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Research Article

Distribution and Classification of Dehydrins in Selected Plant Species Using Bioinformatics Approach

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Background: Plant growth, reproduction and yields are severely damaged under adverse environmental stresses. These stresses can be either biotic or abiotic, and many stress related proteins are expressed in response to these stresses. Among these proteins dehydrins are reported to have a role primarily in the abiotic stresses. Dehydrins are very diverse proteins and a uniform annotation system is needed for their functional characterization in the future research.

Objectives: The aim of the present work is to identify, classify and analyze the expression of dehydrin proteins under different biotic and abiotic stresses in the selected plant species by using different computational tools.

Materials and Methods: Prosite database is used for dehydrin proteins identification, and to conform the location of conserved motifs in selected plant species. The dehydrins extracted from uniprot database were annotated, based on the ensemble plant gene id. Subcellular localization was predicted using PSI predictor tool. Dehydrin expression analyses were retrieved form the genevestigator tool.

Results: Dehydrins were annotated on the basis of dehydrin gene locus and conserved motifs available in different domain databases. Dehydrins were identified and annotated in *Arabidopsis thaliana* (13), *Glycine max* (12), *Zea mays* (05), *Oryza sativa* (11), *Solanum tuberosum* (05), *Solanum lycopersicum* (06), *Triticum aestivum* (32) and *Vitis vinifera* (06). It has been proposed that dehydrins are located primarily in cytosol and nucleus. Based on genevestigater expression analyses the plant species selected for this study contain all the classes of dehydrins, namely Y_nSK_n, K_n, SK_n, and Y_nK_n; except class K_nS.

Conclusions: Dehydrins are diverse proteins and a uniform classification is introduced for their better characterization. The distribution of dehydrins in different tissues and developmental stages suggest an important function throughout plant growth cycle. It has also been concluded that dehydrins expressed particularly in drought, cold and salt stresses, and may have limited role in heat, anoxia, heavy-metal and biotic stresses as well.

Keywords: Bioinformatics; Dehydrins; Stresses; Plant species

1. Background

Plants are sessile in nature and this property often exposes them to different environmental conditions. The mechanisms by which plants deal with these environmental stresses have been explored to know how the growth and reproduction are maintained under these conditions. There are a number of environmental stresses that severely damage plants while working concurrently. Stresses are classified into biotic and abiotic stresses, and both have a great impact on crops' growth and production (1). Generally, the basic mechanism of plant tolerance is the reduction in biological activities and accumulation of reactive oxygen species (ROS), phytoharmones, abscisic acid, salicylic acid, jasmonic acid and ethylene), and activation of specific ion channels utilizing the genetic machinery (2).

Among different stress related proteins, Late Embryogenesis Abundant (LEA) proteins are a group of proteins that are reported to have protective roles in the

higher plants against different environmental stresses. These proteins are found both in plants and animals and were initially characterized in cotton and wheat (3). In higher plants, these proteins have been reported to have role in the maintenance of normal metabolism especially in the severe stress conditions (4, 5).

The structure of LEA proteins is based on particular sequence motifs that are classified into six different groups, which are reported to have resistance against drought, salt, osmotic and low temperature stresses. Dehydrins belong to the group 2 of LEA proteins, with a molecular mass of 9 to 200 KD and lacking cysteine and tryptophan residues but rich in glycine and lysine residues. These proteins are thermo stable and hydrophilic in nature (3). Dehydrins are also found in various other organisms such as fungi, algae, plants and cyanobacteria, and are mostly found in various parts of the cell such as mitochondria, nucleus, vacuole and plasma membrane. Dehydrins are reported to have a strong correlation with drought, cold and salt stress (6). Dehydrins can bind to heavy metals (7) and protect the transcription machinery in the nucleus (8).

Based on their structural features and conserved sequences, they are designated as Y (Tyrosine), S (serine) and K (Lysine). Among these, the K segment is highly conserved which is present on the C-terminus of all dehydrins. The K segment forms the amphipathic alpha helix. The other phosphorylated S-segment facilitates the interaction of dehydrins with specific peptides. The other conservative structure is present on the N-terminus that is known as Y-segment and is similar to the plant and bacterial chaperons. Some other less conservative sequences are also found in dehydrins that are rich with polar amino acid residues (3).

Initially dehydrins are thought to be involved in the water stress and most of the work has been focused in relation to this stress. However, recent studies have shown that its role is also important to overall biotic and abiotic stresses.

2. Objectives

The aim of the present work is to identify and classify the plant dehydrins in the selected plant species to give them uniform annotation system and to explore their putative roles for different stresses in the selected plant species through in silico expression analysis.

3. Materials and Methods

3.1. Identification

Prosite database contains two dehydrin signatures (PS00315, PS00823) for the identification of dehydrin proteins (9). On the basis of these two signatures, dehydrin proteins are extracted from uniport database (http://www.uniprot.org/). The dehydrin proteins were extracted for the selected plant species (*Arabidopsis thaliana, Glycine max, Oryza sativa, Solanum tuberosum, Solanum lycopersicum, Vitis vinifera, Zea mays* and *Triticum aestivum*). After extraction the proteins were further confirmed in Pfam (PF00257) (10) and Interpro (IPR000167, IPR030513) domain databases (11).

3.2. Classification and Localization

The plant dehydrins are classified based on different patterns that are manually created (12) on the basis of previous literature (13). The patterns for Y-motif were [TV]-D-E-Y-G, S-motif were S (7) and that for K-motif were K-(I L)-K-(E D)-K-(L I)-P-G. The FASTA file of the dehydrin proteins for the selected plant species were uploaded against the patterns in the Prosite scan database (9) to conform the location and presence of these conserved sites.

