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Title of thesis: **Isolation, cloning and characterization of abiotic stress responsive genes from *Lepidium latifolium***

Abstract:

Environmental stresses, such as salinity, drought and cold, can induce the expression of a large number of genes. Among these are many transcription factors that regulate the expression of downstream genes. The dehydration-responsive element-binding (DREB) protein/C-Repeat Binding Factors (CBFs) belongs to APETALA2 (AP2) family transcription factors that binds to DRE/CRT cis-element in cold-responsive (COR) genes and induce *COR* genes. CBFs have been isolated and characterized from evolutionarily diverse plant species. CBF pathway is conserved by CBF regulon size varies depending on the cold tolerance of plant species. Hence, cloning of CBFs from highly freezing tolerant plants such as *Lepidium latifolium* L. will be useful in understanding the freezing tolerance of this species. In this study, CBF1 family genes from *L. Latifolium*, designed as *LlDREB1A*, *LlDREB1B* and *LlDREB1C* was cloned by RT-PCR and RACE-PCR. Amino acid sequence analysis showed that putative *LlDREBs* has an AP2 DNA binding domain, a potential CBF type nuclear localization signal (NLS), C-terminal acidic domain and other signature sequences which are typical features of CBF1-type transcription factors. Semi-quantitative RT-PCR expression analysis of *LlDREB1B* revealed that this gene is up-regulated by high salt, dehydration and low temperature stresses whereas remaining two genes are differentially expressed in cold stress and does not response to either salt or drought stress. Microarray data analysis of *Arabidopsis* homologs suggest that all three genes are highly inducible under abiotic stress condition and very low signal ratio against biotic stresses. Our results suggest that *LlDREBs* may play an essential role in regulation of gene expression in response to various abiotic stresses.

Findings: The overall findings of the present research has been summarised as follows.

1. All three full length cDNA viz. *LlDREB1A*, 1B and 1C showed more than 80% homology with CBF/DREB genes from various species belonging to both *Brassica* and other families both at nucleotide and amino acid level. Amino acid sequence analysis showed all cloned cDNA contained a highly conserved 58 amino acid long AP2 domain and DREB1/CBF1 type nuclear localization signal (NLS) consensus sequence PKRPAGRTKFRETRHP, which are specialized feature of CBF/DREB genes.
2. CBF signature sequences DSAWR at the end of AP2 domain and LWSY at the end of C-terminal were also found to be conserved in all three *LlDREBs*. However *LlDREB1A* and 1B protein had LWNY domain instead of LWSY.

3. Three dimensional structure of AP2 domain revealed classic ERF fold consisting of three β-sheets connected by loops and turns and a C-terminal α-helix packed approximate parallel to β-sheets. The two amino acids in β-sheet, 14th valine and 19th glutamic acid play crucial role in the determination of DNA-binding specificity were found to be completely conserved in all three full length cDNAs. In addition to this, other amino acid residues such as Arg6, Arg8, Trp10, Glu16, Arg18, Arg25 and Trp27 of AP2 domain that directly make contact with DNA for DNA-binding activity were also completely conserved except Trp10, which was substituted by amino acid serine.
4. When we compared amino acid sequence of AP2 domain of *LlDREBs* to AtERF, results showed that only 55-60% amino acids are identical but more than 75% amino acids are chemically similar. These differences in amino acid may lead to differences in recognizing DNA sequences and binding specificity of some *cis*-acting elements.
5. Multiple sequence alignment of *LlDREBs* with other DREB1 proteins from different dicot plant species revealed high similarity within AP2 domain and low homology outside AP2 domain. Quite similar results were also observed when compared with monocot DREBs.
6. Phylogenetic tree analysis placed *LlDREB*s genes entirely on separate branch from *Arabidopsis* CBF genes, although they belong to the same family and having more than 80% similarity at amino acid level.
7. Expression analysis of *LlDREB1B* revealed that it expressed under all three abiotic stress condition namely cold, salt and drought. Differentially expression pattern of *LlDREB1A* was noticed under cold stress however did not response to either salt or drought stress. The different expression pattern of both genes was observed under similar cold stress condition.
8. As far as timing of expression is concerned, results suggested that these *LlDREBs* are early responsive transcription factors, induced as early as 30 min after stress and transcript level were remained detectable up to 48 h.
9. When we compared the gene expression pattern of all three genes with expression pattern of *Arabidopsis* homolog under various stimuli and mutants using online microarray database available in website www.genevestigator.com. Microarray data (signal ratio) analysis indicated that all three genes are highest inducible under abiotic stress (cold, salt and drought) and very low signal ratio in response to methyl jasmonate, salicyclic acid and some pathogen like *Pseudomonas syringae*. Therefore we concluded that all three *L. latifolium* DREBs may play important role in abiotic stress tolerance and little or no role in biotic stress signal pathways.