



Review

Beyond Arabidopsis: BBX Regulators in Crop Plants

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Abstract: B-box proteins represent diverse zinc finger transcription factors and regulators forming large families in various plants. A unique domain structure defines them—besides the highly conserved B-box domains, some B-box (BBX) proteins also possess CCT domain and VP motif. Based on the presence of these specific domains, they are mostly classified into five structural groups. The particular members widely differ in structure and fulfill distinct functions in regulating plant growth and development, including seedling photomorphogenesis, the anthocyanins biosynthesis, photoperiodic regulation of flowering, and hormonal pathways. Several BBX proteins are additionally involved in biotic and abiotic stress response. Overexpression of some *BBX* genes stimulates various stress-related genes and enhanced tolerance to different stresses. Moreover, there is evidence of interplay between B-box and the circadian clock mechanism. This review highlights the role of BBX proteins as a part of a broad regulatory network in crop plants, considering their participation in development, physiology, defense, and environmental constraints. A description is also provided of how various BBX regulators involved in stress tolerance were applied in genetic engineering to obtain stress tolerance in transgenic crops.

Keywords: B-box proteins; transcription factors; growth; development; stress response; crop plants



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1. Introduction

Intricate regulation of plant growth and development processes depends mainly on precise spatial and temporal control of gene expression mediated by chromatin modifications in reply to endogenous or external stimuli in the environment. Recognition of the transcriptional profile of genes encoding most plant-specific transcription factors and chromatin regulators is fundamental for understanding and elucidating many plant biological processes. Recent studies have demonstrated significant findings regarding B-box (BBX) proteins, representing a diverse group of zinc finger transcription factors and regulators based on their structure and functions.

The *BBX* genes have been present in all eukaryotic genomes analyzed so far, with the highest number of members within all kingdoms. The availability of complete plant genomic sequences has led to the identification of the B-box (*BBX*) gene family, consisting of 64 *BBX* representatives in apple, 37 in white pear, 32 in Arabidopsis, 30 in rice and potato, 29 in tomato, 25 in pear, and 24 in grapevine [1–8]. Regardless of the species, all BBX family members have one single B-box domain or two arranged in tandem, classified into two types, known as B-box1 (B1) and B-box2 (B2), depending on their consensus sequence and the distance between the zinc-binding residues. Some BBX proteins also possess CCT-domain and several conserved motifs localized outside the domains mentioned above [9,10].

In plants, especially in Arabidopsis, the BBX family has been significantly expanded and functionally well-characterized. Considering the importance of crops, the study of BBX proteins in these plants has become more intense. The diverse functions of BBX in plant growth and development range from the involvement in seedling photomorphogenesis [1,10–17], seed germination, photoperiodic regulation of flowering [18,19],

and shade avoidance [20–22] to responses to biotic and abiotic stresses that have been studied [2,23–26].

Here, we provide a brief story of the functionally characterized B-box-type zinc finger proteins specific to crop plants and emphasize recent evidence supporting their essential functions in plant development and stress response. This review highlights a crucial role of BBX proteins as part of a broad regulatory network in crops.

2. Structural Characteristics of BBX Genes

All of the plant BBX proteins have a standard feature, which is a B-box domain. B-box domains are members of zinc finger (ZF) domains, which are some of the most structurally varied among protein domains [27]. Initially, the B-box domain had been classified as a member of the zinc ribbon fold group. However, based on the currently available sequences and structures, it has been transferred to the RING-like treble clef family [28]. It is worth mentioning that there are no reports regarding structural studies carried out on the B-box so far.

The Function of the B-Box Domains and Conserved Motifs Outside

The structural-level B-box domain has around 40 amino acids in its length, and they fall into two types, 1 and 2. These two types differ in the consensus sequence and the space of the seven or eight Zn(II)-binding residues [29]. The B-box domain has a vital role in protein–protein interaction and mediating transcriptional regulation [30].

Some BBX proteins also have a CCT domain (CONSTANS, CO-LIKE, TIMING OF CAB1: TOC1) [31]. Initially, the presence of a CCT domain was found in CONSTANS (CO), CO-LIKE, and TIMING OF CAB1 (TOC1) proteins in *Arabidopsis thaliana*, which act as critical flowering regulators. This domain has 42–43 amino acid residues localized at the C-terminus. Comparison of the amino acid sequence of BBX proteins in different plant species revealed that the CCT domain is highly conservative [31]. Many reports have shown that the CCT domain has an essential function in transcriptional regulation [32,33]. The nuclear localization signal (NLS) is a part of the CCT domain and plays a crucial role in locating BBX protein in the nucleus [17].

Besides B-box and CCT domains, Holm et al. [9] discovered a binding sequence motif for BBX protein–protein interaction called the VP motif. The VP (VALINE–PROLINE) motif consists of six amino acids with consensus sequence G-I/VV-P-S/T-F, located at the protein molecule's carboxy end, separated by 16–20 amino acids from the CCT domain [9].

