binding factors), which bind to ABRE elements, homologs of *A. thaliana* CBF4/DREB1D transcription activator, which bind to CRT/DRE/LTRE elements, and MYBFs and MYCFs, which bind to MYB and MYC promoter elements. ABA-independent signaling pathways include homologs of *A. thaliana* DREB2A and DREB2B transcription activators, which bind to CRT/DRE/LTRE elements (reviewed in refs 38–42).

On the other hand, some DHNs are able to undergo under stress conditions, posttranslational modifications and mainly phosphorylation.[43-47] This phosphorylation occurring on the

Table 1. Possible functions for selected plant DHNs

Host	DHNs	Domains	Reported activity	Reported phenotype	Reference
Wheat	WCOR410	SK3	Membrane binding	Increased tolerance to cold stress	16, 60
Maize	DHN1	YSK_2	Lipid vesicle binding (PA, PS, PG)	Increased tolerance to ABA and water stress	23
Arabidopsis	ERD10/ERD14	SK3/SK2	Phospholipid vesicles binding	Increased tolerance to dehydration	19
Arabidopsis	ERD10/ERD14	SK3/SK2	Heat protection of ADH, Luciferase, lysozyme, citrate synthase	Increased tolerance to dehydration	19
Barley	P80/DHN5	K9	Cryoprotection of LDH	Increased tolerance to cold stress	81
Peach	PCA 60	Y2K9	Cryoprotection of LDH	Increased tolerance to freeze	26, 82
Wheat	DHN5	YSK_2	Enhanced thermostability of bglG and GOD/POD	Increased tolerance to drought and salt tress	28, 62
Citrus	CuCOR19	SK3	Cryoprotection of Catalase and LDH	Increased tolerance to cold stress	18, 59
Citrus	CuCOR19	SK3	Reduction of lipid peroxidation by scavenging peroxyl and hydroxyl radicals	Increased tolerance to cold stress	83, 84
Citrus	CuCOR15	SK2	Binding to Cu^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+} but not to Mg^{2+}, Ca^{2+} and Mn^{2+}	Increased tolerance to cold stress	31

ADH, alcohol dehydrogenase; bglG, fungal β-glucosidase; GOD, glucose oxidase; LDH, lactate dehydrogenase; PA, phosphatidic acid; PG, phosphatidyglycerol; PS, phosphatidyl-Ser; POD, peroxidase.

S-segment of several DHNs, was shown to be controlled in some cases by casein kinase II (CK2)-type kinases and seems to be associated to their translocation from the cytoplasm to the nucleus (e.g., RAB17 of *Zea mays*).[43] However, nuclear localization has also been reported for some dehydrins that lack the S-segment (e.g., wheat WCS120 or peach PCA60).[25,26] Nonetheless, the phosphorylation of the S segment may have significance in stress tolerance since the wheat stress-inducible dehydrin DHN-5 (YSK_2) shows a differential phosphorylation pattern in two Tunisian durum wheat cultivars with contrasting tolerance to drought and salt stress.[47] In other cases, phosphorylation is necessary for the cation binding properties of DHNs. The phosphorylated forms of the acidic dehydrins COR47, ERD10 and ERD14 of Arabidopsis and of the vacuole-located dehydrin VCaB45 of celery (*Apium graveolens*) bind Ca^{2+} much more efficiently than the dephosphorylated ones.[32,48,49]

Dehydrins as Molecular Markers of Plant Abiotic Stress Tolerance

Several comparative studies on varieties or cultivars (showing marked difference in stress tolerance) of economically important crops provide evidence for a positive correlation between DHN gene expression or DHN protein accumulation, and plant stress tolerance.

It is hence becoming evident that DHNs can be used as plant molecular marker for stress tolerance.[50] Ismail et al.[51] who studied chilling tolerance during the process of seedling emergence in the tropical legume crop *Vigna unguiculata,* revealed a positive correlation between the accumulation of DHN1 protein (Y_2K-type dehydrin of 35 kDa) in seeds of a chilling-tolerant line 1393-2-11, and seedling emergence at 14°C. In contrast, DHN1 was absent in the seeds of a genetically related, but chilling-sensitive line 1393-2-1. Quantitative differences in dehydrin gene expression and dehydrin protein accumulation with respect to the low-temperature stress (cold and frost) have been also largely studied in *Triticeae*, which are grown in temperate climates. In bread wheat (*T. aestivum*), Houde et al.[52] have already described a correlation between the accumulation of DHN proteins belonging to the WCS120 family, and the level of plant-acquired frost tolerance. Furthermore, Vítámvás et al.[53] were able to distinguish at WCS120 protein level, 20 cultivars of different frost-tolerant winter wheat's grown at 17°C or 9°C. Thus, it seems possible to use the level of WCS120 accumulation in wheat plants grown under mild cold temperatures (17–9°C) as a means for estimation of plant winter survival, hence improving the pre-screening procedures in the breeding programmes aimed at improving wheat frost tolerance.

