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 genevestigator expression analyses the plant species selected for this study contain all the classes of dehydrins, namely YnSKn, Kn, SKn, and YnKn; except class KnS.

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), phytohormones, 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 genevestigator 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 genevestigator 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 uniprot 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 uniprot 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 uniprot entries were searched in the plant ensemble databases to retrieve their gene ID. The uniprot 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 species (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 uniprot identifiers (Table 1).

Based on YSK motifs, four dehydrin types (subclasses) have been identified among the selected plant species except K, S. K, subclass has maximum two K segments, SK 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 SK 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. $\alpha$ = PS00315, $\beta$ = PS00823, $\pi$ = PF00257, $\Omega$ = IPR000167, $\Psi$ = IPR030513; PS= Prosite, PF= Pfam, IPR= InterPro, C= Cytosol, N= Nucleus, P= Plasma membrane)

| Species       | Uniport Entry | DHNs     | Ensembl Gene ID | Domain | Class |
|---------------|---------------|----------|----------------|--------|-------|
| *A. thaliana* | P31168 (N)    | AtDHN 1.1| AT1G20440      | $\alpha\beta\Omega\Psi$ | SK2   |
| *A. thaliana* | C0Z2D8 (N)    | AtDHN 1.2| AT1G20440      | $\alpha\beta\Omega\Psi$ | SK2   |
| *A. thaliana* | Q0WL48 (N)    | AtDHN 1.3| AT1G20440      | $\alpha\beta\Omega\Psi$ | SKN   |
| *A. thaliana* | P42759 (N)    | AtDHN 2.1| AT1G20450      | $\alpha\pi\Omega\Psi$  | SK2   |
| *A. thaliana* | F4HST2 (N)    | AtDHN 2.2| AT1G20450      | $\beta\pi\Omega\Psi$  | K2    |
| *A. thaliana* | P42763 (N)    | AtDHN 3  | AT1G76180      | $\alpha\pi$           | SK2   |
| *A. thaliana* | Q96261 (C)    | AtDHN 4  | AT2G12490      | $\beta\Omega\Psi$     | SK    |
| *A. thaliana* | P42758 (C)    | AtDHN 5  | AT3G50970      | $\beta\Omega$          | K     |
| *A. thaliana* | P25863 (N)    | AtDHN 6  | AT3G50980      | $\alpha\pi$           | SK2   |
| *A. thaliana* | Q9SVE4 (N)    | AtDHN 7.1| AT4G38410      | $\beta\Omega\Psi$     | K2    |
| *A. thaliana* | Q0WL48 (N)    | AtDHN 7.2| AT4G38410      | $\beta\Omega\Psi$     | K2    |
| *G. max*      | C6TAX7 (N)    | GmDHN 1  | Glyma_04G009400| $\beta\Omega\Psi$     | SK2   |
| *G. max*      | Q42447 (C)    | GmDHN 2.1| Glyma_07G090400| $\beta\Omega\Psi$     | YK2   |
| *G. max*      | A1KR24 (C)    | GmDHN 2.2| Glyma_07G090400| $\beta\Omega\Psi$     | YK2   |
| *G. max*      | Q70EL9 (C)    | GmDHN 2.3| Glyma_07G090400| $\beta\Omega\Psi$     | YK2   |
| *G. max*      | Q7TCAW0 (C)   | GmDHN 2.4| Glyma_07G090400| $\beta\Omega\Psi$     | YK2   |
| *G. max*      | Q70EL7 (C)    | GmDHN 2.5| Glyma_07G090400| $\beta\Omega\Psi$     | YK2   |
| *Z. mays*     | P12950 (C)    | ZmDHN 1.1| GRMZM2G079440  | $\beta\Omega\Psi$     | SK2   |
| *Z. mays*     | A3KL1 (C)     | ZmDHN 1.2| GRMZM2G079440  | $\beta\Omega\Psi$     | SK2   |
| *Z. mays*     | A3KL10 (C)    | ZmDHN 1.3| GRMZM2G079440  | $\beta\Omega\Psi$     | SK2   |
| *Z. mays*     | C43477 (N)    | ZmDHN 2.1| GRMZM2G079440  | $\beta\Omega\Psi$     | SK2   |
| *Z. mays*     | Q41824 (N)    | ZmDHN 2.2| GRMZM2G079440  | $\beta\Omega\Psi$     | SK2   |
