

Article

Disorder and interactions: What can dehydrins in cereals tell us anymore?

Mouna Choura, Faïçal Brini

Biotechnology and Plant Improvement Laboratory, Centre of Biotechnology of Sfax (CBS)/University of Sfax, B.P “1177” 3018, Sfax Tunisia

E-mail: mouna.choura@cbs.rnrt.tn, faical.brini@cbs.rnrt.tn

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Abstract

Dehydrins (DHNs) are intrinsically disordered proteins that are expressed under conditions of water-related stress. They play a fundamental role in plant response and adaptation to abiotic stresses. The protein architecture of dehydrins can be described by the presence of three types of conserved sequence motifs that have been named the Y-, S- and K- segments. Although, dehydrins are extensively studied, their molecular interactions remain elusive. By combining network analysis with prior knowledge, we provide further insights into the role of some dehydrin disorder in cereals notably in stress tolerance. This work includes a comparative analysis with dehydrins of *Arabidopsis thaliana* to highlight the disorder conservation of dehydrins across evolution.

Keywords *Arabidopsis thaliana*; cereals; dehydrins; interactome; intrinsically disordered proteins.

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1 Introduction

Dehydrins, or group 2 LEA (Late Embryogenesis Abundant) proteins, constitute a class of intrinsically disordered proteins (IDPs) that are expressed under various water-induced stresses. They accumulate typically in maturing seeds or are induced in vegetative tissues under various stress factors that cause cell dehydration including salinity, dehydration, cold, and freezing stress. Several physiological studies focused on plant stress response have reported a positive relationship between the level of accumulation of dehydrin transcripts or proteins and plant stress tolerance notably for wheat and barley (Kosová et al., 2013). Characteristic of the dehydrins are some highly conserved stretches of seven to 17 residues that are repetitively scattered in their sequences, the Y-, S-, and K- segments. The role of the conserved segments is thus not to promote tertiary structure, but to exert their biological function more locally upon interaction with specific biological targets, for example, by acting as beads on a string for specific recognition, interaction with membranes, or intermolecular scaffolding (Mouillon et al., 2006).

While IDPs such as dehydrins are highly disordered *in vitro*, they often gain structure when bound to a target, suggesting that some disordered proteins may be structured *in vivo* in the presence of their cognate ligands such as membranes. Dehydrin sequences, their structures, and the various ligands that bind to this family of proteins were reviewed by (Graether and Boddington, 2014). The structural physico-chemical and functional characterization of plant dehydrins and how these features could be exploited in improving stress tolerance in plants as outlined by (Hanin et al., 2011). Further works have highlighted the multifunctional roles of dehydrins under environmental stress in plants (Liu et al., 2017). Intrinsically disordered proteins (IDPs) conformational flexibility allows them to recognize and interacts with multiple partners that may increase the interaction speed upon stress (Pietrosemoli et al., 2013).

Currently, applications of protein-protein/gene interaction networks play fundamental role in understanding of the complex biological systems, such as investigation of protein functions and related pathways.

Since the development of the microarray and next generation sequencing technology, more and more transcriptome data has become available. The high throughput computational methods have been successfully used to predict some interactomes such as *A. thaliana* (Consortium, 2011), *O. Sativa* (Ho et al., 2012), *Z. mays* (Zhu et al., 2016) and *S. Bicolor* (Tian et al., 2016). Due to the information provided by related databases and public papers, it is a benefit to investigate the available dehydrin interaction networks to get further insights into their role in stress tolerance.

2 Material and Methods

2.1 Protein disorder prediction

The prediction of intrinsic protein disorder was carried out using the reputable and accurate IUPred web server (<http://iupred.enzim.hu/>) (Dosztányi et al., 2005). This server disordered region from amino acid sequence based on pairwise energy content. There are three different prediction types, each using different parameters optimized for slightly different applications. These are: long disorder, short disorder, and structured domains.

