# The Functions of The BBX Family Compared with Other Transcription Factors on Plant Stress Resistance

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**Abstract.** B-box proteins (BBX) are a class of zinc finger proteins with B-box domain, which are transcription factors that regulate various growing processes in plants. The effective cooperation of BBXs and other transcription factors deeply affects the stress resistance of plants. The mechanism of improving plant stress resistance is worth studying for its great potential value in agriculture. In this review, we mainly focus on the 32 BBXs in Arabidopsis and summarize their structural features. We also highlight their vital functions in regulating photomorphogenesis, flowering, and abiotic stress and compare their functions with other transcription factors. In addition, we discuss the current state of research on BBXs in the plant kingdom, then provide novel insights into the future direction of researching plant transcription factors, which suggests investigating their mechanism in engineered Saccharomyces cerevisiae to reconstruct biosynthetic passageways to produce desired metabolites.

Keywords: stress resistance, B-box, transcription factor.

#### 1. Introduction

Transcription factors are essential in controlling gene expression. These regulatory factors are primarily a class of proteins that can specifically bind to DNA [1]. Most of them include one or more DNAbinding domains, directly performing their function by binding to the promoters of target genes to regulate the transcription of the specific genes. Some transcription factors do not bind to the promoters, but indirectly regulate gene transcription by modulating the transcriptional activity of their partner proteins.

In Arabidopsis, there are many transcription factor families, including the MYB superfamily, bHLH family, AP2/EREBP family, bZip family, and C2C2 (Zn)-dof family. The number of members in each family varies considerably, and they play different roles in regulating plant physiological activities. Among them, Zinc-finger proteins constitute a big transcription factors family [2]. One subgroup of zinc finger proteins is known as BBX proteins, which contain one or two B-Box motifs. The critical functions of BBX proteins have been thoroughly researched, including seed germination, seedling photomorphogenesis, thermomorphogenesis, flowering, shade avoidance, and petal senescence. Therefore, the BBX family proteins have a multifaceted impact on regulating the life activities of Arabidopsis thaliana, significantly enhancing the plant's stress resistance. For instance, in Arabidopsis, BBX proteins can regulate photomorphogenesis, adjusting to different light conditions to improve the plant's resistance to light stress [3]. BBX proteins also influence the flowering time, reducing the negative impact of environmental factors on the reproductive process [4]. Overall, the mechanisms of

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stress resistance in plants are crucial, as improving plant stress resistance helps maintain homeostasis under environmental stress, thereby supporting plants' growth and development.

This paper primarily takes the BBX family proteins in Arabidopsis thaliana as an example to briefly introduce their characteristics. Further, it summarizes and analyzes the functions of the BBXs as transcription factors in affecting stress resistance in Arabidopsis and briefly compares them with other transcription factors. Understanding the interactions and functional comparisons between the BBX family and other transcription factors will make our general knowledge of transcription factors in Arabidopsis and plants more transparent and profound. It will help us develop the value of plant transcription factors in agricultural production. Modern biotechnology manipulates transcription factors such as BBX to construct new and superior crop varieties to meet human agricultural production needs. If we can gain a deeper understanding of the mechanisms by which these plant transcription factors interact with each other, there will be more possibilities for us to study and understand the roles of certain plant transcription factors in different plant species and even fungal species.

# 2. Structural Features of BBXs

BBX proteins have several conversed structural features. The B-box domain contained within them is a subclass of zinc finger motif. The B-box domain, characterized by around 40 residues, is categorized into B-box1 and B-box2 based on the number of B-box motifs. The B-box domain is crucial for facilitating interactions between proteins and regulating gene transcription[5]. In animals, the B-box domain often combines with the RING (Really Interesting New Gene) domain and the coiled-coil domain to form RBCC/TRIM proteins. In contrast, RBCC/TRIM proteins do not exist in plants. In plants, the B-box domain exists in two forms: alone or together with the CCT domain (CO, CO-like, TOC1) [4,5]. The CCT domain, located at the C-terminus and consisting of 42-43 amino acids, is present in certain BBX proteins and significantly influences the regulation of DNA transcription by affecting the protein's ability to bind DNA, its transcriptional activity, and its localization within the nucleus [4]. The VP motif, situated between the B-box and CCT domains, serves as a crucial sequence motif for protein-protein interactions and is found in some of the BBX proteins [4,5]. The microproteins AtBBX31 and AtBBX32, which contain an additional C-terminal PF(V/L)FL motif, can interact with the TOPLESS (TPL) protein [5].



