

1-1-2020

Dehydrins: an overview of current approaches and advancement

NESLİHAN SARUHAN GÜLER

RABİYE TERZİ

Follow this and additional works at: <https://journals.tubitak.gov.tr/botany>



Part of the [Botany Commons](#)

Recommended Citation

GÜLER, NESLİHAN SARUHAN and TERZİ, RABİYE (2020) "Dehydrins: an overview of current approaches and advancement," *Turkish Journal of Botany*: Vol. 44: No. 5, Article 1. <https://doi.org/10.3906/bot-2005-78>

Available at: <https://journals.tubitak.gov.tr/botany/vol44/iss5/1>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Botany by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Dehydrins: an overview of current approaches and advancement

Neslihan SARUHAN GÜLER^{1*}, Rabiye TERZİ²

¹Department of Nutrition and Dietetics, Faculty of Health Science, Karadeniz Technical University, Trabzon, Turkey

²Department of Biology, Faculty of Science, Karadeniz Technical University, Trabzon, Turkey

Received: 30.05.2020

Accepted/Published Online: 10.08.2020

Final Version: 23.09.2020

Abstract: Since plants are exposed to diverse environmental stresses in their natural area, numerous reviews have demonstrated many aspects of dehydrins (DHNs), including structural and functional dynamics, and multiple roles such as membrane protection, cryoprotection of enzymes, chaperone feature, and protection from reactive oxygen species. In this review, we have focused on new information, and other promising and emerging topics of DHNs in plants. This review outlines particularly the potential regulatory mechanisms of DHNs associated with stress tolerance and the role of DHNs in morphological responses of the plants exposed to environmental stresses. Besides the discussion of the signaling pathways involved in *DHN* expression under abiotic stress, novel aspects about the effect of *DHN* transcript or the protein accumulation on the tolerance to the stress factors are also presented in transgenic and non-transgenic plants. We hope that this review will help us better understand the role of these impressive proteins in plant stress response mechanisms.

Key words: Dehydrins, stress protein, plant stress tolerance, gene expression, signal pathway

1. Introduction

Adaptation and tolerance mechanisms of plants to environmental stresses attract great interest from researchers around the world. Therefore, it is crucial to further understand mechanisms regulating stress tolerance and those that are involved in developing suitable strategies to promote plant growth under stress conditions. To respond to environmental stresses, plants have developed complex regulatory networks, which include signal perception, transmission, and molecular regulatory mechanisms. Genes stimulated by stress factors protect cells from stress through producing important metabolic proteins and function in gene regulation needed for signal transmission in stress response (Duque et al., 2013).

One of the strategies to increase the stress tolerance is transfer of genes encoding different protein types such as osmoprotectants, chaperones, detoxification enzymes, transcription factors, signal transduction proteins, heat shock proteins, and late-embryogenesis abundant (LEA) proteins into plant genome (Muñoz-Mayor et al., 2012). LEA proteins are a large family that plays important roles in the tolerance of plants to environmental stresses. Due to their overexpression during dehydration stress, dehydration proteins or proteins named dehydrin (DHN)

belong to a subfamily within the LEA family (Malik et al., 2017). The DHNs are categorized as being intrinsically disordered proteins (IDPs) and known as stress proteins involved in formation of protective reactions of plants against dehydration (Liu et al., 2017). Because of their disordered state, they readily bind to other macromolecules, such as membranes, RNA, and DNA. Furthermore, they can function as hub proteins and coordinate cross-talk with cellular signals in response to stress (Zhang et al., 2018). It is postulated that the interaction of DHNs with cellular macromolecules protects them from the dehydration damage that is commonly associated with drought, heat, or cold stress (Zhu, 2001). Since the expression of dehydrins is significantly induced by abiotic stresses, it has been shown that a positive correlation exists between the accumulation of *DHN* transcripts or *DHN* proteins and the stress tolerance in plants (Verma et al., 2017). In fact, transcriptional and proteomic studies on abiotic stress tolerance in several plant species have demonstrated that *DHN* transcripts and *DHN* protein levels increase in tolerant varieties (Hernández-Sánchez et al., 2017). Therefore, DHNs are considered to be a potentially useful marker in the selection of products tolerant to abiotic stress (Kosová et al., 2018). Although the precise functions of many DHNs are unknown, they

* Correspondence: neslihan.saruhan@hotmail.com

have been shown to protect cells from sequestering of ions to stabilize the cell membrane, to eliminate free radicals, to act as molecular chaperone, and to play important roles in enzyme cryoprotection in earlier studies (Halder et al., 2016; Liu et al., 2017). Their roles as protectants of osmoregulation have been also reported (Nylander et al., 2001), and a recent research demonstrated that there is evidence that DHNs provide protection in isolated chloroplasts when added externally during oxidative stress (Halder et al., 2018). In addition, transgenic studies have shown that overexpression or accumulation of many kinds of DHNs enhanced tolerance to abiotic stresses in various plants (Liu et al., 2015; Bao et al., 2017). Considering in vitro experiments, it has been thought that this effect is based on cryoprotective and chaperone activity, as well as metal and reactive oxygen species scavenging functions. However, it can be said that not all DHNs have all of these functions (Hernández-Sánchez et al., 2017).

As mentioned above, although many studies have demonstrated that DHNs play important roles in abiotic stress tolerance, the exact biological and molecular functions of DHNs are still unclear. The following sections will highlight the structure and chemistry of DHNs, the interaction of DHNs with phytohormones, and their close relationship with stress phenomenon, as well as the regulation of *DHN* genes and their molecular signaling with a special focus on the current plant stress tolerance studies. Furthermore, recent developments on DHNs will demonstrate the remarkable ability of these fascinating proteins to increase the stress tolerance in plants.

2. The structure and properties of DHNs

DHNs are a family of proteins considered to be the most important LEA (late embryogenesis abundant) proteins. LEA proteins are thought to act as chaperones preventing misfolding and denaturation of important proteins during water stress (Xiong and Zhu, 2002). As the name suggests, LEA proteins are abundant in the later stages of seed development and protect the seeds against dehydration. It has been known that LEA proteins reported for the first time in embryos of cotton (*Gossypium hirsutum* L.) have been found in many plants and different plant tissues today (Close, 1996). LEA proteins can be divided into seven different groups based on the conserved segments across species (Battaglia et al., 2008). DHNs belong to the group II LEA proteins and they are also known as D-11 family of LEA proteins because one of the first identified DHN proteins is D-11 deposited in the maturing embryo of cotton and the other one is RAB21 induced under dehydration conditions such as salinity and osmotic stress in rice (Close, 1996; Liu et al., 2017; Kosová et al., 2019). DHNs are hydrophilic proteins whose production increases during the last stage of seed ripening and under

water scarcity conditions in vegetative tissues. They contain charged and polar amino acids at a high proportion and nonpolar hydrophobic residues such as the cysteine and tryptophan residues at a low rate; thus, DHNs can have hydrophilic property and boiling-resistant features, similar to other intrinsically disordered proteins (Close, 1996; Eriksson et al., 2011; Yang et al., 2015; Liu et al., 2017). An important feature of DHNs is that they contain a conserved K-segment which is lysine-rich 15-residue motif, EKKGIMDKIKEKLPG. K-segment can be found in 1 to 11 copies near the C terminus of DHN molecules and participate in forming an amphipathic α -helix that may interact with lipid membrane or partially denatured proteins (Close, 1996; Bao et al., 2017; Liu et al., 2017; Yu et al., 2018).

