RESEARCH ARTICLE

DNA Sequencing of Chilling Tolerant *Phaseolus Vulgaris L.* and Chilling Sensitive *Vigna Unguiculata L.*

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Abstract

The response of dehydrin gene in *P. vulgaris* L. and *V. unguiculata* L. to LNT (Low night temperature) stress was studied. It was inferred that dehydrin gene is induced to express the protein which confers an adaptation effect only in P.vulgaris L. but not in *V. unguiculata* L. LNT did not show any genetic variations when compared with a non LNT stress induced plant. Hence *P. vulgaris* L. is considered to be cold tolerant plant and *V. unguiculata* L. is a sensitive plant. *P. vulgaris* L. and *V. unguiculata* L. (LNT treated and control) can be analysed for other genes involved in cold stress. This observation hypothesizes that there is no significant gene level variations between LNT stress induced plant and control plants. It can also be hypothesized that variations may be occurring in its expression level to code for the protein responsible for making the plant tolerant to cold. Moreover the present study also suggested no significant gene level variations between *P. vulgaris* L. and *V. unguiculata* L. plants even though significant biochemical variations were observed when these plants were exposed to LNT stress.

Keywords: Dehydrin Gene; LNT Stress; Cold Tolerance; Gene Expression

Introduction

Crop plants are exposed to a range of external abiotic stress factors such as drought, salinity, cold, freezing, high light sensitivity, nutrient imbalances etc, Numerous cold induced genes have been isolated and characterized in a number plant species.

An increasing number of genes have been revealed to be induced during cold acclimatization [1,2]. Many of these genes encode proteins with known freezing tolerance. They encode newly discovered proteins such as the *Arabidopsis* COR6.6, COR15a and COR78 polypeptides or homologous of LEA proteins such as *Arabidopsis* COR47. The polypeptides encoded by these cold-responsive genes fall into a number of groups based on amino acid sequence similarities, but all share the property of being extremely hydrophilic. In addition, many have relatively simple amino acid compositions (i.e. are composed largely of a few amino acids) have repeated amino acid sequence motifs and remain soluble upon boiling in dilute aqueous buffer. Among the highly expressed cold responsive genes of *Arabidopsis* are the COR genes also designated LTI (low temperature induced), KIN (cold-inducible) and RD (responsive to desiccation). The COR genes comprise four gene families, each of which is composed of two genes that are physically linked in tandem array. The COR78, COR15 and COR6.6 gene pairs encode newly discovered polypeptides and the COR47 gene pair encodes homologous of LEA group II proteins (also known as dehydrins and LEA D11 proteins). Recent studies indicate that COR15a acts in concert with other COR genes to enhance freezing tolerance [3,4].

LEA proteins are expressed at different stages of late embryogenesis in seed embryos and under various conditions of stress including desiccation. LEA proteins are found in different tissues and in all cell types. They have been found to accumulate in cytoplasm and plastids. The very nature of this wide cellular distribution infers a protective function [3]. Furthermore, LEA proteins and heat shock proteins have been shown to be involved in protecting macromolecules such as enzymes and lipids. Dehydrins (LEA D11 family) are proteins that occur in plants due to dehydration, low temperature, and osmotic stress. Earlier Inheritance studies including QTL analysis in crop plants revealed apparent co-segregation of Dhn genes with phenotypes associated with drought and freezing [5,6]. Dhns are unified by the presence of one or more copies of a putative amphipathic α-helix forming domain (The K-segments) which is highly conserved in higher plants. This and other distinct domains of Dhns including a phosphorylatable (the S-segment) and an N- terminal consensus sequence (the Y-segment) are pieced together in a consistent manner, interspersed by other lesser conserved and usually repeated domains (the F-segments) [7,8].

The assembly of domains into numerous, yet consistent permutation has resulted in a range of Dhn polypeptide lengths from 82 to 575 amino acid residues. The number of occurrences of the K-segment varies from one to 11 within a single polypeptide. The bulk of the Dhn polypeptide in most cases contains regions or domains (the F-segments) that are rich in Gly and polar amino acids (especially Thr) and are tandemly repeated between K-segments. But there are contrary examples were the F-segments located between the K-segments are rich in other amino acids or do not exit as tandem repeats. For example, the F-segments located between K- segments in all SK3 Dhns are not Gly rich, but in many cases are rich in Pro and Ala. Because of this distinction and the fact that the SK3 and some other Dhns tend to contain high percentage of acidic residues, it has been proposed that the Dhn family may contain biochemically distinct acidic sub groups. Two studies have clarified the location of Dhns in the cytoplasm [10]. Most Dhns contain putative bipartite nuclear targeting signal sequences. The predicted molecular weights of Dhns based on amino acid sequences are invariably less than their apparent molecular weight SDS-PAGE. This anomaly is also observed with Dhns translated *in-vitro*, retarded migration thus seems to be due at least in part to secondary structure in 0.1% SDS. A ~35 kDa Dhn of *V. unguiculata* L. which is associated with an increment of chilling tolerance during seedling [10]. A simple interpretation of these observation is that Dhns are lipid binding proteins possibly, Dhns and other LEA and COR (cold responsive) proteins function in a lipoprotein environment, at an interface between phospholipids bilayers and aqueous compartment. Dehydrins are a D11 family of late embryogenesis abundant proteins that are induced in vegetative tissues in response to low temperature. The main aim is to isolate the dehydrin gene from cold stress induced *in-vitro* cultured *P. vulgaris* L. and *V. unguiculata* L. plants and to analyse if any sequence variations occurs in the gene level.

Materials and Methods

P. vulgaris L. (Sel.9) and *V. unguiculata* L. (P.152) seedlings were grown under controlled climatic condition (in a polyhouse) by irradiance of 1500 µmol m^{-2}S^{-1} (PAR), temperature of 28 °C and 70% RH for 7 days. The plants were watered regularly. The plants were classified into two groups according to the treatment. One group of plants was continuously grown under ambient irradiance and temperature controlled polyhouse, while the other group of plants were subjected to LNT (low night temperature) treatment of 15 °C by transferring the plants to chilling chamber and treatment was given for 12 h daily (each night from 18:00h to 06:00h) for 5 days. During daytime the plants were grown under polyhouse condition. On 13th day, total RNA was isolated.

