CLONING OF TWO DEHYDRIN GENES FROM THE HALOPHYTES OF THE EGYPTIAN NORTHWEST COASTAL REGION

A. M. K. NADA[1,2], H. M. ABDELHALIM[1] AND A. FAHHAD[2]

1. Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt

2. Faculty of Biotechnology, October University for Modern Science and Arts6 October City, Egypt

Throughout their life cycle, plants are subjected to many adverse environmental stresses such as drought, high temperatures, etc. that dramatically affect plant survival and reduce productivity. To cope with such stresses, plants produce several stress-induced proteins that play a definite role in protecting plants during such severe conditions (Muthalif and Rowland, 1994).

The late embryonic stage represents one of the abiotic stress conditions where the seed starts to lose water content during desiccation. Several molecules have been found to play vital roles in seed development and are thought to help in saving the embryos during desiccation. These include sugars (Koster and Leopold, 1988; Chen and Burris, 1990) and proteins, among which are the late-embryogenesis abundant proteins (LEA) (Blackman *et al.,* 1995; Dure, 1993; Close, 1996; Ingram and Bartels, 1996). LEA proteins were found in the seeds of several plants and in vegetative organs, especially under abiotic stress conditions (Thomashow*,* 1999; Hundertmark and Hincha, 2008). According to amino acid sequence homology, LEA proteins have been separated into different groups (Hundertmark and Hincha, 2008). From these groups the LEA D11 family (LEA type 2 proteins), also known as Dehydrins (Close, 1997), have been estimated to comprise up to 4% of the total seed protein. (Wise and Tunnacliffe, 2004).

Expression of the Dehydrin proteins have been found to be associated with the protection of various types of plant cells from osmotic stresses, such as those caused by desiccation, salt, and low temperatures (Skriver and Mundy, 1990; Close, 1996; Ingram and Bartels, 1996; Allagulova *et al*., 2006). Hyper-osmotic conditions and low temperatures cause cellular dehydration, resulting in the reduction of cytosolic volumes and the alteration of cellular mechanisms. Toward survival, plants accumulate Dehydrin proteins during these conditions in the dehydrating plant tissue, (Abba *et al.,* 2006).

Several studies revealed that Dehydrins are widely distributed in the plant kingdom (Abba *et al.,* 2006), in brown algae (Li *et al*., 1998), in lichen *Selaginella lepidophylla* (Close, 1997) and in cyanobacterium (Close *et al*., 1993).

A number of Dehydrin proteins have been isolated and studied; the physiochemical and/or structural mecha-

nism(s) whereby these proteins function to protect cells from osmotic stress is yet unknown. Dehydrins are hydrophilic proteins that exhibit a recognizable structure (Close, 1996; Soulages *et al*., 2003), and are very rich in glycine residues, while cysteine and tryptophane are lacking (Close, 1997). A key characteristic of Dehydrins is the presence of one or more highly conserved lysine-rich stretches of 15 amino acids, called the K motifs (Dure, 1993; Close, 1996, 1997). Also, Dehydrins can contain two other motifs, an N-terminal Y segment (V/TDE/QYGNP) and a serine-rich S segment which is thought to participate in nuclear localization (Godoy *et al*., 1994; Close, 1997). Dehydrins have a chaperone-like function in stabilizing partially denatured proteins or membranes, coating them with a cohesive water layer and preventing their coagulation during dehydration (Close, 1997; Koag *et al*., 2003). It has been hypothesized that Dehydrins function as surfactant molecules, acting synergistically with compatible solutes to prevent coagulation of colloids and a range of macromolecules (Close, 1997). They are also thought to have the ability to tightly bind and organize water molecules (Soulages *et al*., 2003). This effect could help to reduce water loss from cells under drought conditions (Rinne *et al.,* 1999).

More than 95% of Egyptian land is desert, while the rest is arable land, farms and urban areas. The northwest coastal region is part of this desert and is known to be subjected to several kinds of environmental stresses such as drought, salinity and heat shock. Therefore, plant flora in this region represents a diverse and potential genetic pool for mining genes responsible for abiotic stress tolerance. The work presented here describes the isolation and characterization of two Dehydrin genes from some plants collected from the northwest coastal region of Egypt.

MATERIALS AND METHODS:

Plant material

Young seedlings of stressed *Vicia sativa, Vicia monontha, Vicia cinera, Mesembryanthemum crystallinum* (Ice plant)*, Vicia hirsute* plants were collected from the northwest coastal region of Egypt. The samples of these species growing in the salt marshes and coastal dunes located 25 Km west of Marsa Matrouh (the coastal area of El Qasr) were collected and the green parts of the collected seedlings were immediately frozen in liquid nitrogen to preserve the collected tissues during the trip.