The dehydrins were further annotated based on the ensemble plant gene ID (https://plants.ensembl.org/index.html) and their available transcripts correspond to different uniport entries (http://www.uniprot.org/). These annotations were further confirmed by using Multiple Sequence Alignment using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and more than 95% similar sequences were placed in the same dehydrin group (**Fig. S1**).

Subcellular localizations of the selected dehydrins were identified using PSI predictor (14). PSI predicted tool combines 11 individual predictors (cello, mploc, Predotar, mitoProt, Multiloc, TargetP, wolf PSORT, Subcell predict, iPsort, Yloc and PTS1) and the prediction results give us the sub cellular localization (mitochondria, membrane, plastid, vacuole, golgi, extracellular, cytosol, nucleus, peroxisomes, and endoplasmic reticulum) (bis.zju.edu.cn/psi/).

3.3. Expression Analysis

The expressions of the identified dehydrins were analyzed using genevesigator tool (15) (https://genevestigator.com/) for the selected plant species (*Arabidopsis thaliana, Glycine max, Oryza sativa, Zea mays* and *Triticum aestivum*). Developmental tools have been used to find the expression of dehydrins at different growth stages. Anatomical tool has been used for the expression at different cell lines and tissues. Finally, the perturbation tool has been used to identify dehydrin expression levels under different conditions. Highest p-value and fold change is selected in genevestigtor to

find the most relevant condition at which the dehydrins are expressed.

4. Results

In the present computational study all the available dehydrins were annotated and analyzed for their predicted expression under different stress conditions for the selected plant species.

4.1. Identification of Dehydrins

The raw data of dehydrin proteins in FASTA format was extracted from uniport database on the basis of dehydrin domains, identified in Prosite database (9) and was further validated in the InterPro and Pfam domain databases. After scanning, 1548 dehydrin entries have been identified, among which most of the dehydrins were identified in the plant kingdom. The rest have been distributed in animal, fungi and other prokaryotes (**Fig. S2**).

The present study was limited to eight selected plant species, among which 90 dehydrin entries have been retrieved from the uniport database and PS00823 domain is the most conserved domain identified as it is found in all the selected plant species. The potential dehydrin entries were found in all the selected plant species, i.e., *Arabidopsis thaliana* (13)*, Glycine max* (12)*, Zea mays* (05)*, Oryza sativa* (11)*, Solanum tuberosum* (05)*, Solanum lycopersicum* (06), *Triticum aestivum* (32*)* and *Vitis vinifera* (06) (**Table 1**).

4.2. Classification and Subcellular Localization of Dehydrins

All the uniport entries were searched in the plant ensemble databases to retrieve their gene ID. The uniport entries which have the same gene ID showed that they have more than one transcript. This was confirmed by multiple sequence alignment tool and the transcripts with one gene ID showed more than 95% sequence similarity in each specie (**Fig. S1**). Based on these results, 50 dehydrins were identified that are distributed in the selected plant species, namely: *Arabidopsis thaliana* (08), *Glycine max* (03)*, Zea mays* (02)*, Oryza sativa* (07)*, Solanum tuberosum* (05), *Solanum lycopersicum* (06)*, Triticum aestivum* (17) and *Vitis vinifera* (02). These dehydrins were further divided into different sub groups (DHNs) based on their transcripts as uniport identifiers (**Table 1**).

Based on YSK motifs, four dehydrin types (subclasses) have been identified among the selected plant species except K_nS. K_n subclass has maximum two K segments, SK_n subclass has maximum two S and three K segments, Y_nK_n has two Y and K segments each, Y_nSK_n has two Y and K segments and one S segment, identified according to the patterns created (**Table 1**). *Arabidopsis thaliana* contains four K_n types, seven SK_n and two YK subclass DHNs; *Glycine max* has two K_n, one SK_n, nine Y_nK_n and one Y_nSK_n DHN; *Oryza sativa* DHNs comprise of one K_n, 11 SK_n, one Y_nK_n and two Y_nSK_n; in *Solanum lycopersicum* three DHNs have K_n types, one has SK_n and two have Y_nSK_n; *Solanum tuberosum* contains one K_n, two SK_n, one Y_nK_n and one Y_nSK_n DHNs; *Vitis vinifera* has four DHNs, belonging to the K_n and two to the SK_n types; *Triticum aestivum* contains eight K_n, ten SK_n, one Y_nK_n and 13 Y_nSK_n types DHNs*; while in Zea mays* all five DHNs can be put in to the SK_n type (**Table 1**).

Total putative DHNs extracted from uniprot have been exclusively predicted to be in the cytosol and nucleus except for four dehydrins in *Glycine max* which are localized in plasma membrane. In addition, one of the plasma membrane located dehydrin belongs to the K_n subclass and the rest of the three plasma membranes are in the Y_nK_n subclass (**Table 1**)