As additional motives, DHN proteins also contain Y-segment and/or S-segment due to the presence of tyrosine and serine residues, respectively. Conserved consensus sequence of Y-segment is (V/T)D(E/Q)YGNP that is usually located in 1 to 35 tandem copies in the N terminal of the protein. S segment is formed by a stretch of 4–10 residues, which are a part of a conserved sequence LHRSGS4-10(E/D)3 (Graether and Boddington, 2014; Kosová et al., 2019). Phosphorylation of the S-segment can relocate a DHN from the cytosol to the nucleus (Goday et al., 1994). However, a Kn-type dehydrin and two YnKn-type dehydrins, which does not possess the S-segment, also localize to the cytoplasm and nucleus (Wisniewski et al., 1999; Lin et al., 2012). It is possible that these differences in localization are caused by an undefined sequence element (Graether and Boddington, 2014). Some DHN proteins have been observed to contain less conserved motifs (Φ segments) that are located between the K-segments and rich in glycine and polar amino acids (Campbell and Close, 1997; Graether and Boddington, 2014; Mota et al., 2019). The protein structure of DHNs can be described by the presence of the K-, Y-, and S-segments. DHNs are also divided into five major types as the K_n , SK_n , Y_nSK_n , Y_nK_n , or K_nS , and an intermediate type $SnKS$ according to the presence of K-, Y-, and S-segments and their arrangements in a single polypeptide (Close, 1996; Liu et al., 2012; Zhou et al., 2018). The subscript n represents that the segment can be found more than once. In the K-segment, n is often 2, but it can be up to 13 copies. Generally, the number of copies in the K segment ranges from 1 to 11 but it was reported that one Kn subgroup of DHN member (PtrDHN-1.2) was defined by 13 repeated K-segments, of which a maximum repeated number has previously been documented of being from spinach CAP85 (Liu et al., 2012). Y- and S-segments is often 1 or 2 copies in higher plants or no copies (Malik et al., 2017).

DHNs are found in a wide variety of organisms including, angiosperm, gymnosperm, bryophyte, fungi,

algae, and cyanobacteria (Bao et al., 2017; Yu et al., 2018). Angiosperms contain all five architectures, while gymnosperms only contain Kn and SKn DHNs. Thus, the ancestral DHN in spermatophytes was either Kn or SKn, and the DHN proteins containing Y-segment first appeared in angiosperms (Riley et al., 2019). In many YnSKn-type DHNs, there was a fixed motif (GXGGRRKK (where X can be any amino acid)) between the K-segment and S-segment, which pointed out a potential functional linkage between K- and S-segments. The GXGG motif, highly elastic, takes part in the interaction of negatively charged phosphoserines with K-segment, while RRKK motif is assumed to be a nuclear settlement signal (Yu et al., 2018).

As based on the sequence analysis, Malik et al. (2017) reported that the structure and some biochemical characteristics (isoelectric point, molecular weight, and hydrophobicity level) of DHNs are interdependent and some structures of DHNs are overexpressed during several environmental stress conditions. On the other hand, the molecular weight of the DHNs varies considerably in accordance with their different structure. The lowest molecular weight of the DHNs was 9.6 kDa, while the highest molecular weight was reported to be 200 kDa (Graether and Boddington, 2014).

3. The biological function of DHNs

3.1. The role of DHNs in morphological responses

Drought is a major topic that attracts attention all over the world, as a limiting factor for the yield of the most important crop plants. Effects of drought stress on plants vary depending on plant type, and tolerance ability and adaptability of the plant (Kadioglu et al., 2011). Plants have developed a number of strategies, including morphological, physiological, biochemical and molecular responses, to better adapt to drought conditions. Symptoms describing drought tolerance include cuticular wax accumulation, stomata closing, osmotic adjustment, membrane stabilization at the cellular level, leaf rolling or leaf curling, and enhanced root length at organ level (Kadioglu et al., 2012). Leaf rolling or curling has been identified as one of the most frequent responses to drought stress in many herbs and grains such as rice, maize and sorghum, and is a response to insufficient plant moisture content. When the plant moisture decreases, the plant frequently rolls its leaves to prevent excessive moisture loss. Thus, the plants can avoid from moderate and severe drought conditions through this mechanism. Leaf rolling is a complex phenomenon controlled by multiple genes and gene products (Kadioglu et al., 2012). Although a few rolled-leaf mutants have been identified and some genes controlling leaf rolling have been isolated, the molecular mechanisms of leaf rolling still need to be elucidated.

Furthermore, knowing how leaf rolling is controlled under stress is very important in terms of photosynthetic production and grain yield. Excess rolling or curling can decrease photosynthetic yield because of decreased CO₂ entrance to the cells. For this reason, moderate rolling is desired. Evidence on control of leaf rolling at transcript level was obtained by microarray analysis of differentially expressed genes (DEGs) in rolled maize leaves. One of them was that *DHN* genes interestingly increased between 10 and 250 times in microarray analyses. These microarray analyses indicated that the increase in DHNs was very high and there could be an important relationship between leaf rolling and DHN (Kadioglu et al., 2014). Detailed investigation of interaction between DHNs and leaf rolling will lead to studies to increase photosynthetic efficiency such as obtaining the desired moderate leaf rolling by gene silencing or overexpression of *DHNs* in plants with similar mechanisms under stress conditions.

Although there are not many studies explaining the relation of DHNs with morphological responses in plants exposed to stress, a study was reported on leaf area and the expression level of *DHN* genes in two bread wheat cultivars (Sids and Gmiza) under drought conditions (Hassan et al., 2015). Drought stress increased the transcript level of *DHN* in both cultivars after 3 days of stress by about 2-folds. However, after 6 days of stress, the mRNA level of *DHN* declined slightly in Gmiza but it increased by 3-folds in Sids, the more tolerant cultivar. As seen, the levels of *DHN* gene expression were high in the drought-tolerant wheat cultivar compared with drought-sensitive plants. On the other hand, while there was a progressive increase in the leaf area in both cultivars under normal conditions, the largest increase and the greatest decrease in leaf area were interestingly observed in irrigated and drought-stressed Sids plants, respectively. As remembered, the Sids plants revealed high level of *DHN* expression. Namely, plants capable of expressing high *DHN* showed the greatest decrease in leaf area under drought conditions.

3.2. Interaction with phytohormones

Phytohormones, known as chemical messengers, regulate the growth, development, and cellular activities of plants in all aspects and play a critical role in adapting plants to adverse environmental conditions (Peleg and Blumwald, 2011). At the same time, they cause changes in gene expression by promoting or preventing the degradation of transcriptional regulators (Santner and Estelle, 2009). Hormone signaling networks and their ability to cross-talk make them superior candidates for stress defense responses. DHN biosynthesis is widely under the influence of both dehydration stress (Kovacs et al., 2008) and hormone signaling (Maryan et al., 2019). Phytohormones are crucial factors affecting the accumulation of DHN (Close, 1996). ABA is an elicitor of *DHN* gene transcriptions and *DHNs*

are highly expressed in response to ABA (Saavedra et al., 2006; Liang et al., 2012). Since *DHNs* are one of the genes stimulated by ABA, they are considered ABA-responsive proteins (ABR) (Eriksson et al., 2011) and also significant in plant stress resilience (Graether and Boddington, 2014). However, certain *DHNs* may not be always synthesized in plants by ABA application. In grape, the expression levels of *DHN1* increased in response to various stresses (ABA application as well as drought and low temperature), while neither *DHN3* nor *DHN4* exhibited responsiveness to any of the drought, cold, and ABA application (Yang et al., 2012). Similarly, among the seven *CaDHNs* in pepper, exception for *CaDHN1* and *CaDHN4*, the other pepper *DHN* genes (*CaDHN2*, *CaDHN3*, *CaDHN5*, *CaDHN6*, and *CaDHN7*) were obviously up-regulated by ABA treatment, which indicated that they might be involved in ABA-dependent pathways. *CaDHN1* and *CaDHN4* genes were not induced by exogenous ABA (Jing et al., 2016). Allagulova et al. (2003) reported that the synthesis of basic or neutral YnSK₂-type *DHNs* is induced by drought and ABA. On the other hand, in a study conducted on hybrid cucumber varieties in open field and greenhouse, it was recorded that YnSKn-type *DHN2* gene was notably inducible by water deficit and exogenous ABA treatments. In other respects, when there were no treatments, basal transcription of the *DHN2* gene was lower than that of the SKn-type *DHN3*. *DHN2* was highly induced by both of the treatments. Moreover, transcription of *DHN3* was moderately increased by the water deficit stress in the hybrids, while it was slightly responsive to ABA in all genotypes (Szegő et al., 2019). In addition, it was reported that SnRK2.10 (SNF-1 related protein kinase 2.10), which positively regulates signaling via ABA, could be related to phosphorylation of S-segments in *DHNs* (Vlad et al., 2008).