RNA isolation and Amplification

100 mg of plant tissue was taken for the RNA isolation. The plant tissue was homogenized with 450 µl of RLT buffer and 150 µl of RLC buffer with the help of the homogenizer. The lysate was transferred into the QIA shredder spin column (lilac tube). The spin column was centrifuged at maximum speed for 3 min. After centrifugation, the supernatant of the flow through was transferred to the new microfuge tube without disturbing the cell debris. 225 µl of 100% ethanol was added into the sample and it was mixed immediately by pipetting. Then the sample was applied into the mini spin column and centrifuged for 1 min. at 10,000 rpm. The flow through was discarded and 700 µl of RW1 wash buffer was added to the column. The tube was centrifuged for 1 min. at 10,000 rpm. In the next step the flow through and the collection tube were discarded. The column was placed in a fresh collection tube and 500 µl of RPE was added into the column and centrifuged for 1 min. at 10,000 rpm to wash the column. The column was centrifuged again for 1 min. to dry the membrane to avoid ethanol. The flow through and the collection tube were discarded. Then the column was placed into a new 2 ml collection tube. Then 36 µl of RNase free water was added to the column to elute the RNA in a collection tube and centrifuged for 2 min. at 10,000 rpm. The isolated RNA was visualized in the 1.2% formaldehyde agarose gel electrophoresis. The isolated RNA was amplified by RT-PCR. 12 µl of amplified PCR product was loaded in the 1% agarose gelelectrophoresis [11,12].

Purification of PCR Products (Qiaquick PCR purification kit protocol)

5 volumes of PB buffer and 1 volume of PCR sample were taken in Qiaquick kit tube. Qiaquick spin column was placed in a provided 2ml collection tube. To bind DNA, the sample was applied to the Qiaquick column and centrifuged for 30-60 seconds. The flow through was discarded and the Qiaquick column back was placed into the same tube. 0.75 ml PE buffer was added to the Qiaquick column and centrifuged for 30-60 seconds. The Qiaquick column was placed in a clean 1.5 ml micro centrifuge tube and the DNA was eluted with 40 µl elution buffer.

Cloning and Sequencing

The purified PCR products were cloned using QIAGEN PCR cloning plus kit as described by the manufacturer. Clones were selected and isolated plasmids with insert were sequenced with M13 Sequencing Primers using ABI biosystems automated sequencer (Macrogen Genomics, Korea).

Database Searching

Nucleotide database was searched with the sequences obtained using NCBI BLAST (Blastn) tool [13].

Sequence Analysis

Sequences of both the control and treated *Phaseolus* and *Vigna* species were analyzed using pairwise sequence alignment LALIGN software from EMBNET server [14].

Results

The total RNA isolated from *P. vulgaris* L. and *V. unguiculata* L. (cold stress treated and control) plants produced a distinct band and it was observed in 1.2% FAGE on UV illumination (Figure 1 and 2). Discrete band for *P. vulgaris* L. control dehydrin had the size of 911 bp, while treated dehydrin had 937 bp. In *V. unguiculata* L. control dehydrin (Dhn1) mRNA had 908 bp, while treated dehydrin (Dhn1) mRNA had 982 bp, in 1% agarose gel on UV illumination after loading the RT-PCR product amplified using Dhn primers.

The amplified products were purified and sequenced. The sequences were obtained from automated sequencer. The cold stress induced dehydrin gene of *P. vulgaris* L. and *V. unguiculata* L. and database sequence of control *P. vulgaris* L. and *V. unguiculata* L. plants were genetically analysed using Bioinformatics tools. Pair wise alignment indicated 98% similarity between the cold stresses induced dehydrin gene of *P. vulgaris* L. and *V. unguiculata* L. and database sequence of control *P. vulgaris* L. and *V. unguiculata* L. This genetic analysis concluded that there was no genetic variation between the LNT stress induced *P. vulgaris* L. and *V. unguiculata* L. plant when compared with database sequence of control *P. vulgaris* L. and *V. unguiculata* L.plants.
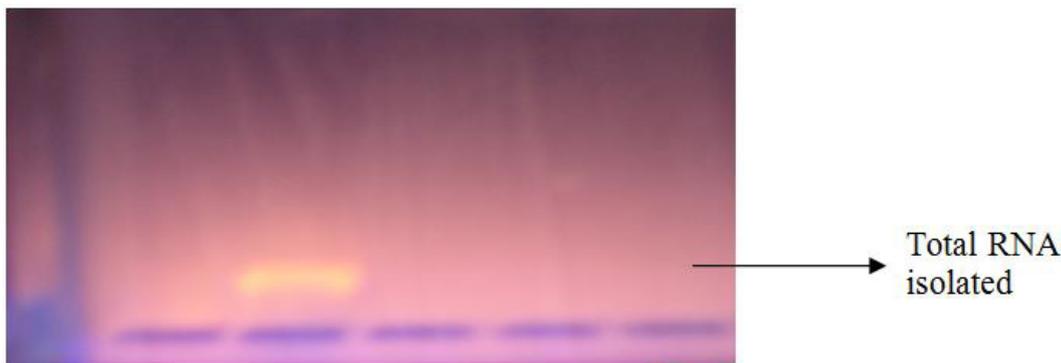
Figure 1: RNA isolated from *P. vulgaris* L. sample

Figure 2: RNA isolated from *V. unguiculata* L. Sample

Lalign output for *P. vulgaris* L. control vs. *P. vulgaris* L. treated

[ISREC-Server] Date: Fri May 4 4:24:26 Europe/Zurich 2007

LALIGN finds the best local alignments between two sequences version 2.0u66

September 1998 resetting to DNA matrix [14]

Comparison of:

(A) ./wwwtmp/lalign/.4415.1.seq Phaseo cont   - 911 nt

(B) ./wwwtmp/lalign/.4415.2.seq Phaseo test   - 937 nt

using matrix file: DNA, gap penalties: -14/-4

92.2% identity in 932 nt overlap; score: 3833 E (10,000): 3.4e-311.