Primer design

Different sequences of Dehydrin genes were downloaded from GenBank and aligned together using CLUSTAL W (version 1.8) software (Thompson *et al*., 1994). Primers were designed according to the conserved region of the genes. The beginning and the ends of these genes were highly conserved; therefore these sequences were useful in designing primers suitable for the amplification of the full length Dehydrin genes.

Isolation of total RNA

Total RNA was extracted using TriPure Isolation Reagent (Roche, Indianapolis, USA) from the leaves of the collected samples. A volume of 100 mg of each frozen tissue was treated with 0.1% (v/v) Diethyl Pyrocarbonate (DEPC), and ground in an autoclaved mortar to a fine powder. A volume of 1 ml TriPure reagent was added to the resulting powder in a polypropylene tube at room temperature. Samples were homogenized and incubated for 5 min at room temperature to ensure the complete dissociation of nucleoproteins. After incubation, 0.2 ml chloroform was added and the tube was vigorously shaken for 15 sec. The tube was incubated at room temperature for 10 min and centrifuged at 12,000 xg for 15 min at 4°C. Isopropanol 0.5 ml, was added to the colorless upper aqueous phase in a new centrifuge tube. The tube was inverted several times to mix thoroughly. To precipitate RNA, samples were incubated for 10 min at 25°C and then centrifuged at 12,000 xg for 10 min at 4°C. The pellet was washed with 1 ml of 75% ethanol. The excess ethanol was removed from the RNA pellet by air-drying. The RNA pellet was resuspended in Diethyl Pyrocarbonate (DEPC) and treated with RNase-free water. RNA was either used immediately or kept at -80°C until further use.

RT-PCR

Isolated RNA was used to detect the presence and expression of the genes under study using the RT-PCR technique. Reverse transcription using a One-Step RT-PCR Kit (Qiagen, Germany) was performed on 2 µg of DNA-free RNA as described by O'Rourke *et al*. (2000). Reaction products were subjected to electrophoresis in a 1.8% agarose gel in 1X TAE buffer as described by Sambrook *et al*. (1989).

Cloning and sequence analysis

The PCR products were cloned in pGEMT Easy plasmid (Promega, Madison, WI) and transformed into *Escherichia coli DH5α*. White colonies were picked and screened for the presence of the cloned fragments by digestion with *EcoRI*. The recombinant clones were sequenced using a Big Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, Foster City, CA) and an ABI PRISM 310 DNA sequencer (Applied Biosystems).

Computer analysis

A homology search was performed using BLASTX against the NCBI protein database (http://www.ncbi.nlm.nih.gov). Sequences of Dehydrin genes that showed similarities with the isolated Dehydrin genes were obtained from the NCBI non-redundant and dbEST data sets using BLASTX or BLASTP version 2.0.10 (Altschul *et al*., 1997). The full amino acid sequences of the proteins were aligned using CLUSTAL W version 1.8 (Thompson *et al*., 1994) and subjected to phylogenetic analysis. Phylogenic trees were constructed using the neighbor-joining (NJ) method (Saitou and Nei, 1987) with parsimony and heuristic search criteria

and 1000 bootstrap replications to assess branching confidence.

During *in silico* mapping, the isolated Dehydrins' sequences were compared to barrel medic BAC/PAC sequences using BLAST (with an e-value threshold of $1e^{-10}$). The barrel medic BAC/PAC that matches the query was used to identify anchored barrel medic markers from the barrel medic genetic linkage map (http://www.tigr.org). The results obtained from this stage were used to construct a comparative map between pea, soybean and barrel medic to identify the tentative chromosomal location of Dehydrin in barrel medic, soybean and pea using comparative mapping strategy (Diab *et al.,* 2007).

Protein expression in prokaryotes

Cloned DNHa Dehydrin in pGEMT Easy plasmid was transformed in BL21(DE3) *E. coli*. Overnight cultures derived from colonies that contained our gene of interest were performed in 5 ml test tubes. The overnight cultures were used to inoculate fresh LB media (25 ml). The culture was grown at 37°C to start the expression experiment. IPTG was used in this experiment to induce protein expression. The starting point to test for IPTG induction should be a well-oxygenated fairly fresh culture, with the desired OD_{600} in the range of 0.5-1.0. The recommended working concentration of IPTG in the bacterial cultures during induction is 0.4 mM, and the recommended time points would be 1, 2 and 3 hrs. At each time point 0.50 ml of suspended cells were removed and centrifuged in an Eppendorf at full speed for 1 min. Then using a micropipette tip, the cell pellet was vigorously mixed, disrupting the cells as much as possible. 20 µl of 4x SDS loading dye were added and mixed well. The mixed sample was heated to 85°C for 3 min to denature the proteins and then the samples were stored (on ice or frozen) until protein gels were ready to run. The samples generated were run on SDS-PAGE gels to see if a new protein of the predicted size would be produced on IPTG induction.

