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BBX proteins and their multifaceted roles in floral regulation

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Abstract

B-box (BBX) proteins, a distinct subgroup of zinc finger transcription factors characterized by the presence of one or two B-box domains, play a pivotal role in regulating plant growth and development, particularly in photomorphogenesis. The role of *BBX* genes in the vegetative-to-reproductive phase transition has gradually been identified in the past few years. *BBX* gene member *BBX1/CONSTANS* is a master regulator of the photoperiod pathway and transcriptionally activates the florigen gene *FLOWERING LOCUS T*. *BBX* genes can generate different transcripts through alternative splicing, which significantly increases their functional diversity and regulatory complexity, and plays an important role in plant flowering regulation. However, a schematic analysis of transcriptional regulations and protein interactions during signaling pathways has not been presented. The review highlights recent progress in the transcriptional regulatory role of *BBX* genes during floral regulation and development. It also provides valuable insights into the structural and functional features of the *BBX* gene family and emphasizes its essential role in the complex regulatory networks underlying floral development in plants.

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Introduction

Floral transition is a critical developmental mechanism in plants that affects their viability and reproductive success. Multiple gene regulatory mechanisms are involved in the reproductive phase transition in plants. Transcription factors (TFs) are master regulators vital for flowering regulatory pathways. TFs directly bind to *cis*-elements in the promoter region, their specific target genes, or through protein-protein interactions[1]. Various TFs such as *MCM1agamous-deficiens-serum response factor* (*MADS*), *WUSCHEL-related homeobox* (*WOX*), *TEOSINTE BRANCHED1/CYCLOIDEA/PCF* (*TCP*), *Auxin Response Factor (ARF)*, *Ethylene Response Factor* (*ERF*), *v-myb myeloblastosis viral oncogene homolog* (*MYB*), *SQUAMOSA Promoter Binding Protein-like* (*SPL*), *basic Helix-Loop-Helix* (*bHLH*), and *Zinc Finger Protein* (*ZFP*) have been identified in plants[2].

Zinc finger proteins are a class of TFs that regulate plant development. The zinc finger protein (ZFP) subfamily *BBX* (*B-box gene family*) contains one or two B-box conserved domains with specific tertiary structures that are stabilized by the binding of zinc ions[3]. The *BBX* gene family is highly conserved among plants. The BBX gene family has been identified in many plant species due to the availability of plant genomes. For example, 32 *BBX* genes have been identified in *Arabidopsis*[3,4].Thirty *BBX* genes in rice (*Oryza sativa*), 22 in tung tree (*Vernicia fordii*), 31 in tomato (*Solanum lycopersicum*), 23 in mung bean (*Vigna radiata*), 28 in alfalfa (*Medicago sativa*)[5−9], and 59 *BBX* genes identified in soybean (*Glycine max*)[10].

BBX genes participate in various developmental mechanisms, such as floral transition, hypocotyl elongation, shade avoidance, circadian rhythm, thermogenesis, and photomorphogenesis[11−14]. *BBX* genes act as integrative factors in flowers and regulate the expression of downstream genes or affect the activities of other proteins[3,15]. *CO* (*CONSTANS*)/*AtBBX1* participates in photoperiod regulation of flowering time in plants[16]. In *Arabidopsis*, *AtBBX28,* and *AtBBX29* promote flowering under long-day (LD) conditions[17]. AtBBX28 and AtBBX29 protein interacted with CO. CO protein is a central player that transcriptionally regulates *FT* (*FLOWERING LOCUS T*) and promotes early flowering in *Arabidopsis*[17]. The CO gene ortholog OsBBX18/OsHd1 (HEADING DATE 1) stimulates early floral induction in rice under short-day (SD) conditions. It regulates the *Rice FT-like 1* (*RFT1*) and *Hd3a* (*HEADING DATE 3a*) genes[18]. Recently, significant progress has been made in *BBX* genes' significant role in flowering and flower development[3]. This complex regulatory mechanism demonstrates the multifaceted role of the *BBX* gene in plant development and floral regulation. This review summarizes the transcriptional regulatory role of *BBX* genes in photoperiod, vernalization, and GA (gibberellins) pathways.

Evolution and structural features of the *BBX* gene family

BBX proteins are characterized by the N-terminal inclusion of one or two B-box domains[3]. *Arabidopsis* BBX proteins are categorized into five groups based on the number of B-box domains and the presence or absence of the CCT (CONSTANS, CO-like, and TOC) domain[4,19]. Group I members are similar to group II in structure, and each member contains one B1(B-boxB1), one B2(B-boxB2/B-boxB2'), and one CCT domain. The B2 structure was slightly different between the group I and group II members (Fig. 1a). Members of group III contained one B1 and one CCT domain. Structural group IV contains one B1, one B2 domain, and no CCT domain. Group V consists of members with only one B1 domain[20]. AtBBX26 and AtBBX27 proteins were previously classified as group V in *Arabidopsis*, which only contains one B1 domain. In contrast, recent findings have shown that AtBBX26 and AtBBX27 contain one B1 and one B2(B-boxB2') domain, and we categorized them as structural group VI (Fig. 1b, c)[4,6]. Meanwhile, the B2 structures in groups VI and IV differ, the same as in groups I and II (Fig. 1a, c)[20].

The green plant ancestor (*Viridiplantae*) possesses the protein containing two B-box domains that originated from branches of groups IV and I/II/III/V/VI (Fig. 1d)[6]. In addition, it was found that AtBBX26 and AtBBX27 lost their CCT domains during recent evolution, thus separating them from group II but retaining their structural group characteristics of B1 and B2[6]. OsBBX25 and OsBBX27 proteins in rice both contain one B1 and one CCT domain, and these