4.3. Expression of Dehydrins

4.3.1. Expression at Developmental Stages

During germination stage in *Arabidopsis thaliana*, the expression of AtDHN1, AtDHN2, AtDHN3, AtDHN5 and AtDHN8 was higher, whereas the expression for AtDHN4, AtDHN6 and AtDHN7 was in the medium range. This expression pattern is the same in the seedling, rosette, bolting, flower and the siliques, except for AtDHN8, whose expression dropped from higher to a medium range. In the senescence, the expression of AtDHN4, AtDHN5, AtDHN6 and AtDHN8 is the highest, whereas the expression of AtDHN1, AtDHN2, AtDHN3 and AtDHN7 is comparatively lower in contrast to the other developmental stages. In *Glycine max* GmDHN1 the expression is in high range while GmDHN2 and GmDHN3 expression is lower in all available developmental stages; although, the expression of GmDHN3 becomes higher during the seed development. In *Zea mays*, the expression of ZmDHN2 is higher comparative to ZmDHN1 in all the developmental stages. In *Oryza sativa*, the expression of OsDHN2 is higher as compared to the other dehydrins in all the selected stages. The expression of all dehydrins becomes higher during the dough stage in *Oryza sativa*. Similarly, the expression of TaDHN6 is comparatively higher and constant in almost all the developmental stages in *Triticum aestivum*. The expression becomes higher for all the dehydrins in wheat in the ripening stage as compared to the other stages (**Table 2**).

Table 1. Putative dehydrins identified through different domain databases and their classification. (Italic uniport entry means that their status has been reviewed. α = PS00315, β = PS00823, π = PF00257, Ω = IPR000167, ʊ = IPR030513; PS= Prosite, PF = Pfam, IPR = InterPro, C= Cytosol, N= Nucleus, P= Plasma membrane)

Species	Uniprot Entry	DHNs	Ensembel Gene ID	Domain	Class
A thaliana	*P31168* (N)	AtDHN 1.1	AT1G20440	αβΩʊ	SK_3
A thaliana	C0Z2D8 (N)	AtDHN 1.2	AT1G20440	αβπΩʊ	SK_2
A thaliana	Q0WL48 (N)	AtDHN 1.3	AT1G20440	αβπΩʊ	SKN
A thaliana	*P42759* (N)	AtDHN 2.1	AT1G20450	αβπ	SK_2
A thaliana	F4HST2 (N)	AtDHN 2.2	AT1G20450	βπΩʊ	K_2
A thaliana	*P42763* (N)	AtDHN 3	AT1G76180	αβπ	SK_2
A thaliana	*Q96261* (C)	AtDHN 4	AT2G21490	αβπΩʊ	SK
A thaliana	*P42758* (C)	AtDHN 5	AT3G50970	βπ	K
A thaliana	*P25863* (N)	AtDHN 6	AT3G50980	αβπ	SK_2
A thaliana	Q9SVE4 (N)	AtDHN 7.1	AT4G38410	βʊ	K_2
A thaliana	Q8GY17 (N)	AtDHN 7.2	AT4G38410	βπΩʊ	K_2
A thaliana	*P30185* (C)	AtDHN 8.1	AT5G66400	αβπ	YK_2
A thaliana	F4K0K7 (C)	AtDHN 8.2	AT5G66400	β	YK_2
G max	C6TAX7 (N)	GmDHN 1	Glyma_04G009400	αβπΩʊ	SK_2
G max	Q42447 (C)	GmDHN 2.1	Glyma_07G090400	βπΩʊ	YK_2
G max	A1KR24 (C)	GmDHN 2.2	Glyma_07G090400	βπΩʊ	Y_2K
G max	Q70EL9 (C)	GmDHN 2.3	Glyma_07G090400	βπΩʊ	Y_2K
G max	Q7XAW0 (C)	GmDHN 2.4	Glyma_07G090400	βπΩʊ	Y_2K
G max	Q70EL7 (C)	GmDHN 2.5	Glym_.07G090400	βπΩʊ	Y_2SK_2
G max	I2E8L4 (C)	GmDHN 3.1	Glyma_09G185500	βπΩʊ	K
G max	I2E8L0 (P)	GmDHN 3.2	Glyma_09G185500	αβʊ	K
G max	Q39876 (P)	GmDHN 3.3	Glyma_09G185500	βπΩʊ	Y_2K
G max	K7LEQ5	GmDHN 3.4	Glyma_09G185500	βπΩʊ	Y_2K
G max	Q70EM0 (P)	GmDHN 3.5	Glyma_09G185500	βπΩʊ	Y_2K
G max	Q39805 (P)	GmDHN 3.6	Glym_.09G185500	βπΩʊ	Y_2K
Z mays	*P12950 (C)*	ZmDHN 1.1	GRMZM2G079440	αβπΩʊ	SK_2
Z mays	A3KLI1 (C)	ZmDHN 1.2	GRMZM2G079440	αβπΩʊ	S_2K
Z mays	A3KLI0 (C)	ZmDHN 1.3	GRMZM2G079440	αβπΩʊ	SK_2
Z mays	C4J477 (N)	ZmDHN 2.1	GRMZM2G373522	αβπΩʊ	SK
Z mays	Q41824 (N)	ZmDHN 2.2	GRMZM2G373522	αβπΩʊ	SK
O sativa	*P30287 (C)*	OsDHN 1.1	Os01g0702500	αβπ	SK_2
O sativa	B9EZ14 (N)	OsDHN 1.2	Os01g0702500	αβπΩʊ	SK_2
O sativa	Q0JK19 (C)	OsDHN 1.3	Os01g0702500	αβπΩʊ	SK_2
O sativa	Q6ESR3 (C)	OsDHN 2.1	Os02g0669100	βπΩʊ	K
O sativa	Q6ESR4 (N)	OsDHN 2.2	Os02g0669100	αβπΩʊ	SK_2
O sativa	Q53JR9 (C)	OsDHN 3	Os11g0451700	αβπΩʊ	YSK_2
O sativa	*Q2R4Z8*	OsDHN 4	Os11g0453900	αβπΩʊ	SK_2
O sativa	*Q2R4Z7**	OsDHN 5	Os11g0454000	αβπΩʊ	$S2K_2$
O sativa	*Q2R4Z5**	OsDHN 6.1	Os11g0454200	αβΩʊ	YK_2
O sativa	B1NEV6* (C)	OsDHN 6.2	Os11g0454200	αβπΩʊ	SK_2
O sativa	*Q2R4Z4 (C)*	OsDHN 7	Os11g0454300	αβπ	YSK_2
S tuberosum	M0ZVK4 (N)	StDHN 1	PGSC0003DMG400003530	αβπΩʊ	K
S tuberosum	M0ZVK5 (C)	StDHN 2	PGSC0003DMG400003531	αβπΩʊ	Y_2SK
S tuberosum	M1AM40 (N)	StDHN 3	PGSC0003DMG400009968	βπ	SK_2
S tuberosum	M1D1X0 (N)	StDHN 4	PGSC0003DMG400030949	βπΩʊ	YK
S tuberosum	M1D7T1 (N)	StDHN 5	PGSC0003DMG400034095	αβπΩʊ	SK
S lycopersicum	K4AWI5 (N)	SlDHN 1	Solyc01g065820.1	βπΩʊ	K