| *O. sativa*   | P307287 (C)   | OsDHN 1.1| Os01g0702500   | $\alpha\Omega$         | SK2   |
| *O. sativa*   | B9EZ14 (N)    | OsDHN 1.2| Os01g0702500   | $\alpha\Omega$         | SK2   |
| *O. sativa*   | Q6E3R4 (C)    | OsDHN 1.3| Os01g0702500   | $\alpha\Omega$         | SK2   |
| *O. sativa*   | Q6E3R4 (C)    | OsDHN 2.1| Os02g0669100   | $\beta\Omega\Psi$     | K     |
| *O. sativa*   | Q53397 (C)    | OsDHN 2.2| Os02g0669100   | $\beta\Omega\Psi$     | SK2   |
| *O. sativa*   | Q53397 (C)    | OsDHN 3  | Os11g0451700   | $\beta\Omega\Psi$     | YSK   |
| *O. sativa*   | Q2R4Z7 (N)    | OsDHN 4  | Os11g0453900   | $\beta\Omega\Psi$     | SK2   |
| *O. sativa*   | Q2R4Z7 (P)    | OsDHN 5  | Os11g0454000   | $\beta\Omega\Psi$     | YK2   |
| *O. sativa*   | Q2R4Z7* (P)   | OsDHN 6  | Os11g0454200   | $\beta\Omega\Psi$     | YK2   |
| *O. sativa*   | BINEV6* (C)   | OsDHN 6.2| Os11g0454200   | $\beta\Omega\Psi$     | SK2   |
| *O. sativa*   | Q2R4Z4 (C)    | OsDHN 7  | Os11g0454300   | $\beta\Omega\Psi$     | SK2   |
| *S. tuberosum*| M0ZVK4 (N)    | StDHN 1  | PGSC0003DMG040003530| $\beta\Omega\Psi$   | YK2   |
| *S. tuberosum*| M0ZVK5 (C)    | StDHN 2  | PGSC0003DMG040003531| $\beta\Omega\Psi$   | YK2   |
| *S. tuberosum*| M1AM40 (N)    | StDHN 3  | PGSC0003DMG040009968| $\beta\Omega\Psi$   | SK2   |
| *S. tuberosum*| M1D1X0 (N)    | StDHN 4  | PGSC0003DMG040030949| $\beta\Omega\Psi$   | YK2   |
| *S. tuberosum*| M1DTT1 (N)    | StDHN 5  | PGSC0003DMG040034695| $\beta\Omega\Psi$   | SK2   |
| *S. lycopersicum*| K4AW15 (N) | SIDHN 1  | Solyc01g065820.1| $\beta\Omega\Psi$   | K     |
Continued Table 1. Putative dehydrins identified through different domain databases and their classification. (Italic uniprot entry means that their status has been reviewed. α = PS00315, β = PS00823, π = PF00257, Ω = IPR000167, Ω = IPR035013; PS = Prosite, PF = Pfam, IPR = InterPro, C= Cytosol, N= Nucleus, P= Plasma membrane)

| Species | UniProt Entry | DHNs | Ensembl Gene ID | Domain | Class |
|---------|---------------|------|----------------|--------|-------|
| **S lycopersicum** | K4B3K5 (C) | SIDHN 2 | Solyc01g109920.2 | αβπΩƱ | YSK |
| **S lycopersicum** | K4B6C8 (N) | SIDHN 3 | Solyc02g062390.2 | αβπΩƱ | K |
| **S lycopersicum** | K4BAZ9 (C) | SIDHN 4 | Solyc02g084840.2 | αβπΩƱ | Y, S |
| **S lycopersicum** | P22240 (C) | SIDHN 5 | Solyc02g084850.2 | αβπΩƱ | K |
| **S lycopersicum** | K4BVU7 (N) | SIDHN 6 | Solyc04g082200.2 | βαπΩƱ | SK_2 |
| **T aestivum** | W5DSZ6 (N) | TaDH N 1.1 | TRIAE_CS42_3AL_TGACv1_195928_AA0655770 | αβπΩƱ | YSK |
| **T aestivum** | D0PRB6 (N) | TaDH N 1.2 | TRIAE_CS42_3AL_TGACv1_195928_AA0655770 | αβπΩƱ | YSK |
| **T aestivum** | A0A077SSJ5 (C) | TaDH N 2 | TRIAE_CS42_3B_TGACv1_224725_AA0800670 | αβπΩƱ | SK_2 |
| **T aestivum** | W5ERW2 (N) | TaDH N 3 | TRIAE_CS42_4DS_TGACv1_361015_AA1158770 | αβπΩƱ | YSK |
| **T aestivum** | Q00742 (C) | TaDH N 4.1 | TRIAE_CS42_5AL_TGACv1_375535_AA1253650 | αβπΩƱ | SK_2 |
| **T aestivum** | A0A0FH4MAT1 (C) | TaDH N 4.2 | TRIAE_CS42_5AL_TGACv1_375535_AA1253650 | βαπΩƱ | K |
| **T aestivum** | W5FSQB (C) | TaDH N 5.1 | TRIAE_CS42_5DL_TGACv1_433513_AA1415270 | αβπΩƱ | K_2 |
| **T aestivum** | P46524 (C) | TaDH N 5.2 | TRIAE_CS42_5DL_TGACv1_433513_AA1415270 | αβπΩƱ | SK_2 |
| **T aestivum** | P93608 (N) | TaDH N 6.1 | TRIAE_CS42_6AL_TGACv1_475158_AA1511400 | αβπΩƱ | SK |
| **T aestivum** | W5G4Q9 (C) | TaDH N 6.2 | TRIAE_CS42_6AL_TGACv1_475158_AA1511400 | αβπΩƱ | SK |