Figure 1. Phylogenetic Analysis of BBX Proteins in Plants

The 32 proteins in Arabidopsis, considering the number of B-box and CCT domains, can be classified into five distinct groups. Among the 32 BBX proteins in Arabidopsis, AtBBX1 to AtBBX6 belong to Group I, consisting of two tandem B-box domains(B-box1 and B-box2) and a CCT domain. AtBBX7 to AtBBX13 belong to Group II, carrying B1, B2', and a CCT domain. AtBBX14 to AtBBX17 belong to Group III, which has both a B-box domain and a CCT domain. AtBBX18 to AtBBX25 belong to Group IV, consisting of B1 and B2 domains, while there is only one B-box domain in each protein in Group V, which contains AtBBX26 to AtBBX32. BBX proteins, found in a variety of plants, can also be categorized into five similar types based on their structure and domains(as shown in **Figure 1**). This conservation highlights the evolutionary significance of the B-box domain in plant development and stress responses.

# 3. The Functions of BBXs in Regulating Plant Stress Resistance

BBXs are deeply involved in the process of photomorphogenesis In Arabidopsis [6]. Under light conditions, plants experience photomorphogenesis, which is characterized by shortened hypocotyls, open and expanded cotyledons, green chloroplasts, and photoautotrophic growth [4]. Under red light, the Phytochrome B (phyB) - PHYTOCHROME INTERACTING FACTOR 3 (PIF3) module requires the participation of AtBBX4. phyB and AtBBX4 directly interact to promote the accumulation of AtBBX4 [7]. AtBBX4 inhibits the transcriptional activity of PIF3, thereby promoting photomorphogenesis under red light. The interaction between AtBBX20, AtBBX21, AtBBX22, AtBBX23, and HY5 significantly enhances HY5's transcriptional activity, ultimately facilitating the promotion of photomorphogenesis [8]. The transcriptional feedback loop composed of AtBBX28, AtBBX29, AtBBX30, AtBBX31, and HY5 inhibits photomorphogenesis [9]. Therefore, BBX proteins receive signals from photoreceptors under different light conditions, further cooperating with other proteins to affect the plant's adaptive response to the light environment and enhance plant stress resistance.



Figure 2. The Mechanisms of BBXs Regulating Flowering

BBXs also have a significant role in controlling flowering [4]. In Arabidopsis, AtBBX1, AtBBX6, and AtBBX24 can promote flowering. In contrast, AtBBX4, AtBBX5, AtBBX7, AtBBX10, AtBBX19, AtBBX28, AtBBX30, AtBBX31, and AtBBX32 are involved in delaying flowering. AtBBX1/CO interacts with the FLOWERING LOCUS T (FT) promoter to activate FT, promoting flowering. The CO-FT module is differentially influenced by various BBXs. These BBXs mainly affect the transcription

level of CO/AtBBX1 and the binding of CO/AtBBX1 to FT. AtBBX19 binds to CO to form a heterodimer, inhibiting the activity of CO on FT. AtBBX28 inhibits flowering by physically interacting with CO and inhibiting CO binding to the FT locus. AtBBX30 and AtBBX31 interact with CO and use an additional PFVFL motif to attract TPL/TPR corepressor proteins, effectively reducing the function of CO. Overexpression of AtBBX30 and AtBBX31 results in a significant delay in flowering. Through these mechanisms(as shown in **Figure 2**), BBX proteins can strictly regulate flowering time, ensuring seed development under favorable environmental conditions. This series of mechanisms is crucial for reproductive success and can also enhance plant stress resistance [10].

As plants grow, they face a range of environmental stress factors, including drought, salinity, flooding, extreme temperatures, and other abiotic stresses. Beyond their involvement in growth and development, research has indicated that BBX proteins also participate in signaling pathways triggered by both abiotic and biotic stress conditions [4,5]. AtBBX24, known as a salt tolerance protein (STO), participates in salt stress signaling [11]. AtBBX25 and AtBBX21/STH2 are salt-tolerance homologs (STH). They enhance plant salt tolerance. In Arabidopsis, the ability to tolerate freezing is directly associated with the biosynthesis and metabolism of anthocyanins, flavonoids, and aromatic compounds. AtBBX7 and AtBBX8 improve freezing tolerance by controlling the genes that are involved in the synthesis and metabolism of these substances [12]. AtBBX18 and AtBBX23 promote thermomorphogenesis by reducing the accumulation of ELF3. Under high temperatures, the transcription levels of AtBBX18 and AtBBX23 are elevated. Although this promotes thermomorphogenesis, overexpression of AtBBX18 inhibits heat tolerance in plants [13, 14].

BBX proteins significantly contribute to regulating the plant life cycle and boosting plant resistance to stress, including but not limited to the processes mentioned above of photomorphogenesis, flowering, and environmental stress. As transcription factors, BBX proteins directly participate in regulating gene expression, fundamentally influencing plant traits.