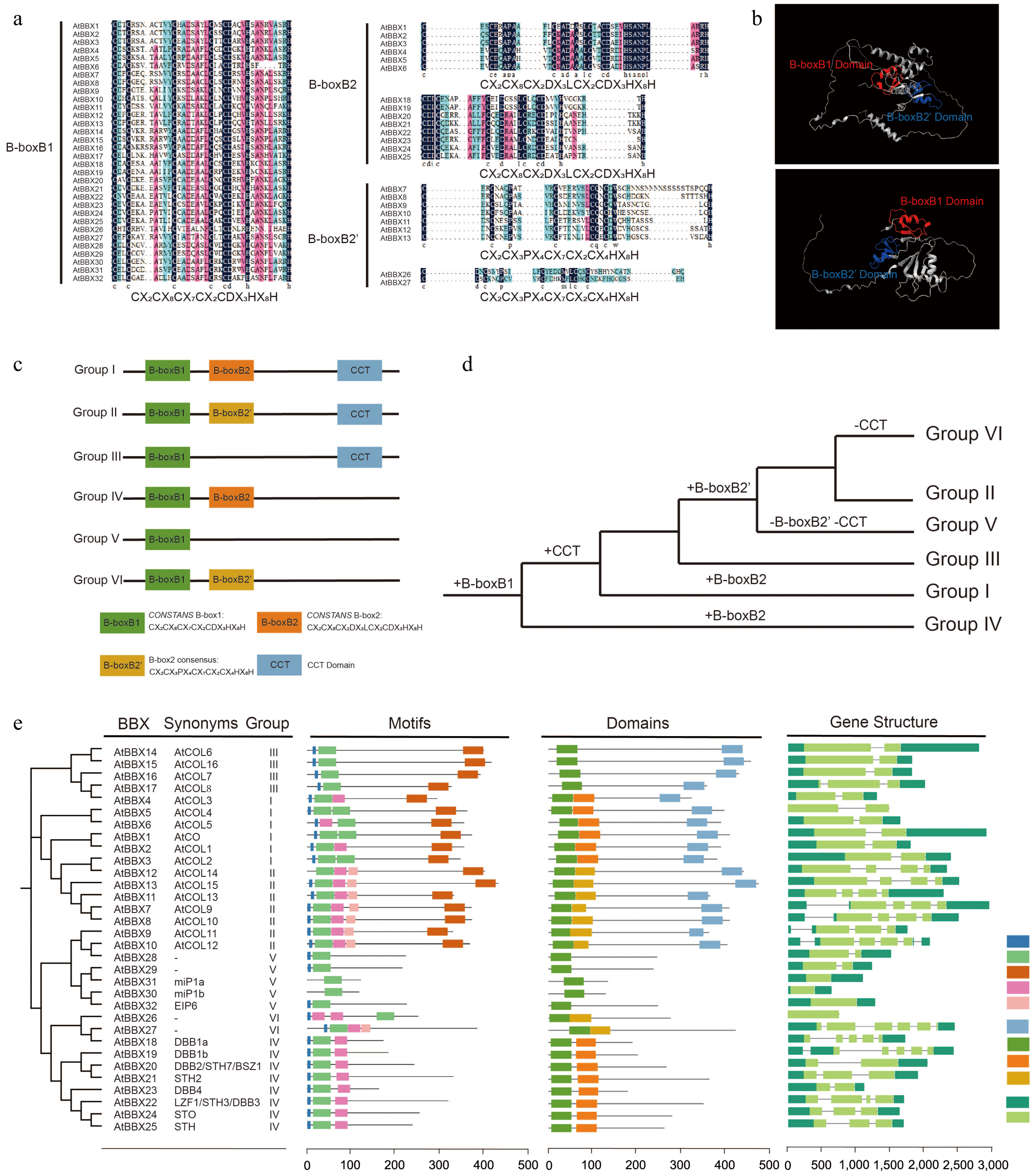

Fig. 1 Structural features of the *AtBBX* gene family. (a) Multiple sequence alignment analysis of *AtBBX* gene family member domains. B-boxB1 of groups I–VI, B-boxB2 of groups I and IV, and B-boxB2' of groups II and VI are indicated. The black color indicates 100% similarity. Red and blue indicate 75% and 50% similarity, respectively. (b) Tertiary structures of AtBBX26 and AtBBX27 (Group VI) proteins were retrieved from observations by Cheng & Wang[69]. Homology modeling of two proteins was constructed using the AlphaFold (https://alphafold.ebi.ac.uk) online server. Visualization with Chimera X. B-boxB1 and B-boxB2' domains are shown with red and blue colors, respectively. (c) Classification of *AtBBX* gene family structural groups (Groups I–VI). (d) Evolutionary hypothesis of BBX protein domains. (e) Characterization of *Arabidopsis AtBBX* genes. Neighbor-joining trees, synonyms, conserved motifs, domains, and exon-intron structures are indicated. '–' signifies that it is not available. Five color boxes indicate different motifs. Four color boxes indicate different domains. The dark green icon means UTR (untranslated region), the light green icon means CDS (coding sequence), and the line between UTR and CDS means intron (a non-coding part of a gene or mRNA molecule).

two proteins evolved through internal deletion of the B2 domain, similar to the evolutionary pattern observed in *Arabidopsis*[5]. In this study, 32 AtBBX proteins were analyzed and found to consist of various sequence-specific binding sites, which may indicate their multifaceted role in plant development (Fig. 1e)[4].

Transcriptional regulatory role of BBX proteins

Structural characterization of the BBX protein was based on its B-box and CCT domains (Figs 2, 3). Some members also have VP and PF (V/L) FL motifs, which play significant roles in transcriptional regulation and mediating protein-protein interactions[21]. In pear (*Pyrus pyrifolia Nakai*), PpBBX18 forms a heterodimer through two B-box domains with the bZIP domain at the C-terminal end of PpHY5 (ELONGATED HYPOCOTYL 5) protein in which PpHY5 binds to the G-box motifs of *PpMYB10*, and PpBBX18 provides the transactivation site, which activates the transcription of *PpMYB10* and induces anthocyanin accumulation in the light[22]. In cabbage (*Brassica rapa*), the BrBBX32 protein N-terminus interacts with BrAGL24 (AGAMOUS-LIKE 24) and promotes flowering[23]. The B-box domain can participate in BBX protein interactions among different species. The B-box domain of AtBBX32 in soybean mediates its binding to GmBBX62. Deletion of the first 17 amino acids in the N-terminal B-box motif of AtBBX32 reduced its binding to GmBBX62 by 80%. However, deleting outside the B-box region does not affect its binding to GmBBX62[24]. Cysteine mutations in the N-terminal B-box domain of AtBBX28 significantly impair heterodimerization between AtBBX28 and CO[25]. Replacing the conserved Cys-25 in the *Arabidopsis* AtBBX19 Box1 motif with Ser eliminated the interaction of AtBBX19 with CO protein and the inhibitory effect of AtBBX19 on flowering. In contrast, the C76S replacement in the Box2 motif is not helpful[26].

The CCT domain consists of 42–43 amino acid residues and is highly conserved among different plants[15]. Several BBX proteins with CCT domains have been reported to play essential roles in transcriptional regulation. The CCT domain of CO has been shown to mediate flowering in *Arabidopsis* by directly binding to the *FT* promoter and activating its expression[16]. In addition, the CCT domain of tomato TCOL1 and *Arabidopsis* CO proteins has been shown to approach target genes in DNA and regulate gene expression by interacting with the HAP5/NF-YC subunit of the DNA-binding protein CBF (C-REPEAT BINDING FACTOR). Mutations in the CCT domain of TCOL causes disruptions in its interaction with tomato THAP5 protein and similar mutations in CO also impair its function and delay flowering in *Arabidopsis*[16,27]. In subsequent studies, the B-box domain of CO could recruit the B-box domains of three additional CO proteins to form a homotetramer, and each of the four CO proteins, through their CCT domains, formed a complex with NF-YB/YC, which non-specifically recognized DNA along with the CCT domain to bind to the TGTG motif of the *FT* promoter, and the TGTG motif was shared by P1, P2, CORE1 and CORE2 elements (Figs 2, 3)[27−31].

The VP (Val-Pro) motif consists of six amino acids with a consistent G-I/VV-P-S/T-F sequence. It is located at the C-terminus of BBX proteins[3,21]. The VP motif was previously thought to be specific to group I. In contrast, VP also pairs at the C-terminus in individual members of groups III, IV, and V[15,21]. Sixteen−20 amino acids separate the VP motif from the CCT domain. They are essential for mediating the interactions of some BBX proteins with other proteins[21,32]. The replacement of the VP motif with alanine eliminated the interaction of STH (AtBBX25) and HY5 with COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1) proteins[32]. The VP motif and WD40 domain are also crucial for interacting AtBBX4 with COP1 and regulating the degradation of the HY5 protein in plants[32,33]. In addition, the VP motif of CO is essential for its interaction with the SPA1 protein[34]. The PF(V/L)FL (Phe-Val/Leu-Phe-Leu) motif has also been reported to play a crucial role in some BBX proteins, which mediate the interaction of the AtBBX30/AtBBX31 (miP1a/b) microproteins with the TPL/TPR (TOPLESS/TOPLESS-RELATED) proteins as well as formation with the CO trimeric deterrent complex to delay floral transition[35,36].