Additionally, seven new motifs (M1–M7) specific to each structural group were identified [31]. However, their role has not yet been defined, except for the M6 motif, which can significantly impact the functional determination of BBX proteins belonging to the same structural group. The conversion of motive M6 from AtBBX21 to M6 from AtBBX24 has abolished its function in promoting photomorphogenesis [34].

3. Look into the Genomes: BBX Family from Arabidopsis to Crops

Arabidopsis, as a model plant, has been extensively investigated for BBX proteins. A transcription factor, CONSTANS (CO), involved in the flowering pathway, was the first identified B-box protein [35]. The B-box family in *Arabidopsis thaliana* consists of 32 proteins. Characterizations of 16 *COL* (*CONSTANS-like*) genes and 8 *DBB* (*Double B-box*) genes by Robson et al. [32] and Kumagai et al. [27], respectively, have made an outstanding contribution in collecting members of the B-box family in this model plant. To provide a uniform nomenclature for the B-box protein family, Khanna et al. [1] published a complete set of all *Arabidopsis* genes with B-box motifs. Since that report, B-box protein families have been rapidly identified and characterized in other plant species. A genome-wide survey of BBX genes considered chromosome localization, gene structures, conserved domains, phylogenetic relationships, subcellular localizations, promoter *cis*-regulatory elements, and expression patterns under the diurnal cycle and stress or hormone treatments.

3.1. Classification of BBX Genes in Crops

The *BBX* gene family can be divided into five structural groups depending on one or two B-box domains and the CCT domain (Figure 1). The first and second groups consist of proteins with two B-box domains and a CCT domain. Additionally, the VP motif is composed of six amino acid residues, localized in C termini, and has been established as belonging in the first group. In the third group, proteins have one B-box and one CCT domain. The fourth group consists of two tandem B-box domains, and in the fifth group, proteins have a single B-box domain. Crocco and Botto [31] conducted a comprehensive evolutionary analysis of the *BBX* protein family in 12 plant species that started from green algae and ended with dicots. The results showed that each of the five *BBX* protein groups evolved independently during plant evolution. Some literature has distinguished *BBX* proteins in separate subfamilies, including *COL* (CO-like) and *DBB* (double B-box). Indeed, the *COL* family contains proteins with double B-box and CCT domains, and they are homologs of the *CONSTANS* protein. The *DBB* proteins lack the CCT domain and have two tandem-localized B-box domains in the sequence.

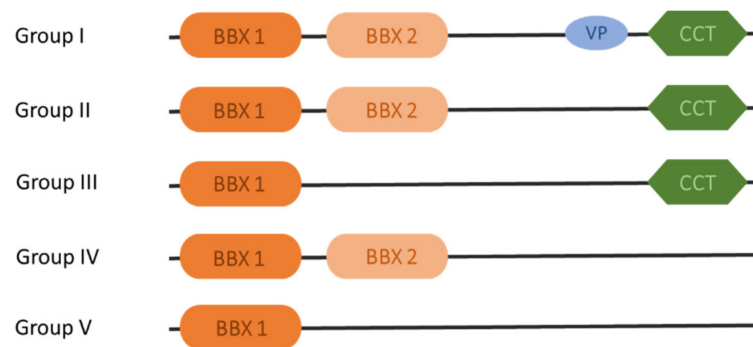


Figure 1. Scheme of B-box (*BBX*) proteins with main domains in each structure group.

3.1.1. Cereal Crops

Rice was the first crop to have the whole B-box protein family identified [2]. In this plant, 30 *OsBBX* genes have been identified and named according to rice chromosomes position. *OsBBX* genes are distributed in all chromosomes, omitting chromosomes 10 and 11 (Table 1). A segmental duplication analysis showed that 18 *OsBBXs* are located in the chromosomes duplicated segmental regions [2].

Table 1. *BBX* gene distribution on crop plants chromosomes.

Plant Species	No. of <i>BBX</i> Genes	No. of Chromosomes Having <i>BBX</i> /No. of Plant Chromosomes	Chromosome with no <i>BBX</i>	References
Rice (<i>Oryza sativa</i>)	30	10/12	10 and 11	[2]
Maize (<i>Zea mays</i>)	31	9/10	8	[36,37]
Apple (<i>Malus domestica</i>)	64	15/17	4 and 15	[6]
Pear (<i>Pyrus bretschneideri</i>)	25	12/17	1,2,4,7,12	[5]
Pear (<i>Pyrus pyrifolia</i>)	39	nd/17	nd	[38]
Rose (<i>Rosa chinensis</i>)	22	6/7	1	[39]
Peach (<i>Prunus persica</i>)	20	6/8	3 and 6	[39]
Strawberry (<i>Fragaria vesca</i>)	21	6/7	7	[39]
Sweet cherry (<i>Prunus avium</i>)	22	6/8	2 and 6	[39]
Black raspberry (<i>Rubus occidentalis</i>)	20	6/7	7	[39]
Tomato (<i>Solanum lycopersicum</i>)	29	11/12	11	[3]

Table 1. Cont.