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                    10        20        30        40        50        60

Phaseo  TNNNTCCGTTNCACTCTTTTNCTAGTTTGTTATTGTGATCCGATCATGGCAGAGGAAACC

        :     ::   : ::::::::    :::::::::::::::::::    :::::::::::::

Phaseo  TACTNCCNGTTCACTCTTTT---AGTTTGTTATTGTGATCCGA--ATGGCAGAGGAAACC
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             20          30          40          50          60          70

             70          80          90          100         110         120
Phaseo  CAGAACAAGTATGAGCGCAGAGAGCAGTGAGGTGGAGGTCCAGGATCGTGGAGTTTTGAC
        :::::::::::::::::::::::::::::::::::::    :::::::::::::::::::::::::
Phaseo  CAGAACAAGTATGAGCGCAGAGAGCAGTGAGGTG--GGTCCAGGATCGTGGAGTTTTGAC
             80          90          100         110         120

             130         140         150         160         170
Phaseo  TTTCTCGGTAAGGGAAAAAGCC-GTAGGAAGAAGAG----AAGCCAGGAAGAGGTGATCG
        :::::::::::::::        ::  :::::::::::    :::::::::::::::::::::
Phaseo  TTTCTCGGTAAGGGAAGCCGCTAGTAGGAAGAAGAGCCGGAAGCCAGGAAGAGGTGATCG
           130         140         150         160         170         180

             180         190         200         210         220         230
Phaseo  TCACCGAGTTTGAAAAGATCACAGTGTCAGAGGAGAGAAGGAAGAGGAAGAGAAGAA---
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::: :
Phaseo  TCACCGAGTTTGAAAAGATCACAGTGTCAGAGGAGAGAAGGAAGAGGAAGAGAAGCACAG
           190         200         210         220         230         240

               240         250         260         270         280         290
Phaseo  GCACAGCCTTTTAGAAAAGCTTCACGATCTGACAAGCCGGTTATCCCTGATAGCTCTTCA
        : :      :::::::::::::::::::::::::::::::::::::::::::  ::::::::::
Phaseo  GGAACTCCTTTTAGAAAAGCTTCACGATCTGACAAGCCGGTTATCCCTG--AGCTCTTCA
           250         260         270         280         290         300

             300         310         320         330         340
Phaseo  AGCGAAGGA-----AGGAGAAGACGGGGAGAAAAAGAAGAAGAAGAAGAAGGAGAAGAGG
        :::::::::       :::::::::::::::::::::::::::::::::::::::::::::::
Phaseo  AGCGAAGGATTCAGAGGAGAAGACGGGGAGAAAAAGAAGAAGAAGAAGAAGGAGAAGAGG
           310         320         330         340         350         360

             350         360         370         380         390         400
Phaseo  AGAAGAAGAAGATTACGAGAGAAAATAGAGGGTTATCACAAGGAAGACACGAGTGTTCCA
        :::::::::       :::::::::::::::::::::::::::::::::::::::::::::::
Phaseo  AGAAGAAGA----TACGAGAGAAAATAGAGGGTTATCACAAGGAAGACACGAGTGTTCCA
           370         380         390         400         410         420

             410         420         430         440         450         460
Phaseo  GTGGAGAAAGTGAGGTTGTGGAAGGGAAAAGAAGGGG-GCCATTCCTGGAGAAGATTAAG
        ::::::::::::::::::::::::::::::::::::::  ::::: : :::::::::::::::::
Phaseo  GTGGAGAAAGTGAGGTTGTGGAAGGGAAA-GAAGGGCCGACATTCCTGGAGAAGATTAAG
             430         440         450         460         470         480
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        470        480        490        500        510        520
Phaseo  GAGAAGCTACCAGGGCACAAGAATGAGGAGGCGGCAGCTCCTCCTCCGCCACCACCTGCT
        :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Phaseo  GAGAAGCTACCAGGGCACAAGAATGAGGAGGCGGCAGCTCCTCCTCCGCCACCACCTGCT
           490        500        510        520        530        540


        530        540        550        560        570        580
Phaseo  GCCACCTCATTGATCGGAACATGAAGGAGGCGAAGGAGAAGAAAGTATATTGGAGAAGAT
        :::::::::::::::::::::::::::::::::::::::::::::: :::::::::::::::
Phaseo  GCCACCTCATTGATCGGAACATGAAGGAGGCGAAGGAGAAGAAA-TATATTGGAGAAGAT
           550        560        570        580        590        600


           590        600        610        620        630        640
Phaseo  ---AAAAGAGAAGCTTCCTGGTTACCACTCAAAGACAGAGGAGGACGTAGAAAGAAAAGC
        :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Phaseo  TATAAAAGAGAAGCTTCCTGGTTACCACTCAAAGACAGAGGAGGACGTAGAAAGAAAAGC
              610        620        630        640        650        660


           650        660        670        680        690        700
Phaseo  GGGAGATG----GGTCACTGAGATTGAGAGAAGGGTTGTGTTGTGGGGTTTGGTTGGATG
        ::::::::      ::::::::::::::::::::::::::::::::::::::::::::::::
Phaseo  GGGAGATGCTAGGGTCACTGAGATTGAGAGAAGGGTTGTGTTGTGGGGTTTGGTTGGATG
              670        680        690        700        710        720


           710        720        730        740        750        760
Phaseo  ATTGTGGTGTGCTTTGTTTTCATTTCATCATATCAAACACTTGTGGGCTTTCTTATCTTT
        :::::::::::::::::::::::::::::::::::::::::  :::::::::::::::::::
Phaseo  ATTGTGGTGTGCTTTGTTTTCATTTCATCATATCAAAC--TTGTGGGCTTTCTTATCTTT
              730        740        750        760        770


           770        780        790        800        810
Phaseo  TGTGCTTCTTCTTCATGCATACTTTACTTTTTAGCGG---ATTATTATTATTGTGTATGT
        ::::::::::::::::::::::::::::::::::::::   :::::::: ::::::::::
Phaseo  TGTGCTTCTTCTTCATGCATACTTTACTTTTTAGCGGCCAATTATTATT-TTGTGTATGT
           780        790        800        810        820        830