| **T aestivum** | T1VYS7 (N) | TaDH N 6.3 | TRIAE_CS42_6AL_TGACv1_475158_AA1511400 | αβπΩƱ | SK |
| **T aestivum** | P93607 (C) | TaDH N 6.4 | TRIAE_CS42_6AL_TGACv1_475158_AA1511400 | αβπΩƱ | SK |
| **T aestivum** | A5C8L5 (C) | TaDH N 7 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | K_2 |
| **T aestivum** | P46526 (C) | TaDH N 7.1 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | K_2 |
| **T aestivum** | O65216 (C) | TaDH N 7.2 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | SK_2 |
| **T aestivum** | W5GAN3 (N) | TaDH N 7.3 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | B0LXL4 (C) | TaDH N 7.4 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | W5DSQ9 (C) | TaDH N 7.5 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | SK_2 |
| **T aestivum** | W5GW81 (C) | TaDH N 7.6 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | SK_2 |
| **T aestivum** | W5G4Q9 (C) | TaDH N 7.7 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | SK_2 |
| **T aestivum** | O65216 (C) | TaDH N 7.8 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | W5GW81 (C) | TaDH N 7.9 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | W5GW81 (C) | TaDH N 7.10 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | SK_2 |
| **T aestivum** | Q41579 (C) | TaDH N 7.11 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | Q8W192 (C) | TaDH N 7.12 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | W5GYW6 (C) | TaDH N 7.13 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | W5GV89 (C) | TaDH N 7.14 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | Q8LP43 (C) | TaDH N 7.15 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | Q0K1W1 (C) | TaDH N 7.16 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | U677L2 (C) | TaDH N 7.17 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | D2TE72 (C) | TaDH N 7.18 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | P93610 (C) | TaDH N 7.19 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | W5FU7 (C) | TaDH N 7.20 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **T aestivum** | W5FA07 (C) | TaDH N 7.21 | TRIAE_CS42_6AL_TGACv1_475170_AA1513200 | βαπΩƱ | YSK |
| **V vinifera** | F6I0M9 (C) | VvDHN 1.1 | VIT_0300038g04390 | αβπΩƱ | SK |
| **V vinifera** | F6HC4 (N) | VvDHN 1.2 | VIT_0300038g04390 | αβπΩƱ | SK |
| **V vinifera** | Q3ZL41 (C) | VvDHN 2.1 | VIT_040023g02480 | βαπΩƱ | K |
| **V vinifera** | A3REN2 (N) | VvDHN 2.2 | VIT_040023g02480 | βαπΩƱ | K |
| **V vinifera** | H9A0H0 (C) | VvDHN 2.3 | VIT_040023g02480 | βαπΩƱ | K |
| **V vinifera** | A5C8L5 (C) | VvDHN 2.4 | VIT_040023g02480 | βαπΩƱ | K |
Table 2. Expression of dehydrin genes using Genevestigator developmental and anatomy tool.

| Developmental stages | Tissues and cell lines |
|----------------------|------------------------|
| **DHNs**             | **A thaliana**          | **G max**               | **Z mays**              | **O sativa**             |
|                      | **Expression threshold:** L= 6-8, M=8-12, H= 12-18, | **Expression threshold:** L=7-8, M=8-12, H= 12-20, | **Expression threshold:** L=0-1, M=1-4, H,4-10, | **Expression threshold:** L=7-8, M=8-12, H,12-21, |
|                      | **No of Samples**       | **H** H H M M M M | **H** M M M | **H** M H H H M | **H** M H M M H |
| Germinated Seed      | 515                     | H H H M M M M M | 61          | H M M | - |
| Seedling             | 2785                    | H H H L H L M M | 618         | H M M | - |
| young rosette        | 836                     | H H H L H L M M | 3           | H M M | - |