RESULTS AND DISCUSSION

As mentioned earlier, the northwest coastal region of Egypt is an environmentally stressed area. Therefore, plants grown in this area are exposed to severe abiotic stress. Total RNA was isolated from *Vicia sativa, Vicia monantha, Vicia cinera, Mesembryanthemum crystallinum* (Ice plant), and *Vicia hirsute* plants collected from this region. RT-PCR was performed to screen for the presence of expressed Dehydrin genes using specific primers (Fig. 1).

As shown in Fig. (1), several Dehydrin genes were found to be expressed in all collected plants, one gene was found in *Vicia sativa*, three in *Vicia monantha* and *Vicia cinera,* six in *Mesembryanthemum crystallinum* (Ice plant), and three were found in *Vicia hirsute*. The amplified genes varied in size ranging from ≅ 300 to 900 bp. These results are in agreement with Roberton and Chandler (1992),

where they raised an antiserum against Dehydrin isolated from maize (*Zea mays*) which recognized several polypeptides in extracts of pea (*Pisum sativum*) cotyledons.

RT-PCR was repeated for *Vicia monantha* to verify the results and it was chosen to be a target for the current studies (Fig. 2). The Dehydrin primers successfully amplified three fragments of ≅ 900, 588 and 417 bp, respectively with RT-PCR. Two amplified cDNA fragments (588 and 417 bp) were purified, cloned into the pGEM®-T Easy Vector, and sequenced. The nucleotide sequences proved that we successfully isolated two cDNA clones with 588 and 417 bp. These two 588 and 417 bp genes were named DHNa and DHNb, respectively.

The isolated sequences were subjected to the BLASTX analysis to confirm the presence of the key characteristic sequences of the Dehydrins – the highly conserved amino acid motifs- (Dure, 1993; Close, 1996, 1997). The results of these analysis revealed that, two K motifs and one N-terminal Y segment (V/TDE/QYGNP) were conserved in DHNa. While, one K motifs and one N-terminal Y segment (consensus V/TDE/QYGNP) are conserved in DHNb. BLASTX results also showed 91% similarity between the DHNa isolated gene and Dehydrin 1 gene isolated from *Pisum sativum* (Roberton and Chandler 1992), the nearest sequence to DHNa, 75% between DHNa and Dehydrin 3 isolated from *Pisum sativum* (Grosselindemann *et al.,* 1998) and 70% between DHNa and Dehydrin 2 isolated from *Pisum sativum* (Roberton and Chandler 1992). On the other hand, BLASTX results showed 88% similarity between to DHNb isolated gene and Dehydrin 1 gene isolated from *Pisum sativum* (Roberton and Chandler 1992), the nearest sequence to DHNb, 83% between DHNb and Dehydrin 3 isolated from *Pisum sativum* (Grosselindemann *et al.,* 1998) and 80% between DHNa and Dehydrin 2 isolated from *Pisum sativum* (Grosselindemann *et al.,* 1998). Finally, the BLASTX results revealed that we have two different new genes DHNa and DHNb. They have a similarity to the closest Dehydrins (Dehydrin 1) about 91% and 88% respectively. The isolated sequences were submitted to GenBank under the accession numbers (AB506694 and AB506695) for DHNa and DHNb, respectively.

Computation analysis using expasy online analysis tools (http://au.expasy.org/tools/protparam.html) of various physical and chemical parameters for DHNa and DHNb revealed that DHNa has a molecular weight of 20.2 KDa and a theoretical PI of 5.97, while DHNb has a molecular weight of 14.19 KDa and a theoretical PI of 5.9.

To determine the evolutionary relatedness of DHNa and DHNb to other Dehydrin proteins, the neighbor-joining method (NJ) was used to generate a phylogenetic tree based on amino acid sequence homology (Fig. 3). The tree showed that DHNa and DHNb proteins

form a distinct clade with various Dehydrin sequences isolated from *Pisum sativum.* This is due to the fact that both *Vicia monantha* and *Pisum sativum* have the same classification tree (Subkingdom, Tracheobionta, Superdivision, Spermatophyta, Division: Magnoliophyta, Class Magnoliopsida, Subclass: Rosidae, Order: Fabales and Family: Fabaceae). They differ only in the genus where *Vicia monantha* belongs to *Vicia* L., while *Pisum sativum* belongs to *Pisum* L. This means that they have the same ancestors, which was reflected on the phylogenetic tree. Percentage of similarity between DHNa, DHNb and Dehydrins isolated from *Pisum sativum* was previously discussed in the BLASTX analysis results.

