Biochemical and Biophysical Research Communications xxx (2018) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Transcriptional regulation of bHLH during plant response to stress

Xi Sun [1], Yu Wang [1], Na Sui*

Shandong Provincial Key Laboratory of Plant Stress, College of Life Sciences, Shandong Normal University, Jinan, Shandong, 250014, PR China

A R T I C L E I N F O

Article history:
Received 16 July 2018
Accepted 24 July 2018
Available online xxx

Keywords:
bHLH
Transcriptional regulation
Plant
Stress response

A B S T R A C T

Basic helix-loop-helix protein (bHLH) is the most extensive class of transcription factors in eukaryotes, which can regulate gene expression through interaction with specific motif in target genes. bHLH transcription factor is not only universally involved in plant growth and metabolism, including photomorphogenesis, light signal transduction and secondary metabolism, but also plays an important role in plant response to stress. In this review, we discuss the role of bHLH in plants in response to stresses such as drought, salt and cold stress. To provide a strong evidence for the important role of bHLH in plant stress response, in order to provide new ideas and targets for the prevention and treatment of plant stress resistance.

1. Introduction

In nature, the growth and development of plants is inevitably affected by many biotic and abiotic stresses [1–5]. In order to survive and optimize living conditions, plants themselves must use a wide range of physiological and biochemical processes to respond to various stresses [6–12]. In general, these responses are regulated by activating or inhibiting gene-specific expression [13–26]. One of the genes-specific expression pathways is that transcription factors (TFs) interact with *cis*-acting elements to specifically express genes involved in environmental stress to maintain normal life activities of plants [27]. Among these TFs, there are six gene families that control stress responses [28]. Among the families of transcription factors related to plant stress resistance, there are four major categories of bZIP, WRKY, AP2/EREBP and MYB [29]. The bHLH family is the second largest family in plants after the MYB family [13]. But in plant stress response, studies on the bHLH transcription factor family have lagged behind.

The bHLH family is first discovered in murine muscle development studies [30] and subsequently is identified in all eukaryotes including animals, plants and fungi [31]. 162 bHLH members are identified in Arabidopsis [32] and 167 members are identified in rice [33]. In recent years, genome-wide analysis of bHLH transcription factors has been carried out in plants such as peanuts, apples, and *Brachypodium* to understand the more functions of the bHLH family in plants. Although most eukaryotes have the bHLH family of proteins, many of the biological functions of this family have not been studied in depth [34].

1.1. *Structure of bhlh transcription factors*

bHLH, as its name suggests, has a highly conserved basic/helix-loop-helix special structural domain consisting of two parts, one part is the basic amino acid region and the other part is the helix-loop-helix region (HLH) (Fig. 1). These two parts are completely different in function and have a total of about 50–60 amino acids. The basic amino acid region is located at the N-terminus of the bHLH domain and contains approximately 15 amino acids, including about 6 basic residues, the main function of which is to recognize and specifically bind the DNA motif in the target gene promoter [35]. The helix-loop-helix region is located at the C-terminus of the bHLH domain and contains approximately 40–50 amino acids, including two amphipathic alpha helices consisting of hydrophobic residues linked by a hydrophobic ring, the main function of which is to carry out the domain dimerization to promote protein-protein interactions and allows the formation of homodimeric or heterodimeric complexes to control gene transcription [36]. The Leu23/64, the Leu/Iso54 and the Val61 in this region are highly conserved, and the Leu23 and Leu52 are indispensable for the formation of dimers [37]. Studies have shown that the bHLH protein mainly binds to the core DNA sequence motif called E-box (5-CANNTG 3), of which G-box (5-CACGTG-3) is the most common form [38], determines several conserved amino acids in the basic amino acid region that are recognized for the core

---

* Corresponding author.
E-mail addresses: suina800101@163.com, 184961053@qq.com (N. Sui).

[1] Xi Sun and Yu Wang have contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.07.123
0006-291X/

Please cite this article in press as: X. Sun, et al., Transcriptional regulation of bHLH during plant response to stress, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.123

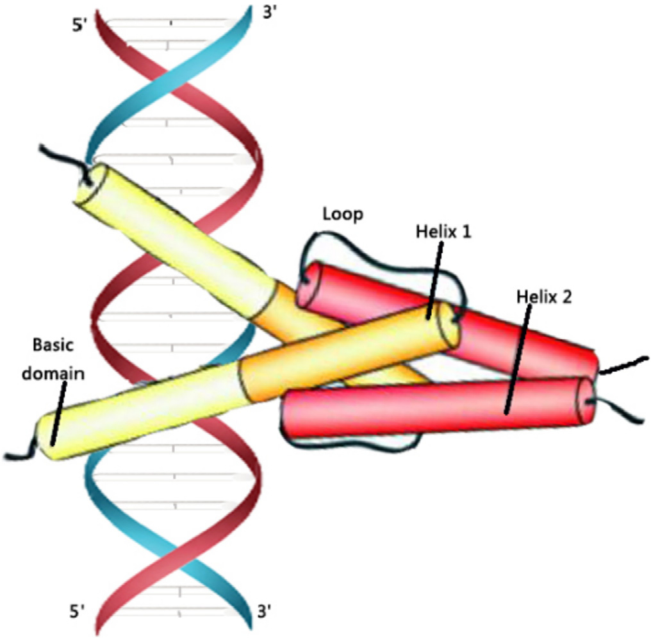

Fig. 1. Complex formed between bHLH motif and its target DNA [40].

consensus sites of different boxes [39]. However, sequences other than the highly conserved bHLH domain are less conserved and often differ.