Salicylic acid, as a key phytohormone, is known to be an inducer of many stress-related proteins. These proteins were demonstrated to be involved in the SA-mediated tolerance to environmental stresses (Zhang et al., 2006; Sun et al., 2009). In recent years, it has been emphasized that the expression of *DHNs* was associated with SA. Sun et al. (2006) observed that drought-induced *DHN* expression increased when low concentrations of SA (up to 0.25 mM) were applied to barley seedlings under drought conditions. Conversely, higher concentrations of SA (0.25–0.50 mM) have led to the decrease of *DHN* expression under the same growth conditions. A heavy metal responsive *DHN* gene *PvSR3* in bean was found to be upregulated by exogenous SA, indicating that SA-mediated metal tolerance in plants results from expression of *DHN* (Zhang et al., 2006). Jing et al. (2016) reported that most of the *CaDHN* genes in pepper were induced by SA application. In the same study, out of seven pepper *DHN* genes, five genes were induced

by SA with the exception of *CaDHN2* and *CaDHN5*, which were relatively stable in their expression (Jing et al., 2016). Likewise, Bao et al. (2017) reported that after 2 mM SA treatment, *Prunus mume* *DHN* genes (*PmLEA8*, *PmLEA19*, and *PmLEA20*) were increased by more than 4 times relative to the control treatment; thus, these *DHNs* may play an important role in increasing the drought and cold tolerance in *P. mume*.

Similar to ABA and SA, other hormone signaling pathways and physiological activators associated with plant defense mechanism, such as jasmonic acid (JA), ethylene (ET), auxin, cytokinin, gibberellin (GA), and proline, could induce the expression of *DHN* genes, which means that there is a potential interaction between *DHNs* and multiple stresses signaling in plants. For example, in *Boea crassifolia*, YnSK₂-type *DHN* like gene *BcDh2* is noted to enhance stress tolerance due to mechanical injury mediated by JA and SA signaling pathways (Shen et al., 2004). Moreover, the induction by JA and methyljasmonate (MeJA) of Y₂K dehydrin CpDHN1 from *Cicer pinnatifidum* and S₈K₄-type dehydrin PgDHN1 from white spruce *Picea glauca* was reported in earlier studies (Richard et al., 2000; Bhattarai and Fettig, 2005). Shekhawat et al. (2011) showed that *MusaDHN-1*, a SK₃-type *DHN* gene in banana, contributed positively towards drought and salt stress tolerance, and responses to ABA, ethylene, and MeJA. Similarly, SK₃-type *DHN* gene in wheat *WZY1-2* could be induced by dehydration, ABA, SA, MeJA, indole-3-acetic acid, and GA (Zhu et al., 2014). In another study, *DHN* expression was investigated in ABA-, SA-, and MeJA-treated leaves, in order to determine whether the response of pepper *DHNs* under stress conditions is related to these molecules. According to the results obtained in this study, by exogenous ABA treatment, most of the *DHN* genes were significantly up-regulated, especially *CaDHN3* and *CaDHN5*. However, the expression of *CaDHN1* gene did not change significantly and *CaDHN4* was down-regulated. On the other hand, for SA application, all the genes were up-regulated. Finally, most genes were rapidly up-regulated after MeJA application, especially *CaDHN1* and *CaDHN3* increased by more than 10-fold. However, the expression of *CaDHN6* gene showed a slight down-regulation (Jing et al., 2016). The distinct SKY motifs of the *DHNs* induced by wounding indicate that these *DHNs* play different roles in the response to wounding. An earlier study in *Arabidopsis* showed that an induction of *HbDHN1* and *HbDHN2*, identified as SKY-type *DHN*, may be an indicator of dehydration in the wound area after mechanical injury. Moreover, it was found out that both *HbDHN1* and *HbDHN2* from *Hevea brasiliensis* were strikingly induced by several stress phenomena such as ABA, wounding, cold, PEG, and ethylene, but not induced by JA. Thus, it was reported that *DHNs* are not specific for

wounding response but function commonly under various abiotic stress conditions (Cao et al., 2017).

In addition to the abovementioned hormones, brassinolides (BL) have also been reported to participate in cold signaling pathway by inducing the expression of few genes including SSK₂-type II DHN (Li et al., 2012). On the other hand, *DHN* genes have been shown to be highly sensitive to polyamines. Li et al. (2015) showed that exogenous spermine induced expressions of three *DHN* genes; thus, ameliorating drought damages through spermine treatment could be associated with DHN synthesis in two different white clover cultivars. In particular, expressions of *DHN* genes (Y₂SK, Y₂K, and SK₂) were found to be significantly increased by the exogenous spermidine. In addition, GA was shown to play a role in the regulation of *DHN* gene expression during seed germination and seedling growth of cereal grains (An and Lin, 2011).

3.2. Interaction with reactive oxygen species

DHNs can reduce the level of ROS because amino acids such as histidine, glycine, and lysine, which are targets of radical-mediated oxidation, are high in most DHNs. In particular, the presence of the K segment in the structure of the DHNs protects against oxidative damage, possibly with a "shielding effect" as previously reported (Liu et al., 2017). This "shielding effect" of the DHNs may be due to the number and structure of amino acids present in their structure as stated above, since numerous histidine residues allow the sequestration of transition metal ions, making them unusable for Fenton reaction (Halder et al., 2018). DHNs can also function as antioxidants by binding free metal ions and preventing excessive production of ROS in the cell compartments since free metal ions act as catalysts of ROS production (Cao et al., 2017). Previous studies also supported the idea that DHNs play a protective role against oxidative damage caused by excess ROS and free metal ions (Zhang et al., 2006). A novel SbDHN2 (SK₃-type) protein in *Sorghum* was reported to have radical scavenging function as well as a metal binding one. Under stress conditions, the free radical scavenging function of the SbDHN2 protein is estimated to be due to the percent of highly polar (charged) amino acid residues and the physicochemical properties of the protein (Halder et al., 2016). Likewise, overexpression of SK2-type *HbDHNs* obtained from *Hevea brasiliensis* in *Arabidopsis thaliana* was shown to increase antioxidant enzymes and decrease ROS level. This finding suggested that *HbDHNs* also potentially act as ROS scavenging molecules, directly or indirectly regulating the redox status and affecting lactifer differentiation (Cao et al., 2017). Increased expression levels of *DHN* genes under environmental stress conditions may reduce the production of ROS due to their metal binding properties (Liu et al., 2017). Similar

results were reported in a study of transgenic tobacco plants overexpressing *SbDHN1* and *SbDHN2*, wherein transgenic tobacco plants had lower H₂O₂ and superoxide radical levels and the higher expression of ROS scavenging enzymes in comparison with wild-type tobacco under oxidative stress conditions. An increase in proline content positively associated with other antioxidants in transgenic lines may also play a significant role in alleviating oxidative stress. Experimental evidence has demonstrated that DHNs provide protection in collaboration with other defense mechanisms under stress conditions (Halder et al., 2018). On the other hand, it has been also demonstrated that DHNs can be induced by exogenous ROS. Heavy metal response *DHN* gene *PvSR3* isolated from bean is rich in lysine residues and its expression is responsive to exogenous H₂O₂, indicating that H₂O₂ may regulate the expression of *PvSR3*. This also means that *PvSR3* is a radical scavenging protein and might enhance metal tolerance in plants by alleviating oxidative damage (Zhang et al., 2006). Additionally, it was found that the transcript levels of *OsDHN RAB16D* (YSK2-type dehydrin) in rice seedlings were induced in response to H₂O₂ and drought exposure (Tiwari et al., 2019).