2.2 Protein binding region prediction

The binding regions involved in protein-protein interaction were predicted using ANCHOR tool (<http://anchor.enzim.hu/>) (Dosztányi et al., 2009), which is based on the IUPred program mentioned above. Anchor predicts binding regions located in disordered proteins from the amino acid sequence.

2.3 Disorder evaluation in protein-protein interaction

We extracted data on binary protein-protein interactions in *O.sativa*, *Z.Mays*, *S.bicolor* and *A.thaliana* from the STRING database (Szklarczyk et al., 2015).

3 Results and Discussion

3.1 The structural characteristics of dehydrins: disorder and function

Dehydrins share a number of structural features. One of the most notable features is the presence, in their central region, of a continuous run of five to nine serines followed by a cluster of charged residues. Such a region has been found in all known dehydrins so far with the exception of pea dehydrins. A second conserved feature is the presence of two copies of K-segments. The first copy is located just after the cluster of charged residues that follows the poly-serine region and the second copy is found at the C-terminal extremity. The presence of the K segment raises the question of whether DHNs bind lipids, bilayers, or phospholipid vesicles. DHN1 in *Zea mays* (*ZmDHN1* or *RAB17*) can bind to lipid vesicles that contain acidic phospholipids. It was reported that *ZmDHN1* binds more favourably to vesicles of smaller diameter than to larger vesicles, and that the association of DHN1 with vesicles results in an apparent increase of α -helicity of the protein.

Thus, the role of the K-segment may be hydrophobic interaction with partially denatured proteins and protect the cell membranes, especially under the stressed conditions (Koag et al., 2003, 2009).

Rice (*O. sativa* L.) is susceptible to drought-induced stress as compared to other cereals (Mostajeran AND Rahimi-Eichi, 2009), resulting in poor seedling vigor (Bouman et al., 2005), fertility and adversely affecting the crop yield. It has been reported that *OsDHN1* gene overexpressed in rice confers high tolerance to drought and salt stress (Kumar et al., 2014). Further, rice dehydrin K-segments are responsible for the antibacterial activities against Gram positive bacteria (Zhai et al., 2011). Previous data showed that YSK2 type dehydrin from *Sorghum bicolor* (*SbDhn1*) play a protective role under high temperature and osmotic stress. *SbDhn2* possessed metal binding as well as radical scavenging activity (Halder et al., 2017) (Halder et al., 2018). Another study revealed that the K-segments of the wheat dehydrin DHN-5 are essential for the protection of enzyme activities *in vitro*. In addition, the K-segments have antibacterial and antifungal activities against Gram-positive and Gram-negative bacteria and fungi (Drira et al., 2015).

3.2 Dehydrins: disorder and interactions

The analysis of the amino composition of the dehydrin family (Pfam00257) reveals that dehydrins (DHN1) in *O. sativa*, *Z. mays*, *S. bicolor*, *T. aestivum*, *T. turgidum* and *A. thaliana* have similar disorder percentage (Fig. 1). Moreover, similar disordered binding regions (DBR) were noted (Table 1). Interestingly, these regions are involved in DNA, metal ions or lipid vesicles bindings under stress (Liu et al., 2017). This may explain that no physical partner was found in the network. In addition, the *A. thaliana* interactome is more studied than those of cereals.