It could also be observed from the constructed phylogenetic tree that DHNa and DHNb showed a degree of similarity with the Dehydrin genes isolated from *Medicago truncatula* (32% and 39%), *Medicago sativa* subsp. falcata (49% and 66%), *Medicago sativa* (32% and 38%), *Cicer pinnatifidum* (47% and 42%), *Solanum commersonii* (49% and 58%) and *Nicotiana tabacum*; (50% and 63%) respectively. Results of the phylogenetic tree and sequence homology was in agreement with the phylogenetic study of Chung *et al.* (2003) and Abba *et al.* (2006), where they analyzed the evolutionary distance between Dehydrin isolated from *Pisum sativum* and other Dehydrins from other species like *Solanum commersonii.*

Due to the insufficient genomic information about the *Vicia* genus, *in silico* mapping of DHNa and DHNb was performed on pea genome as it is closely related to the *Vicia* genus in the taxonomy tree and shares a common ancestory. *In silico* mapping of VmDEHYDRINa,b revealed that Dehydrin genes are located on barrel medic chromosomes 4 and 3 respectively, Soybean chromosome C2 and Pea chromosomes 7 and 3, respectively (Figs 4 and 5).

DHNa was found to be linked to the marker h2_9n11a on chromosome 4 in the barrel medic. APX marker (which is less than 12 cM away from the h2_9n11a marker) on chromosome 4 of the barrel medic is linked to the marker Apx on chromosome 7 of the pea. According to the fact that there is less than 20cM in distance between 2 markers, then they are physically linked and are moved together. It can be assumed that h2_9n11a marker could have a match near the Apx marker on the pea chromosome 7. Two other markers (DK413L and h2_116a3b) are cross-linked to (K365_1 and A538_1) respectively. This could mean that C2 of the soybean corresponds to chromosome 4 in the barrel medic but in opposite directions, and that the h2_9n11a marker could be present upstream from the DK413L marker.

In case of DHNb, 005G05 marker on chromosome 3 of the barrel medic (http://www.tigr.org/db.shtml). Two markers were linked between chromo-

some 3 of the barrel and chromosome C2 of the soybean. .Position 34 cM on chromosome 3 of the barrel medic was linked to the A635_1 marker on C2 of the soybean, while DK473L marker (which is present in the same position as that of the 005G05 marker) from barrel matched the A059_2 marker from the soybean. However, they are inverted and a similar assumption would be made as that done for DHNa linkage analysis on C2 of soybean and that the 005G05 marker could be present upstream from the A059_2 marker. On the other hand, a marker called NPAC positioned ~8 cM upstream from 005G05 marker, is linked on chromosome 3 of the pea. It could therefore predict the presence of 005G05 marker in the pea on chromosome 3.

The linkage of markers that could be related to both genes, Dehydrin a and b, of the same chromosome (C2) would probably assure their presence on the same chromosome in soybean. This assumption is supported by the similar function and relation to the same gene family of both genes.

However, as map data accumulate, it becomes increasingly difficult to find segments in which gene content and order are strictly parallel in the three genomes, due in part to experimental error, but also to high rates of insertion and deletion of small regions of chromosomes.

In order to test the expression of the DHNa gene, shake flask fermentations were performed. At the starting point, induction culture was split into two identical growth flasks. One had IPTG added to induce T7 RNA polymerase, and the other flask was a control with no IPTG added. The two cultures were grown at 37°C. Samples were taken after 1, 2 and 3 hours. As shown in Fig. (6), a band of molecular weight 20 KDa started to be induced after 2 and 3 hours. This result is compatible with computation analysis results revealing that DHNa has a molecular weight of 20.2 KDa. In future, the induction experiment needs to be optimized to produce more protein products for protein analysis purposes. The same experiment will be conducted for the other isolated Dehydrin DHNb gene.

SUMMARY

The current study represents the isolation of two dehydrin genes from wild halophytes (*Vicia monantha*) grown naturally in the northwest costal region of Egypt. Specific primers were designed on the basis of sequence homology of Dehydrin genes. The designed PCR primers were subsequently used for RT-PCR using RNA isolated from *Vicia monantha*. The amplified products (DHNa and DHNb with molecular weight 588 and 417 bp respectively) were cloned, sequenced and submitted to the GenBank. The nucleotide sequences of the amplified fragments were aligned with their corresponding genes using BLASTX. The BLASTX results revealed that we have two different new genes DHNa and DHNb with a similarity to the closest Dehydrins (Dehydrin 1 from *Pisum sativum*) about 91% and 88% respectively. The phylogenetic tree showed

that DHNa and DHNb proteins form a distinct clade with various Dehydrin sequences isolated from *Pisum sativum. In silico* mapping revealed that DHNa and DHNb genes are located on barrel medic chromosomes 4 and 3 respectively, Soybean chromosome C2 and Pea chromosomes 7 and 3, respectively. IPTG was used to induce DHNa gene in *E. coli* where a 20 KDa protein band started to be induced during 3 hours induction.