3.3. DHNs and other proteins' interactions

Protein-protein interactions play crucial roles in all biological processes. DHNs have a highly flexible structure because they consist of charged and polar amino acids and are therefore considered intrinsically disordered proteins (IDPs). IDPs are naturally unstructured proteins that interact with multiple partners, providing significant advantages in molecular recognition through protein-protein interactions (Sun et al., 2013). Only a few (limited) parts of DHN molecules can adopt secondary structure and almost no part can adopt tertiary structure. Therefore, they are described as "protein clouds" (Uversky, 2016). Recently, it has been reported that DHNs may act as a positive regulator by interaction with other proteins associated with drought stress signaling mediated by ABA and function as a protective protein (Tiwari et al., 2019). In a recent study on this subject, the interaction between YKS-type DHN and FK506 binding proteins in the nucleus was investigated. DHNs are often known to act as chaperones to cope with stress. FK506 binding proteins localized in the nucleus also function as a chaperone and are associated with the abiotic stress factor. In this study, it was found that DHNs not only act as drought-resistant protein, but also interact with FK506 binding protein in the nucleus and acts as regulatory protein for drought tolerance by regulating the ABA responsive signaling pathway (Tiwari et al., 2019). DHNs have been recognized as molecular protectors for functionality of some proteins such as lactate dehydrogenase and malate dehydrogenase in response to environmental stresses, as supported by previous

studies (Zhou et al., 2017; Lv et al., 2018). Misfolding of proteins that are toxic to plants is triggered by drought stress, so protein quality control is critical to plant quality. DHNs protect target proteins from degradation under environmental stress conditions. In addition, there are protein degradation systems to remove damaged proteins to protect plants from severe stress conditions. In a recent study, a new function has been identified: DHNs play an important role in autophagic degradation under severe stress conditions. Autophagy is not only a housekeeping mechanism but also an adaptive process involved in the adaptation of plants to biotic and abiotic stresses (Nolan et al., 2017). The function of MtCAS31 DHN protein (Y2K4-type) in *Medicago*, which was previously defined as a positive regulator of drought response (Xie et al., 2012), in protein quality control under severe drought stress was investigated. DHN MtCAS31 was found to act as a cargo receptor to participate in drought-induced autophagic degradation, thereby reducing water loss and increasing drought tolerance under drought stress (Li et al., 2020).

3.4. Interaction with phospholipids

DHNs are disordered proteins expressed as a response to stress in plants as previously stated. The molecular function and structural effect of DHNs is still unclear, but increasing evidence suggests that they play a role in maintaining the composition and functional dynamics of cell membranes (Eriksson et al., 2016). DHNs are located close to the membranes and their interactions change certain membrane properties, such as the phase transition temperature and water content (Eriksson et al., 2011; Gupta et al., 2019) DHNs can also bind to anionic phospholipids and the binding requires the presence of basic amino acids such as arginine and lysine in the binding sites of DHNs (Kooijman et al., 2007; Koag et al., 2009). The interaction of DHNs with charged lipids in the membranes occurs through electrostatic interactions (Koag et al., 2009; Eriksson et al., 2011). DHNs have at least one copy of the highly conserved K segment, which is regarded as a membrane binding motif. A good example is cold-induced Lti30, a positively charged dehydrin from *Arabidopsis thaliana*. Lti30 contains six K segments each containing two contiguous histidines and shows high expression during stress conditions. It interacts with the membrane electrostatically by pH-dependent protonation of histidines and binds to membranes by recognizing the negatively charged head groups of phospholipids (Eriksson et al., 2011). Further studies have shown that Lti30 is bound to the lipid bilayer by electrostatic bonds, which limits the mobility of lipids and membrane-bound proteins (Eriksson et al., 2016). Lipid binding at low pH was found to induce protein aggregation as well as microdomain formation. It has also been shown that Lti30

and other membrane-binding DHNs can maintain cell integrity by modulating the membrane structure during stress and possibly by lipid "crosslinking" (Gupta et al., 2019).

4. The molecular functions of DHNs

4.1. Signaling pathways involved in DHNs gene expression

The expression of DHNs is strongly influenced by adverse environmental stress conditions. The accumulation of DHNs in plants can be triggered by abiotic stress factors through multiple signal pathways (Yu et al., 2018). The level of plant DHN gene expression may vary depending on the type of stress that the plant is exposed to and the subclass to which DHN belongs (Abedini et al., 2017). Gene regulation under various environmental stress conditions is associated with interactions between cis-regulatory elements and trans-acting factors (TFs) and their overexpression has been proved to be useful for improving stress tolerance in plants. Many cis-regulatory elements of DHNs promoters respond to one or more environmental signals in plants. These cis-regulatory elements contain ABA-responsive elements (ABREs), C repeats (CRT), low temperature/dehydration sensitive elements (LTRE/DRE), and light-responsive elements, which respond to low temperature, drought, and high salinity. The cis-regulatory elements in promoters play a critical role in the regulation of gene transcription. Promoter regions are important entry points for understanding the transcriptional regulatory mechanism and gene expression patterns (Zhang et al., 2018). Yamaguchi-Shinozaki and Shinozaki (2005) stated that cis-acting regulatory elements are key molecules involved in the transcriptional regulation of gene activities controlling many abiotic stresses, hormones, and developmental processes. Many genes of DHNs and their promoter regions have been reported and their regulatory roles are well known (Zhu et al., 2014; Lv et al., 2017). For instance, Zhu et al. (2014) reported that the promoter of the SK₃-type DHN gene, called WZY1-2, contained many elements such as ABRE, LTRE, Gibberellin responsive elements (GARE), Salicylic acid responsiveness (SARE) and TGACG (involved in the MeJA-responsiveness). They pointed out that WZY1-2 can be induced by cold and ABA, and that the cis-regulatory elements could play a critical role in expression of gene under stress. Moreover, Liu et al. (2019) pointed out that the YSK₂-type DHN gene, WZY2 isolated from wheat, could be positively regulated by transcription factors, and WZY2 might play an important role in the ABA signaling pathway through its interaction with protein phosphatase 2C (PP2C) to regulate stress response gene expression.