           820        830        840        850            860        870
Phaseo  ATCATCTCTTGCATATACAAAAAGCTTTCGTACC-----TTTCCTC-TTTCTTTTTCTCA
        ::::::::::::::::::::::::::::::::::::     :::::::: :::: :::::::
Phaseo  ATCATCTCTTGCATATACAAAAAGCTTTCGTACCAGGTCTTTCCTCNTTTCNNTTTCTCA
           840        850        860        870        880        890
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                          880              890              900
          Phaseo  TAACTTGTTTGATGT---AATGTATGGANANA
                  :::::::::::::::    ::::::::::: : :
          Phaseo  TAACTTGTTTGATGTNANAATGTATGGAAAGA
                  900              910              920
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54.7% identity in 802 nt overlap; score: 383 E(10,000): 1.6e-23

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                 90         100        110        120        130
   Phaseo  GAGAGCAGTGAGGTG-GAGGTCCAGGATCGTGGAGTTTTGACTTTCT-CGGTAAGGGAAA
           :::  ::::  :::   :  :::::     ::  ::  :::   :  ::  :::    :  :     ::  ::
   Phaseo  GAGCGCAGAGAGCAGTGAGGT---GGGTCCAGGATCGTGGAGTTTTGACTTTCTCGGTAA
                 90         100        110        120        130


                 140        150        160        170        180        190
   Phaseo  ---AAGCCG-TAGGAAGAAGAGAAGCCAGGAAG--AGGTGATCGTCACCGAGTTTGAA--
              :::::: :::  : :::::   :::: ::::: :::       :: : ::      ::
   Phaseo  GGGAAGCCGCTAGTAGGAAGAAGAGCC-GGAAGCCAGGAAGAGGTGATCGTCACCGAGTT
              140        150        160        170        180        190


                 200        210        220        230        240
   Phaseo  --AAGATCACAGTGTCAGAGGAGAGAAGGAAGAGGAAGAGAAGAAGCACAGCCTTTTAGA
             :: :   ::    ::      ::   ::::  : : :: :: ::  :::::::::        ::
   Phaseo  TGAAAAGATCACAGTGTCAGAGGAGA-GAAGGAAGAGGAAGAGAAGCACAGG------GA
             200        210        220        230        240        250


                 250        260        270        280        290        300
   Phaseo  AAAGCTTCACGATCTG----ACAAGCCGGTTATCCC-TGATAGCTCTTCAAGCGAAG-GA
              :    :::   ::     :       ::  :  :     : ::   : ::    ::    :          ::: ::
   Phaseo  ACTCCTTTTAGAAAAGCTTCACGATCTGACAAGCCGGTTATCCCTGAGCTCTTCAAGCGA
                 260        270        280        290        300        310


                 310        320        330        340        350
   Phaseo  AGGA------GAAGACGGGGAGAAAAAGAAGAAGAAGAAGAAGGAGAAGAGGAGAAGAAG
           ::::      : :::  :  :  :  :  ::  :::::::::::::::: ::  :::  :::  :::::
   Phaseo  AGGATTCAGAGGAGAAGACGGGGAGAAAAAGAAGAAGAAGAAGAAGGAGAAGAGGAGAAG
                 320        330        340        350        360        370


                 360        370        380        390        400        410
   Phaseo  AAGATTACGAGAGAAAATAGAGGGTTATCACA-AGGAAGACACGAGTGTTCCAGTGGAGA
           :::::      ::::  ::  :  ::  :          :   ::  : :  :  :  :  ::   :  ::         ::
   Phaseo  AAGATA---CGAGAGAAAATAGAGGGTTATCACAAGGA-AGACACGAGT----GTTCCAG
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                380        390        400        410        420

         420        430        440        450        460        470
Phaseo AAGTGAGGTTGTGGAAG-GGAAAAGAAGG--GGGCCATTCCTGGAGAAGATTAAGGAGAA
        : ::     :: ::   : :::::  ::: :  :::::     :   : :::  :   :: :
Phaseo TGGAGAAAGTGAGGTTGTGGAAGGGAAAGAAGGGCCGACATTCCTGGAGAAGATTAAGGA
           430        440        450        460        470        480


         480        490        500        510        520        530
Phaseo GCTACCAGGGCACAAGAATGAGGAGGCGGCAGCTCCTCCTCCGCCACCACCTGCTGCCAC
        :     : :      : ::   : :::: ::: ::  ::::::: :: ::::: :::: :
Phaseo GAAGCTACCAGGGCACAAGAATGAGGAGGCGGCAGCTCCTCCTCCGCCACCACCTGCTGC
           490        500        510        520        530        540


         540        550        560        570        580
Phaseo CTCATTGATCGGAACATGAAGGAGGCGAAGGAGAAGAAAGTATATTGGAGAAGA---TAA
        :: ::: :   : :  :       :: ::   : :::  :::::  :  : :  :: ::   : :
Phaseo  AAAAGA-GAAGCTTCCTGGTTAC-CACTCAAAGACAGAGGAGGACGTAGAAAGAAAAGCG
           610        620        630        640        650        660


         650        660        670        680        690        700
Phaseo AGATGGGTCACTGAGATTGAGAGA-AGGGTTGTGTTGTGGGGTTTGGTT-GGATGATTGT
        ::    :   :   :   : :::::   :: :   : :::::::   ::   :::: :: ::   ::
Phaseo GGAGATGCTAGGGTCACTGAGATTGAGAGAAGGGTTGTGTTGTGGGGTTTGGTTGGATGA
           670        680        690        700        710        720


         710        720        730        740        750        760
Phaseo GGTGTGCTTTGTTTTCATTTCATCATATCAAA-CACTTGTGGGCTTTCTTATCTTTTGTG
        :::: : :: :::   ::::::    :::: : ::       :: :    : :   : : :: ::
Phaseo T-TGTGGTGTGCTTTGTTTTCATTTCATCATATCAAACTTGTGGGCTTTCTTATCTTTTG
           730        740        750        760        770        780


         770        780        790        800        810
Phaseo CTTCTTCTTCATGCATACTTTACTTT--TTAGCGGATT---ATTATTATTGTGTATGTAT
        : :::::::: : ::: : : :::::  ::      :           ::::::::::: ::: : : :
Phaseo -TGCTTCTTC-TTCATGCAT-ACTTTACTTTTTAGCGGCCAATTATTATTTTGTGTATGT
           790        800        810        820        830