Continued Table 1. Putative dehydrins identified through different domain databases and their classification. (Italic uniport entry means that their status has been reviewed. α = PS00315, β = PS00823, π = PF00257, Ω = IPR000167, ʊ = IPR030513; PS= Prosite, PF = Pfam, IPR = InterPro, C= Cytosol, N= Nucleus, P= Plasma membrane)

Species	Uniprot Entry	DHNs	Ensembel Gene ID	Domain	Class
S lycopersicum	K4B3K5 (C)	SlDHN 2	Solyc01g109920.2	αβπΩʊ	YSK
S lycopersicum	K4B6C8 (N)	SlDHN 3	Solyc02g062390.2	αβπΩʊ	K
S lycopersicum	K4BAZ9 (C)	SlDHN 4	Solyc02g084840.2	αβπΩʊ	Y_2SK_2
S lycopersicum	*P22240 (C)*	SlDHN 5	Solyc02g084850.2	αβπΩʊ	K
S lycopersicum	K4BVU7 (N)	SlDHN 6	Solyc04g082200.2	βπΩʊ	SK_2
T aestivum	W5D5Z6 (N)	TaDHN 1.1	TRIAE_CS42_3AL_TGACv1_195928_AA0655770	αβπΩʊ	YSK_2
T aestivum	D0PRB6 (N)	TaDHN 1.2	TRIAE_CS42_3AL_TGACv1_195928_AA0655770	αβπΩʊ	YSK
T aestivum	A0A077S5J5 (C)	TaDHN 2	TRIAE_CS42_3B_TGACv1_224725_AA0800670	αβπΩʊ	SK_2
T aestivum	W5ERW2 (N)	TaDHN 3	TRIAE_CS42_4DS_TGACv1_361015_AA1158770	αβπΩʊ	YSK_2
T aestivum	*Q00742 (C)*	TaDHN 4.1	TRIAE_CS42_5AL_TGACv1_378535_AA1253650	αβπ	YSK_2
T aestivum	A0A0F7WA67 (C)	TaDHN 4.2	TRIAE_CS42_5AL_TGACv1_378535_AA1253650	βπΩʊ	K
T aestivum	A0A0H4MAT1 (C)	TaDHN 5.1	TRIAE_CS42_5DL_TGACv1_433513_AA1415270	αβπΩʊ	K_2
T aestivum	W5FQI8 (C)	TaDHN 5.2	TRIAE_CS42_5DL_TGACv1_433513_AA1415270	αβπΩʊ	SK_2
T aestivum	*P46524 (C)*	TaDHN 6.1	TRIAE_CS42_6AL_TGACv1_471582_AA1511400	αβπΩʊ	SK
T aestivum	P93608 (N)	TaDHN 6.2	TRIAE_CS42_6AL_TGACv1_471582_AA1511400	αβπΩʊ	SK_2
T aestivum	W5G4Z9 (C)	TaDHN 6.3	TRIAE_CS42_6AL_TGACv1_471582_AA1511400	αβπΩʊ	SK
T aestivum	T1VYS7 (N)	TaDHN 6.4	TRIAE_CS42_6AL_TGACv1_471582_AA1511400	αβπΩʊ	SK
T aestivum	P93607 (C)	TaDHN 6.5	TRIAE_CS42_6AL_TGACv1_471582_AA1511400	αβπΩʊ	SK
T aestivum	A8CWL2 (C)	TaDHN 6.6	TRIAE_CS42_6AL_TGACv1_471582_AA1511400	αβπΩʊ	SK
T aestivum	*P46525 (C)*	TaDHN 7.1	TRIAE_CS42_6AL_TGACv1_471708_AA1513200	βπΩʊ	K_2
T aestivum	*P46526 (C)*	TaDHN 7.2	TRIAE_CS42_6AL_TGACv1_471708_AA1513200	βπΩʊ	K_2
T aestivum	O65216 (C)	TaDHN 7.3	TRIAE_CS42_6AL_TGACv1_471708_AA1513200	βπΩʊ	SK_2
T aestivum	W5GAN3 (N)	TaDHN 8	TRIAE_CS42_6AL_TGACv1_472740_AA1525450	βπΩʊ	YSK
T aestivum	B0LXL4 (C)	TaDHN 9	TRIAE_CS42_6AL_TGACv1_473324_AA1530160	αβπΩʊ	YSK_2
T aestivum	*W5GD49 (C)*	TaDHN 10	TRIAE_CS42_6AS_TGACv1_486309_AA1559560	αβπΩʊ	SK_2
T aestivum	W5GW81 (C)	TaDHN 11.1	TRIAE_CS42_6BL_TGACv1_500727_AA1608920	αβπΩʊ	YSK_2
T aestivum	Q41579 (C)	TaDHN 11.2	TRIAE_CS42_6BL_TGACv1_500727_AA1608920	αβπΩʊ	YSK_2
T aestivum	Q8W192 (C)	TaDHN 12	TRIAE_CS42_6BL_TGACv1_503721_AA1628330	αβπΩʊ	YSK_2
T aestivum	W5GYW6 (C)	TaDHN 13	TRIAE_CS42_6DL_TGACv1_526795_AA1692050	αβπΩʊ	YSK_2
T aestivum	W5GVC9 (N)	TaDHN 14	TRIAE_CS42_6DL_TGACv1_527031_AA1697460	αβπΩʊ	YSK_2
T aestivum	Q8LP43 (C)	TaDHN 15.1	TRIAE_CS42_7AL_TGACv1_556589_AA1766430	βπΩʊ	K
T aestivum	Q0KIW1 (C)	TaDHN 15.2	TRIAE_CS42_7AL_TGACv1_556589_AA1766430	βπΩʊ	K
T aestivum	U6C7L2 (C)	TaDHN 15.3	TRIAE_CS42_7AL_TGACv1_556589_AA1766430	βπΩʊ	K
T aestivum	D2TE72 (C)	TaDHN 15.4	TRIAE_CS42_7AL_TGACv1_556589_AA1766430	βπΩʊ	YSK
T aestivum	P93610 (C)	TaDHN 16	TRIAE_CS42_7BL_TGACv1_578284_AA1892310	βπΩʊ	K
T aestivum	W5FJU7 (C)	TaDHN 17.1	TRIAE_CS42_U_TGACv1_641024_AA2082530	βπΩʊ	YK_2
T aestivum	W5FA07 (C)	TaDHN 18	TRIAE_CS42_5BL_TGACv1_404666_AA1307790	αβπΩʊ	YSK_2
V vinifera	F6I0M9 (C)	VvDHN1.1	VIT_03s0038g04390	αβπΩʊ	SK
V vinifera	F6H0C4 (N)	VvDHN 1.2	VIT_03s0038g04390	αβπΩʊ	S_2K
V vinifera	Q3ZNL4 (C)	VvDHN 2.1	VIT_04s0023g02480	βπΩʊ	K
V vinifera	A3REN2 (N)	VvDHN 2.2	VIT_04s0023g02480	βπΩʊ	K
V vinifera	H9A0H3 (C)	VvDHN 2.3	VIT_04s0023g02480	βπΩʊ	K
V vinifera	A5C8L5 (C)	VvDHN 2.4	VIT_04s0023g02480	βπΩʊ	K