1.2. Classification of bhlh transcription factors

The study of bHLH in animals is more in-depth than plants [35]. Atchley et al. (1999) [35] classify the bHLH family in animals into six categories, A-F, based on evolutionary relationships, binding patterns with DNA, and their own functional properties. Members of group A can be combined with E-box with CACCTG or CAGCTG characteristics; group B members can be combined with E-box with CACGTG or CATGTG characteristics, including Myc family; group C members have 1–2 PAS domains (Drosophila Period-human Arnt-Drosophila Single-minded domains); members of group D have no basic DNA binding region; members of group E are easy to bind to N-box with CACGCG or CACGAG bases; and group F bHLH has only one family of COE (collier/olfactory-1/early B-cell factor) [40]. Although the structural characteristics of most bHLH transcription factors in plants are similar to those of animal B, they can also be combined with the same characteristics of E-box. The classification of plant bHLH transcription factors is very different from that of animals, depending on their structure and functional differences. The phylogenetic tree of plant bHLH is first constructed in Arabidopsis, and the *AtbHLH* family is divided into 12 subfamilies [41]. In rice, the phylogenetic tree of the bHLH family is divided into 22 subfamilies [33]. With the development of molecular biology and the increase of genomic sequences, more and more bHLH transcription factors have been identified. For example, the genomes of peanut, *Brachypodium*, apple and common beans respectively contain 261, 146, 188 and 155 bHLH genes [28,42–44]. At the same time, the classification of the family in plants is becoming increasingly clear.

According to current studies on the function of bHLH proteins, plant bHLH transcription factors regulate the expression of a large number of genes involved in a wide range of overlapping and specific regulatory pathways [45]. For example, the bHLH transcription factor regulates carpel, anther, epidermal cell and stomatal development [46], early stage of root hair cell differentiation, seed germination [13], flowering time control [45] and root hair formation [47]. In addition, bHLHs are involved in the regulation of metabolic processes, including the biosynthesis of alkaloids and nicotine [48]. Many bHLHs play a role in light signal regulation [45] and are also involved in the function of signaling hormones such as abscisic acid (ABA) [49], brassinosteroids (BRs) [50], ethylene, gibberellin and jasmonic acid [51]. In addition, bHLH transcription factors are also involved in plant responses to stress, such as hypothermia, drought, salinity, and iron-deficient abiotic stress [52]. Taken together, the bHLH transcription factor is involved in plant responses to biotic and abiotic stresses.

1.3. Response of bhlh to drought stress

Drought stress is one of the major environmental factors that can cause physiological and biochemical effects on plants, mainly in the aspects of growth, photosynthesis [53] and yield, hormone metabolism, enzyme system, etc., causing irreversible damage to plants. The bHLH transcription factor plays an active role in plant response to drought stress.

The bHLH1 gene in *Tamarix* can improve abiotic stress tolerance by inducing expression of stress tolerance-related genes by increasing osmotic potential, increasing ROS clearance and enhancing second messenger in the stress signaling cascade [54]. Under salt and drought stress, *VvbHLH1* in grapes confers a dominant effect on salinity and drought tolerance by increasing the accumulation of flavonoids and ABA signaling in transgenic *Arabidopsis thaliana*. The *VvbHLH1* gene may be used to increase the content of valuable flavonoids and improve the tolerance of Arabidopsis and other plants under abiotic stress [55]. *PebHLH35*, which is present in the nucleus of *Populus euphratica*, is overexpressed under drought stress, which can regulate stomatal density, stomatal pore size, photosynthesis and growth, and ultimately improve plant tolerance to drought [56]. In addition, it was also found in tea plants that the 22 and 39 *CsbHLH* genes are up-regulated in response to drought stress under drought stress induction [57].

1.4. Research progress of bhlh in plants response to salt risistance

Soil salinization is an major environmental factor in the worldwide [58–60]. In China, more than one million acres of agricultural land are affected by salt [61–63]. Salt stress can affect plant growth, metabolism, and reproduction, such as photosynthesis [64–71], ion homeostasis [72–79], seed germination [80–90], membrane permeability [91–95], etc.. In recent years, more and more studies have indicated that bHLH transcription factors are involved in plant response to abiotic stress and improve plant stress tolerance.

Overexpression of *bHLH39* in wheat improved drought, salt and freeze tolerance of transgenic Arabidopsis. Under abiotic stress conditions, the survival rate of transgenic seedlings is significantly higher than that of wild type, and the soluble sugar and proline content in plants increase. In addition, the percentage of electrolyte leakage measured in transgenic plants is lower compared to wild type plants, thereby increasing the tolerance of transgenic plants to abiotic stresses. Under salt stress, *bHLH39* in wheat plants regulates the expression level of stress-responsive genes, which makes them show higher expression levels, thus increasing the tolerance of over-expressed plants to salt [96]. In addition, bHLH was also found to be involved in salt stress responses in poplar [97] and *Chenopodium glaucum* [98]. Under abiotic stress conditions, ROS is highly accumulated in the guard cells of plants, which activates the ROS-induced stomatal closure pathway to reduce stomatal opening

Please cite this article in press as: X. Sun, et al., Transcriptional regulation of bHLH during plant response to stress, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.123