CO proteins form homodimers with each other and heterodimerize with specific AtBBX proteins[37]. The interaction procedure may require the presence of at least three B-box domains between

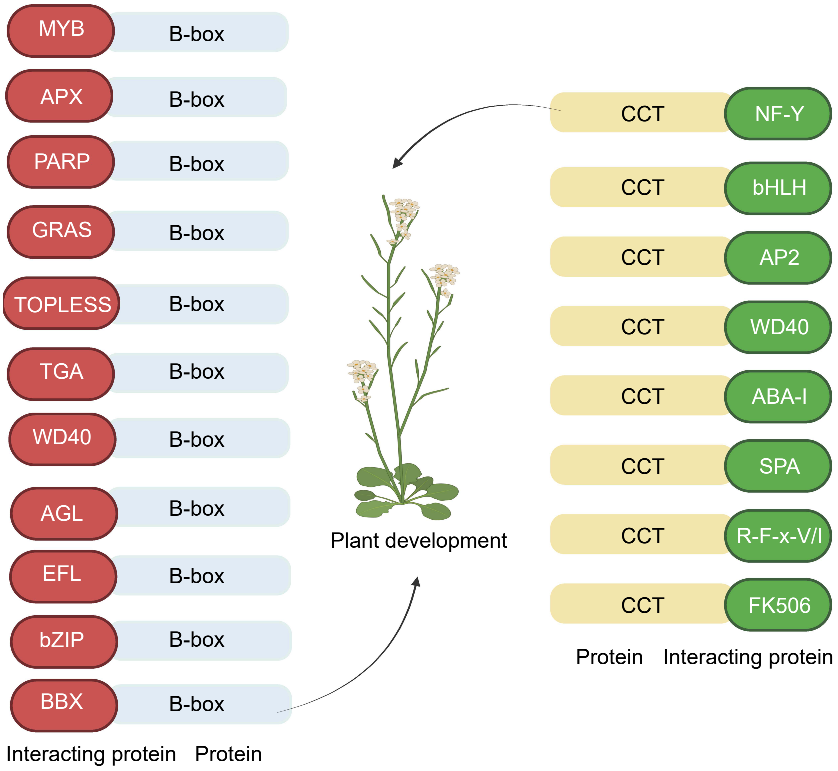

Fig. 2 Protein interactions of B-box and CCT domain in plants. Letters in the ellipses represent acronyms for the type of proteins interacting with the domain. B-box interacting proteins: BBX (AtBBX32-GmBBX62)[24], bZIP (PpBBX18-PpHY5)[13], EFL (AtBBX19-EFL3)[38], AGL (BrBBX32-BrAGL24)[23], WD40 (AtBBX19-COP1)[38], TGA (CO-TGA4)[39], TOPLESS (CO-TPL/TPR)[35], GRAS (AtBBX4-DELLA)[40], PARP (CmBBX8-CmRCD1)[41], APX (AtBBX18-APX1)[42] MYB (CO-AS1)[43]. Interacting proteins of CCT: FK506 (CO-FKBP12)[44], R-F-x-V/I (WNK-OSR1)[45], SPA (CO-SPA1)[34], ABA-I (CO-ABI3)[46], WD40 (CO-COP1)[47], AP2 (CO-TOE1/2)[48], bHLH (CO-CIB1)[49], NF-Y (CO-NF-YB/YC)[28].

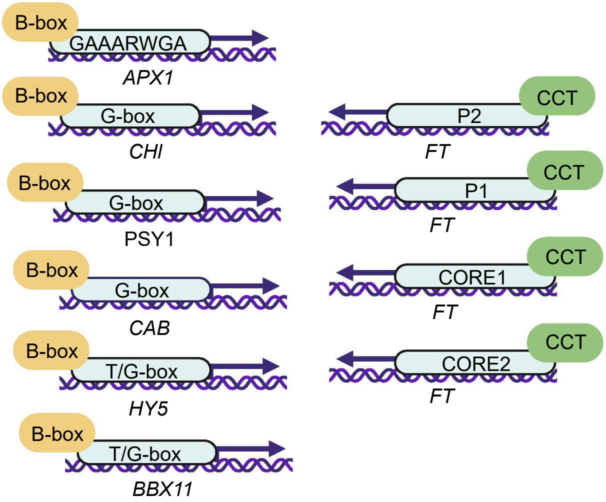

Fig. 3 Transcriptional regulation of B-box and CCT domain in plants. Letters in ellipses with arrows represent promoter binding site abbreviations. Transcriptional regulation of B-box: GAAARWGA (AtBBX18-*APX1*)[42], G-box (AtBBX21/22-*CHI*)[50], G-box (SlBBX20-*PSY1*)[51], G-box (AtBBX22-*CAB*)[50], T/G-box (AtBBX21-*HY5*)[52], T/G-box (AtBBX21-*AtBBX11*)[53]. Transcriptional regulation of CCT: P1/P2/CORE1/CORE2 (CO-*FT*)[29,54].

two proteins[37]. The CCT domain promotes their binding to the *FT* target site, thereby affecting *Arabidopsis* flowering[37]. In summary, B-box, and CCT domains are crucial functional domains in plant growth and development (Fig. 3).

Alternative splicing of *BBX* genes

BBX genes can produce multiple transcripts through alternative splicing to regulate plant development[55−57]. The *CO* gene produces two RNA variants through intron-retentive (IR) splicing: full-length COα with intact functionality and truncated COβ with a C-terminal deletion of the CCT domain. *COα* gene overexpression induces early flowering, whereas *COβ* gene overexpression delays flowering. *COβ* gene reduces the binding of the COα to the *FT* promoter by interacting with COα protein, thereby inhibiting flowering in *COα-COβ* co-expression plants[55]. Further studies revealed that COβ protein does not interact with E3 ubiquitin ligases (COP1, HOS1, HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE1, FKS1, FLAVIN-BINDING KELCH REPEAT F-BOX 1), but regulates the interaction between COα and E3 ubiquitin ligases. This regulation destabilizes the COα protein and promotes degradation[55].

PeCOL13 gene(*Phyllostachys edulis*) produces full-length PeCOL13α and PeCOL13β lacks the CCT domain through IR splicing[56]. In contrast, the PeCOL13β protein diminishes *PeCOL13α*-repressed flowering by competing with PeCOL13α for binding to the *PeFT* promoter[56]. While the homologous protein AtCOL13 has two B-box domains (B-boxB1, B-boxB2'), PeCOL13 has only one B-boxB2' domain. It contains distinct amino acid residues from the second B-box domain, potentially owing to their opposing functions (Figs 1e, 4)[56]. IR RNA variant transgenic plants of *AtBBX24* and *AtBBX22* exhibited photosensitivity and acted as regulators of photomorphogenesis[57]. In summary, the complex splicing pattern of the *BBX* gene enriches its molecular functional diversity and provides fine regulatory mechanisms for plant development and environmental adaptation.