REFERENCES

Abba, S., S. Ghignone and P. Bonfante (2006). A dehydration-inducible gene in the truffle Tuber borchii identifies a novel group of Dehydrins. BMC Genomics Research article, 7: 39-54.

Allagulova, C. R., F. R. Gimalov, F. M. Shakirova and S. Abba (2006). The plant Dehydrins: structure and putative functions. Biochemistry – Moscow, 68: 945-951.

Altschul, S. F., T. L. Madden, A. A. Schaffer, J. H. Zhang, Z. Zhang, W. Miller and D. J. Lipman (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, 25: 3389-3402.

Blackman, S. A., R. L. Obendorf and A. C. Leopold (1995). Desiccation tolerance in developing soybean seeds: the role of stress proteins. Physiol. Plant, 93: 630-638.

Chen, Y., J. S. Burris (1990). Role of carbohydrates in desiccation tolerance and membrane behavior in maturing maize seeds. Crop Sci., 30: 971-975.

Chung, E., S. Y. Kim, Y. S. Yi, and D. Choi (2003). *Capsicum annuum* Dehydrin, an Osmotic-Stress Gene in Hot Pepper Plants. Mol. Cells, 15(3):327-332

Close, T. J. (1996). Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. Physiol. Plant, 97: 795-803.

Close, T. J. (1997). Dehydrins: a commonality in the response of plants to dehydration and low temperature. Physiol. Plant, 100: 291-296.

Close, T. J., R. D. Fenton and F. Moonan (1993). A view of plant Dehydrins using antibodies specific to the carboxy terminal peptide. Plant Mol. Biol., 23: 279-286.

Diab, A. A., A. Ageez, B. A. Abdelgawad and T. Z. Salem (2007). Cloning, sequence analysis and in silico mapping of a drought-inducible gene coding for s-adenosylmethionine decarboxylase from durum wheat. World Applied Sciences Journal, 2: 333-340.

Dure, L. I. (1993). Structural motifs in LEA proteins of higher plants. Response of Plants to Cellular Dehydration during Environmental

Stress. American Society of Plant Physiologists, Rockville, MD, p 91-103.

Godoy, J. A., R. Lunar, S. Torres-Schumann, J. Moreno, M. Rodrigo and J. A. Pintor-Toro (1994). Expression, tissue distribution and subcellular localization of Dehydrin TAS14 in salt-stressed tomato plants. Plant Mol. Biol., 26: 1921-1934.

Grosselindemann, E., M. obertson, J. A. Wilmer, and P. M. Chandler (1998) Genetic variation in pea (Pisum) Dehydrins: sequence elements responsible for length differences between Dehydrin alleles and between Dehydrin loci in *Pisum sativum* L. Theor. Appl. Genet., 96: 1186-1192.

Hundertmark, M. and D. K. Hincha (2008). LEA (Late Embryogenesis Abundant) proteins and their encoding genes in *Arabidopsis thaliana*. BMC Genomics Research article ,9: 1-22.

Ingram, J. and D. Bartels (1996). The molecular basis of dehydration tolerance in plants. Annu Rev. Plant Physiol. Plant Mol. Biol., 47: 377-403.

Koag, M. C., R. D. Fenton, S. Wilkens and T. J. Close (2003). The binding of maize DHN1 to lipid vesicles: gain of structure and lipid specificity. Plant Physiology, 131: 309-316.

Koster, K. L. and A. C. Leopold (1988). Sugars and desiccation tolerance in seeds. Plant Physiol., 88: 829-832.

Li, R., S. H. Brawley and T. J. Close (1998). Proteins immunologically related to Dehydrins in fucoud algae. J. Phycol., 34: 642-650.

Muthalif, M. M. and L. J. Rowland (1994). ldentification of Dehydrin-Like Proteins Responsive to Chilling in Floral Buds of Blueberry (Vaccinium, section Cyanococcus). Plant Physiol., 104: 1439-1447.

O'Rourke, E. J., C. Chevalier, S. Boiteux, A. Labigne, L. Ielpi and J. P. Radicella (2000). A novel 3-methyladenine DNA glycosylase from *Helicobacter pylori* defines a new class within the endonuclease III family of base excision repair glycosylases. J. Biol. Chem., 275: 20077-20083.

Rinne, P. L. H., P. L. M. Kaikuranta, L. H. W. van der Plas and C. van der Schoot (1999). Dehydrins in cold-acclimated apices of birch (*Betula pubescens Ehrh*.): production, localization and potential role in rescuing enzyme function during dehydration. Planta, 209: 377-388.

Roberton, M. and P. M. Chandler (1992). Pea Dehydrins: identification,

characterization and expression. Plant Mol. Biol., 19: 1031-1044.