      820        830        840
Phaseo CATC-TCTTGCATATACAAAAA
        ::: :::       ::: : :::
Phaseo -ATCATCTCTTGCATATACAAA
          840        850
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55.4% identity in 578 nt overlap; score: 258 E(10,000): 4.3e-13

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              290        300        310        320        330        340
Phaseo  TTCAAGCGA-AGGAAGGAGAAGACGGGGAGAAAAAGAAGAAGAAGAAGAAGGAGAAGAGG
                ::::   :: : :: :: ::   :       : :::: :::::::::: :::::: :::::::: :
Phaseo  TTCAGAGGAGAAGACGGGGAGAAAAAGAAGAAGAAGAAGAAGGAGAAGA-GGAGAAGAAG
               320        330        340        350        360        370


           350        360        370        380        390        400
Phaseo  AGAAGA-AGAAGATTACGAGAGAAAATAGAGGGTTATCACAAGGAAGACACGAGTGTTCC
             : : :: :::: ::     ::: :  :  : : ::     ::: ::         : ::::
Phaseo  ATACGAGAGAAAAT--AGAGGGTTATCACAAGGAAGACACGAGTG---TTCCAGTGGAGA
              380        390        400        410        420


           410        420        430        440          450        460
Phaseo  AGTGGAGAAAGTG-AGGTTGTGGAAGGGAAAAGAA----GGGGGCCATTCCTGGAGAAGA
             :    :::   ::: : :       ::::::   : :      :: :     :::   :::::::
Phaseo  AAGTGAGGTTGTGGAAGGGAAAGAAGGGCCGACATTCCTGGAGAAGATTAA-GGAGAAGC
         430        440        450        460        470        480


             470        480        490        500        510        520
Phaseo  TTAAGGAGAAGCTACCAGGGCACAAGAATGAGGAGGCGGCAGCTCCTCCTCCGCCACCAC
             :  :  ::  :  :: ::      ::  : :::: ::: ::  :::::::  :: :::::
Phaseo  T--ACCAG--GGCACAAG-----AATGAGGAGGCGGCAGCTCCTCCTCCGCCACCACCTG
            490        500        510        520        530


                530        540        550        560        570
Phaseo  CTGCTGCCACCTCATT--GATCG----GAACATGAAGGAGGCGAAGGAGAAGAAAGTATA
             ::::  :: : :   :  :: :      : :   ::::::: :::  : :     :: : :
Phaseo  CTGCCACCTCATTGATCGGAACATGAAGGAGGCGAAGGAGAAGAAATATATTGGAGAAGA
         540        550        560        570        580        590


             580        590        600        610        620        630
Phaseo  TTGGAGAAGATAAAAGAGAAGCTTCCTGGTTACCACTCAAAGACAGAGGAGGACGTA-GA
             ::   : :::::  ::       : :: :    :   ::     : :::  ::::: :   : : ::
Phaseo  TTATAAAAGAGAAGCTTCCTGGTTACCACT---CAAAGACAGAG-GAGGACGTAGAAAGA
         600        610        620        630        640        650


             640        650        660        670        680
Phaseo  AA-----GAAAAGC--GGGAGA-TGGGTCACTGAGATTGAGAGAAGGGTTGTGTTGTGGG
             ::      :: : ::  ::: : :: :  : :::::    ::  :  : :::::: :: :
Phaseo  AAAGCGGGAGATGCTAGGGTCACTGAG--ATTGAGAGA-AGGGTTGTGTTGTGGGGTTTG
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             660        670        680        690        700        710

             690        700        710        720        730        740
     Phaseo  GTTTGGTTGGATGAT-TGTGGTGTGCTTTGTTTTCATTTCATCATATCAAACACTTGTGG
             :::  :: ::    ::    :::: : :: :::  ::::::   :::: :      :: :
     Phaseo  GTT-GGATGATTGTGGTGTGCTTTGTTTTCATTTCATCATATCAAACTTG-----TGGGC
                720        730        740        750        760

             750        760        770        780        790        800
     Phaseo  GCTTTCTTATCTTTTG-TGCTTCTTCTT-CATGCAT-ACTTTACTTTTTAGCGG---ATT
              : : : : :: ::  : :::::::: : ::: : : ::::: ::      ::     :::
     Phaseo  TTTCTTATCTTTTGTGCTTCTTCTTCATGCATACTTTACTTT--TTAGCGGCCAATTATT
               770        780        790        800        810        820

                810        820        830
     Phaseo  ATTATTATTGTGTATGTATCATCTCTTGCATATACAAA
              ::: :    : ::::: ::: :::    ::: : :::
     Phaseo  ATTTGTGTATGTATC-ATC-TCTTGCATATACAAAAA
                830        840        850        860
```

Lalign output for *V. unguiculata* L. control vs. *V. unguiculata* L. treated

[ISREC-Server] Date: Fri May 4 4:14:27 Europe/Zurich 2007

LALIGN finds the best local alignments between two sequences version 2.0u66

September 1998 resetting to DNA matrix [14]

381 resetting to DNA matrix

Comparison of:

(A) ./wwwtmp/lalign/.4659.1.seq Vigna cont   - 908 nt

(B) ./wwwtmp/lalign/.4659.2.seq Vigna test   - 982 nt

 using matrix file: DNA, gap penalties: -14/-4

96.0% identity in 916 nt overlap; score: 4137 E(10,000):   0

```
              20         30         40         50         60         70
     Vigna   GAAACTGATG--AATATGGCAACCCGGTTC-ATGCAGCAAGTGTCCTCGGGCCTCCACCA
             :::::::::   :::::::::::::::::: ::::::::::::::::::::::::::::::::
     Vigna   GAAACTGATGNNAATATGGCAACCCGGTTCNATGCAGCAAGTGTCCTCGGGCCTCCACCA
              20         30         40         50         60         70


              80         90        100        110        120        130
     Vigna   CCGGTGGTCTTGGCCTGGATGACACTAACAAGCAACATGATACCAGTAATGTCTACGGTG
             :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
     Vigna   CCGGTGGTCTTGGCCTGGATGACACTAACAAGCAACATGATACCAGTAATGTCTACGGTG
              80         90        100        110        120        130
```