Table 2. Expression of dehydrin genes using Genevestigator developmental and anatomy tool.

DHNs		1	2	3	4	5	6	7	8	10	13	17	18
Developmental stages	No of Samples	***A thaliana*** Expression threshold: L= 6-8, M=8-11, H,11-20											
Germinated Seed	515	H	H	H	M	H	M	M	H				
Seedling	2785	H	H	H	L	H	L	M	M				
young rosette	836	H	H	H	L	H	L	M	M				
Developed rosette	2196	H	H	H	L	H	L	M	M				
Blotting	369	H	H	H	L	M	L	M	M				
Young flower	720	H	H	H	L	H	L	M	M				
Developed flowerer	1038	H	H	H	L	M	L	M	M				
Flowers and siliques	274	H	H	H	M	M	M	M	M				
Mature siliques	93	H	H	H	H	H	H	M	H				
Senescence	18	M	M	M	H	H	H	M	H				
Tissues and cell lines		L=6-8, M=8-12, H= 12-18,											
Callus	31	H	H	H	L	M	L	M	M				
Callus culture	714	H	H	H	L	H	L	M	M				
Seedling	2345	H	H	H	L	H	L	M	M				
Inflorescence	801	H	H	H	M	M	M	M	M				
Shoot	4580	H	H	H	L	M	L	M	M				
Root	1081	H	H	H	L	H	L	M	M				
Developmental stages		***G max*** Expression threshold: L= 6-8, M=8-12.5, H,12-19											
Germination	61	H	M	M									
Main shoot growth	618	H	M	M									
Flowering	3	H	M	M									
Fruit formation	63	H	M	M									
Bean development	169	H	M	H									
Tissues and cell lines		Expression threshold: L=7-8, M=8-12, H,12-20											
Callus culture	25	H	M	M									
Seedling	57	H	M	H									
Inflorescence	237	H	M	H									
Shoot	432	H	M	M									
Root	2872	H	M	M									
Developmental stages		***Z mays*** Expression threshold: L=0-1, M=1-4, H,4-10											
Germination	225	H	H										
Seedling	774	H	M										
Stem elongation	387	H	M										
Inflorescence	48	H	M										
Anthesis	86	H	M										
Fruit formation	658	H	M										
Dough	138	H	H										
Tissues and cell lines		Expression threshold: L=0-1, M=1-4, H,4-10											
Callus culture	6	M	H										
Seedling	233	M	H										
Inflorescence	1057	M	H										
Shoot	976	M	H										
Root	107	M	H										
Developmental stages		***O sativa*** Expression threshold: L=7-8, M=8-12, H,12-21											
Germination	361	M	H	M	M	H	H	H					
Seedling	996	M	H	M	M	H	M	H					
Tillering	304	M	H	M	M	M	M	H					
Elongation	89	M	H	M	M	H	M	H					
Booting	126	M	H	M	M	H	M	H					
Heading	355	M	H	M	M	H	M	H					
Flowering	109	M	H	M	M	M	M	H					
Milk	87	H	H	M	M	H	M	H					
Dough	13	H	H	H	H	H	H	H					
Tissues and cell lines		Expression threshold: L=7-8, M=8-12, H,12-19											
Callus	73	H	H	M	H	H	H	H					
Callus culture	3	H	M	M	M	M	M	M					
Seedling	428	M	H	M	M	H	M	H					
Inflorescence	503	H	H	M	M	H	H	H					