Saitou, N. and M. Nei (1987). The neighborjoining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

Sambrook, J.; E. F. Fritsch, and T. Maniatis, (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Skriver, K., J. Mundy (1990). Gene expression in response to abscisic acid and osmotic stress. The Plant Cell, 2: 503-512.

Soulages, J. L., K. Kim, E. L. Arrese, C. Walters and J. C. Cushman (2003). Conformation of a group 2 late embryogenesis abundant protein from soybean: evidence of poly (L-proline)-type II structure. Plant Physiol., 131: 963-975.

Thomashow, M. F. (1999). Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. Ann. Rev. Plant Physiol., 50: 571-599.

Thompson, J. D., D. G. Higgins and T. J. Gibson (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22: 4673-4680.

Wise, M. J. and A. Tunnacliffe (2004). POPP the question: what do LEA proteins do?. Plant Sci., 9: 13-17.

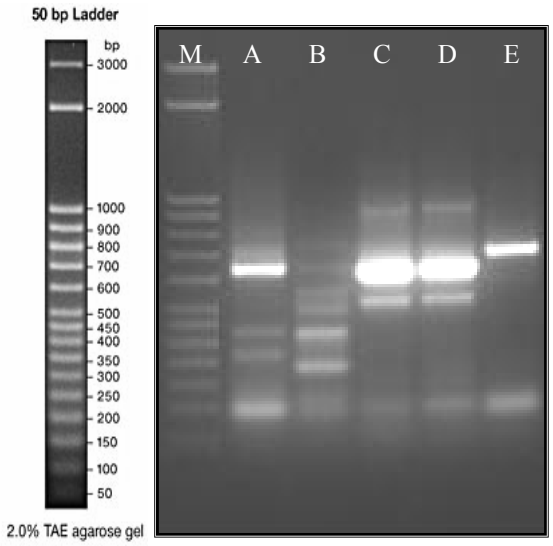

Fig. (1): Amplification of Dehydrin genes using RT-PCR from (A) *Vicia hirsute*, (B) *Mesembryanthemum crystallinum* (Ice plant), (C) *Vicia cinera*, (D) *Vicia monantha* and (E) *Vicia sativa*. M: marker 50 bp.

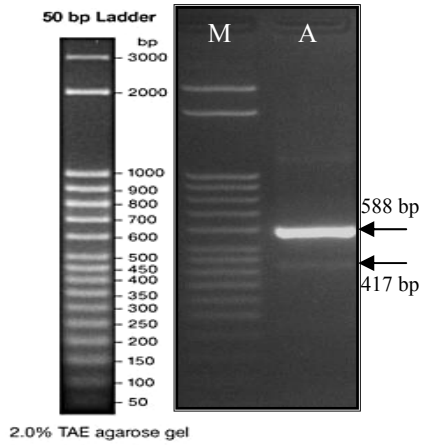

Fig. (2): A re-amplification of Dehydrin genes using RT-PCR from *Vicia monantha*. M: marker 50 bp.

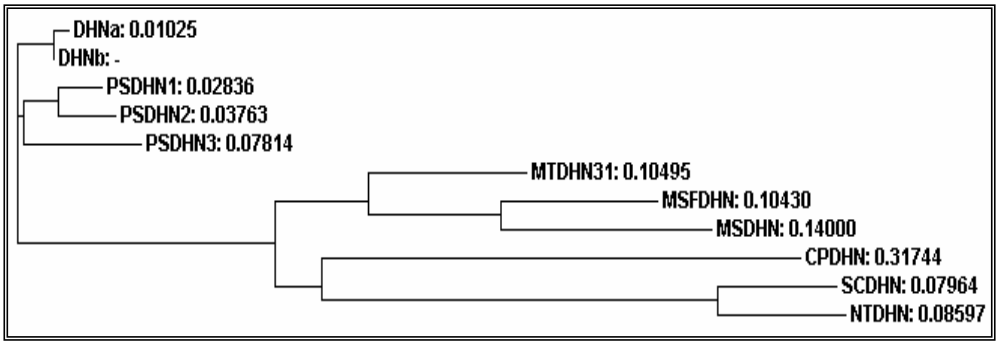

Fig. (3): Phylogenetic cladogram of plant Dehydrins. Tree based on deduced amino acid sequences of Dehydrins constructed using the ClustalW: (www.ebi.ac.uk/clustalw/). Dehydrins are: DHNa and DHNb isolated in this study, PDDHN1 (*Pisum sativum*; P28639), PDDHN2 (*Pisum sativum*; P28640), PDDHN3 (*Pisum sativum*; AAB51381), MTDHN31 (*Medicago truncatula*: ABX80067), MSFDHN (*Medicago sativa* subsp. falcata; ABX80061), MSDHN (*Medicago sativa*; AAL15651), CPDHN (*Cicer pinnatifidum*; AAN77521), SCDHN (*Solanum commersonii*; CAA75798) and NTDHN (*Nicotiana tabacum*; BAD13498).