```
             140        150        160        170        180        190
Vigna    CAGACACCCGTAGACACGGAACTTTGGCATAGGTGACACCGGTAGACAGCACGGAACTAC
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Vigna    CAGACACCCGTAGACACGGAACTTTGGCATAGGTGACACCGGTAGACAGCACGGAACTAC
             140        150        160        170        180        190


             200        210        220        230        240        250
Vigna    CGGTGGTTTTACTGGTGACACCGGTAGACAATATGGCACTACCGGAGGCTTTACCGGTGA
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Vigna    CGGTGGTTTTACTGGTGACACCGGTAGACAATATGGCACTACCGGAGGCTTTACCGGTGA
             200        210        220        230        240        250



             260        270        280        290        300
Vigna    CACCGGGAGACAACATGGGACTACCGGTGGTTTTACCGGTGAC----ACTGGGTAACAAC
         :::::::::::::::::::::::::::::::::::::::::::       ::::::::::::
Vigna    CACCGGGAGACAACATGGGACTACCGGTGGTTTTACCGGTGACCTTCACTGGGTAACAAC
             260        270        280        290        300        310


         310        320        330        340        350        360
Vigna    ATGGGACTACCGGTGGTTTTACGTGACACTGGGAGACAACATGGGACTACTGGTGGTTTT
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
 Vigna   ATGGGACTACCGGTGGTTTTACGTGACACTGGGAGACAACATGGGACTACTGGTGGTTTT
             320        330        340        350        360        370


         370        380        390        400        410        420
Vigna    AGGTGACACTGGGAGACAACATGGCCGACTACCGGTGACACTGGGAGACAACATGCGACT
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Vigna    AGGTGACACTGGGAGACAACATGGCCGACTACCGGTGACACTGGGAGACAACATGCGACT
             380        390        400        410        420        430


         430                    440        450        460        470
Vigna    ACCGGC-----------GGCTTTACTGGTGGTGACACTGGTCTGGGAGGCCCTTACGTTG
         ::::::            ::::::::::::::::::::::::::::::::::::::::::
Vigna    ACCGGCCGTCCGAAAGCGGCTTTACTGGTGGTGACACTGGTCTGGGAGGCCCTTACGTTG
                 440        450        460        470        480        490


         480        490        500        510        520        530
Vigna    GAGCCAACACCGCACACAGGGACTGGTCCTAGAAGTGGCACAGGTGGCAGCGCCTATGGA
         :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Vigna    ---CCAACACCGCACACAGGGACTGGTCCTAGAAGTGGCACAGGTGGCAGCGCCTATGGA
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                  500          510          520          530          540

           540          550          560          570          580          590

Vigna   TCGGGTGGTTATGGATGTGGAATCAGCTGGAGCTGGGTATGGTATGAACACGGGGGGAGC

        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::

Vigna   TCGGGTGGTTATGGATGTGGAATCAGCTGGAGCTGGGTATGGTATGAACACGGGGGGAGC

           550          560          570          580          590          600


           600          610          620          630          640          650


Vigna   ACACTCAGTGATGAAAGGTATGGAAGGGAGTATCGTGAGCAATATGGGTAGTCTCGTGGA

        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::

Vigna   ACACTCAGTGATGAAAGGTATGGAAGGGAGTATCGTGAGCAATATGGGTAGTCTCGTGGA

           610          620          630          640          650          660


           660          670          680          690          700          710


Vigna   AAGATCATGACAAGAAAGGGATAGTGGACAAGATTAAGGAGAACCCGGAGGACACAGTGA

        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::

           670          680          690          700          710          720


           720          730          740          750          760          770


Vigna   CAANCAAGTACATCATGGGTGTGCATGCATGCGTATATATACGGGTAGTATAATTAAAG-

        :::  :::::::::::::::::::::::::::::::::::::::::::::::::::::::::

Vigna   CAA-CAAGTACATCATGGGTGTGCATGCATGCGTATATATACGGGTAGTATAATTAAAGG

           730          740          750          760          770          780


                        780          790          800          810          820


Vigna   ----------ATGTTATATTGTTGTGTTTTTGAATAAGTTTGCTGCATATATACGTACTC

        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::

Vigna   GGGGTTTACTATGTTATATTGTTGTGTTTTTGAATAAGTTTGCTGCATATATACGTACTC

           790          800          810          820          830          840


             830          840          850          860          870          880


Vigna   GTACACTGTCGTTCTCGTGTAGGNTATGTGGTGGATCTTGTATATNGGTTNATAGTAAAG

        :::::::::::::::::::::::: :::::::::::::::::::::: :::: :::::::::