BBX genes involved in flowering regulatory pathways

Flowering is a complex biological process that involves multiple regulatory pathways, such as vernalization, photoperiod, autonomous, gibberellins (GA), and age-dependent pathways[58]. These regulatory pathways comprise a complex network of genes and

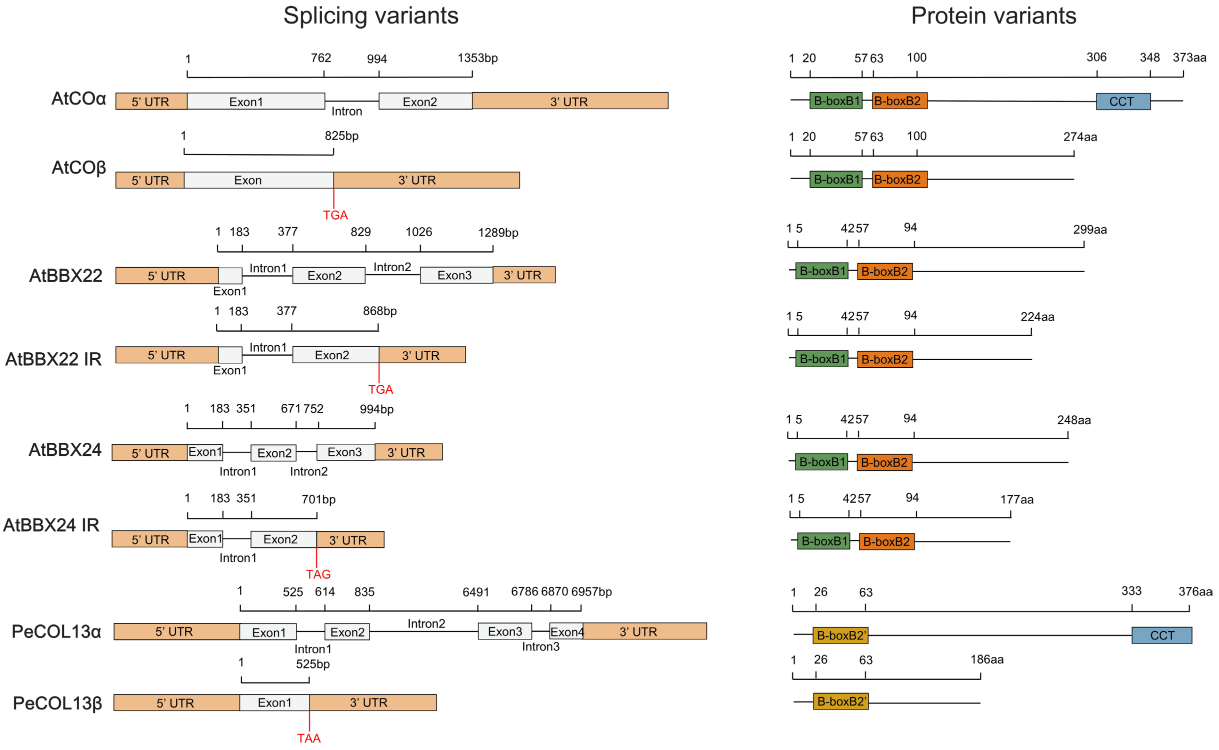

Fig. 4 Sequence structures of mRNA and protein isoforms of four *BBX* genes. The black horizontal line indicates the number of base pairs and amino acids. Yellow boxes indicate 5'/3' UTRs, grey boxes indicate exons, and black lines indicate excised introns. Four colour boxes indicate different domains.

transcription factors. *Arabidopsis AtBBX* genes play essential roles in floral regulation and development (Table 1, Fig. 5). However, *AtBBX* gene orthologues in other plant species are conserved in functions, such as *CmBBX8* (*Chrysanthemum morifolium*), *FaCO* (*Fragaria* × *ananassa*), and *BvBBX19* (*Beta vulgaris*) promote plant flowering. On the other hand, *OsCOL4*, *GmCOL1a* (*Glycine max*), and *AcBBX5* (*Ananas comosus*) inhibited flowering. *OsHd1* acts as a floral promoter and repressor (Table 2). *BBX* genes and their role in development are highly expressed in plant flower organs[59–61]. *AcBBX5* was expressed explicitly in the stamens and petals of pineapple (*Ananas comosus*), and the transgenic *Arabidopsis* plant's petal and sepal widths were enlarged[59]. BBX genes regulate the miRNA-lncRNA-TF module and trigger floral organ development[62]. For example, lch-lnc3939-*AtBBX19* and lch-lnc6617-*AtBBX24* are involved in floral organ development[26,62,63]. OsBBX11 and OsBBX19 protein interaction regulates the expression of *OsBTB97* (*OsBBX11*) and controls the growth of flower organs in rice[64].

Role of *CO* in the photoperiodic flowering

CO was the first BBX protein identified in plants[16] and plays a central role in photoperiod-mediated flowering. The *FT* gene receives signals from *CO* under LD conditions to induce the transition from vegetative to reproductive growth in *Arabidopsis*. In contrast, it did not affect *CO* under SD conditions. However, the *CO* gene can suppress *FT*-induced flowering under SD conditions by promoting the expression of the *FT* repressor gene *TFL1* (*TERMINAL FLOWER1*)[16,28,65]. In particular, TFL1 interacts with FD (FLOWERING LOCUS D) protein to negatively regulate *FD*-dependent target gene transcription and form the TFL1-FD complex to delay flowering. *FD* gene is vital to the FD-FT protein complex, promoting *Arabidopsis* flowering[106]. Most AtBBX proteins play a role in flowering and are regulated under the photoperiodic pathway by interacting with CO (Fig. 5). For example, AtBBX14, AtBBX15, AtBBX16, and AtBBX28 interact with CO protein in the nucleus to inhibit flowering by preventing the ability of CO to activate *FT* and reducing *FT* expression[25,72].

Interestingly, the interaction between AtBBX28 and CO did not affect the transcriptional activation activity of CO protein[25]. Similar to *Arabidopsis*, one rice *CO* homolog, *OsHd1*, promotes flowering under SD conditions and represses flowering under LD conditions[81]. *OsHd1* regulation of rice tasseling involves only the enhancement and reduction in the expression of *Hd3a* (*HEADING DATE 3A*) and *RFT1* (*RICE FLOWERING LOCUS T 1*), homologs of the *FT* gene[18]. Recent studies have identified some *OsBBX genes* that affect rice tasseling through the photoperiodic pathway (Fig. 6). Among these, *OsBBX5* (*OsCOL4*), *OsBBX7* (*OsCOL9*), *OsBBX10* (*OsCOL10*), and

Table 1. Functions of *AtBBX* genes in inducing floral transition in *Arabidopsis*.