| Developed rosette    | 2196                    | H H H L H L M M | 3           | H M M | - |
| Blotting             | 369                     | H H H L M L M M | 3           | H M M | - |
| Young flower         | 720                     | H H H L H L M M | 3           | H M M | - |
| Developed flowerer   | 1038                    | H H H L L M M M | 63          | H M M | - |
| Flowers and siliques | 274                     | H H H M M M M M | 169         | H M M | - |
| Mature siliques      | 93                      | H H H H H H M H | 233         | M H | - |
| Senescence           | 18                      | M M M H H H H M | 107         | M H | - |
|                       | Callus                  | 31 H H H L M L M M | 1081       | H H L H L M M M | - |
| Callus culture       | 714                     | H H H L L M M M | 2345        | H H H L H L M M | - |
| Seedling             | 2345                    | H H H L H L M M | 801         | H H H M M M M M | - |
| Inflorescence        | 801                     | H H H M M M M M | 4580        | H H H L M L M M | - |
| Shoot                | 4580                    | H H H L M L M M | 1081        | H H H L L M M M | - |
| Root                 | 1081                    | H H H L L L M M | - | H M | - |
| Germination          | 61                      | H M M | - | H M M | - |
| Main shoot growth    | 618                     | H M M | - | H M M | - |
| Flowering            | 3                       | H M M | - | H M M | - |
| Fruit formation      | 63                      | H M M | - | H M M | - |
| Bean development     | 169                     | H M M | - | H M M | - |
| Callus culture       | 25                      | H M M | - | H M M | - |
| Seedling             | 57                      | H M H | - | H M H | - |
| Inflorescence        | 237                     | H M H | - | H M H | - |
| Shoot                | 432                     | H M M | - | H M M | - |
| Root                 | 2872                    | H M M | - | H M M | - |
| Germination          | 225                     | H H | - | H H | - |
| Seedling             | 774                     | H M | - | H M | - |
| Stem elongation      | 387                     | H M | - | H M | - |
| Inflorescence        | 48                      | H M | - | H M | - |
| Anthesis             | 86                      | H M | - | H M | - |
| Fruit formation      | 658                     | H M | - | H M | - |
| Dough                | 138                     | H H | - | H H | - |
| Callus culture       | 6                       | M H | - | M H | - |
| Seedling             | 233                     | M H | - | M H | - |
| Inflorescence        | 1057                    | M H | - | M H | - |
| Shoot                | 976                     | M H | - | M H | - |
| Root                 | 107                     | M H | - | M H | - |
| Germination          | 361                     | M H M M H H H H | 361         | M H M M H H H H | - |
| Seedling             | 996                     | M H M M H M M H | 996         | M H M M H M M H | - |
| Tillering            | 304                     | M H M M M M M H | 304         | M H M M M M M H | - |
| Elongation           | 89                      | M H M M H M M H | 89          | M H M M H M M H | - |
| Booting              | 126                     | M H M M H M M H | 126         | M H M M H M M H | - |
| Heading              | 355                     | M H M M H M M H | 355         | M H M M H M M H | - |