Vigna   GTACACTGTCGTTCTCGTGTAGG-TATGTGGTGGATCTTGTATAT-GGTT-ATAGTAAAG

           850          860          870          880          890          900


             890          900

Vigna   ANGGAATAATTGCATG
```

```
                   :  ::::::::::::::
        Vigna    A-GGAATAATTGCATG
                      910
```

66.1% identity in 599 nt overlap; score: 868 E(10,000): 6.1e-64

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               170        180        190        200        210        220
 Vigna    GACACCGGTAGACAGCACGGAACTACCGGTGGTTTTACTGGTGACACCGGTAGACAATAT
          ::::::  :::::::     :::::::      : :    ::  :::::::::::::::::  :
 Vigna    GACACCCGTAGACA---CGGAACT-----TTGGCATA--GGTGACACCGGTAGACAGCAC
               140        150            160        170        180


               230        240        250        260        270        280
 Vigna    GGCACTACCGGAGGCTTTACCGGTGACACCGGGAGACAACATGGGACTACCGGTGGTTTT
          ::  :::::::: :: ::::: ::::::::::: ::::::  :::: :::::::: :: :::
 Vigna    GGAACTACCGGTGGTTTTACTGGTGACACCGGTAGACAATATGGCACTACCGGAGGCTTT
               190        200        210        220        230        240


               290        300        310        320        330        340
 Vigna    ACCGGTGACACTGGGTAACAACATGGGACTACCGGTGGTTTTAC--GTGAC----ACTGG
          :::::::::: :::   :::::::::::::::::::::::::::  :::::    :::::
 Vigna    ACCGGTGACACCGGGAGACAACATGGGACTACCGGTGGTTTTACCGGTGACCTTCACTGG
               250        260        270        280        290        300


                350        360        370        380        390        400
 Vigna    GAGACAACATGGGACTACTGGTGGTTTTAGGTGACACTGGGAGACAACATGGCCGACTAC
          :  :::::::::::::::: :::::::::: :::::::::::::::::::::::  ::::::
 Vigna    GTAACAACATGGGACTACCGGTGGTTTTACGTGACACTGGGAGACAACATGG--GACTAC
               310        320        330        340        350        360


                          410        420        430        440
 Vigna    CGGTG----------ACACTGGGAGACAACATG--CGACTACCGGCGGCTTT-----ACT
          ::::          ::::::::::::::::::: ::::::::::: : :      ::
 Vigna    TGGTGGTTTTAGGTGACACTGGGAGACAACATGGCCGACTACCGGTGACACTGGGAGACA
               370        380        390        400        410        420


               450        460        470        480        490
 Vigna    GGTGGTGAC-ACTGG---TCTGGGAG-GCCCTTACGTTGGAGCCAACACCGCACAC--AG
          :  ::: :: :: ::   :: :  :: : : :::: ::: :   :::: :  :     ::
 Vigna    ACATGCGACTACCGGCCGTCCGAAAGCGGCTTTAC--TGGTGGTGACACTGGTCTGGGAG
               430        440        450        460        470
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             500         510         520         530             540
Vigna   GGACT---GGTCCTAGAAGTGG-CACAGGTGGCAGCGCCTA----TGGATCGGGTGGTTA
        :  ::    :  : : :  :  : :::::: : : :  ::::    ::: : :::::
Vigna   GCCCTTACGTTGCCAACACCGCACACAGG-GACTGGTCCTAGAAGTGGCACAGGTGGCAG
          480        490         500         510        520         530


             550         560         570         580         590         600
Vigna   TGGATGTGGAATCAGCTGGAGCTGGGTATGGTATGAACACGGGGGGAGCACACTCAGTGA
         :   : :::: :: : :::    ::: : ::: :: :      :  :::::    :  : :
Vigna   CGCCTATGGA-TCGGGTGGTTATGGATGTGGAATCA-----GCTGGAGCTGGGTATGGTA
         540         550         560         570         580         590


             610         620         630         640         650         660
Vigna   TGAAAGGTATGGAAGGGAGTATCGTGAGCAAT-ATGGGTAGTCTCGTGGAAAG-ATCATG
        ::::     : ::  :::::: :    : ::  :: :   ::::   : : :  :: ::: ::
Vigna   TGAA---CACGGG-GGGAGCACACTCAGTGATGAAAGGTA---TGGAAGGGAGTATCGTG
                 600         610         620         630         640


             670         680         690         700         710         720
Vigna   A-CAAGAAAGGGA---TAGTGGACAAGATTAAGGAGAACCCGGAGGACACAGTG-ACAA
        : ::: :   :: :    : :::::  :::::  : :    ::  : : :  : :::: ::::
Vigna   AGCAATATGGGTAGTCTCGTGGA-AAGATCATG----ACAAGAAAGGGATAGTGGACAA
          650         660         670         680         690
```