Plant Species	No. of <i>BBX</i> Genes	No. of Chromosomes Having <i>BBX</i> /No. of Plant Chromosomes	Chromosome with no <i>BBX</i>	References
Potato (<i>Solanum tuberosum</i>)	30	10/12	11	[4]
Cotton (<i>Gossypium hirsutum</i>)	42	18/18	-	[40]
Grapevine (<i>Vitis vinifera</i>)	24	11/19	2,6,8,10,13,15,16,17	[8]
Wild peanut (<i>Arachis duranensis</i>)	24	9/10	2	[41]
Banana (<i>Musa acuminata</i>) *	25	7/11	4,5,6,8	[42]
Soyabean (<i>Glycine max</i>) *	26	nd/10	nd	[43]
Leek (<i>Allium porrum</i>) *	17	nd/8	nd	[44]
Sugar beet (<i>Beta vulgaris</i>) *	10	nd/9	nd	[45]

* only *COL* genes; nd—no data available.

Maize (*Zea mays*) includes 19 *COL* [36] and 12 *DBB* [37] genes in its genome. All *BBX* genes are distributed on 9 of 10 maize chromosomes, except chromosome 8, and their nomenclature refers to their position on chromosomes (Table 1). Eight *ZmCOL* gene pairs have been identified to be involved in segmental duplications. Simultaneously, the expansion of the maize *ZmDBB* gene family occurred at the same duplication event.

3.1.2. Rosaceae Species

According to phylogenetic relationships and domains, 64 *BBX* genes in the apple genome are divided into five groups. This number of genes is significantly large compared to *BBX* genes in other plants, and it suggests that tandem, segmental, or genome-wide duplication in apple might cause this phenomenon [6]. A total of 50 genes have been mapped into 15 of 17 apple chromosomes (Table 1). Identifying the chromosome position for the remaining 14 genes was not successful, probably caused by incorrect assembling of genomic sequences.

In the pear genome (*Pyrus bretschneideri* Rehd.), 25 *BBX* genes have been identified, clustered in five groups, and sequentially named [5]. All the *PbBBX* genes are distributed among 12 of the total 17 pear chromosomes. The presence of segmental duplication for 13 gene pairs and no single tandem duplication is characteristic of *BBX* genes in this species. By contrast, in other *Pyrus* species, *Pyrus pyrifolia*, a total of 39 *BBX* family members were identified and were named according to the chromosomal distribution [38].

Shalmani et al. [39] have recently identified the *BBX* gene family in other *Rosaceae* species like rose, peach, strawberry, sweet cherry, and black raspberry with 22, 20, 21, 22, and 20 *BBX* members, respectively.

3.1.3. Solanaceae Species

In tomato, 29 putative *BBX* genes have been identified and named according to their homology to *Arabidopsis* *BBX* genes. The whole family is distributed within all chromosomes except for chromosome 11. The nuclear location of most tomato *BBX* proteins have been envisaged using in silico analysis, and has been confirmed for seven of them by *Arabidopsis* mesophyll protoplast assay [3].

A comparable number of *BBX* genes, 30, have been discovered in potato, and numbered based on *BBX* and *CCT* domains length and presence. Except for chromosome 11, potato *StBBX* genes are widely distributed in the whole genome [4].

3.1.4. Other Crops

In cotton, 42 *GhCOL* genes were identified in the genome, distributed unevenly along 18 different chromosomes. Phylogenetic analysis clustered them into three groups, whereby 14 *COL* genes in group I showed conserved structure compared with other plants. Analysis

of gene expression patterns in group I concluded that these genes are potentially involved in photoperiodic flowering and light signaling regulation [40].

Comprehensive bioinformatics analysis of whole genomes of grapevines led to the detection of 24 *BBX* genes, of which 22 genes are evenly distributed in 11 of the 19 chromosomes, while the two genes are not assigned to any position [8].

The same number of *BBX* genes, 24, were detected on nine of the ten chromosomes in a wild peanut [41].

In bananas, 25 *COL* genes belong to group I–III. Nine genes from group I were investigated by Chaurasia et al. and the results showed that those genes are highly conserved in structure compared to members in other plants [42].

In soybeans, 26 *CO*-like genes are classified into three clades, comprising 13 homologous pairs [43]. On the contrary, only 17 putative *COL* genes were identified in leek, a herbaceous plant belonging to the *Amaryllidaceae* family [44]. Four of these leek *COL* genes show high sequence similarity with key factors modulating the heading date in barley and rice.

Sugar beets have been demonstrated to possess at least 10 *CONSTANS-LIKE* genes. However, these data are based on ESTs collection availability, whereas the sugar beet genome sequence was published a few years after that [45]. Therefore, it is expected that a larger number of genes may be identified.