Continued Table 2. Expression of dehydrin genes using Genevestigator developmental and anatomy tool.

DHNs		1	2	3	4	5	6	7	8	10	13	17	18
Shoot	1501	M	H	M	M	M	M	H					
Root	321	M	H	M	M	M	M	M					
Developmental stages		***T aestivum*** Expression threshold: L=7-8, M=8-12, H,12-19											
Germination	68			H			H			M	H	H	H
Seedling	428			M			H			L	M	M	M
Tillering	71			L			H			M	M	M	H
Stem elongation	36			M			H			M	M	M	M
Booting	31			M			H			M	H	H	H
Inflorescence	40			L			H			M	M	M	M
Anthesis	649			L			H			M	H	M	M
Milk development,	363			M			H			M	M	M	M
Dough development	276			H			H			H	H	H	H
Ripening	20			H			H			H	H	H	H
Tissues and cell lines		Expression threshold: L=7-8, M=8-12, H,12-18											
Seedling	238			M			H			M	M	M	M
Inflorescence	1070			M			H			M	H	M	M
Shoot	674			M			H			M	M	M	M
Root	80			M			H			M	H	H	H

4.3.2. Expression in Tissue and Cell Lines

At the tissue level the expression of AtDHN1, AtDHN2 and AtDHN3 is higher in all the tissues (**Table 2**), whereas the expression of AtDHN4 is comparatively lower in all the selected tissues. AtDHN5 expression is higher in roots as compared to the other tissues. AtDHN6, AtDHN7 and AtDHN8 expressions are in the medium range in all the tissues. In *Glycine max* the expression of GmDHN1 is higher as compared to GmDHN2 and GmDHN3. In *Zea mays* the expression of ZmDHN2 is higher while ZmDHN1 expression is medium in all the tissues. In *Oryza sativa* OsDHN1 is highly expressed in callus and in inflorescence. OsDHN2 expression is higher in all the tissues except in the callus culture. OsDHN3 and OsDHN4 expressions are medium in all the selected tissues. OsDHN5 expression is relatively higher in the callus, seedling, inflorescences and shoots as compared to the other tissues. OsDHN6 expression is higher in callus and inflorescence, whereas OsDHN7 expression is higher in callus, seedling, inflorescence and shoots. In *Triticum aestivum* TaDHN6 expression is higher, whereas TaDHN3 and TaDHN10 expressions are medium in all the tissues. The expression of TaDHN13 is higher in roots and inflorescence, whereas TaDHN17 and TaDHN18 expressions are higher in roots as compared to their expression in other tissues.

4.3.3. Expression under Biotic and Abiotic Stresses

During biotic stresses, AtDHN1, AtDHN2 and AtDHN5 are upregulated after treating *Arabidopsis thaliana* with *Liriomyza huidobrensis*. However, AtDHN1, AtDHN2, AtDHN4, AtDHN5 and AtDHN8 are downregulated during different perturbations in biotic stimulus. AtDHN3, AtDHN6, and AtDHN5 perturbations are not available at the selected threshold (**Table 3**). Most of the Arabidopsis dehydrins are upregulated during drought, cold, salt and ABA stresses. During low temperature AtDHN4 and AtDHN8 are downregulated whereas AtDHN1, AtDHN2 and AtDHN3 are upregulated (**Table 4**).

At the selected threshold level in *Glycine max*, only GmDHN3 appears to be upregulated after incubation with *Phtophthora sojae*. Abiotic stimulus results did not retrieve at the selected filter criteria for *Glycine max* (Table 4). Exposed to biotic stimulus, the ZmDHN1is upregulated after treatment with *Colletotrichum graminicola* and *Fusarium verticillioides*, whereas ZmDHN2 showed upregulation in the presence of *Colletotrichum graminicola* and *Rhopalosiphum maidis*. During abiotic stress both ZmDHN1 and ZmDHN2 appear to be upregulated by drought, cold and heat (**Tables 3 and 4**).

In *Oryza sativa* specie, both OsDHN6 and OsDHN7 appeared to be either up or downregulated after treatment with *Xanthomonas campestris* and *Xanthomonas oryzae*. OsDHN1 and OsDHN2 are upregulated after incubation with *Xanthomonas campestris*. Similarly, OsDHN5 and OsDHN7 are upregulated when treated with *Xanthomonas oryzae* and *Nilapervata lugens* respectively. Biotic perturbation data reveals that OsDHN4 is downregulated after incubation with *Xanthomonas oryzae*. OsDHN3 did not retrieve the biotic stress data at the selected filter criteria. During abiotic stress the perturbation results show that all

Table 3. Dehydrins expression during different conditions (biotic stress) using Genevestigator perturbation tool. (↑= Upregulated, ↓= Downregulated, Number in brackets= number of perturbations).