61.3% identity in 802 nt overlap; score: 865 E(10,000): 1.1e-63

```
              140         150         160         170         180
Vigna   GACACCCGTAGACA---CGGAACTT----TGGCATA---GGTGACACCGGTAGACAGCAC
        ::::::: :::::::     :::::::      ::: :      :::::::::::::::::   :
Vigna   GACACCGGTAGACAGCACGGAACTACCGGTGGTTTTACTGGTGACACCGGTAGACAATAT
           170         180         190         200         210         220


              190         200         210         220         230         240
Vigna   GGAACTACCGGTGGTTTTACTGGTGACACCGGTAGACAATATGGCACTACCGGAGGCTTT
        :: :::::::: :: ::::: :::::::::: :::::: :::: :::::::: :: :::
Vigna   GGCACTACCGGAGGCTTTACCGGTGACACCGGGAGACAACATGGGACTACCGGTGGTTTT
          230         240         250         260         270         280


              250         260         270         280         290         300
Vigna   ACCGGTGAC----ACCGGGAGACAACATGGGACTACCGGTGGTTTTACCGGTGACACTGG
        ::::::::    :: :::  ::::::::::::::::::::::::::: ::::::::::
Vigna   ACCGGTGACCTTCACTGGGTAACAACATGGGACTACCGGTGGTTTTAC--GTGACACTGG
          230         240         250         260         270         280
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              290         300         310         320         330         340

                    310         320         330         340         350
Vigna   GTAACAACATGGGACTACCGGTGGTTTTACGTGACACTGGGAGACAACATGG--GACTAC
        :  :::::::::::::: :::::::::: :::::::::::::::::::::::  ::::::
Vigna   GAGACAACATGGGACTACTGGTGGTTTTAGGTGACACTGGGAGACAACATGGCCGACTAC
              350         360         370         380         390         400

            360         370         380         390         400         410
Vigna   TGGTGGTTTTAGGTGACACTGGGAGACAACATGGCCGACTACCGGTGACACTGGGAGACA
          :              :::::::::::::::::::: ::::::::::     : :    :: :
Vigna   CG----------GTGACACTGGGAGACAACATG--CGACTACCGG-----CCGTCCGAAA
                            410         420         430         440

          420         430         440         450         460         470
Vigna    ACATGCGACT--ACCGGCGGCTTTACTGGT---GGTGACACTG--GTCTGGGAGGCCCTT
            ::: ::  :: :: ::      ::::::    :: : ::    :: ::  :    ::
Vigna    ----GCGGCTTTACTGGTGGTGACACTGGTCTGGGAGGCCCTTACGT-TGCCAACACCGC
              450         460         470         480         490         500

            360         370         380         390         400         410
Vigna   TGGTGGTTTTAGGTGACACTGGGAGACAACATGGCCGACTACCGGTGACACTGGGAGACA
          :              :::::::::::::::::::: ::::::::::     : :    :: :
Vigna   CG----------GTGACACTGGGAGACAACATG--CGACTACCGG-----CCGTCCGAAA
                            410         420         430         440

          420         430         440         450         460         470
Vigna    ACATGCGACT--ACCGGCGGCTTTACTGGT---GGTGACACTG--GTCTGGGAGGCCCTT
            ::: ::  :: :: ::      ::::::    :: : ::    :: ::  :    ::
Vigna    ----GCGGCTTTACTGGTGGTGACACTGGTCTGGGAGGCCCTTACGT-TGCCAACACCGC
              450         460         470         480         490         500

              480         490         500         510         520
Vigna    ACGTTGGAGC-----CAACACCGCACACAGG-GACTGGTCCTAGAAGTGGCACAGGTGGC
         ::    ::  :      : : :      ::::::: : :   ::::     :::   : :::::
Vigna    ACACAGGGACTGGTCCTAGAAGTGGCACAGGTGGCAGCGCCTA----TGGATCGGGTGGT
              510         520         530         540         550

            530         540         550         560         570         580
Vigna   AGCGCCTATGGA-TCGGGTGGTTATGGATGTGGAATCAGCTGGAGCTGGGTATGGTATGA
          :   : ::::: :: : :::    ::: : ::: ::        :: :  ::    ::    :
Vigna   TATGGATGTGGAATCAGCTGGAGCTGGGTATGGTAT------GAACACGG---GGGGAGC
        560         570         580         590         600
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                  710         720         730         740         750
        Vigna  GGAGGACACAGTGACAANCAAG----TACAT-CATGGGTGTGCATGCATGC-GTATATAT
                 : :     :::      :::    :: :       : ::: :::: :: :   :: :   :  :::::  :
        Vigna  AGTGACAACAAGTACA-TCATGGGTGTGCATGCATGCGTATATACGGGTAGTATAATT
                  730         740         750         760         770         780


                  760         770         780         790         800         810
        Vigna  AC-GGGTAGTATAATTAAAGATGTTATATTGTTGTGTTTTTGAATAAGTT--TGC-TGCA
                 : ::: :: :: :  : : : :    : :::: :: ::::   :  :   ::   ::: :   :
        Vigna  AAAGGGGGGTTTACT--ATGTTATATTGTTGT-GT-TTTTGAATAAGTTTGCTGCATATA
                  790         800         810         820         830
                  710         720         730         740         750
        Vigna  GGAGGACACAGTGACAANCAAG----TACAT-CATGGGTGTGCATGCATGC-GTATATAT
                 : :     :::      :::    :: :       : ::: :::: :: :   :: :   :  :::::  :
        Vigna  AGTGACAACAAGTACA-TCATGGGTGTGCATGCATGCGTATATACGGGTAGTATAATT
                  730         740         750         760         770         780


                  760         770         780         790         800         810
        Vigna  AC-GGGTAGTATAATTAAAGATGTTATATTGTTGTGTTTTTGAATAAGTT--TGC-TGCA
                 : ::: :: :: :  : : : :    : :::: :: ::::   :  :   ::   ::: :   :
        Vigna  AAAGGGGGGTTTACT--ATGTTATATTGTTGT-GT-TTTTGAATAAGTTTGCTGCATATA
                  790         800         810         820         830


                  820         830         840         850         860         870
        Vigna  TATATAC--GTACTCGTACACTGTCGTTCTCGTGTAGGNTATGTGGTGGATCTTGTATAT
                 ::   :::  :::: :      :   : :::::     :: :  : :      :   :: :: :  :: :::
         Vigna  TACGTACTCGTACACTGTCGTTCTCGTGTAGGTATGTGGTGGATCTTGTATATGGT-TAT
                840         850         860         870         880         890


                    880         890
         Vigna  NGGTTNATAGTAAAGANGGAAT
                  : :   : ::  ::    :    : ::
         Vigna  AG-TAAAGAGGAATAATTGCAT
                  900         910
```

Discussion

A growing number of genes have been shown to be induced during cold acclimatization. The cold stress induced dehydrin gene of *P. vulgaris* L. and *V. unguiculata* L. and database sequence of control *P. vulgaris* L. and *V. unguiculata* L. plants were genetically analysed using Bioinformatics tools. Pair wise alignment indicated 98% similarity between the cold stress induced dehydrin gene of *P. vulgaris* L. and *V. unguiculata* L. and database sequence of control *P. vulgaris* L. and *V. unguiculata* L. Dehydrin gene enhances tolerance to freezing stress in *Arabidopsis* and in Transgenic Arabidopsis plants over expressing multiple dehydrins were generated [7,15]. These findings showed that over expression of dehydrin results in increased freezing tolerance. This genetic analysis concluded that there was no genetic variation between the LNT stress induced *P. vulgaris* L. and *V. unguiculata* L. plant when compared with database sequence of control *P. vulgaris* L. and *V. unguiculata* L. plants.

This observation hypothesizes that there is no significant gene level variations between LNT stresses induced plants and control plants. It can also be hypothesized that variations may be occurring in its expression level to code for the protein responsible for making the plant tolerant to cold. Dehydrin gene produces the protein in response to LNT stress [16]. Moreover the present study also suggested no significant gene level variations between *P. vulgaris* L. and *V. unguiculata* L. plants even though significant biochemical variations were observed when these plants were exposed to LNT stress.

Conclusion

In this study, we have attempted to study the response of dehydrin gene in *P. vulgaris* L. and *V. unguiculata* L. to LNT stress. From this study, it can be inferred that dehydrin gene is induced to express the protein which confers an adaptation effect only in *P. vulgaris* L. but not in *V. unguiculata* L. LNT does not show any genetic variations when compared with a non LNT stress induced plant. Hence *P. vulgaris* L. is considered to be cold tolerant plant and *V. unguiculata* L. is a sensitive plant. *P. vulgaris* L. and *V. unguiculata* L. (LNT treated and control) can be analysed for other genes involved in cold stress. The Dhn gene can be cloned and expressed. The expressed protein characteristics can also be studied. The Dhn gene can be genetically transferred to plants using gene transformation methods to produce stress tolerant plants. This will be very useful for agriculturists to increase crop productivity which will be a great boon for a country like India with agriculture as prime occupation.

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