In studies dealing with drought stress, Pelah et al.[54] found a correlation between drought tolerance and accumulation of dehydrin proteins in *Populus popularis*. Park et al.[55] have found a correlation between *Dhn3* and *Dhn4* transcript accumulation and several traits associated with drought tolerance (relative water content RWC, Drought yield index) in a set of Korean barley cultivars. Similarly, Labhilili et al. found a correlation between the level of dehydrin transcript accumulation and drought tolerance in two differently tolerant cultivars of durum wheat (*T. turgidum* ssp. *durum*).

Use of Dehydrins for Improvement of Plant Tolerance to Abiotic Stress

Numerous transgenic studies revealed a positive effect of dehydrin gene expression on plant stress tolerance (**Table 1**). Studies performed by Saavedra et al.[22] on the moss *Physcomitrella patens*,

have shown that a *P. patens* knockout mutant, which has its only dehydrin gene, *PpdhnA*, disrupted, reveals an impaired ability to recover after salt and osmotic stress. Peng et al.[57] have shown that Arabidopsis transgenic plants overexpressing *RcDHN5* (an SK2 acidic dehydrin from frost-tolerant *Rhododendron catawbiense*) show enhanced frost tolerance. Similarly, Yin et al.[58] concluded that the expression of DHN24 protein from wild potato *Solanum sogarandinum* in cucumber (*Cucumis sativus*) resulted in improved frost tolerance. Similarly, studies that used a dehydrin transgene expressed in a stress-sensitive plant, have reported enhanced tolerance to stress in the transformed plant. For example, Hara et al.[59] have shown that the expression of CuCOR19 from *Citrus unshiu* in tobacco mitochondria led to a reduced lipid peroxidation. Houde et al.[60] have found out that expression of *WCOR*410 from common wheat in strawberry led to the enhancement of strawberry frost tolerance. Kaye et al.[61] reported that tobacco plants expressing spinach *CAP*85 and *CAP*160 DHNs revealed a lower level of electrolyte leakage after a frost test, which indicates a reduction of freezing injury in the transformants. Brini et al.[62] have observed that the expression of the durum wheat (*Triticum turgidum* ssp. *durum*) DHN-5 in *A. thaliana* led to an increase in salt and osmotic stress tolerance. Likewise, RoyChoudhury et al.[63] observed enhanced tolerance to drought and salt stress in tobacco plants transformed with *Rab16A* (=*Rab21*) gene from salt-tolerant Indica rice variety Pokkali. Similarly, Cheng et al.[64] have shown that overexpression of the wheat dehydrin *PMA*80 (as well as the *LEA*I protein PMA1959) enhances rice tolerance to drought and salt stress. Figueras et al.[65] have also reported that *A. thaliana* plants transgenic for the maize *Rab*17 are more tolerant to osmotic stress. Park et al.[55] have observed that Arabidopsis plants overexpressing barley *Dhn3* and *Dhn4* genes stayed green and viable on a growth medium containing 500 mM mannitol, a concentration at which wild-type plants are unable to grow.

On the other hand, Xu et al.[66] found out that expression of *BjDHN2* and *BjDHN3* proteins from *Brassica juncea* in transgenic tobacco plants resulted in higher tolerance to heavy-metal (Cd^{2+} and Zn^{2+}) stress. The transgenic plants showed lower electrolyte leakage and malondialdehyde production, suggesting that *BjDHN2* and *BjDHN3* could enhance tolerance to heavy metals by attenuating lipid peroxidation and protecting cellular membranes.

It is worth mentioning that plants overexpressing DHNs are more tolerant to abiotic stress, is not a general rule. Several LEA proteins including dehydrins from desiccation tolerant resurrection plant *Craterostigma plantagineum* failed to increase drought tolerance in tobacco.[67] Similarly, the overexpression of *RAB18* in Arabidopsis did not improve freeze and drought tolerance.[68] Whereas, the co-expression of the same gene together with another DHN (*Cor47)* led to improved freeze tolerance but not drought tolerance.[69] These authors found also that Arabidopsis transgenics for both DHNs *Lti29* and *Lti30,* stand better freezing conditions than lines overexpressing single DHN genes. These findings suggest that in some circumstances some DHN can act in synergistic way to improve freeze tolerance.