4. Time to Switch from Vegetative to Generative Development

Strict regulation of flowering time is essential for plant reproductive success, enabling seed development completion in beneficial environmental conditions [46]. The photoperiodic flowering induction mechanism has been best recognized and characterized in *Arabidopsis thaliana*, where the FLOWERING LOCUS T (FT) and the CONSTANS (CO/*BBX1*) are the critical elements [47–49]. Research has shown that the *AtBBX1*, the first identified and characterized protein belonging to the *BBX* family, plays an essential role in regulating flowering time and flower development [19]. Besides, several other proteins belonging to the *BBX* family also perform a crucial role in regulatory networks, controlling floral transition and flower formation in *Arabidopsis*, including *AtBBX4/COL3* [50], *AtBBX6/COL5* [51], *AtBBX7/COL9* [52], *AtBBX10/COL12* [53], and *AtBBX17/COL8* [54].

Undoubtedly, less is known about the function of *BBX* proteins in crop growth and development. However, many *BBX* proteins in plants other than *Arabidopsis* are also likely to play a role in these processes. In rice, the short day (SD) plant, the GI–CO–FT regulatory pathway is conserved and flowering time is mutually regulated by two different photoperiodic pathways, in which several *BBX* members act as flowering inducers or repressors (Figure 2). The rice *CO* ortholog, *Hd1* (HEADING DATE 1)/*OsBBX18*, promotes flowering under inductive short-day conditions by regulating the *Heading date 3a* (*Hd3a*) and *Rice FT-like 1* (*RFT1*) florigen genes [55]. *Hd3a* is also induced by another flowering activator, *Ehd1* (Early heading date 1), which functions independently of *Hd1* under SD conditions. Meanwhile, under noninductive long-day conditions, *Hd1* turns into a flowering repressor and affects the expression of *Hd3a*. Another key repressor of flowering in rice is a small protein termed *Ghd7* (Grain number, plant height, and heading date 7), which acts as an LD-specific repressor of *Ehd1* expression. So far, other proteins belonging to the *BBX* family in rice that may negatively affect flowering under two different photoperiodic conditions have been identified (Figure 2). Among them, *OsBBX5*(*OsCOL4*), *OsBBX7*(*OsCOL9*), *OsBBX10*(*OsCOL10*), and *OsBBX23*(*OsCOL13*) repress flowering by reducing the expression of *FT*-like genes and heading date through *Ehd1* (Early heading date 1) [56–59]. Moreover, some *BBX* proteins, including *OsBBX10*(*OsCOL10*) and *OsBBX26*(*OsCOL15*), act downstream of *Ghd7* repressor, reducing expression of *Ehd1* [58–60]. In maize, a typical SD plant, the most critical period in the whole development is the flowering time that determines the size of the cob formed by the plant and its filling with grain to a significant extent. In this species, the B-box-type gene corresponding to the *Arabidopsis CO*, called *Conz1*, activates the *FT*-like *ZCN8*, which functions as a floral inductor involved in photoperiod sensitivity in maize [61,62]. The *AtCO* gene homologs, *SbHd1*, *HvCO1*, and *HvCO9*, have also been

found in other cereal crops, such as sorghum and barley, respectively, representing the long-day (LD) plants [63–65]. Under LD conditions, *SbHd1* activates flowering by inducing *SbCN8* and *SbCN12* (orthologs of maize *ZCN8* and *ZCN12*, respectively) [63], while *HvCO1* and *HvCO9* are involved in the activation of *FT*-like genes required for flowering induction in barley [65,66].

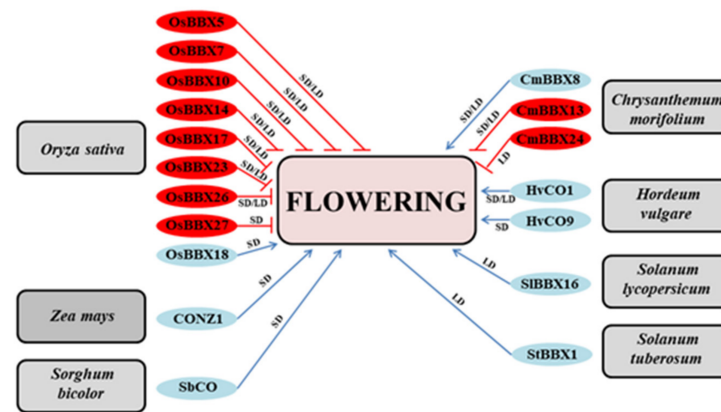


Figure 2. Involvement of B-box proteins as positive (blue) and negative (red) regulators of flowering in various crop plants including *O. sativa*: OsBBX5 (ID: Os02g0610500) [56], OsBBX7 (ID: Os02g0724000) [57], OsBBX10 (ID: Os03g0711100) [58], OsBBX14 (ID: Os05g0204600) [67], OsBBX17 (ID: Os06g0264200) [68], OsBBX18 (Os06g0275000) [55], OsBBX23 (ID: Os07g0667300) [59], OsBBX26 (ID: Os08g0536300) [60], OsBBX27 (ID: Os09g0240200) [69]; *Z. mays*: CONZ1 (ID: ABW82153) [61]; *S. bicolor*: SbCO (ID: Sb10g010050) [63]; *C. morifolium*: CmBBX24 (ID: KF385866) [64], CmBBX13 (ID: KP963935) [70], CmBBX8 (ID: KP96393) [71]; *H. vulgare*: HvCO1 (ID: AF490467) [65], HvCO9 (ID: AY082965) [66]; *S. lycopersicum*: SIBBX16 (ID: Solyc12g005750.1) [72]; *S. tuberosum* (ID: AM888389.1) [73]. LD—long day, SD—short day.