| Flowering            | 109                     | M H M M M M M H | 109         | M H M M M M M H | - |
| Milk                 | 87                      | H H H M M M H M | 87          | H H H M M M H M | - |
| Dough                | 13                      | H H H H H H H H | 13          | H H H H H H H H | - |
| Callus               | 73                      | H H M H H H H H | 73          | H H M H H H H H | - |
| Callus culture       | 3                       | H M M M M M M M | 3           | H M M M M M M M | - |
| Seedling             | 428                     | M H M M M M M H | 428         | M H M M M M M H | - |
| Inflorescence        | 503                     | H H M M H H H H | 503         | H H M M H 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 ZmDHN1 is 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).

| Conditions      | DHN1 | DHN2 | DHN3 | DHN4 | DHN5 | DHN6 | DHN7 | DHN8 | DHN13 | DHN17 | DHN18 |
|-----------------|------|------|------|------|------|------|------|------|-------|-------|-------|
| *A. thaliana*    |      |      |      |      |      |      |      |      |       |       |       |
| *L. huidobrensis* | ↑    | ↑    |     | ↑    | ↑    |     | ↑    | ↑    |       |       |       |
| *P. cumeferina*  | ↓    |     |     |     |     |     |     |     |       |       |       |
| *P. syringae*    | ↓    |     |     |     |     |     |     |     |       |       |       |
| *S. sclerotiorum*| ↓    |     |     |     |     |     |     |     |       |       |       |
| *G. cichoracearum* |     |     |     |     |     |     |     |     |       |       |       |
| *G. orontii*     |     |     |     |     |     |     |     |     |       |       |       |
| *M. incognita*   |     |     |     |     |     |     |     |     |       |       |       |
| *Z. mays*        |      |      |      |      |      |      |      |      |       |       |       |
| *C. graminicola* | ↑    |     |     |     |     |     |     |     |       |       |       |
| *R. maidis*      |      |     |     |     |     |     |     |     |       |       | (5)   |
| *F. verticillioides* |     |     |     |     |     |     |     |     |       |       |       |
| *P. sojae*       |      |     |     |     |     |     |     |     |       | (4)   |       |
| *T. aestivum*    |      |      |      |      |      |      |      |      |       |       |       |
| *A. caliginosa*  |      |      |      |      |      |      |      |      |       |       |       |
| *F. graminearum* |      |      |      |      |      |      |      |      |       |       |       |
| *G. graminis*    |      |      |      |      |      |      |      |      |       |       |       |
| *X. translucens* |      |      |      |      |      |      |      |      |       |       |       |
| *P. triticina*   |      |      |      |      |      |      |      |      |       |       |       |
| *G. max*         |      |      |      |      |      |      |      |      |       |       |       |
| *Z. mays*        |      |      |      |      |      |      |      |      |       |       |       |
| *T. aestivum*    |      |      |      |      |      |      |      |      |       |       |       |
| *O. sativa*      |      |      |      |      |      |      |      |      |       |       |       |
| *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)| ↑ (1)| ↑ (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*       |      |      |      |      |      |      |      |      |       |       |       |