Although it is known that DHNs play an important role in defense response against stress, it is still unclear whether their functions are hormone-dependent or not. Many

studies indicate the view that DHNs, particularly those with the S segment, are ABA-regulatory proteins, as evidenced by the presence of ABA-dependent and ABA-independent pathway-related motifs in the promoter of several *DHN* genes. In barley, 10 out of 13 *DHN* genes are induced by low temperature and drought stress as well as by ABA due to an occurrence of several ABRE elements in their promoters (Tommasini et al., 2008). Genetic expression studies have shown that majority of well-characterized stress-induced proteins are the product of ABA-responsive gene. In bermudagrass, it was demonstrated that the expression of a *DHN* gene, *CdDHN4*, was induced by drought stress and ABA application, and *CdDHN4* was regulated via ABA-mediated signal transduction. In that paper, the ABA sensitivity of *CdDHN4* indicated that bermudagrass could be an important mechanism enhancing drought tolerance and thus preventing excessive accumulation of ABA (Lv et al., 2017). In a recent study in cucumber, it has been seen that expression levels of the *CsDHN2*, *CsDHN3*, and *CsDHN4* genes, which represent four putative DHN family members, are regulated by an ABA-dependent signal. It was stated that these genes contained 2-6 ABA sensitive elements, and the number of ABRE, one of these elements, was associated with the transcripts of *DHN* genes in response to ABA (Zhou et al., 2018). YnSKn-type dehydrins in wheat and Kn-type dehydrin DHN-5 in barley were noted to be stimulated by ABA, drought, and cold due to the availability of many ABRE elements in their promoters (Wang et al., 2014). In addition, different cis-acting regulatory elements involved in phytohormone responsiveness were identified in *BnDHN* promoter and their coexpressed genes (Maryan et al., 2019). On the other hand, specific hormone signaling networks determine the induction of different groups of DHNs. For instance, Maryan et al. (2019) stated that KnS-type DHNs were under the control of auxin, MeJA, and ethylene signaling network. Similarly, the overexpression of *AdDHN1*, a SKn-type DHN gene from *Arachis duranensis* in *Arabidopsis* lines, was found to show enhanced tolerance to combined abiotic stress factors while increasing susceptibility to the biotrophic root node nematode. This contradictory role of *AdDHN1* in response to abiotic and biotic stresses supported the idea that it was associated with the overexpression of *DHN* in the ABA and JA signaling pathways (Mota et al., 2019). In another study, to understand the function of *CaDHN4* isolated from pepper in cold and salt tolerance and sensitivity to ABA, expression of *CaDHN4* was reduced using a virus-induced gene silencing and overexpressed in *Arabidopsis*. It has been found that silencing *CaDHN4* reduced tolerance to cold and salt stresses of the plants; however, *CaDHN4* overexpressed to *Arabidopsis* provided stronger tolerance to different stress conditions. Furthermore, it has currently been reported that transgenic *Arabidopsis*, which

expresses the *CaDHN4* promoter, showed more intensive GUS staining under treatment with exogenous hormones such as ABA, JA, and SA than under control conditions (Zhang et al., 2020). Moreover, it has been determined that exogenous SA in wheat caused an increase in the transcript accumulation of SKn-type *wzy1-2* dehydrin promoter and this increase was achieved in two ways, either directly by SA synthesis or by synthesis of other phytohormones independent of de novo SA synthesis (Zhu et al., 2014). Similarly, in a study of wheat leaves, it was found that GA induced the accumulation of *wzy1-2* mRNA and positively regulated the *wzy1-2* promoter via a cis-acting element (Zhu et al., 2014). Therefore, we can say that the results of the studies on the *DHN* gene expression and transgenic plants are different depending on the plant species and DHN types.

4.2. Potential role of using DHNs in production of stress-resistant plants

As well as the protective roles of DHNs in plant adaptation against environmental stress factors, researching the potentials of using DHNs in increasing the stress tolerance of economically important plants has recently gained importance. At the present, little is known about the potential roles of DHNs in crop yields under stress conditions. For example, there are only a few studies on the use of *DHN* genes isolated from legume species to increase stress tolerance in crops with transgenic approaches (Xie et al., 2012). In addition, identification of new editions of legumen genes, including the last gene sequence of two *Arachis* species (Bertioli et al., 2016), can add a new perspective to the molecular functions of DHNs in the legumens (Mota et al., 2019). These studies include both biotechnological approaches and selection of crops with high levels of *DHN* expression due to increased stress tolerance. Stress-resistant and stress-sensitive plant varieties can be determined according to the levels of *DHN* expression not only under stress conditions, but also in stress-free environments or in the early stages of stress (Kosová et al., 2019). Transgenic research on DHNs in the cultivation of stress-resistant mutants has far-reaching effects in plant biology. Majority of DHN species and their functions develop throughout the evolutionary process of species. The function of homologous proteins in the same family is determined by the expression of heterologous of different types of DHNs. For example, in agronomic species such as rice, heterologous expression of *DHN* genes is related to drought, cold, and salt tolerance. Such studies play a critical role in the selection of transgenic plants containing the *DHN* gene to increase agricultural production. Moreover, identifying new DHNs associated with stress tolerance can provide useful markers for selection of stress-tolerant genotypes with breeding or transformation technologies.

4.3. Transgenic approaches on DHNs

Various studies on both wild-type (nontransgenic) and transgenic plants show that DHN transcript or protein (expression) accumulation has a positive effect on plant tolerance to various stress factors. This effect is supported by recent studies. For example, it has been reported that overexpression of *Physcomitrella patens* two DHN genes, *PpDHNA* and *PpDHNC*, improved tolerance to salt and drought stresses in transgenic *A. thaliana*. In that paper, the increase in stress tolerance of *PpDHN* transgenic plants has been shown to be associated with an increase in ROS scavenging ability and maintenance of membrane integrity (Li et al., 2017). In another study, SiDHN, a dehydrin member in *Saussurea involucrata*, a dicotyl plant resistant to harsh stress conditions, was found to significantly increase the tolerance of the transgenic tobacco plants to low temperature and drought stresses (Guo et al., 2019). Moreover, *OesDHN*, a DHN gene from oleaster (*Oleaster europaea* L.), a wild olive form and a drought-resistant plant in the Mediterranean region, has been shown to increase drought tolerance when overexpressed in *Arabidopsis* (Chiappetta et al., 2015). Similarly, overexpression of four DHN genes in *Prunus mume*, a plant resistant to low temperatures, in *E. coli* and tobacco has been reported to increase the freezing resistance (Bao et al., 2017). A study

from Cao et al. (2017) demonstrated that two DHN genes, *HbDHN1* and *HbDHN2* from *Hevea brasiliensis*, can cause a significant increase in tolerance to drought, salt, and osmotic stress when overexpressed in *Arabidopsis*. It has been found that *CdDHN4* gene isolated from bermudagrass (*Cynodon dactylon* L.) also indicated a strong expression level when plants were exposed to drought, salt, high/low temperature, and ABA applications (Lv et al., 2017). Halder et al. (2017) reported that *SbDHN1*, YSK2-type DHN from *Sorghum bicolor*, demonstrated enhanced transcript level when exposed to high temperature and osmotic stress conditions, and its overexpression in tobacco plants under stress created a protective effect. Additionally, the expression level of *IpDHN*, a DHN gene associated with salt tolerance, was reported to increase after salt, cold, osmotic, and oxidative stress and ABA application (Zhang et al., 2018). Moreover, overexpression of a DHN gene *AddHN1* isolated from *Arachis duranensis* known as a wild species in South America conferred enhanced tolerance to both drought and low temperature in transgenic *Arabidopsis* plants. Maryan et al. (2019) indicated that three different types of *BnDHN* such as SKn (*BnLEA10* and *BnLEA18*), YnKn (*BnLEA90*) and YnSKn (*BnLEA104*) were strongly expressed in the cold-stressed *Brassica napus* and DHN gene expression increased under