	DHN1	DHN2	DHN3	DHN4	DHN5	DHN6	DHN7	DHN8	DHN13	DHN17	DHN18
					A thaliana						
L huidobrensis	↑	↑			↑						
P cucumerina	↓										
P. syringae	↓				↓ (2)						
S sclerotiorum	↓	↓		↓				↓			
G cichoracearum					↓						
G orontii					↓						
M incognita		↓			↓						
					Z mays						
C graminicola	↑	↑									
R maidis		↑ (5)									
F verticillioides	↑										
					G max						
P sojae			↑ (4)								
					T aestivim						
A caliginosa										↓ (2)	
F graminearum									↓ (2)	↑	↑ (3)
G graminis										↓	
X translucens									↓	↓	↓
P triticina									↑		
T caries									↓		
					O sativa						
X campestris	↑ (5)	↑ (3)				↑↓	↑↓				
X oryzae				↓	↑	↑ (5), ↓ (3)	↑ (4), ↓ (3				
N lugens							↑				

Table 4. Dehydrins expression during different conditions (abiotic stress) using Genevestigator perturbation tool. (↑= Upregulated, ↓= Downregulated, Number in brackets= number of perturbations).

Conditions	DHN1	DHN2	DHN3	DHN4	DHN5	DHN6	DHN7	DHN8	DHN13	DHN17	DHN18
					A thaliana						
Draught	↑ (8)	↑ (8)	↑ (2)	↑ (12)	↑ (12)	↑ (3)	↑ (5)	↑ (12)			
Cold	↑ (8)	↑ (8)	↑ (1)	↑ (2)	↑ (10)			↑ (2)			
Salt	↑ (1)	↑ (1)		↑ (5)	↑ (3)	↑ (1)	↑ (25)	↑ (5)			
Temp (28-19)	↑ (4)	↑ (5)	↑ (2)	↓				↓			
Hormone (ABA)	↑ (4)	↑ (06)	↑ (4)	↑ (2)		↑ (9)	↑ (5)	↑ (13)			
					Z mays						
Drought	↑ (5)	↑ (4)									
Cold	↑ (2)	↑ (2)									
Heat	↑ (2)	↑ (3)									
					T aestivum						
Draught						↑ (3)			↑ (14)	↑ (13)	↑ (14)
Cold			↑						↑ (7)	↑	↑ (5)
Salt									↑ (3)	↑ (5)	↑ (3)
Hormone (ABA)			↑							↑ (2)	
					Oryza sativa						
Draught	↑ (15)	↑ (7)	↑ (12)	↑ (15)	↑ (20)	↑ (8)	↑ (19)				
Cold	↑ (1)	↑ (14)				↑					
Salt	↑ (4)	↑ (1)	↑ (1)	↑ (5)	↑ (7)	↑ (7)	↑ (4)				
Heat	↑ (4)			↑ (4)	↑ (4)	↑ (8)					
Anoxia	↑ (4)	↑ (4)	↑ (4)	↑ (4)	↑ (4)	↑ (4)	↑ (2), ↓ (6)				
Arsenic	↑			↑		↑	↑ (2)				
Chromium					↑	↑					
Hormone (ABA)						↑ (4)					

the rice dehydrins are upregulated during drought and salt stress. OsDHN1, OsDHN2 and OsDHN6 are upregulated during cold stress, while OsDHN1, OsDHN4, OsDHN5 and OsDHN6 are upregulated after heat stress, and OsDHN6 is upregulated after treatment with ABA. During heavy metal stress OsDHN6 is upregulated after Cr and Ar stress, whereas OsDHN1, OsDHN4 and OsDHN7 appear to be upregulated during Ar stress while OsDHN5 is upregulated after treating *Oryza sativa* with Cr. During anoxia stress the trends show that all the rice dehydrins are downregulated (**Tables 3 and 4**).

The biotic stress perturbation data shows that TaDHN13 is downregulated after incubation with *Fusarium graminearum*, *Xanthomonas translucens* and *Tellatia caries*, and upregulated after treatment with *Puccinia triticina*. TaDHN17 is downregulated when wheat is exposed to *Aporrectodea caliginosa*, *Gaeumannomyces graminis* and *Xanthomonas translucens*; and upregulated after the treatment with *Fusarium graminearum*. TaDHN18 is downregulated by *Xanthomonas translucens* and upregulated by *Fusarium graminearum*. During the drought stress, TaDHN6, TaDHN13, TaDHN17 and TaDHN18 are upregulated, while the cold stress resulted in the upregulation of TaDHN3, TaDHN13, TaDHN17 and TaDHN18. Salt stress has resulted in the upregulation of TaDHN13, TaDHN17 and TaDHN18, while ABA stress results in the upregulation of TasDHN3 and TaDHN17 (**Tables 3 and 4**).

5. Discussion

In the present study dehydrins were extracted from the selected plant species from uniprot database, based on the available signatures in Prosite, Pfam and Interpro databases. The identified dehydrins in the uniprot database show that there are many transcripts available for a single gene (**Table 1**). Further, there is still no agreed upon classification available for dehydrin proteins. Dehydrins is a diverse class of proteins and there is not much similarity found in them except the conserved signature motifs (13). In the present study, classification is based on the gene locus (**Table 1**). The previously known dehydrin protein annotations in each species is different in the uniport database, i.e., COR47, ERD10, ERD14, Dehydrin LEA, Xero2, Xero1, Cold regulated protein, Rab18 in *Arabidopsis thaliana* (http://www.uniprot.org/) have been named DHN1,2,3,4,5,6,7 and 8 respectively. In *Zea mays* DHN1 and dehydrin 3 have been annotated as DHN1 and DHN2 respectively. Similarly, in *Oryza sativa* dehydrin has been annotated as DHN1 (Rab25), DHN2 (DHN1, DIP1, LIP9), DHN3, DHN4 (Rab16D), DHN5 (Rab16C), DHN6 (Rab16B), DHN7 (Rab21). In other selected species, most of the dehydrin proteins are not characterized and the annotations are not uniform as well. So, annotation of dehydrin proteins on the basis of their unique conserved motifs, gene locus and deferent transcripts is providing a uniform classification that can be used in future for the rest of the plant kingdom.