Dehydrins Responsive to Biotic Stresses

The role of DHNs in abiotic stress tolerance was largely documented. In contrast, whether these proteins are involved in biotic stress response remains an open question. Nonetheless, wounding was reported to induce the expression of specific DHNs such as *BcDh2* of *Boea crassifolia*.[70] It is worth to note that plant wounding which is a common biotic stress exerted by insects or herbivores is also regarded as a dehydration stress because it is associated with cellular damage that leads to water loss. Interestingly, as wounding activates jasmonate and salicylic acid stress signaling pathways, the wound-induced expression of *BcDh2* is mediated by these hormonal signals. The induction by jasmonic acid and methyl jasmonate of CpDHN1 (Y_2K dehydrin) from *Cicer pinnatifidum* and of PgDHN1 (S_8K_4 dehydrin) from white spruce *Picea glauca* was reported in references 71 and 72. Rouse et al.[73] performed a promoter analysis of *A. thaliana* cold-inducible Kn-type dehydrin gene *Lti30* (*Xero 2*) using GUS reporter gene and they concluded that the *Lti30* promoter also displayed wounding-induction among other treatments. Sun et al.[74] have observed a positive effect of low concentrations of exogenous salicylic acid (up to 0.25 mM) on the expression of drought-inducible dehydrins in barley seedlings subjected to drought stress. In contrast, higher concentrations of salicylic acid (0.25–0.50 mM) have led to the decrease of dehydrin expression under the same growth conditions (water stress).

On the other hand, Turco et al.[75] have reported the expression of several dehydrin-like proteins in drought-tolerant oak species *Quercus ilex* in response to infection with *Phytophthora cinnamomi*.

Modulation of Plant Defense Responses by Dehydrins

Recently, it has been reported that in addition to its contribution in enhancing osmotic stress tolerance, the wheat DHN-5 seems to have a pleiotropic effect on stress responses in Arabidopsis.[76] Transcriptome profiling revealed that DHN-5 overexpression affects the expression of not only genes involved in abiotic stress tolerance (i.e., LEA, RD29B, RAB18 and LTI30), but also those related to defense responses: several genes coding for PR (pathogenesis related) proteins were transcriptionally activated by DHN-5. Most interestingly, the authors found that DHN-5 interferes with jasmonate (JA) signaling. Overexpression of DHN-5 resulted in downregulation of genes encoding 3 members of JAZ (jasmonate-ZIM domain) proteins, which are negative regulators of JA signaling.[77] However, DHN-5 transgenic plants were more resistant to JA than wild type. Moreover, as in the case of a JA insensitive mutant *jai3-1*, these transgenic lines showed compromised expression of a subset of JA- and wound-responsive genes (which are regulated by the MYC2 transcription factor),[78] but activation of the genes responsive to pathogen responses (**Fig. 1**). Considering the role of JA as a key signaling molecule in defense mechanisms against pathogens, it would be therefore interesting to explore whether DHN-5 can influence (via the alteration of MYC2-dependent JA responsive genes) the

level of plant resistance to pathogen attacks. It remains however unclear whether DHNs confer plant resistance to pathogens. Nevertheless, such an assumption should be well thought-out especially since few DHNs were reported to have antibacterial activities. The overexpression of an SK3-type dehydrin ERD10 of Arabidopsis in *E. coli* leads to a bacterial growth inhibition which seems to be linked to the K-segments.[79] These observations were reinforced this year by similar findings with RR46, another SK3-type from rice which beside *E. coli* can inhibit the growth of a number of Gram$^+$ bacteria.[80] Interestingly, these authors showed that synthetic K-segments (i.e., KKK KGL KEK IKE KLP GHK) are still also able to exert inhibitory effects but limited to some Gram$^+$ bacteria. They claim that amino acids in K-segments can in some cases adopt a transmembrane structure, similar to that found in other antimicrobial peptides which use this property to interact with bacterial cell membranes, and hence causing bacterial growth inhibition. Based on these DHN related antibacterial activities, it is therefore attractive to speculate that some DHNs can somehow inhibit the growth of pathogens hence improving plant resistance against them. Knowing their association to abiotic stresses, some DHNs can then act as a connection node in the cross talk between biotic and abiotic stress signaling pathways. It is generally thought that abiotic stress response is prioritized by the plant over the biotic stress response. In this respect, perhaps DHNs may be selected along evolution as part of a defense mechanism in plants against opportunistic bacterial infections usually present during water scarcity periods. Alternatively, some DHNs can via their antibacterial activity alleviate the biotic stress response even when common biotic stress signaling pathways are ineffective and therefore might serve as the last intracellular fort once the pathogen invades the plant cell.