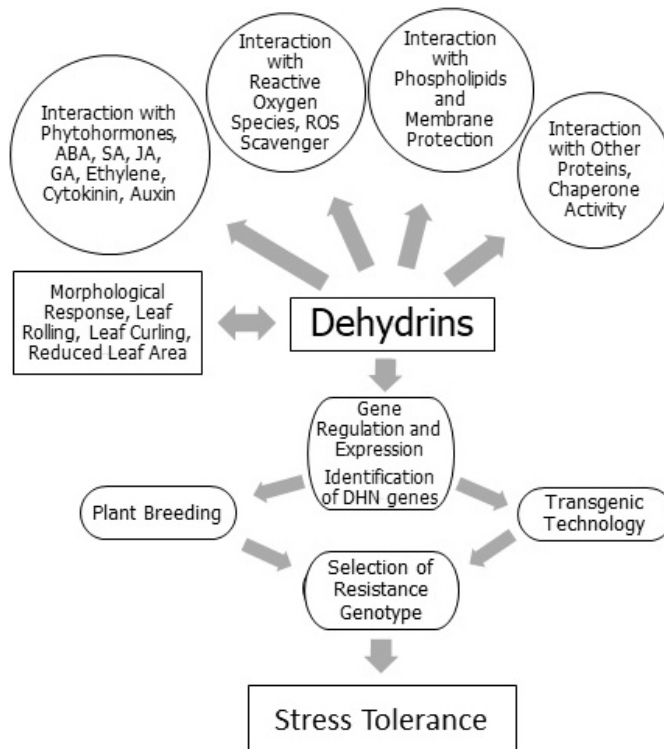


Figure. The multifunctional role of dehydrins (DHNs) in revealing stress tolerance through possible morphological, biological, and molecular interactions in plants.

cold stress in the tolerant canola genotype. On the other hand, DHNs are known to play a protective role in several plant developmental responses such as seed desiccation. A recent study assumed that seed-specific MdoDHN11 in apple was thought to play a protective role against dehydration in the seed development stage. In this context, transgenic *Arabidopsis* plants expressing *MdoDHN11* were produced and subjected to a severe water deficiency stress, aiming to mimic a situation that could occur during seed development. All of the transgenic lines were found to show greater tolerance to water deficiency in comparison with wild-type plants. It has been shown that MdoDHN11, which is specific for seed, plays an important role in dehydration during seed development, and its mechanism of action interacts with protein and other components in the cell (da Silveira Falavigna et al., 2019). As can be understood from the above studies, the differences in plant stress tolerance are much more often determined by quantitative differences in *DHN* gene expression or DHN protein accumulation (Kosová et al., 2019). Based on these studies, it is clear that DHNs can be used as indirect indicators of plant stress tolerance; however, DHNs are also still thought to have unknown functions.

5. Conclusion remarks

As shown in Figure, DHNs are a sophisticated protein group with multifunctional mission such as chaperone

activity, membrane protection, ROS scavenger, stress tolerance as well as interactions with phytohormones and other proteins. Moreover, there is probably an interaction between DHN accumulation and morphological responses such as leaf rolling, leaf curling, and reduced leaf area in plants under stress condition (Figure). Studies investigating the relationship between DHNs and plant stress response have attracted great attention in recent years, as it is crucial for further understanding the mechanisms that regulate stress tolerance and explain how plants respond to environmental stresses. Realization of the signal pathways of various DHNs under stress conditions will provide more detailed insights into plants' stress tolerance. Further studies are needed to clarify the unknown functions of DHNs, and understanding the true potential of these fascinating proteins is especially important for plants under stress conditions. Therefore, investigating the advanced functions and mechanisms of DHNs will make significant contributions to the development of stress-resistant products in genetic breeding research.

The interaction between DHNs and morphological responses in plants under stress condition can be one of the essential points in revealing the stress response. Therefore, further studies of *DHN* gene expression associated with morphological responses and hormones in plants exposed to environmental stresses in the near future will be useful for understanding stress tolerance mechanisms.

References

- Abedini R, GhaneGolmohammadi F, PishkamRad R, Pourabed E, Jafarnejhad A et al. (2017). Plant dehydrins: shedding light on structure and expression patterns of dehydrin gene family in barley. *Journal of Plant Research* 130 (4): 747-763.
- Allagulova CR, Gimalov FR, Shakirova FM, Vakhitov VA (2003). The plant dehydrins: structure and putative functions. *Biochemistry (Moscow)* 68 (9): 945-951.
- An YQ, Lin L (2011). Transcriptional regulatory programs underlying barley germination and regulatory functions of gibberellin and abscisic acid. *BMC Plant Biology* 11 (1): 105.
- Bao F, Du D, An Y, Yang W, Wang J et al. (2017). Overexpression of *Prunus mume* dehydrin genes in tobacco enhances tolerance to cold and drought. *Frontiers in Plant Science* 8: 151.
- Battaglia M, Olvera-Carrillo Y, Garciarubio A, Campos F, Covarrubias AA (2008). The enigmatic LEA proteins and other hydrophilins. *Plant Physiology*, 148(1): 6-24.
- Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD et al. (2016). The genome sequences of *Arachis duranensis* and *Arachis ipaensis*, the diploid ancestors of cultivated peanut. *Nature Genetics* 48 (4): 438.
- Bhattarai T, Fetting S (2005). Isolation and characterization of a dehydrin gene from *Cicer pinnatifidum*, a drought-resistant wild relative of chickpea. *Physiologia Plantarum* 123(4): 452-458.
- Campbell SA, Close TJ (1997). Dehydrins: genes, proteins, and associations with phenotypic traits. *New Phytologist* 137(1): 61-74.
- Cao Y, Xiang X, Geng M, You Q, Huang X (2017). Effect of *HbDHN1* and *HbDHN2* genes on abiotic stress responses in *Arabidopsis*. *Frontiers in Plant Science* 8: 470.
- Chiappetta A, Muto A, Bruno L, Woloszynska M, Van Lijsebettens M et al. (2015). A dehydrin gene isolated from feral olive enhances drought tolerance in *Arabidopsis* transgenic plants. *Frontiers in Plant Science* 6: 392.
- Close TJ (1996). Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. *Physiologia Plantarum* 97 (4): 795-803.
- da Silveira Falavigna V, Malabarba J, Silveira CP, Buffon V, de Araújo Mariath JE et al. (2019). Characterization of the nucellus-specific dehydrin MdoDHN11 demonstrates its involvement in the tolerance to water deficit. *Plant Cell Reports* 38 (9): 1099-1107.
- Duque AS, de Almeida AM, da Silva AB, da Silva JM, Farinha AP et al. (2013). Abiotic Stress Responses in Plants: Unraveling the Complexity of Genes and Networks to Survive. In: Vahdati K, Leslie C (editors). *Abiotic Stress-Plant Responses and Applications in Agriculture*, Intech, Pijeka, pp. 49-101.