Species	BBX	Gene ID	Function	Ref.
Arabidopsis thaliana	AtBBX1	Q39057	*CO* is involved in the photoperiodic regulation of flowering under LD conditions by binding to the *FT* promoter and activating its expression over a longer time; Phytochrome B delays flowering in plants under SD conditions by directly reducing CO protein activity or indirectly antagonizing its effect on flowering time; *CO* can inhibit *FT* induction of flowering by affecting *TFL1* expression under SD conditions	[16,65]
	AtBBX4	Q9SK53	Interaction of AtBBX32 with AtCOL3 under LD conditions enables AtCOL3 protein to bind the *FT* promoter and repress its transcription	[66]
	AtBBX5	Q940T9	AtCOL4 interacts with CO proteins to inhibit flowering under LD and SD conditions in plants	[67]
	AtBBX6	Q9FHH8	*AtCOL5* overexpression plants flower early under SD conditions, and *AtCOL5* mutant plants do not affect flowering	[68]
	AtBBX7	Q9SSE5	*AtCOL9* regulates flowering time under LD conditions by repressing *CO* expression while reducing *FT* expression and delaying the floral transition	[69]
	AtBBX10	Q9LJ44	AtCOL12 physically interacts with CO in vivo to inhibit flowering under LD conditions by inhibiting CO protein function while reducing *FT* expression	[70]
	AtBBX13	Q9C7E8	AtCOL15 interacts with CO protein and represses CO-mediated transcriptional activation of *FT* in LD conditions; AtCOL15 can also compete with CO protein and directly bind to the CORE2 motif on the *FT* promoter to repress flowering in *Arabidopsis*	[71]
	AtBBX14	Q8LG76	AtBBX14 interacts with CO in the nucleus and disrupts CO binding to the *FT* promoter, preventing the ability of CO to activate *FT* and inhibiting flowering under LD conditions	[72]
	AtBBX15	Q8RWD0	AtBBX15 interacts with CO in the nucleus and disrupts CO binding to the *FT* promoter, preventing the ability of CO to activate *FT* and inhibiting flowering under LD conditions	[72]
	AtBBX16	Q8RWD0	AtBBX16 interacts with CO in the nucleus and disrupts CO binding to the *FT* promoter, preventing the ability of CO to activate *FT* and inhibiting flowering under LD conditions	[72]
	AtBBX17	Q9M9B3	Overexpression of *AtCOL8* causes late flowering under LD conditions and AtBBX17 protein inhibits *FT* transcription by interacting with CO	[73]
	AtBBX19	C0SVM5	*AtBBX19* inhibits flowering by consuming CO and ultimately inhibiting *FT* as the primary pathway for regulating *SOC1*	[26]
	AtBBX24	Q96288	Overexpression of *AtBBX24* accelerated flowering under LD and SD conditions, whereas mutant *AtBBX24* delayed flowering only under SD conditions *AtBBX24* not only repressed *FLC* expression but also competed with *FLC* to regulate *FT* and *SOC1* expression, thereby promoting flowering	[63]
	AtBBX28	NP_194461	The interaction of AtBBX28 with CO reduced the binding of CO to *FT* promoter and inhibited flowering under LD conditions; *AtBBX28-AtBBX29* double mutant plants have reduced transcriptional activation activity of CO to *FT* promoter, and the interaction of AtBBX28 with CO reduces CO binding to the *FT* promoter and represses flowering under LD and low-temperature conditions	[17,25]
	AtBBX29	NP_200258	*AtBBX28-AtBBX29* double mutant plants have reduced transcriptional activation activity of CO to *FT* promoter, and the interaction of AtBBX29 with CO reduces CO binding to the *FT* promoter and represses flowering under LD and low-temperature conditions	[17]
	AtBBX30	Q1G3I2	The miP1b interacts with TPL and forms a trimeric deterrent complex with CO to delay floral transition under LD conditions	[35,36]
	AtBBX31	Q9LRM4	The miP1a interacts with TPL and forms a trimeric deterrent complex with CO to delay floral transition under LD conditions	[35,36]
	AtBBX32	Q9LJB7	Interaction of AtBBX32 with COL3 under LD conditions enables AtCOL3 to bind the *FT* promoter and repress its transcription	[66]

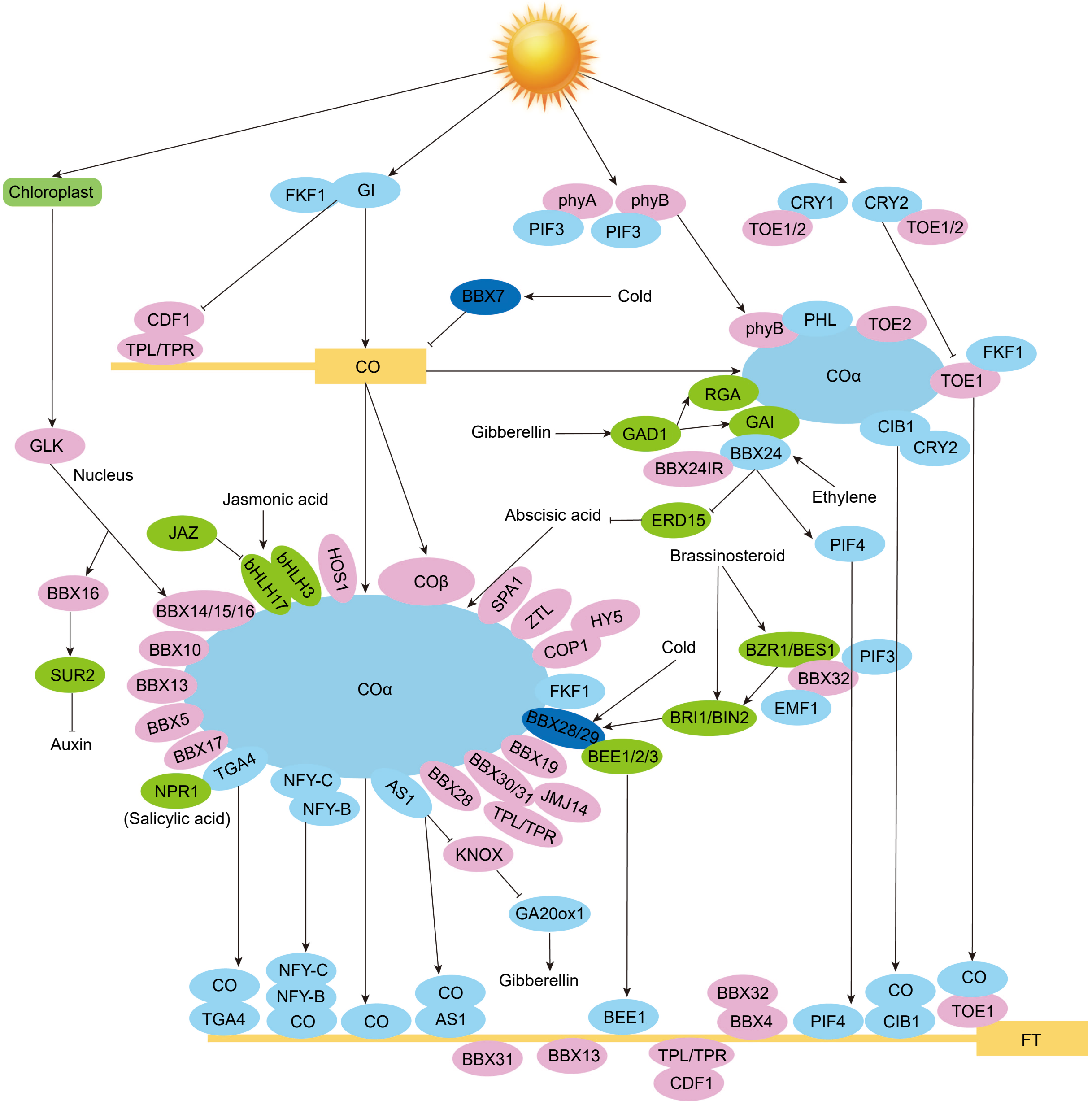

Fig. 5 Mechanism of *AtBBX* genes in reproductive phase transition in *Arabidopsis*. The letters in the boxes represent short names of genes, and the letters in the ellipses represent short names of proteins. Positive regulators are shown as light blue circles. Negative regulators are in pink, and hormone-responsive proteins are in green. Cold-responsive proteins are shown in dark blue. Solid lines with arrows represent facilitation, and solid lines with flat ends represent inhibition[1,3,12,14,15,21,30,31,40,48,49,58,63,113−118].