The subcellular localization for all the dehydrins in the selected plant tissues are primarily into the cytosol, nucleus and some of them are also located in the plasma membrane in the *Glycine max* based on the prediction tool used (**Table 1**). Studies have shown that DHN1 in *Zea mays*, WCS120 in *Triticum aestivum* and PCA60 in peach are localized both to cytosol and nucleus (16, 17). Some other dehydrins such as Rab21 in *Oryza sativa* (18) and WCOR410 in wheat (19) are reported to be localized in the cytosol and plasma membrane. Studies have also shown the localization of dehydrins in mitochondria, chloroplast and endoplasmic reticulum (17, 20, 21). The prediction tool gives the localization of dehydrins in the chloroplast, mitochondria, endoplasmic reticulum and even in golgi bodies but their results have been excluded as the scores are less than that of cytosol, nucleus and plasma membrane.

The expression analysis has been used at different developmental stages and in different cell lines and tissues with the help of developmental and anatomical tools in genevestigator (**Table 2**). The expression analysis showed that dehydrins are distributed throughout the developmental stages, i.e., from seedling emergence to the maturation of the plants and flowers. The same expression analyses have been shown for different tissues and cell lines i.e., callus, seedling, inflorescence, shoots and roots. These results have also shown that more than one dehydrin can be localized in the same tissues and they may have an important function throughout the plant growth and that is to cope with different stresses. Different studies have conformed the distribution of dehydrins in different tissues during plant growth and development. Rab18 in *Arabidopsis thaliana* and Rab17 in *Zea mays* has been shown to accumulate in the embryo and in the endosperm of the mature seeds (22, 23). It has also been demonstrated that ERD14 and ERD10 are localized in the roots, stems, leaves and flowers (24). Similarly, PCA60 dehydrin is accumulated in all the tissues of the shoots and WCOR410 in wheat is localized in the tissues of roots, leaves and crowns (17, 19).

According to the combination of YSK motifs dehydrins have been subdivided into five classes: Y_nSK_n, K_n, K_nS, SK_n, and Y_nK_n (13, 25). The plant species selected for

this study contain all the classes previously identified, except K_nS (Table 1). The SK_n was identified as the most abundant class, whereas the Y_nSK_n has been reported previously as the most abundant class (3). Y_nSK_n class has not been identified in *Arabidopsis thaliana*, *Zea mays* and *Vitis venifira*. Further, Y_nK_n in *Zea mays* and *Vitis venifira* while K_n is absent in *Zea mays* (**Table 1**). Plant dehydrins have versatile function in plant tolerance including drought, ABA, cold, salt and even in heavy metal stresses (26), which is confirmed by our in-silico expression analysis. In *Arabidopsis thaliana*, dehydrins are mostly upregulated or in few perturbations downregulated in drought, cold, and salt stress, ABA treatment, heat stress, anoxia, heavy metal stress and up to some extent, in biotic stress (**Table 3,4**). It has been proposed previously that Y_nSK_n class dehydrins are induced by drought or ABA treatment, but their expression remains unchanged during cold stress (3, 27). The expression analysis done by using genevestigator tool reveals that Y_nSK_n type dehydrins are expressed during cold, salt, heat, anoxia as well as during biotic stress (**Table 3,4**). Similarly, based on previous studies K_n class dehydrins have been shown to be involved in cold stress (7, 28-30) and up to some extent are induced during drought stress and during ABA treatment (13). The expression analysis in this study has also identified that K_n type dehydrins are also expressed during salt, anoxia and biotic stresses (Table 3,4). Y_nK_n and SK_n class have been shown to be associated mainly with the cold tolerance (19, 25, 31). In addition, SK_n dehydrin accumulates during low temperature, drought salinity, wound stress and with certain hormones treatments (32). The expression analysis using genevestigator shows that both Y_nK_n and SK_n are expressed after the plant dealing with drought, cold, and salt stresses, anoxia, heat stress, ABA, heavy metals and during the biotic stress as well (**Table 3, 4**). As the K_nS class is absent from the selected plant species, which has a role to reduce the metal toxicity (25), this suggests that metal detoxification can still be overcome by the expression of Y_nK_n and SK_n class of dehydrins. Moreover, as different classes of dehydrins are located in the same tissue at developmental stages, it is concluded that different classes of dehydrins have redundant function and cannot exhibit distinct functions as suggested previously (3, 25).

6. Conclusions

Dehydrins annotation is based on the conserved motifs, gene locus and their transcripts. This classification provides a uniform system for better characterization of dehydrin proteins. It has also been predicted that the distribution of dehydrins in the different tissues and developmental stages suggest an important function throughout the plant's growth cycle. It was also concluded that dehydrins express particularly in drought, cold and salt stresses, but may have limited role in heat, anoxia, heavy metal and biotic stresses.

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Supplementary material:

Figure S1: Similarity index after multiple sequence alignment for the classification of dehydrin genes.
Figure S2: Putative dehydrins in different kingdoms

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