

# Homeobox leucine zipper proteins and cotton improvement

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## ABSTRACT

Transcription factors play key roles in plant development and stress responses through their interaction with cis-elements and/or other transcription factors. Homeodomain associated leucine zipper proteins (HD-Zip) constitute a family of transcription factors that are characterized by the presence of a DNA-binding domain closely linked with leucine zipper motif functioning in dimer formation. This type of association is unique to plants and considered as an excellent candidate to activate developmental responses to altering environmental conditions. Cotton is the most important fiber plant with a lot of local and commercial uses in the world. HD-Zip proteins not only have key roles in different stages of vascular and inter-fascicular fiber differentiation of cotton but also are suggested to have an important role against abiotic stress that is one of the key factors limiting cotton productivity. Plants have developed various strategies to manage stress conditions through a combination of metabolic, physiological and morphological adaptations. These adaptive changes rely largely on alterations in gene expression. Therefore, transcriptional regulators play a crucial role in stress tolerance. Being a transcription factor HD-Zip might be a useful target for genetic engineering to generate multiple stress tolerance in susceptible plants. In the following chapter, we discussed how the HD-Zip proteins would play a useful role for cotton development both in fiber production and stress adaptation.

**Keywords:** Cotton; Stress; Transcription Factor; HD-Zip Proteins; Homeobox Leucine Zipper

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## 1. ENVIRONMENTAL STRAINS AND PLANTS

Plants are exposed to a variety of stress factors that prevent them from attaining their full genetic potential. This can be due to insects, fungal infections, weeds, bacteria or viruses, all of which are known as biotic factors. The abiotic stress factors include drought, salinity, flooding, oxidative stress, heavy metal, cold and high temperature [1,2]. Abiotic stress, in fact, is the major cause of crop failure worldwide. It dips average yields for most major crops by more than 50% [3].

When plants are subjected to the stress, they respond through various cellular signal transduction pathways, which result in accumulation of certain differentially expressed gene products that can be classified as functional and regulatory proteins. Functional proteins include water channel proteins, key enzymes for osmolyte biosynthesis, chaperones, LEA (late embryogenesis abundant) proteins, proteinases and detoxicating enzymes. Regulatory proteins include transcription factors, protein kinases, and phospholipases. Regulatory proteins are involved in the further regulation of signal transduction and gene expression of stress tolerant proteins [4-7]. Improving the crop plant potential to endure different abiotic stresses will lead to more yields by either enhancing the crop set or expanding crop cultivation in the areas previously refuted due to stress intolerance.

## 2. GOSSYPIUM (COTTON)

Cotton is an important cash crop known as white gold due to its valuable fiber production and oilseeds [8]. A large number of ginning factories and textile mills greatly depend upon cotton. However, Cotton yield is greatly affected by many factors, such as the variety grown, cultivation method, environmental and climatic conditions,

amount and application strategy of fertilizers, time of sowing and availability of irrigation water [9].

There are 50 diverse species of the genus *Gossypium*. Four species are cultivated, *G. hirsutum* L. and *G. barbadense* L., that are tetraploid ( $2n = 4x = 52$ ), and *G. arboreum* L. and *G. herbaceum* L., that are diploid ( $2n = 2x = 26$ ). The most extensively cultivated species throughout the world is *G. hirsutum*. Whereas the diploid cotton species are a pool for important disease resistance and pest control genes, and improved agronomic and fiber qualities but also have better opportunities for structural and functional studies of genes through advanced systems of gene knockouts [10]. Asiatic *G. arboreum* L. (Desi cotton.) has built-in desirable genes for drought tolerance and resistance to insect pests like bollworms, aphids and diseases like black arm, root rot, reddening of leaves and most importantly, highly destructive leaf curl disease of cotton. Its diploid genome makes it a good choice for the identification of novel genes in genus *Gossypium* [11].

Some of the cotton genotypes are more tolerant through an intricate set of genetic parameters including sensing, signal transduction and response. Due to the large number of genes participating in response to an external stress, improvement through conventional breeding is very difficult. Conventional breeding has developed many new cultivars and varieties; however it has some limitations like thousands of genes getting transferred in each cross and the barriers for gene transfer through incompatibility and species differences [12]. Genetic engineering technology has made possible the insertion of desired foreign gene(s) to overcome problems of sexual incompatibility and species barriers between organisms. This technology helps the breeders and molecular biologists to introduce only the gene of interest with more selective modification and represents a significant advance [13]. In this background we require those cotton varieties which resist these biotic and abiotic stresses. This resistance in cotton to various stresses can be gained by improving the cotton plant through stress resistant genes with special emphasis on stress responsive transcription factors controlling the multiple genes involved in stresses [14]. Several stress responsive transcription factor genes have been identified in *G. hirsutum* and *G. arboreum* such as WRKY, EREBP, NAC, HD-Zip and DREB genes [15-19].