| Draught         | ↑ (3)|       |       |       |       |       |       |       |       |       |       |
| Cold            | ↑ (7)|       |       |       |       |       |       |       |       |       |       |
| Salt            | ↑ (3)|       |       |       |       |       |       |       |       |       |       |
| Hormone (ABA)   |       |       |       |       |       |       |       |       |       |       |       |
| *T. aestivum*   |      |      |      |      |      |      |      |      |       |       |       |
| Draught         | ↑ (15)| ↑ (7)| ↑ (12)| ↑ (15)| ↑ (20)| ↑ (8)| ↑ (19)|       |       |       |       |
| Cold            | ↑ (1)| ↑ (14)|     |       |       |       |       |       |       |       |       |
| Salt            | ↑ (4)| ↑ (1)|     |       |     |       |       |       |       |       |       |
| Heat            | ↑ (4)| ↑ (4)|     |       |     |       |       |       |       |       |       |
| Anoxia          | ↑ (4)| ↑ (4)|     |     |     |     |     |     |       |       |       |
| Arsenic         |       | ↑   |     |     |     |     |     |     |       |       |       |
| Chromium        | ↑     |     |     |     |     |     |     |     |       |       |       |
| Hormone (ABA)   |       | ↑   |     |     |     |     |     |     |       |       |       |
| *O. sativa*     |      |      |      |      |      |      |      |      |       |       |       |
| Draught         | ↑ (15)| ↑ (7)| ↑ (12)| ↑ (15)| ↑ (20)| ↑ (8)| ↑ (19)|       |       |       |       |
| Cold            | ↑ (1)| ↑ (14)|     |       |       |       |       |       |       |       |       |
| Salt            | ↑ (4)| ↑ (1)|     |       |     |       |       |       |       |       |       |
| Heat            | ↑ (4)| ↑ (4)|     |       |     |       |       |       |       |       |       |
| Anoxia          | ↑ (4)| ↑ (4)|     |     |     |     |     |     |       |       |       |
| Arsenic         | ↑     |     |     |     |     |     |     |     |       |       |       |
| Chromium        | ↑     |     |     |     |     |     |     |     |       |       |       |
| Hormone (ABA)   |       | ↑   |     |     |     |     |     |     |       |       |       |

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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 uniprot database, i.e., COR47, ERD10, ERD14, Dehydrin LEA, Xero2, Xero1, Cold regulated protein, Rab18 in *Arabidopsis thaliana* ([http://www.uniprot.org/](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: YSK, K, K, SK, and YK (13, 25). The plant species selected for
In addition, SKn dehydrin accumulates during 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 YnSKn 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 YnSKn type dehydrins are expressed during cold, salt, heat, anoxia as well as during biotic stress (Table 3,4). Similarly, based on previous studies Kn 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 Kn type dehydrins are also expressed during salt, anoxia and biotic stresses (Table 3,4). YnK and SKn class have been shown to be associated mainly with the cold tolerance (19, 25, 31). In addition, SKn dehydrin accumulates during low temperature, drought salinity, wound stress and with certain hormones treatments (32). The expression analysis using genevestigator shows that both YnK and SKn 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 KnS 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 YnK and SKn 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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