- Eriksson SK, Kutzer M, Procek J, Gröbner G, Harryson P (2011). Tunable membrane binding of the intrinsically disordered dehydrin Lti30, a cold-induced plant stress protein. *The Plant Cell* 23 (6): 2391-2404.
- Eriksson S, Eremina N, Barth A, Danielsson J, Harryson P (2016). Membrane-induced folding of the plant stress dehydrin Lti30. *Plant Physiology* 171 (2): 932-943.
- Graether SP, Boddington KF (2014). Disorder and function: a review of the dehydrin protein family. *Frontiers in Plant Science* 5: 576.
- Goday A, Jensen B, Culiáñez-Macià F, Mar Albà M, Figueras M et al. 1994. The maize abscisic acid-responsive protein Rab17 is located in the nucleus and interacts with nuclear localization signals. *Plant Cell* 6: 351-360.
- Guo X, Zhang L, Wang X, Zhang M, Xi Y et al. (2019). Overexpression of *Saussurea involucrata* dehydrin gene *SiDHN* promotes cold and drought tolerance in transgenic tomato plants. *PloS One* 14 (11).
- Gupta A, Marzinek JK, Jefferies D, Bond PJ, Harryson P et al. (2019). The disordered plant dehydrin Lti30 protects the membrane during water-related stress by cross-linking lipids. *Journal of Biological Chemistry* 294 (16): 6468-6482.
- Halder T, Agarwal T, Ray S (2016). Isolation, cloning, and characterization of a novel *Sorghum* dehydrin (*SbDhn2*) protein. *Protoplasma* 253 (6): 1475-1488.
- Halder T, Upadhyaya G, Ray S (2017). YSK2 type dehydrin (*SbDhn1*) from *Sorghum bicolor* showed improved protection under high temperature and osmotic stress condition. *Frontiers in Plant Science* 8: 918.
- Halder T, Upadhyaya G, Basak C, Das A, Chakraborty C et al. (2018). Dehydrins impart protection against oxidative stress in transgenic tobacco plants. *Frontiers in Plant Science* 9: 136.
- Hassan NM, El-Bastawisy ZM, El-Sayed AK, Ebeed HT, Alla MMN (2015). Roles of dehydrin genes in wheat tolerance to drought stress. *Journal of Advanced Research*, 6 (2): 179-188.
- Hernández-Sánchez IE, Maruri-López I, Graether SP, Jiménez-Bremont JF (2017). In vivo evidence for homo- and heterodimeric interactions of *Arabidopsis thaliana* dehydrins *AtCOR47*, *AtERD10*, and *AtRAB18*. *Scientific Reports* 7 (1): 1-13.
- Jing H, Li C, Ma F, Ma JH, Khan A et al. (2016). Genome-wide identification, expression diversification of dehydrin gene family and characterization of *CaDHN3* in pepper (*Capsicum annuum* L.). *PloS One* 11 (8).
- Kadioglu A, Saruhan N, Sağlam A, Terzi R, Acet T (2011). Exogenous salicylic acid alleviates effects of long term drought stress and delays leaf rolling by inducing antioxidant system. *Plant Growth Regulation* 64 (1): 27-37.
- Kadioglu A, Terzi R, Saruhan N, Sağlam A (2012). Current advances in the investigation of leaf rolling caused by biotic and abiotic stress factors. *Plant Science* 182: 42-48.
- Kadioglu A, Sağlam A, Terzi R (2014). Effect of leaf rolling on gene expression in plants under drought stress. In: 3rd International Molecular Biology and Biotechnology Congress; Sarajevo, Bosnia and Herzegovina. p. 203.
- Koag MC, Wilkens S, Fenton RD, Resnik J, Vo E et al. (2009). The K-segment of maize DHN1 mediates binding to anionic phospholipid vesicles and concomitant structural changes. *Plant Physiology* 150: 1503-1514.
- Kooijman EE, Tieleman DP, Testerink C, Munnik T, Rijkers DT et al. (2007). An electrostatic/hydrogen bond switch as the basis for the specific interaction of phosphatidic acid with proteins. *The Journal of Biological Chemistry* 282: 11356-11364.
- Kosová K, Vítámvás P, Urban MO, Prášil IT, Renaut J (2018). Plant abiotic stress proteomics: The major factors determining alterations in cellular proteome. *Frontiers in Plant Science* 9: 122.
- Kosová K, Prášil IT, Vítámvás P (2019). Role of dehydrins in plant stress response. In: Pessaraki M (editor): *Handbook of Plant and Crop Stress*. 4th Ed. CRC Press, Taylor & Francis, Boca Raton, pp. 175-196.
- Kovacs D, Kalmar E, Torok Z, Tompa P (2008). Chaperone activity of ERD10 and ERD14, two disordered stress-related plant proteins. *Plant Physiology* 147 (1): 381-390.
- Li B, Zhang C, Cao B, Qin G, Wang W et al. (2012). Brassinolide enhances cold stress tolerance of fruit by regulating plasma membrane proteins and lipids. *Amino Acids* 43 (6): 2469-2480.
- Li Z, Jing W, Peng Y, Zhang XQ, Ma X et al. (2015). Spermine alleviates drought stress in white clover with different resistance by influencing carbohydrate metabolism and dehydrins synthesis. *PLoS One* 10 (4).
- Li Q, Zhang X, Lv Q, Zhu D, Qiu T et al. (2017). *Physcomitrella patens* dehydrins (PpDHNA and PpDHNC) confer salinity and drought tolerance to transgenic *Arabidopsis* plants. *Frontiers in Plant Science* 8: 1316.
- Li X, Liu Q, Feng H, Deng J, Zhang R et al. (2020). Dehydrin *MtCAS31* promotes autophagic degradation under drought stress. *Autophagy* 16 (5): 862-877.
- Liang D, Xia H, Wu S, Ma F (2012). Genome-wide identification and expression profiling of dehydrin gene family in *Malus domestica*. *Molecular Biology Reports* 39 (12): 10759-10768.
- Lin CH, Peng PH, Ko CY, Markhart AH, Lin TY (2012). Characterization of a novel Y2K-type dehydrin *vrdsn1* from *Vigna radiata*. *Plant and Cell Physiology* 53: 930-942.
- Liu CC, Li CM, Liu BG, Ge SJ, Dong XM et al. (2012). Genome-wide identification and characterization of a dehydrin gene family in poplar (*Populus trichocarpa*). *Plant Molecular Biology Reporter* 30: 848-859.
- Liu H, Yu C, Li H, Ouyang B, Wang T et al. (2015). Overexpression of *ShDHN*, a dehydrin gene from *Solanum habrochaites* enhances tolerance to multiple abiotic stresses in tomato. *Plant Science* 231: 198-211.
- Liu Y, Song Q, Li D, Yang X, Li D (2017). Multifunctional roles of plant dehydrins in response to environmental stresses. *Frontiers in Plant Science* 8: 1018.
- Liu H, Yang Y, Zhang L (2019). Identification of upstream transcription factors and an interacting PP2C protein of dehydrin *WZY2* gene in wheat. *Plant Signaling & Behavior* 14 (12): 1678370.