OsBBX23 (*OsCOL13*) inhibit flowering by decreasing the expression of the rice *RFT1* gene through *Ehd1* (*EARLY IN SHORT DAYS 1*)[69,74,76,80].

In addition, the *OsBBX10* gene functions downstream of *Ghd7* (*GRAIN NUMBER, PLANT HEIGHT,* and *HEADING DATE 7*) to delay rice tasseling by reducing the expression of *Ehd1*[76,82]. Furthermore, *Ghd7* expression is regulated by *SE5* (*PHOTOPERIOD SENSITIVITY 5*)[76]. Interestingly, *Ghd7* and *Ehd1* in rice do not exist as homologous genes in *Arabidopsis*, and their presence largely changes the GI-CO-FT-mediated photoperiodic pathway in rice[107]. Ghd7 could form multimers with Oshd1, Oshd1-DTH8 (DAYS TO HEADING 8), and Oshd1-DTH8-PRR37 (PSEUDO-RESPONSE REGULATOR 37) protein complex, respectively, and changes their activities to inhibit flowering under LD conditions[108]. *Ghd7* gene expression is repressed under SD conditions, resulting in the release of the *Oshd1* gene to achieve Oshd1-Hd3a-mediated early flowering[108]. The *Ehd1* gene is similarly highly expressed and regulated by multiple *BBX* genes to achieve early flowering[108]. In addition to rice, *CO* homologs are also found in other plants, such as *SbCO*, *ZmCONZ1*, and *HvCO1*, which can affect photomorphogenesis to regulate plant flowering through different pathways[96,100,109]. Three members of the sorghum (*Sorghum bicolor* L.) PEBP (PHOSPHATIDYLETHANOLAMINE-BINDING PROTEIN FAMILY) family, *SbCN8*, *SbCN12*, and *SbCN15*, have been shown to affect flowering through the classical GI-CO-FT regulatory pathway[18,100,101]. The *SbCO* gene can indirectly activate *SbCN8* and *SbCN12* to induce flowering by promoting *SbEhd1* expression under SD conditions[100]. In addition, *SbEhd1* expression is suppressed by *SbPRR37* and *SbGhd7* under LD conditions[100].

AtBBX32 inhibits the expression of *EMF1* (*EMBRYONIC FLOWER 1*) by interacting with it through the N-terminal B-box domain and delays floral formation in *Arabidopsis*[110]. Under SD conditions,

Table 2. Functions of *BBX* genes in inducing floral transition in different plant species.

Species	BBX	Gene ID	Function	Ref.
Oryza sativa	OsCOL4	BAS79707	*OsCOL4* inhibits rice flowering under SD and LD conditions	[74]
	OsCOL9	BAS84196	*OsCOL9* suppresses *Ehd1* expression and inhibits rice flowering under SD and LD conditions	[75]
	OsCOL10	BAS86019	*OsCOL10* is a flowering repressor linking *Ghd7* and *Ehd1* in rice and inhibits flowering in rice under SD and LD conditions	[76]
	OsBBX14	BAS92741	*OsBBX14* acts as a floral repressor by promoting *OsHd1* expression under LD conditions; *OsBBX14* delayed flowering under SD conditions by acting as a repressor of *Ehd1*	[77]
	OsCCT19	XP_015642185	Transgenic plants of *OsCCT19* with delayed tasseling in LD conditions	[78]
	OsCOL16	BAS97134	*OsCOL16* up-regulated the expression of *Ghd7*, which in turn down-regulated the expression of *Ehd1*, *Hd3a*, and *RFT1*, leading to late tasseling under both LD and SD conditions	[79]
	OsCOL13	BAT03112	*OsCOL13* functions as a negative regulator downstream of *OsphyB* and upstream of *Ehd1* in rice, resulting in late tasseling under LD and SD conditions	[80]
	OsHd1	BAS97223	*OsHd1* acted as a promoter of tasseling under SD conditions and as a suppressor of tasseling under LD conditions	[81]
	OsCOL15	BAT06449	*OsCOL15* inhibited flowering under LD and SD conditions by up-regulating *Ghd7* and down-regulating *RID1* expression, thereby down-regulating *Ehd1*, *Hd3a*, and *RFT* expression	[82]
	OsCO3	BAT06983	*OsCO3* inhibits *Hd3a* and *FTL* expression to delay flowering under SD conditions	[83]
	OsDTH2	BAS97360	*OsDTH2* is independent of *OsHd1* and *Ehd1* and directly represses the expression of *Hd3a* and *RFT1* to induce rice tasseling under LD conditions	[84]
Chrysanthemum morifolium	CmBBX7	AMO42717	CmBBX7 and CmBBX8 interact with each other to positively regulate *CmFTL1* expression by binding to its promoter to accelerate flowering under LD conditions	[85]
	CmBBX5	–	CmBBX5 interacts with CmBBX8 to inhibit *CmFTL1* regulation of chrysanthemum flowering under LD and SD conditions	[86]
	CmBBX8	AMO42713	CmBBX8 protein accelerates plant flowering under LD conditions by directly targeting *CmFTL1* by interacting with CmERF3 or CmRCD1; CmBBX8 and CmBBX7 interact with each other to positively regulate *CmFTL1* expression by binding to its promoter to accelerate flowering under LD conditions	[41,85,87]
	CmBBX13	KP963935	*CmBBX13* delays flowering in plants under SD and LD conditions in a photoperiod-independent pathway	[88]
	CmBBX24	KF385866	*CmBBX24* inhibited flowering by affecting the photoperiod and GA pathways, and under LD conditions, *CmBBX24* regulated flowering mainly by affecting the GA pathway	[89]
Fragaria × *ananassa*	FaBBXx28c1	QOI16737	Overexpression of *FaBBX28c1* showed a late-flowering phenomenon in LD conditions	[90]
	FvCO	WBW02120	Overexpression of *FvCO* plants under LD resulted in slightly early flowering, whereas overexpression under SD induced early flowering	[91]
Glycine max	GmCOL1a	Glyma08g28370	Overexpression of *GmCOL1a* leads to plants flowering late under LD conditions	[92]
Beta vulgaris	BvBBX19	XP_019107108	BvBBX19 and BvBTC1 interact to form a heterodimer and bind the *BvFT2* promoter to activate flowering under LD conditions	[93,94]
	BvBTC1	BBH85249	BvBTC1 and BvBBX19 interact to form a heterodimer and bind the *BvFT2* promoter to activate flowering under LD conditions	[93,94]
	BvCOL1	ACC95129	Transgenic *BvCOL1* plants compensate for the late-flowering phenotype of *Athaliana* co-2 mutants with a positional/gene-dose effect	[95]
Hordeum vulgare	HvCO1	AF490467	*HvCO1* activates *HvFT1* to induce flowering under LD and SD conditions	[96]
	HvCO9	AY082965	Overexpression of *HvCO9* plants flowering late in LD and SD conditions	[97]
	HvCO2	XP_044950638	Overexpression of *HvCO2* promotes flowering induction by Ppd-*H1* and *HvFT1* expression in LD and SD conditions	[98]
Vitis vinifera	VvCO	XP_059589686	*VvCO* expression is associated with seasonal flowering induction in latent buds	[99]
Sorghum bicolor	SbCO	EER88227	*SbCO* promotes flowering by inducing *SbEhd1* and *SbFTL* genes under LD conditions	[100]
Zea mays	ZmCONZ1	ABW82153	ZmConz1 activates *ZmZCN8*, which acts as a floral inducer involved in photoperiod sensitivity in maize	[101]
Ananas comosus	AcBBX5	XP_020107577	AcBBX5 protein binds the *AcFT* promoter and reduces its expression, thereby delaying flowering under LD conditions	[59]
Rosa rugosa	RcCO	RcChr2g0164091	Interaction between RcCO and RcCOL4 promotes the binding of RcCO protein to the CORE motif in the *RcFT* promoter and induces *RcFT*, accelerating flowering under LD and SD conditions	[102]
	RcCOL4	RcChr6g0299051	Interaction between RcCOL4 and RcCO promotes the binding of RcCO protein to the CORE motif in the *RcFT* promoter and induces *RcFT*, accelerating flowering under LD and SD conditions	[102]
Mangifera indica	MiCOL2A	WED40957	Overexpression of *MiCOL2A* in Arabidopsis delays flowering of transgenic plants under LD and SD conditions	[103]
	MiCOL2B	WED40958	Overexpression of *MiCOL2B* in Arabidopsis delays flowering of transgenic plants under LD and SD conditions	[103]
	MiCOL6	WED40966	*MiCOL6* promotes early flowering in transgenic plants under LD and SD conditions	[104]
	MiCOL7A	WED40967	*MiCOL7A* inhibits flowering under LD and SD conditions by reducing *AtFT* and *AtSOC1* expression	[104]
	MiCOL7B	WED40968	*MiCOL7B* inhibits flowering under LD and SD conditions by reducing *AtFT* and *AtSOC1* expression	[104]
Solanum lycopersicum	SlBBX4	Solyc08g006530	The *SlBBX4* mutant showed delayed flowering in both LD and SD conditions	[105]