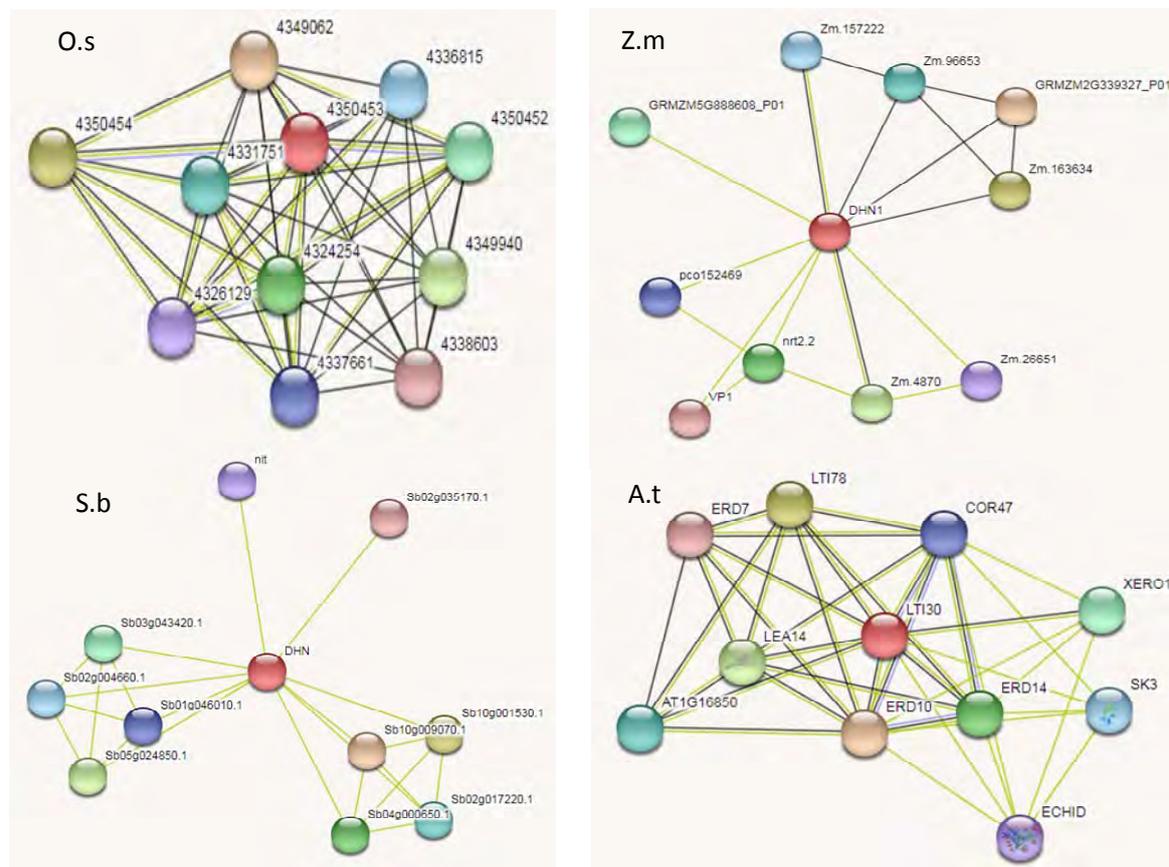


Fig. 1 Protein-Protein interaction networks of *DHN1* in *O. sativa*, *Z. mays*, *S. bicolor* and *A. thaliana* generated by STRING software (<http://string-db.org/>). Functional partners are detailed in Table 1.

Table 1 List of some dehydrins, disorder percentages, the number of disorder binding regions and the predicted functional partners.