- Lv A, Fan N, Xie J, Yuan S, An Y et al. (2017). Expression of *CdDHN4*, a novel YSK2-type dehydrin gene from bermudagrass, responses to drought stress through the ABA-dependent signal pathway. *Frontiers in Plant Science* 8: 748.
- Lv A, Su L, Liu X, Xing Q, Huang B et al. (2018). Characterization of dehydrin protein, *CdDHN4-L* and *CdDHN4-S*, and their differential protective roles against abiotic stress in vitro. *BMC Plant Biology* 18 (1): 299.
- Malik AA, Veltri M, Boddington KF, Singh KK, Graether SP (2017). Genome analysis of conserved dehydrin motifs in vascular plants. *Frontiers in Plant Science* 8: 709.
- Maryan KE, Lahiji HS, Farrokhi N, Komeleh HH (2019). Analysis of *Brassica napus* dehydrins and their co-expression regulatory networks in relation to cold stress. *Gene Expression Patterns* 31: 7-17.
- Mota APZ, Oliveira TN, Vinson CC, Williams TCR, Costa MMD C et al. (2019). Contrasting effects of wild *Arachis* dehydrin under abiotic and biotic stresses. *Frontiers in Plant Science* 10: 497.
- Muñoz-Mayor A, Pineda B, Garcia-Abellán JO, Antón T, Garcia-Sogo B et al. (2012) Overexpression of dehydrin *tas14* gene improves the osmotic stress imposed by drought and salinity in tomato. *Journal of Plant Physiology* 169 (5): 459-468.
- Nolan TM, Brennan B, Yang M, Chen J, Zhang M et al. (2017). Selective autophagy of BES1 mediated by DSK2 balances plant growth and survival. *Developmental Cell* 41 (1): 33-46.
- Nylander M, Svensson J, Palva ET, Welin BV (2001). Stress-induced accumulation and tissue-specific localization of dehydrins in *Arabidopsis thaliana*. *Plant Molecular Biology* 45 (3): 263-279.
- Peleg Z, Blumwald E (2011). Hormone balance and abiotic stress tolerance in crop plants. *Current Opinion in Plant Biology* 14 (3): 290-295.
- Richard S, Morency MJ, Drevet C, Jouanin L, Séguin A (2000). Isolation and characterization of a dehydrin gene from white spruce induced upon wounding, drought and cold stresses. *Plant Molecular Biology* 43 (1): 1-10.
- Riley AC, Ashlock DA, Graether SP (2019). Evolution of the modular, disordered stress proteins known as dehydrins. *PLoS One* 14 (2).
- Saavedra L, Svensson J, Carballo V, Izemendi D, Welin B (2006). A dehydrin gene in *Physcomitrella patens* is required for salt and osmotic stress tolerance. *The Plant Journal* 45: 237-249.
- Santner A, Estelle M (2009). Recent advances and emerging trends in plant hormone signalling. *Nature* 459 (7250): 1071-1078.
- Shekhawat UKS, Srinivas L, Ganapathi TR (2011). *MusaDHN-1*, a novel multiple stress-inducible SK3-type dehydrin gene, contributes affirmatively to drought-and salt-stress tolerance in banana. *Planta* 234 (5): 915.
- Shen Y, Tang MJ, Hu YL, Lin ZP (2004). Isolation and characterization of a dehydrin-like gene from drought-tolerant *Boea crassifolia*. *Plant Science* 166 (5): 1167-1175.
- Sun X, Yuan S, Lin HH (2006). Salicylic acid decreases the levels of dehydrin-like proteins in Tibetan hullless barley leaves under water stress. *Zeitschrift Für Naturforschung C* 61 (3-4): 245-250.
- Sun X, Xi DH, Feng H, Du JB, Lei T et al. (2009). The dual effects salicylic acid on dehydrin accumulation in water-stressed barley seedlings. *Russian Journal of Plant Physiology* 56 (3): 348-354.
- Sun X, Rikkerink EH, Jones WT, Uversky VN (2013). Multifarious roles of intrinsic disorder in proteins illustrate its broad impact on plant biology. *Plant Cell* 25: 38-55.
- Szegő A, Badics E, Gubala D, Oszlányi R, Oyuntogtokh BE (2019). Diverse responsiveness of dehydrin genes to abscisic acid and water stress treatments in cucumber F1 cultivar hybrids. *The Journal of Horticultural Science and Biotechnology* 94 (6): 726-734.
- Tiwari P, Indoliya Y, Singh PK, Singh PC, Chauhan PS (2019). Role of dehydrin-FK506-binding protein complex in enhancing drought tolerance through the ABA-mediated signaling pathway. *Environmental and Experimental Botany* 158: 136-149.
- Tommasini L, Svensson JT, Rodriguez, EM, Wahid A, Malatrasi M et al. (2008). Dehydrin gene expression provides an indicator of low temperature and drought stress: transcriptome-based analysis of Barley (*Hordeum vulgare* L.). *Functional & Integrative Genomics* 8: 387-405.
- Uversky VN (2016). Dancing protein clouds: The strange biology and chaotic physics of intrinsically disordered proteins. *The Journal of Biological Chemistry* 291: 6681-6688.
- Verma G, Dhar YV, Srivastava D, Kidwai M, Chauhan PS (2017). Genome-wide analysis of rice dehydrin gene family: Its evolutionary conservedness and expression pattern in response to PEG induced dehydration stress. *PLoS One* 12 (5).
- Vlad F, Turk BE, Peynot P, Leung J, Merlot S (2008). A versatile strategy to define the phosphorylation preferences of plant protein kinases and screen for putative substrates. *The Plant Journal* 55 (1): 104-117.
- Wang Y, Xu H, Zhu H, Tao Y, Zhang G et al. (2014). Classification and expression diversification of wheat dehydrin genes. *Plant Science* 214: 113-120.
- Wisniewski M, Webb R, Balsamo R, Close TJ, Yu XM et al. (1999). Purification, immunolocalization, cryoprotective, and antifreeze activity of PCA60: A dehydrin from peach (*Prunus persica*). *Physiologia Plantarum* 105: 600-608.
- Xie C, Zhang R, Qu Y, Miao, Z., Zhang, Y et al. (2012). Overexpression of *MtCAS31* enhances drought tolerance in transgenic *Arabidopsis* by reducing stomatal density. *New Phytologist* 195 (1): 124-135.
- Xiong L, Zhu JK (2002). Molecular and genetic aspects of plant responses to osmotic stress. *Plant, Cell & Environment* 25: 131-139.
- Yamaguchi-Shinozaki K, Shinozaki K (2005). Organization of cis-acting regulatory elements in osmotic-and cold-stress-responsive promoters. *Trends in Plant Science* 10 (2): 88-94.
- Yang Y, He M, Zhu Z, Li S, Xu Y et al. (2012). Identification of the dehydrin gene family from grapevine species and analysis of their responsiveness to various forms of abiotic and biotic stress. *BMC Plant Biology* 12: 140.

- Yang W, Zhang L, Lv H, Li H, Zhang Y et al. (2015). The K-segments of wheat dehydrin WZY2 are essential for its protective functions under temperature stress. *Frontiers in Plant Science* 6: 406.
- Yu Z, Wang X, Zhang L (2018). Structural and functional dynamics of dehydrins: a plant protector protein under abiotic stress. *International Journal of Molecular Sciences* 19 (11): 3420.
- Zhang H, Zheng J, Su H, Xia K, Jian S et al. (2018). Molecular cloning and functional characterization of the dehydrin (*IpDHN*) gene from *Ipomoea pes-caprae*. *Frontiers in Plant Science* 9: 1454.
- Zhang HF, Liu SY, Ma JH, Wang XK, Meng YC (2020). CaDHN4, a salt and cold stress-responsive dehydrin gene from pepper decreases abscisic acid sensitivity in *Arabidopsis*. *International Journal of Molecular Sciences* 21 (1): 26.
- Zhang Y, Li J, Yu F, Cong L, Wang L et al. (2006). Cloning and expression analysis of SKn-type dehydrin gene from bean in response to heavy metals. *Molecular Biotechnology* 32 (3): 205-217.
- Zhou Y, He P, Xu Y, Liu Q, Yang Y et al. (2017). Overexpression of *CsLEA11*, a Y_3SK_2 -type dehydrin gene from cucumber (*Cucumis sativus*), enhances tolerance to heat and cold in *Escherichia coli*. *AMB Express* 7 (1): 1-9.
- Zhou Y, Hu L, Xu S, Jiang L, Liu S (2018). Identification and transcriptional analysis of dehydrin gene family in cucumber (*Cucumis sativus*). *Acta Physiologiae Plantarum* 40 (8): 144.
- Zhu JK (2001). Plant salt tolerance. *Trends in Plant Science* 6 (2): 66-71.
- Zhu W, Zhang D, Lu X, Zhang L, Yu Z et al. (2014). Characterisation of an SKn-type dehydrin promoter from wheat and its responsiveness to various abiotic and biotic stresses. *Plant Molecular Biology Reporter* 32 (3): 664-678.