'–' signifies that it is not available.

AtBBX6 can lead to early flowering of plants by promoting the expression of *FT* and *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*), and *AtBBX6*-overexpression plants in *CO* mutants can inhibit the later-flowering effect to a certain extent. In contrast, the time of floral formation was not altered in *AtBBX6*-deficient mutants, and the molecular mechanism associated with *CO* has not been clarified[68]. Cotton (*Gossypium hirsutum*) GhBBX5, GhBBX8, GhBBX23, and GhBBX26 interact with GhFT to regulate cotton

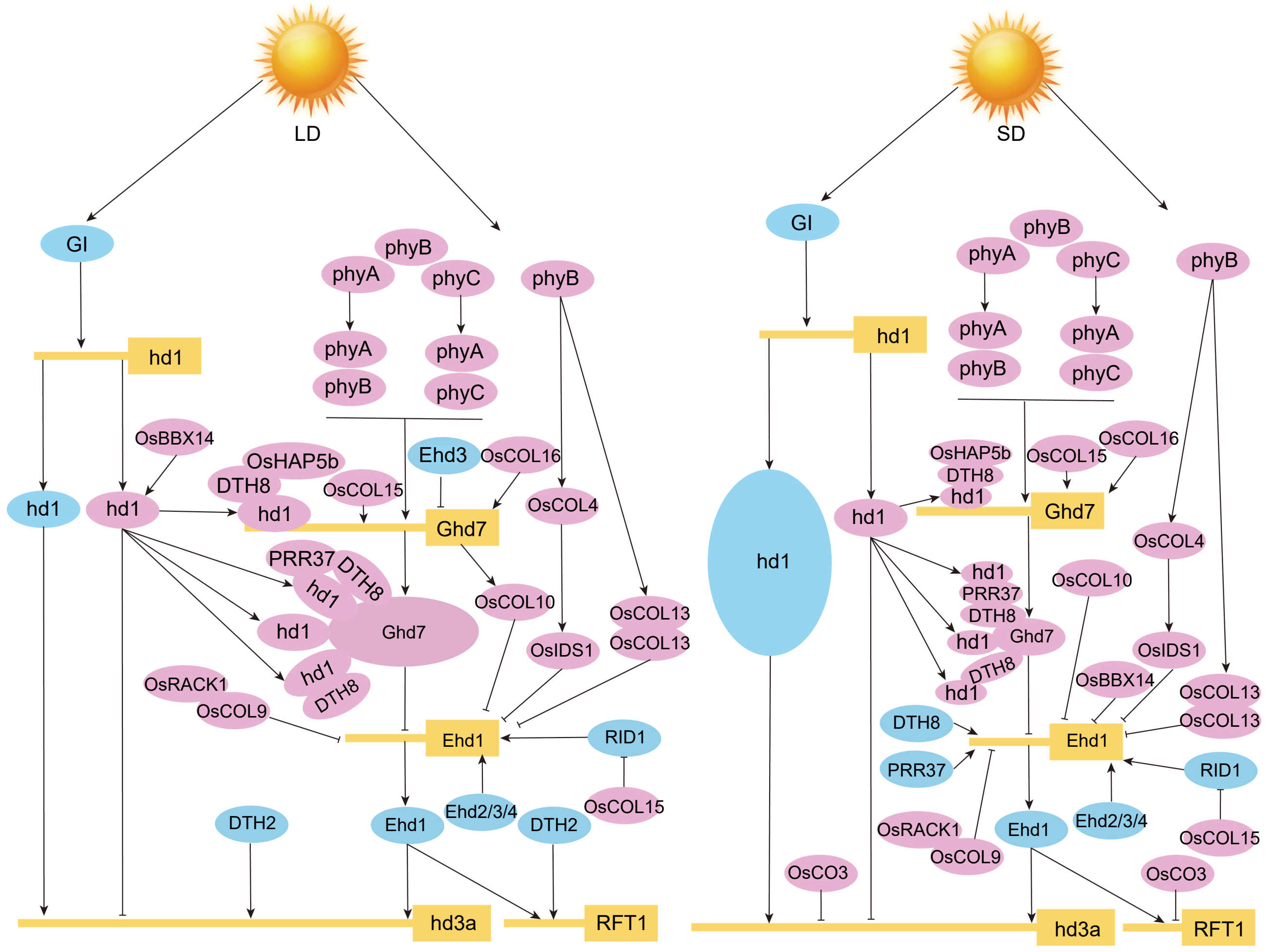

Fig. 6 Rice *OsBBX* genes regulatory network during photoperiodic flowering. The left plot represents LD conditions, and the right plot represents SD conditions. The letters in the boxes represent short names of genes, and the letters in the ellipses represent short names of proteins. Positive regulators are shown in light blue circles, and negative regulators are shown in pink. Solid lines with arrows represent facilitation, and solid lines with flat ends represent inhibition[74−77,79,80,82−84,119−122].

flowering[61]. Thus, some BBX proteins can act on flower genes to regulate plant flowering directly.