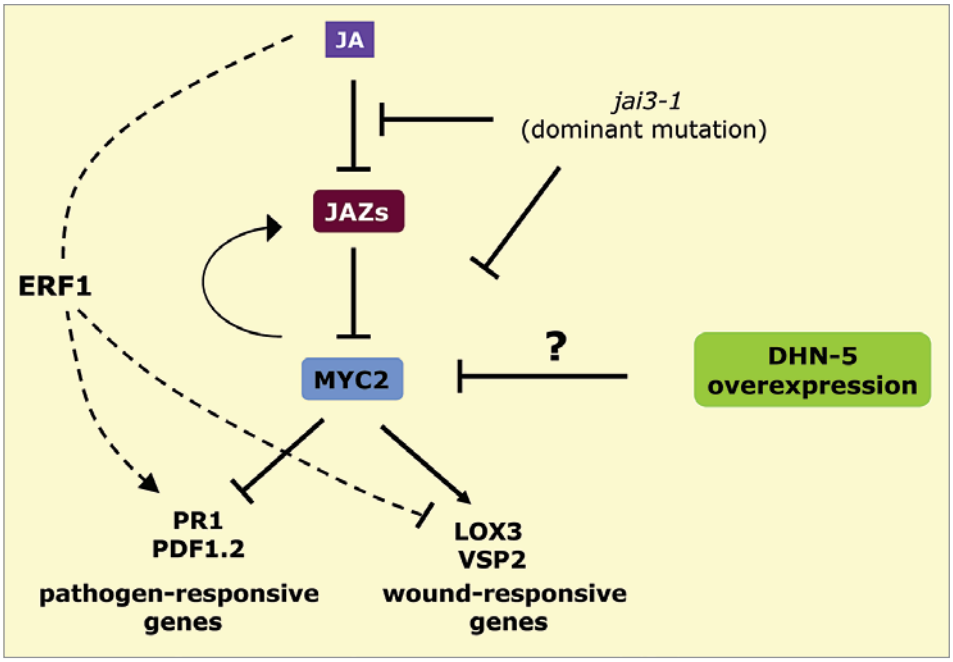

Figure 1. DHN-5 interferes with JA signaling pathway. Overexpression of DHN-5 causes decreased sensitivity to jasmonate (JA), and affects a subset of JA-responsive genes, downregulation of wound-induced genes and upregulation of pathogen-responsive genes in *Arabidopsis thaliana*. JAZ (jasmonate ZIM-domain) proteins are negative regulators of JA-signaling. *JAI3* encodes a member of JAZ protein, JAZ3. Myc2 (JAI1) is a bHLH-type transcription factor. ERF1 is a transcription factor, which mediates responses to ethylene and JA, antagonistic to MYC2 function. How DHN-5 affects JA signaling is unknown. For details, see references 76–78.

Conclusions and Perspectives

With increasing data from diverse research fields, DHNs appear to be an amazingly versatile group of LEA proteins presumably due to their intrinsically unstructured character. They exhibit myriads of functions (e.g., chaperone, cryoprotective, antifreeze, radical-scavenging, ion-binding functions) when exposed to various stress factors, including drought, high-salinity stress, low temperature stress, heavy-metal stress, and perhaps also to biotic stresses. Despite the relevant progress made toward understanding the role of DHNs, the molecular mechanisms through which they can enhance stress tolerance remain unknown. Nevertheless, the recent report of Brini et al.[76] provides insights into this complex question. It is plausible that some DHNs can have a regulatory function in stress responses. In the case of DHN-5, the regulatory role may be attributed to its potential capacity to shuttle between the cytoplasm and the nucleus,[47] perhaps according to its phosphorylation status as was previously reported on the maize counterpart RAB17.[45] Finally, these findings provide for the first time that a DHN might contribute to understanding the mechanism that regulates the plant defense responses. Future work should broadly examine other DHNs to learn whether DHN-dependent regulatory mechanisms modulate pathogen responses.

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