# 4. The Interaction and Comparison of BBXs and Other Transcription Factors

BBXs are involved in a complex regulatory network with other transcription factors. As transcription factors, they can interact with each other through protein-protein interactions to influence the transcription of target genes; they can also bind to each other's promoters, thereby affecting the expression of target transcription factor genes. Therefore, the function of BBXs relies heavily on the actions of other essential transcription factors.

HY5, a bZIP protein and transcription factor, enhances photomorphogenesis by regulating the expression of more than 3000 genes in response to various light conditions. BBXs and HY5 interact and regulate each other. In Arabidopsis, AtBBX21, AtBBX22, AtBBX23, AtBBX24, AtBBX25, and AtBBX28 directly bind to HY5, regulating its transcription process, thereby controlling photomorphogenesis [15,16,17,18,19]. Additionally, the T/G-box cis-element in the HY5 promoter can directly bind to AtBBX21, activating HY5 transcription. HY5 protein can also interact with AtBBX22 promoter, activating AtBBX22 expression [20]. Conversely, the promoters of AtBBX30 and AtBBX31 are bound by HY5, leading to the repression of their transcription [21]. Overall, the AtBBX family and HY5 form an intricate and complex transcriptional regulatory network, primarily playing a role in mutual regulation during photomorphogenesis, which affects plant growth and development and enhances plant stress resistance.

ABI5 is another member of the bZIP family and acts as a transcription factor influencing seedling growth post-germination. It has a strong connection with two microproteins in Arabidopsis, AtBBX30 and AtBBX31. ABI5 protein can directly bind to the promoters of AtBBX30 and AtBBX31, regulating their transcription. Additionally, ABI5 can enhance its stability by binding with AtBBX30 and AtBBX31. Although AtBBX30 and AtBBX31 do not directly interact with the ABI5 promoter, they can promote the binding of ABI5 to its binding domains, thereby enhancing the function of ABI5 [22]. Moreover, AtBBX19 can bind to the GT1-motif in the ABI5 promoter, inducing ABI5 transcription and subsequently inhibiting seed germination [23]. AtBBX21 can bind to both HY5 and ABI5, inhibiting its transcription by preventing the binding of ABI5 to its promoter [24].

Therefore, the interactions between BBXs and other transcription factors are intricate, complex, and crucial. It can be summarized that most BBX family proteins primarily function as transcriptional regulators. They do not directly interact with the promoters of functional target genes to activate their transcription; instead, they bind to other transcription factors or the promoters of other transcription factors, regulating the activity of other transcription factor proteins to achieve their ultimate function, thereby enhancing plant stress resistance.

# 5. Conclusion

BBX proteins, as essential transcription factors, interact with other transcription factors to impact plant growth. They play a vital role in enhancing plant stress resistance by regulating stress-responsive genes and pathways. However, the mechanisms of BBX in different plant species, especially crops, still need to be fully understood. Nonetheless, the critical roles of BBX in photomorphogenesis, flowering, drought resistance, cold resistance, heat resistance, and the biosynthesis of anthocyanins and other compounds demonstrate its significant potential in developing high-yield, stress-resistant transgenic crops. Preliminary successes have already been achieved in this area. Recent studies suggest that SIBBX20 plays a vital role in regulating carotenoid biosynthesis, positioning it as a novel target for the genetic enhancement of the nutritional quality of tomato fruit [25]. The COL protein OsCOL9 is potentially a pathogen-responsive transcription factor in rice, as demonstrated by transgenic rice plants with overexpressed OsCOL9 exhibiting notably increased resistance to rice blast disease. Conversely, plants with the oscol9 gene knocked out show increased susceptibility [26]. The interaction mechanisms between BBXs and other plant transcription factors still need to be confirmed. Once effective results are achieved, modern biotechnological approaches such as transgenics and CRISPR-mediated genome editing could be used to manipulate BBX genes, potentially leading to the development of superior crop varieties to meet agricultural production needs.

Saccharomyces cerevisiae is an ideal platform for reconstructing various biosynthetic pathways, producing plant metabolites, including phytohormones like carvacrol and gibberellin. The current approach to engineering yeast for the production of plant metabolites mainly involves the direct insertion of functional protein genes. A deeper understanding of the interaction mechanisms of transcription factors like BBX might allow exogenous transcription factor genes to be inserted into yeast. Utilizing exogenous transcription factors to influence yeast gene expression could establish efficient biosynthetic pathways to obtain desired metabolites. This could potentially lead to a new direction in the research of engineering yeast, supporting the further development of cell factories for plant metabolites. Consequently, the value of BBX family proteins could be exploited not only in the plant kingdom but also in exploring their potential value in fungi, especially in yeast.

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