Organism	name	Accession	% of Disorder	DBR	Nb of partners	Predicted functional partners
<i>A. thaliana</i>	Xero2	P42758	33	4	10	-ERD10: <i>Dehydrin ERD10</i> -LTI78: LOW-TEMPERATURE-INDUCED 78 -LEA14: LATE EMBRYOGENESIS ABUNDANT 14 -ERD14: Dehydrin ERD14 -Dehydrin XERO1 - AT1G16850 : uncharacterized protein -SK3 : <i>SKP1-like 3</i> - COR47 :Dehydrin COR47 -ECHID: <i>Naphthoate synthase</i> -ERD7:EARLY-RESPONSIVE TO DEHYDRATION 7
<i>O. sativa</i>	Rab16B	Q2R4Z5	83	4	10	- 4349062 :Expressed protein - 4350454 :Dehydrin putative - 4349940 :expressed protein - 4324254 : Late embryogenesis abundant protein, group 3, putative, expressed - 4350452: Dehydrin putative expressed - 4331751: Embryonic protein DC-8, putative, expressed - 4336815 : Late embryogenesis abundant group 1, putative, expressed - 4337661 : Putative low temperature and salt responsive protein, expressed - 4326129: Late embryogenesis abundant protein, group 3, putative, expressed - 4338603 : Eukaryotic peptide chain release factor subunit 1-1, putative, expressed
<i>T.aestivum</i>	LEA2	U6C7L2	85	3	-	
<i>T.turgidum</i>	Dehydrin	Q6IVU6	74.01	4	-	
<i>Z.mays</i>	ZmDHN1 (RAB17)	P12950	92	4	10	-GRMZM2G339327_P01: Putative uncharacterized protein -Zm.163634: Putative uncharacterized protein - Zm.4870 : dehydrin - nrt2.2 : Putative high affinity nitrate transporter - GRMZM5G888608_P01: Putative uncharacterized protein - Zm.96653 : Putative uncharacterized protein - Zm.157222: Putative uncharacterized protein - pco152469 :stress protein - Zm.26651 : Late embryogenesis abundant protein -VP1: Regulatory protein viviparous-1
<i>S.bicolor</i>	SbDHN	C5YX70	100	4	10	- Sb10g009070 : Putative uncharacterized protein - Sb10g001530 : Putative uncharacterized protein - Sb04g000650.1 : Putative uncharacterized protein - Sb03g043420.1 : Putative uncharacterized protein - Sb02g017220.1 : Putative uncharacterized protein - Sb02g004660.1: putative glycosyl-hydrolase - Sb01g046010 :putative sugar transport protein - nit: nitralse - Sb02g035170.1 : putative translation initiation factor SUI1

3.3 Gene interaction network

Currently, applications of protein-protein/gene interaction networks play fundamental role in understanding of the complex biological systems, such as in investigating of protein functions and related pathways. Intrinsically disordered proteins (IDPs) conformational flexibility allows them to recognize and interacts with multiple partners, that may increase the interaction speed (Pietrosemoli et al., 2013). Based on genomic context, high-throughput experiments, co-expression and text mining, gene co-expression networks of *OsDHN1*, *ZmDHN1*, *SbDHN1*, *LEA2* and *Xero2* were constructed (Fig. 2). In the network, nodes represent genes and edges represent the interaction. The degree of a node is the number of edges connected to this node. The clustering coefficient of a node is the ratio of the observed number of direct connections between the node's immediate network neighbours over the maximum possible number of such connections.