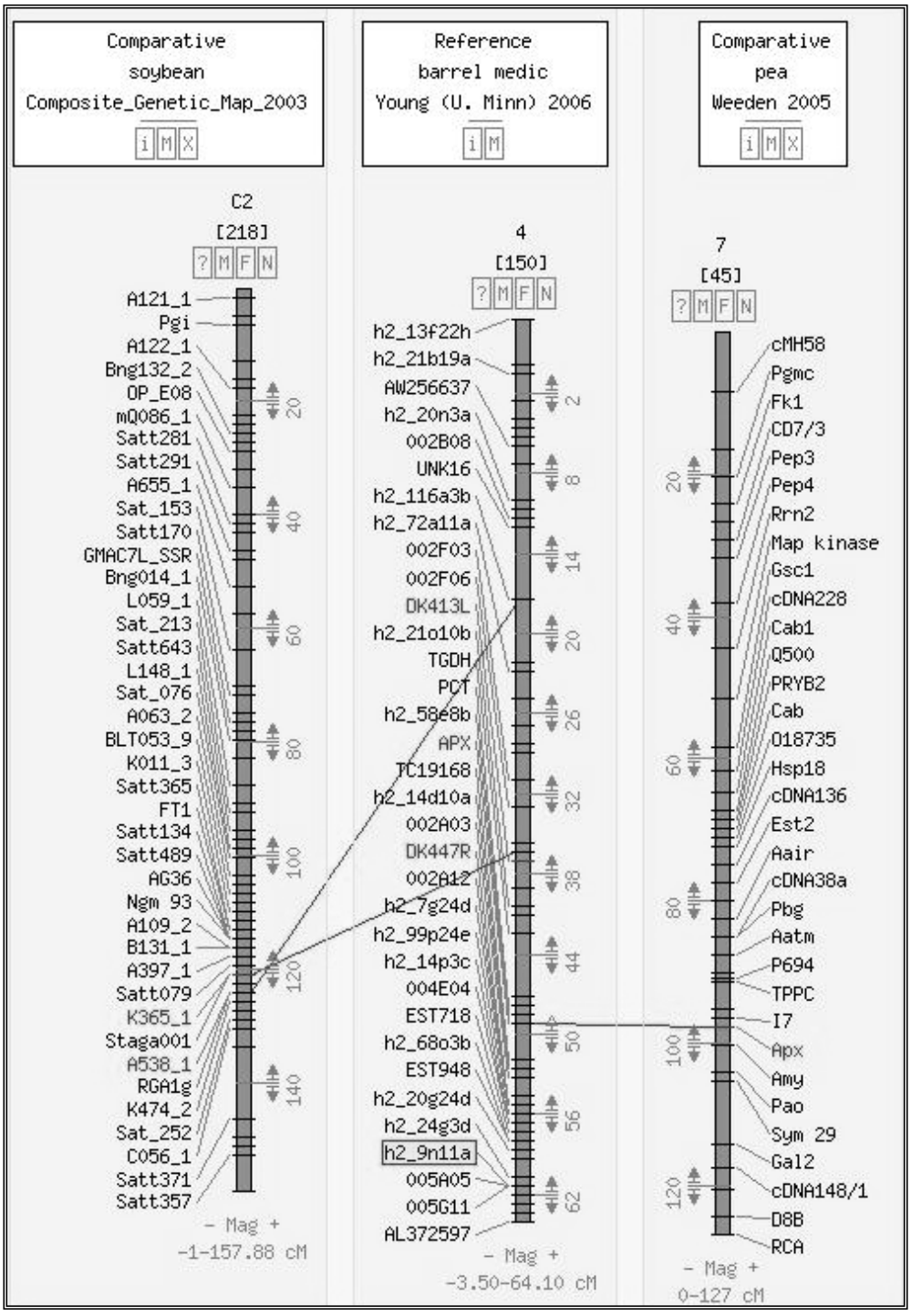

Fig. (4): *In silico* mapping of VmDEHYDRINa revealed that DHNa gene is located on barrel medic chromosome 4, Soybean chromosome C2 and Pea chromosome 7.