AtCO homologs in different species, including potato [74], ryegrass [75], grape [76], and alfalfa [77], are also presumably involved in photoperiodic flowering induction. Additionally, in potatoes, *StCO* regulates photoperiodic tuberization in a graft-transmissible manner [78]. The genes corresponding to the tomato *FT* homolog, designated *StSP6A* and *StSPD3*, have been identified in wild potato species *S. tuberosum ssp. andigena* [73,79,80]. As shown, the *StSP6A* encodes a protein promoting tuber formation [73], while *StSPD3* encodes a protein promoting floral development [79,80]. Potato *StCOL1* (BBX1) protein controls *StSP6A* expression through direct activation of an additional *FT* family member, while *StSP5G*, which acts as a repressor of *StSP6A* in leaves, mediates the strict short-day (SD) requirement of *andigena* plants for tuberization [73].

Other *BBX* genes, apart from *BBX1/CO*, are implicated in regulating flowering time in crops as well. In tomato *SIBBX16*, the closest homolog of Arabidopsis microprotein 1b interacts with *TCMP-2*, a small family member of tomato cystine-knot proteins, and affects tomato flowering [81]. Arabidopsis plants ectopically overexpressing the *TCMP-2* exhibited an increased level of FLOWERING LOCUS T (*FT*) mRNA and anticipated flowering. Previously, the same authors revealed that transgenic tomato plants *pTCMP-2::TCMP-1* with increased *TCMP-2* expression in flower buds showed accelerated termination of the sympodial units [72]. In chrysanthemum, three B-box proteins have been identified so far that play a positive or negative role in regulating flowering time. One of them, CmBBX8, stimulates flowering in summer-flowering chrysanthemum grown under LD conditions [71], while CmBBX13 and CmBBX24 proteins cause late flowering under long- and/or short-day conditions [64–66,70,72–81].

Understanding the flowering mechanisms and the role of B-box proteins in the photoperiodic flowering pathways in various crops is a crucial interest. Although the regulatory network triggering flowering is conserved in many species, the function of BBX acting

downstream of the photoperiod response to accelerate or prevent floral initiation may vary significantly among plants.

5. Crops *BBX* Genes in the Anthocyanins Biosynthesis

Anthocyanins are pigments responsible for the red to black color of plant organs, such as flowers or fruits. Besides visual effects and market value, the accumulation of anthocyanins in organs is connected with biotic or abiotic stress, such as viral pathogens, wounding, or drought [82,83]. Those pigments also take part in protection against photo-oxidative and heat damage [84]. A few apple *BBX* proteins, including *MdBBX1*, *MdBBX20*, and *MdBBX33/MdCOL11* are regulator factors of anthocyanin synthesis. *MdBBX33* is a close homolog of Arabidopsis *AtBBX22*, and its overexpression causes an increased anthocyanin level in Arabidopsis seedlings [85]. *MdBBX33* protein regulates anthocyanin accumulation, influencing the red skin color of apples in a light and temperature-dependent manner. Both low temperature and UV-B light correlate with upregulation of *MdBBX33* expression and positively affect anthocyanin accumulation in apple fruits [85]. Furthermore, the expression of two anthocyanin accumulation-responsible genes, *MdMYBA* and *MdbHLH*, increase in the fruit ripening stage, which is associated with the increase of *MdBBX33* transcript level.

On the contrary, another *BBX* gene, *MdBBX1*, when overexpressed in apple, does not directly increase anthocyanin accumulation. However, *MdBBX1* may activate two essential genes—an MYB activator, *MYB10*, and the anthocyanin biosynthetic gene *DRF* (*DIHYDROFLAVONOL 4-REDUCTASE*)—by binding to a CCAAT motif present in their promoter region, ultimately leading to increased anthocyanin levels [86]. Moreover, expression patterns of some other *BBX* genes in apples, such as *MdBBX15*, *MdBBX17*, *MdBBX35*, *MdBBX51*, and *MdBBX54*, are correlated with anthocyanin induction in apple fruit skin [86]. Transactivation assays on the *MYB10* promoter revealed that these *BBX* proteins could function as activators via direct induction of the apple anthocyanin-regulating *MYB10* [86].