### 3. TRANSCRIPTION FACTOR (TF) AGAINST STRESS

Transcription factors are the sequence-specific DNA binding proteins that control the transfer of genetic information from DNA to mRNA [20]. These are the first line of defense against stress stimuli that in turn activate the expression of other stress responsive genes. These transcription factors bind to the specific elements in the pro-

motor regions called *cis*-acting elements and the transcription factors that bind to these elements are known as *trans*-acting factors. Several *cis*-acting promoter elements and their subsequent binding proteins, each containing a distinct type of DNA binding domain, such as AP2/ERF, basic leucine zipper, HD-ZIP, MYB, MYC, and several classes of zinc finger domains, have been involved in plant stress responses due to their variable expression under different stress conditions [21]. Combinatorial interactions of promoters DNA *cis*-acting elements with *trans*-acting protein factors are chief processes governing spatio-temporal gene expression [22].

Most of the transcription factors are common among different plants in their motif structure and mode of action [23]. These are potent targets for genetic engineering of stress tolerance because a transcription factor is encoded by a single gene but regulates the expression of several other genes leading to the activation of complex adaptive mechanisms. Therefore, in transgenic plants transcription factors can confer better stress tolerance than a single gene transfer. This opens an excellent opportunity to develop stress tolerant crops in future that can contribute to sustainable food and fiber production in the world [24].

Several transcription factor proteins have been identified from different *Gossypium* species and analyzed for their role in diverse stress and development conditions. A leucine zipper-containing WRKY protein named GaWRKY1 was isolated from *G. arboreum* using the CAD1-A, (a gene contributing in cotton sesquiterpene biosynthesis) promoter. In transgenic *Arabidopsis* plants and transiently transformed tobacco leaves expression of GaWRKY1 triggered expression of the CAD1-A promoter, and interruption of the W-box abolished the activation [15]. Duan *et al.* [16] isolated two EREBP (ethylene response element binding protein) genes named GhEREB2 and GhEREB3 suggesting their role as the positive transcription factors in biotic stress (ethylene and jasmonic acid) signal transduction pathways. A DRE-binding protein, GhDBP2, was isolated from *G. hirsutum* seedlings that participate in the activation of down-stream genes in response to environmental stresses and ABA treatment [17]. Another DRE binding protein, GhDREB, containing a conserved AP2/EREBP domain reported in *G. hirsutum* that is induced by drought, high salt and cold stresses in seedlings. GhDREB accumulates higher levels of soluble sugar and chlorophyll in leaves following to drought, high salt, and freezing stress treatments in transgenic wheat plants conferring enhanced tolerance [25]. From the NAC (NAM, ATAF1, -2, and CUC2) gene family, six full-length, intact putative transcription factors were isolated from *G. hirsutum* (GhNAC1-GhNAC6) that showed differential gene regulation under dehydration, high salt, cold and ABA

treatments [18].

#### 4. HD-ZIP PROTEINS

HDZip proteins are characterized by the presence of a DNA-binding homeodomain (about 60 amino acid long) with a closely linked leucine zipper motif functioning in dimer formation [26]. The leucine zipper motif adjoining to the C-terminal of the homeodomain is assumed to form an amphipathic  $\alpha$ -helix with a series of leucine residues responsible for dimerization of a pair of target DNA contacting surfaces [27]. Leucine zippers are responsible for the interaction of HD-Zip proteins among each other and with other leucine zipper proteins [28]. Homo- and heterodimer interactions may have important role in the function of these proteins [29]. This type of homeodomain association is present only in plants and it is considered that HD-Zip genes originated in plant lineage by exon exchange between a homeodomain gene and a leucine zipper containing sequence [30]. None of the nearly 350 homeobox genes examined in animal system contains a leucine zipper [31].

So far, the homeodomain-leucine zipper proteins have been identified in many plants such as sunflower [32,33], carrot [34], soybean [35], tomato [36], rice [37] and Arabidopsis [38]. These proteins have been suggested as excellent candidates to activate developmental responses to altering environmental conditions, a characteristic feature of plants. Numerous authors have suggested that expression of HD-Zip transcription factors family is regulated by diverse external factors such as illumination or drought. HD-Zip proteins are categorized into four classes (I - IV) based on gene structure, presence of unique domains and function [39]. A few HDZip family members are supposed to control the development of particular plant regions, such as the vascular system is controlled by (ATHB8, class III, [40]; Oshox1, class II, [41] Vahox1, class I, [42], and root hairs and trichomes (ATHB10, class IV, [43].