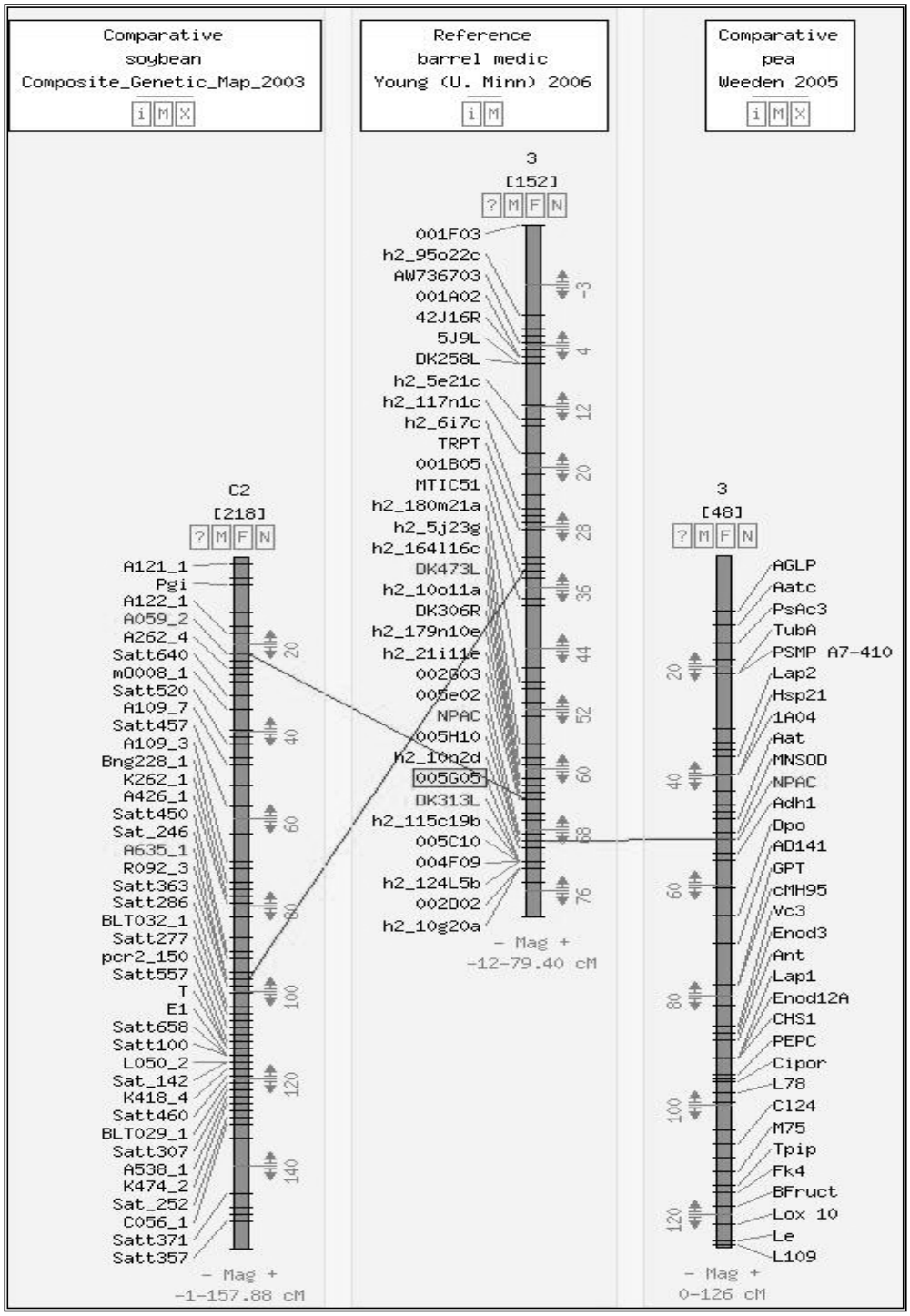

Fig. (5): *In silico* mapping of VmDEHYDRIN b revealed that DHNb gene is located on barrel medic chromosome 3, Soybean chromosome C2 and Pea chromosomes3.

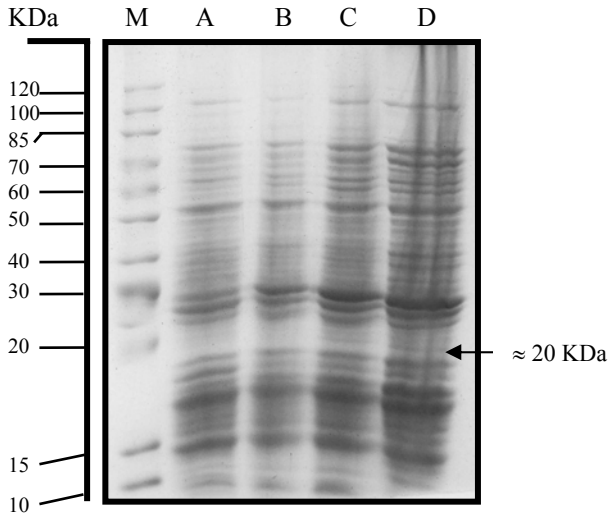

Fig. (6): Protein expression of DHNa gene; (A) control (without IPTG), (B) after one hour induction, (C) after two hours induction, (D) after three hours induction. M: marker.