Studies on apples revealed that ultraviolet treatments promote some *BBX* transcription factors, which activate the expression of main anthocyanin biosynthetic genes and ultimately lead to increased anthocyanin levels. One of them is *MdBBX20*, which stimulates anthocyanin accumulation under ultraviolet radiation and low-temperature conditions. Overexpression of *MdBBX20* caused increased anthocyanin accumulation in transformed calli [87]. Furthermore, *MdBBX20* interacts by its B-box2 domain with transcription factor *HY5*, and in complexes, it regulates transcription of *MdMYB1/MdMYB10*, the anthocyanin key regulator concentrations, by binding its G-box *cis*-element. *MdBBX22* is another UV-inducible protein that directly interacts with *MdHY5* and enhances the binding to key anthocyanin synthesis factors, *MdMYB10* and *MdCHS*. Overexpression of *MdBBX22* has been shown to induce anthocyanin biosynthesis [88,89]. Interestingly, *MdBBX24*, *MdBBX33*, *MdBBX37*, and *MdBBX48* also interact with *MdHY5*, suggesting that numerous *BBX* might be entangled with anthocyanins synthesis [88,89].

It is also worth mentioning that some *BBX* members in apples, including *MdBBX20*, *MdBBX22*, *MdBBX23*, *MdBBX24*, *MdBBX25*, *MdBBX33*, and *MdBBX43*, interact with *MdBT2* protein, known as a negative regulator of the UV-B-induced anthocyanin biosynthesis. An et al. [88] revealed that *MdBT2* degrades *MdBBX22* protein through the 26S proteasome pathway and the other members of the *BBX* family might be ubiquitination substrates for *MdBT2*.

So far, two *BBX* proteins have been identified that act as positive regulators of anthocyanin accumulation in a red pear. One of them is nuclear-localized protein, *PpBBX16*, a close homolog of *AtBBX22*, that favorably controls anthocyanin production in light-induced conditions via activating *PpMYB10* [38]. However, *PpBBX16* cannot directly bind the promoter of *PpMYB10* and requires the presence of *PpHY5* to achieve complete functionality. Moreover, *PpBBX16* can promote the expression level of anthocyanin-related genes, such as *PpCHI*, *PpCHS*, and *PpDFR*, as was shown in the dual-luciferase assay introduced in tobacco. Overexpression of *PpBBX16* in Arabidopsis seedlings increased anthocyanin content in the hypocotyls and tops of flower stalks. Furthermore, other *BBX* protein

PpBBX18 also physically interacted with PpHY5, thus inducing transcription of *PpMYB10* and consequently regulating anthocyanin biosynthesis in *Arabidopsis* and pear [38].

Besides positive regulators of anthocyanins biosynthesis, BBXs also play a role as negative regulators. In apples, MdBBX37 was indicated as an inhibitor of anthocyanin biosynthesis. Its interactions with pivotal positive regulators MdMYB1 and MdMYB9 block the binding to their target genes. Also, it acts as a suppressor of *MdHY5* expression by binding to its promoter [88,89]. Meanwhile, in pears, PpBBX21 protein directly interacts with PpBBX18 or PpHY5, inhibits PpBBX18-PpHY5 complex formation, and represses anthocyanin biosynthesis [90].

BBX proteins are involved in the precise control of anthocyanin synthesis by binding to HY5 and transcriptional regulation of *MYB10*. Likewise, modulation of expression of other essential genes involved in anthocyanin production provides new insights into the multifunctionality of these factors. However, many questions remain to be answered to fill the knowledge gaps on light-induced anthocyanin biosynthesis.

6. Involvement of the BBX Proteins in Stress Response and Hormonal Pathways

Many reports have indicated that BBX proteins are involved in the signaling pathway induced by abiotic stresses, including low temperature, high salinity, drought, and heat. Some BBX proteins might be engaged in responses to several abiotic stress factors. In *Arabidopsis*, AtBBX18 negatively regulates thermotolerance through modulation of the expression of heat-stress-responsive genes, such as *DGD1*, *Hsp70*, *Hsp101*, and *APX2* [25]. Another *Arabidopsis* B-box protein, AtBBX24/STO, enhances the growth of roots in high salt conditions [24]. Regulation of gene expression at the transcriptional level is mostly mediated by sequence-specific binding of transcription factors to the *cis*-acting promoter elements. Numerous BBX genes contain several putative stress-related *cis*-acting elements, such as MBS, ARE, LTR, and HSE. The transcript level of many BBX genes are altered under different stress conditions, as shown by transcription profiling (Table 2). Thus BBX proteins seem to be essential factors that integrate various signal transduction pathways, replying to diverse stresses and engaging in many cellular processes. However, only a few BBX proteins have been proven to be associated with responses to stress factors so far.

Table 2. BBX genes in various crop species and their known transcript positive response to various abiotic stresses and hormones.