Many BBX proteins play a role in flowering by affecting the COP1-HY5 module[13]. The ubiquitin ligase COP1/SPA (CONSTITUTIVELY PHOTOMORPHOGENIC 1/SUPPRESSOR OF PHYA-105) in *Arabidopsis* mediates its degradation under dark conditions by interacting with CO and AtCOL12, which is hypothesized to compete with CO for the binding site of COP1, thus indirectly affecting plant flowering[70,111]. Similar to AtCOL12, AtBBX28 functions in COP1-AtBBX28-HY5 and AtBBX28-CO-FT by interacting with proteins[25,111,112]. AtBBX28 interacts with COP1 and mediates its degradation in the dark through the 26S proteasome, whereas AtBBX28 also interacts with HY5 in a dose-dependent manner, negatively regulating HY5 expression[112]. AtBBX4, AtBBX30, EIP6, AtBBX32, and OsBBX14 proteins participate in floral regulation by affecting the COP1-HY5 and CO-FT modules[13,33,77].

Role of *BBX* genes in hormone signaling pathway

The *BBX* genes play an essential role in the GA pathway during floral regulation (Fig. 5). *CmBBX24* negatively regulates GA biosynthesis by affecting the regular expression of GA biosynthesis genes *GA20ox* (*GA 20-OXIDASE*) and *GA3ox* (*GA 3-OXIDASE*) and inhibits flowering. It also acts as a flowering repressor by reducing the expression of *GI* (*GIGANTEA*), *PRR5*, *CO*, *FT*, *FTL,* and *SOC1* genes in the photoperiodic pathway[89]. The expression pattern of *CmBBX24* in the overexpression plants was consistent with that of the florigen-resistant gene *CmAFT* (*ANTI-FLORIGEN TRANSPORTER*). In contrast, the expression levels of *CmFTL3* and *CmSOC1* were significantly increased by silencing *CmBBX24*[89]. *CmBBX24* transgenic plants showed suppression of *CmBBX24* expression after treatment with GA4/7. GA may negatively inhibit the regulatory process of *CmBBX24* in chrysanthemum flower formation[89]. *AtBBX24* can compete with *FLC* (*Flowering Locus C*) to regulate *FT* and *SOC1* expression, promote flowering in *Arabidopsis,* and interact with GAI (GIBBERELLIN INSENSITIVE) protein in the gibberellin pathway[40]. CO protein could also interact with GAI, RGL1 (RGA-LIKE1), and RGA (REPRESSOR OF GA1-3), and RGA inhibited CO interaction with NF-YB2 *in vitro*[116].

BBX genes also participate in other plant growth regulators, such as ethylene (ETH) and abscisic acid (ABA), promoting flowering. The *CmBBX8* gene affects flowering time in response to ETH signaling, and further studies found that CmBBX8 reduces the expression of *CmFTL1* by interacting with CmERF3 (ETHYLENE RESPONSIVE TRANSCRIPTION FACTOR) or CmRCD1 (RADICAL-INDUCED CELL DEATH 1) protein[41,123]. CmBBX5 interacts with CmBBX8 protein to inhibit the regulation of *CmFTL1* in chrysanthemum flowering under LD and SD conditions via a mechanism different from CmERF3[86]. Similarly, cabbage *BcERF070* repressed the expression of downstream *BcFT* genes by directly binding to the promoter of *BcBBX29*, resulting in the inhibition of flowering under ETH conditions[87].

The synthesis and accumulation of ABA can induce the transcriptional activation activity of *GI* and *CO*, affecting the expression of their downstream *FT* genes to promote floral transition[124]. CO proteins also interact with TGA4 (TGACG MOTIF-BINDING FACTOR 4), which not only binds to *NPR1* (*NON-EXPRESSOR OF PR1*) to regulate the expression of defense genes mediated by salicylic acid (SA) signaling but also directly binds to the *FT* promoter and regulates its expression to control the flowering time of the plant[39,125]. In summary, BBX proteins are essential in multiple hormone signaling pathways that affect plant flowering. Thus, *BBX* genes play a vital role in many aspects of the hormone pathway that regulate plant flowering through transcriptional and post-transcriptional regulation.

BBX genes and vernalization signaling pathway

Some BBX proteins act as regulators of low-temperature signaling and participate in the vernalization pathway to control plant floral formation. There is an interaction between beet BvBBX19 and BTC1, and the two BBX proteins interact *in vivo* to form a heterodimer that acquires a CO-like function and binds to the *BvFT2* promoter to activate twitching and F_2 plants with mutations in both genes do not twitch even after vernalization[93,94]. *BrBBX32* in Chinese cabbage regulates flowering time by interacting with BrAGL24 protein, which governs the interaction between BrAGL24 and BrSOC2. In contrast, *Arabidopsis AtAGL24* and *SOC1* upregulate the expression of each other and mediate vernalization to promote flowering in an *FLC*-independent manner[23].

AtBBX28 nor *AtBBX29* single mutants possess flowering-related phenotypes, whereas *AtBBX28* and *AtBBX29* double mutant plants exhibit significantly delayed flowering phenotypes at low temperatures (16 °C)[17]. Low temperature reduced the expression of *FT*, *CO*, and *TSF* (*TWIN SISTER OF FT*), which may have resulted from the mutation of *AtBBX28* and *AtBBX29,* affecting the transcriptional activation activity of CO on the *FT* promoter. In contrast, a delayed flowering phenotype appeared in *AtBBX28* overexpression plants, which may be related to *AtBBX28's* response to temperature changes[25]. The cold-induced expression of *AtBBX7* and *AtBBX8* is positively regulated upstream by *CRY2* (*CRYPTOCHROME 2*) and *HY5* but suppressed by *COP1*. In addition, *AtBBX7* and *AtBBX8* positively regulate cold resistance by regulating the expression of downstream *COR* (*Cold-Responsive*) genes[126]. *OsBBX7*, *OsBBX17*, and *CmBBX24* have also been shown to be highly expressed under low-temperature conditions while affecting the flowering time of plants. However, a specific association has not been clarified[60,89]. These studies have confirmed the central role of *BBX* genes in floral induction and floral development in plants.

Conclusions

BBX genes are essential regulators of reproductive phase transition in plants. *BBX* genes trigger the transcriptional activity of downstream target genes and BBX proteins interact with other TFs and regulate flowering regulatory pathways, such as photoperiodic, GA, and vernalization pathways (Figs 5, 6). These regulatory mechanisms interact with each other to synergize and regulate the plant flowering process to ensure that the plants flower well and adaptively. This study explored the interactions, domain specificity, and transcriptional regulation mechanisms between BBX proteins and other regulatory factors to reveal their delicate regulatory mechanisms and potential application values in optimizing floral regulation in plants. Flowering is directly linked to fruit and commercial production. An in-depth investigation of the function of *BBX* genes in the flowering transition of plants will provide a basis for understanding flowering to meet the needs of agricultural production and obtain desirable agronomic traits.

Author contributions

The authors confirm contribution to the paper as follows: writing—original draft preparation: Xu Z; data curation and software: Xu Z, Wang C; writing—review and editing: Khan FS; supervision and conceptualization: Khan FS, Zhang H; resources and investigation: Khan FS, Ouyang Y; funding acquisition: Zhang H. All authors have read and agreed to the published version of the manuscript.

Data availability

The data are available on request from the corresponding author.

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Conflict of interest

The authors declare that they have no conflict of interest.

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