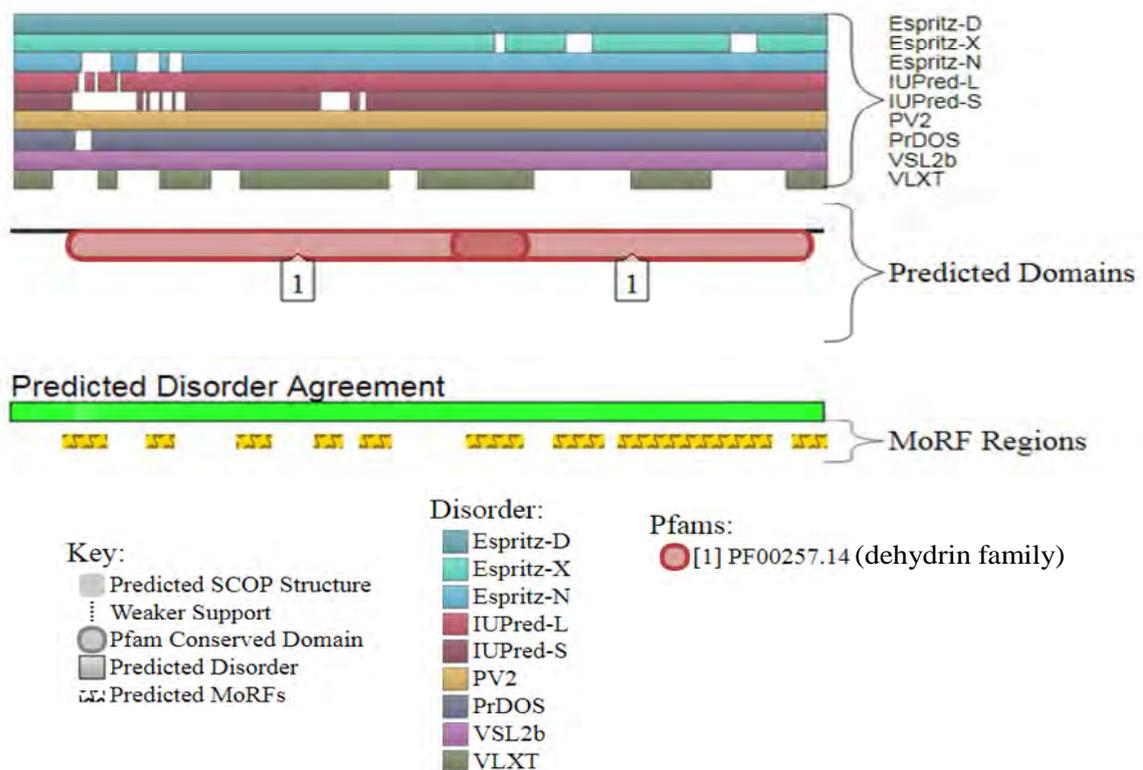


Fig. 2 Intrinsic disorder propensity and some important disorder-related functional information generated for dehydrin in wheat by the D2P2 database (<http://d2p2.pro/>) (Oates et al., 2013). Here, the green bar in the middle of the plot shows the predicted disorder agreement between nine predictors. Yellow bars show the location of the predicted disorder-based binding sites (molecular recognition features, MoRFs).

The comparison of networks indicated similar topological features (Table 2). Moreover, functional features of networks revealed that dehydrins are mainly involved in water stress (GO: 0009415) and in response to stress (GO: 0006950) (Table 3). This functional conservation should guide further effort to explore the role of dehydrin in cereals. Although the molecular function of the dehydrins still unknown, dehydrins bind to various molecules such as water, phospholipids in maize and in metals in *A. thaliana*. These features explain the proposed functions of dehydrins notably protective activity, membrane stability and metal binding involving interaction to the cellular targets often without partners.

Table 2 Network statistics.

Dehydrin	Nb of nodes	Nb of edges	Avg.node degree	Avg. local clustering coefficient
OsDHN1	11	49	8.91	0.904
ZmDHN1	11	18	3.27	0.819
SbDHN	11	22	4	0.933
Xero2	11	39	7.09	0.847

Table 3 The gene ontology (GO) molecular functions and biological processes of dehydrins in *A.thaliana*, *O.sativa*, *Z. mays*, *S.bicolor*, *T.aestivum*, *H.vulgare*.

Organism	Molecular Function	Biological process
<i>A.thaliana</i>	cobalt ion binding copper ion binding nickel cation binding zinc ion binding	cold acclimation defense response to fungus response to abscisic acid response to cold response to water deprivation
<i>O.sativa</i>	unknown	cold acclimation response to abscisic acid response to water deprivation
<i>Z. mays</i>	phosphatidic acid binding phosphatidylglycerol binding phosphatidylinositol binding phosphatidylserine binding	Response to stress Response to water
<i>S.bicolor</i>	unknown	Response to stress Response to water
<i>T.aestivum</i>	unknown	Response to stress Response to water
<i>H.vulgare</i>	unknown	Response to stress Response to water

4 Conclusion

This study has focused on some dehydrins in cereals and *A. thaliana* in order to investigate the potential roles of disorder in the function and molecular interactions. Leveraging to their high structure flexibility, they stabilize membrane and cellular targets under extreme environmental conditions such as low temperatures and drought. Although the data limitations of some cereal interactomes such as wheat and barley, the network based approach provides a global view of relationships between dehydrins and their potential partners. Function similarities between dehydrins in cereals and *A. thaliana* may suggest similar partners. The large data observed to date predicts that many novel dehydrin partners remains to be identified and validated.

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