Gene	Accession No./ID	Response to Abiotic Stress and Hormones									Species	References
		Cold/ Chilling	Drought	Salt	Dehydration	Heat	ABA	GA	SA	MeJA		
<i>VvZFPL</i>	HQ179976	+									<i>Vitis vinifera</i>	[91]
<i>SsBBX24</i>	ABC25454	+		+							<i>Solanum sogarandinum</i>	[26]
<i>MdBBX10</i>	MDP0000733075			+							<i>Malus domestica</i>	[92]
<i>OsBBX1</i>	Os01g0202500	+	+	+							<i>Oryza sativa</i>	[93]
<i>OsBBX2</i>	Os02g0176000	+	+	+			+	+	+		<i>Oryza sativa</i>	[93]
<i>OsBBX7</i>	Os02g0724000						+	+	+		<i>Oryza sativa</i>	[93]
<i>OsBBX8</i>	Os02g0731700	+	+	+							<i>Oryza sativa</i>	[93,94]
<i>OsBBX14</i>	Os05g0204600						+				<i>Oryza sativa</i>	[93]
<i>OsBBX17</i>	Os06g0264200							+	+	+	<i>Oryza sativa</i>	[93]
<i>OsBBX19</i>	Os06g0298200	+	+	+				+	+	+	<i>Oryza sativa</i>	[93]
<i>OsBBX24</i>	Os08g0178800	+	+	+				+	+	+	<i>Oryza sativa</i>	[93]
<i>SIBBX1</i> *	Solyc02g089520.1	+									<i>Solanum lycopersicum</i>	[3]
<i>SIBBX7</i> **	Solyc12g006240.1					+					<i>Solanum lycopersicum</i>	[3]
<i>SIBBX16</i> ***	Solyc12g005750.1			+							<i>Solanum lycopersicum</i>	[3]
<i>MaCOL1</i>	JQ314345	+									<i>Musa nana</i>	[95]
<i>Cm-BBX24</i>	KF385866	+			+						<i>Chrysanthemum morifolium</i>	[64]
<i>IbBBX24</i>	MH813941									+	<i>Ipomoea batatas</i>	[96]

Solanum lycopersicum BBX genes with the same type of responses are marked with asterisks: * *SIBBX3* (Solyc02g089540.2), * *SIBBX9* (Solyc07g045180.2), * *SIBBX19* (Solyc01g110370.2), * *SIBBX21* (Solyc04g081020.2), * *SIBBX28* (Solyc12g005660.1), * *SIBBX29* (Solyc02g079430.2); ** *SIBBX11* (Solyc09g0074560.2), ** *SIBBX12* (Solyc05g024010.2), ** *SIBBX15* (Solyc05g009310.2); *** *SIBBX17* (Solyc07g062620.1), *** *SIBBX18* (Solyc02g084420.2), *** *SIBBX24* (Solyc06g073180.2).

Plants in nature are also exposed to biotic stresses covering a broad spectrum of plant pathogens. Present knowledge indicates that BBX regulators may also participate in the control of plant defense responses. Unfortunately, the understanding of the role of BBX proteins in this process is still in its infancy. The expression of a rice gene, *OsCOL9*, encoding a BBX protein belonging to group II of the COL protein family, has been shown to be enhanced at the mRNA level after *Magnaporthe oryzae* infection. Moreover, transgenic *OsCOL9* knock-out rice plants showed increased pathogen susceptibility [97]. The expression of a banana gene, *MaCOL1*, increased after infection by *Colletotrichum musae* [95]. Overexpression of *IbBBX24* gene significantly increased *Fusarium* wilt disease resistance in cultivated sweet potatoes [96].

Some BBX family members also play essential roles in hormone signaling pathways. There are many reports documenting the response of Arabidopsis *BBX* genes to plant hormones and the involvement of these proteins in many hormonal pathways [98]. Moreover, the transcript accumulation of several *BBX* genes in crops is elevated in response to exogenous treatment of phytohormones, including ABA, GA, JA, and SA (Table 2). Most of these genes possess one or more well-defined hormone-responsive elements in their promoter sequences, like ABRE (ABA—responsive element), ERE (ethylene responsive element), CGTCA-motif and TGACG-motif (MeJA responsive elements), which respond to different hormonal pathways [2,3]. Interestingly, in bananas, *MaCOL1* protein can mediate cross-talk between signaling pathways in response to biotic and abiotic stresses since the accumulation of *MaCOL1* transcript was enhanced by chilling and pathogen infection [95].

Thus, transcriptomic analyses using macro- and microarray approaches are excellent tools for identifying new genes related to plant responses to different stresses and exogenous hormone treatments. However, the alternations in gene expression are frequently not reflected at the protein level. Therefore, the dynamic coordination of transcription seems essential to verify observed changes in expression profiles in response to external and internal signals.