The Arabidopsis genes *Athb2* and *Athb4* (both class II) are highly induced by far-red light, indicating a role in the shade avoidance response [31]; *Athb6*, *Athb7* and *Athb12* are inducible by drought as well as ABA, implying their putative function in dehydration responses [44, 45]. From *C. plantagineum*, two HDZip genes (*CPHB-1* and *CPHB-2*, class II) are dehydration-inducible, and one of them is ABA-inducible (*CPHB-2*) [46]. Therefore, they are thought to be involved in regulation of dehydration responses through different branches of the dehydration-induced signalling network, ABA-independent or ABA-dependent. Similar overexpression was observed in five families of *Craterostigma plantagineum* homeobox leucine zipper genes (*CPHB*) that were isolated by Deng *et al.* [47]. All families of *CPHB* genes modulate their expression against dehydration in leaves and roots. Aka-

shi *et al.* [48] isolated an HD-Zip gene from Wild watermelon (*Citrullus lanatus sp.*) differentially expressed under drought stress. Expression and functional studies on the sunflower Hd-Zip II subfamily with special emphasis on *Hahb-10* from sunflower indicated that the members are expressed primarily in mature photosynthetic tissues, and up-regulated by etiolation and gibberellins in seedlings [49].

*In vitro* and *in vivo* binding assays have demonstrated that HDZip proteins from Arabidopsis, *C. plantagineum*, sunflower and rice preferentially bind to two 9-bp pseudopalindromic sequences, CAAT (A/T) ATTG (HDE1) and CAAT(G/C)ATTG (HDE2) [50]. A few other binding sequences relevant to homeodomain proteins were reported in plants like: A soybean homeodomain leucine zipper proteins bind to CATTAAATTAG sequence present in the phosphate response domain of VspB promoter [51] and ATHB6 of plant-specific HD-Zip class targets the core motif (CAATTATTA) present in its own promoter that mediated ABA-dependent gene expression [52]. With the help of *cis*-acting elements, efforts to identify target genes in planta will contribute greatly to the understanding of HDZip function.

This is important to provide fundamental molecular information towards understanding of the biological roles of the HD-Zip proteins in cotton and present a valuable source for improving cotton varieties with resistance to abiotic stresses.

#### 5. COTTON HD-ZIP PROTEINS

A number of homeodomain leucine zipper protein of different classes have been identified in different species of cotton such as GbHB1 from *G. barbadense* and GaHOX1 and GaHOX2 from *G. arboreum* that plays a role in fiber development [53] whereas *G. hirsutum* GhHB1 is involved in root development and salt stress [19]. *G. arboreum* GaHDZ protein was identified as ABRE binding protein. It showed enhanced expression under salt, heavy metals and drought treatments (Author unpublished data). Recently three HD-Zip proteins designated as GhHB2, GhHB3 and GhHB4 were isolated from cotton cDNA library. All these proteins are suggested to be involved in early seedling development whereas expression of these Hb proteins was up-regulated in response to gibberellin signaling [54]. Another HD-Zip IV family transcription factor, Meristem Layer 1 (GbML1) was isolated and characterized from *G. barbadense* that interacted with a key regulator of cotton fiber development. When expressed in Arabidopsis, GbML1 increased the number of trichomes on stems and leaves and increased the accumulation of anthocyanin in leaves [55]. L1 layer-specific HD-ZIP gene from tetraploid *G. hirsutum* GhHD-1 is expressed in trichomes and early fibres thus might play a role in cotton fibre initiation. Further microarray analysis of

GhHD-1 lines indicated that it potentially regulates the levels of ethylene and reactive oxidation species (ROS) through a WRKY transcription factor and calcium-signalling pathway genes to activate downstream genes necessary for cell expansion and elongation [56].

## 6. CONCLUSION

Plants respond and adapt to environmental stresses through not only physiological and biochemical processes but also molecular and cellular processes. Several genes with various functions are induced by drought and cold stresses, and those various transcription factors are involved in the regulation of these stress-inducible genes through their specific binding to the *cis*-acting elements of their promoters. Gaining an understanding of the mechanisms that regulate the expression of these genes is a fundamental issue in plant biology and will be necessary for the genetic improvement of plants cultivated in extreme environments. There have been extensive studies regarding the role of transcription and its regulation by promoter elements during abiotic stress. Cotton is best known for its fiber development but it suffers badly due to abiotic stresses. Several transcription factors have been reported that confer resistance to cotton is against these stresses. In the last decade HD-Zip proteins have been found in different resistant varieties that could not only support the cotton plants to withstand stress period but also specifically involved in efficient fiber development. These characteristics made the HD-Zip proteins an efficient target for cotton genetic engineering to develop better varieties.

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