7. Stress Response of Transgenic Plants Overexpressing the *BBX* Regulators

Recognition of plants' genetic and molecular resistance mechanisms to environmental stimuli allows researchers to design the new strategies to improve plants' stress tolerance. Although abiotic stress tolerance is a polygenic trait, single genes encoding crucial transcriptional regulators can improve plant adaptation to various stresses by turning regulatory gene networks on and off. The significance of some BBX proteins in stress tolerance has been revealed by manipulating the genes encoding such proteins in transgenic economically essential plants to obtain desirable agronomic characteristics and stress resistance. Many studies revealed the potential of manipulating *BBX* genes to confer enhanced tolerance to various stresses. Changing the *BBX* gene expression enhanced stress tolerance in Arabidopsis, chrysanthemums, apples, and rice (Table 3) [64,91,92,94,99,100].

Table 3. Stress response of transgenic plants overexpressing *BBX* genes.

Gene	Accession No./ID	From	To	Phenotypes	References
<i>MdBBX10</i>	MDP0000733075	<i>Malus domestica</i>	<i>Arabidopsis thaliana</i>	Salt and drought tolerance	[92]
<i>VpSBP16</i>	nd	<i>Vitis pseudoreticulata</i>	<i>Arabidopsis thaliana</i>	Salt and drought tolerance	[99]
<i>Cm-BBX24</i>	KF385866	<i>Chrysanthemum morifolium</i>	<i>Chrysanthemum morifolium</i>	Tolerance to freezing and drought	[64]
<i>VvZFPL</i>	HQ179976	<i>Vitis vinifera</i>	<i>Arabidopsis thaliana</i>	Cold tolerance	[91]
<i>AtBBX29</i>	At5g54470	<i>Arabidopsis thaliana</i>	<i>Saccharum</i>	Drought tolerance	[101]
<i>MdBBX37</i>	MDP0000157816	<i>Malus hupehensis</i>	<i>Malus hupehensis</i>	Cold tolerance	[100]
<i>Ghd2(OsBBX8)</i>	Os02g0731700	<i>Oryza sativa</i>	<i>Oryza sativa</i>	Drought tolerance	[94]

nd—no data available.

Although analyses of *BBX* gene overexpression in response to defined stress are very informative, studies focusing on crop productivity will provide answers regarding the transgenic plants' improvements in stress tolerance and yield under field conditions.

8. The Interplay between BBX Proteins and the Circadian Clock

Most processes in living organisms evolve cyclically. The rhythmic course of phenomena is the result of organisms' adaptation to periodically changing conditions on Earth. In plants, the synchronization of the development cycle with cyclical changes in the environment is possible by developing an endogenous mechanism of the biological clock, which generates rhythms of a ~24 h period [102]. Since the expression of numerous genes in crops is controlled at the transcript level by the biological clock, it indicates that the circadian oscillator affects agricultural importance traits. Several oscillator components have been identified as essential determinants of yield-related traits [103].

Many genes, the expressions of which are controlled by the biological clock, encode proteins containing the B-box zinc finger domain [2,4,26,27]. In fact, in Arabidopsis, some *BBX* proteins involved in flowering are under circadian clock control. Thus the expressions of *AtBBX1/CONSTANS* and *AtBBX32* are regulated by the biological clock [27,50]. Moreover, transcriptional analysis of other *BBX* genes in Arabidopsis revealed circadian-dependent regulation of *AtBBX18*, *AtBBX19*, *AtBBX22*, *AtBBX24*, and *AtBBX25* [17,27]. In the promoter regions of clock controlled genes, the specific *cis*-elements "CAANNATC" associated with the circadian regulation were found [4,104]. The transcription factor *StZPR1*, belonging to the zinc finger family type C₄, has been identified recently, which binds to the "CAACAGCATC" motive defined by the term CIRC (circadian regulated) in the *StBBX24* gene promoter in *Solanum tuberosum*. Moreover, in potato transgenic plants with silenced *StZPR1* expression, there are disturbances of some *BBX* genes daily oscillations, such as *StBBX5*, *StBBX9*, *StBBX18*, *StBBX24* and *StBBX27* [105]. It is also noteworthy that the circadian clock is able to interrupt an effect of external stimuli on some *BBX* expressions. This interruption allows plants perform temporal gating in response to environmental constraints, thus triggering appropriate reactions for stress at a more suitable time of a day [26].

The full extent of the mechanisms by which plant keep the clock is still under investigation. Moreover, understanding the multiplatform link between the clock genes and cell-level circadian responses involving large *BBX* gene networks remains unexplained.

9. Summary and Prospects

BBX proteins constitute a complex regulatory network in planta [17,21,98,106]. Despite considerable progress in understanding B-box proteins' function in growth and development and stress responses in crops, the physiological role and the molecular mechanisms for many of them remain still unknown. Knowledge of protein partners for B-box proteins under different circadian cycles and environmental conditions and identifying critical regulators of their transcription will provide insight into molecular relationships between structure and function of this family. More information regarding the functions of *BBX* might help to understand the complexity of signaling pathways generated by the biological clock. However, to provide new insights into the role of *BBX* proteins in plants, more time-consuming experimental *in vivo* data, as gene overexpression and knock-outs, are required.

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