SI Appendix

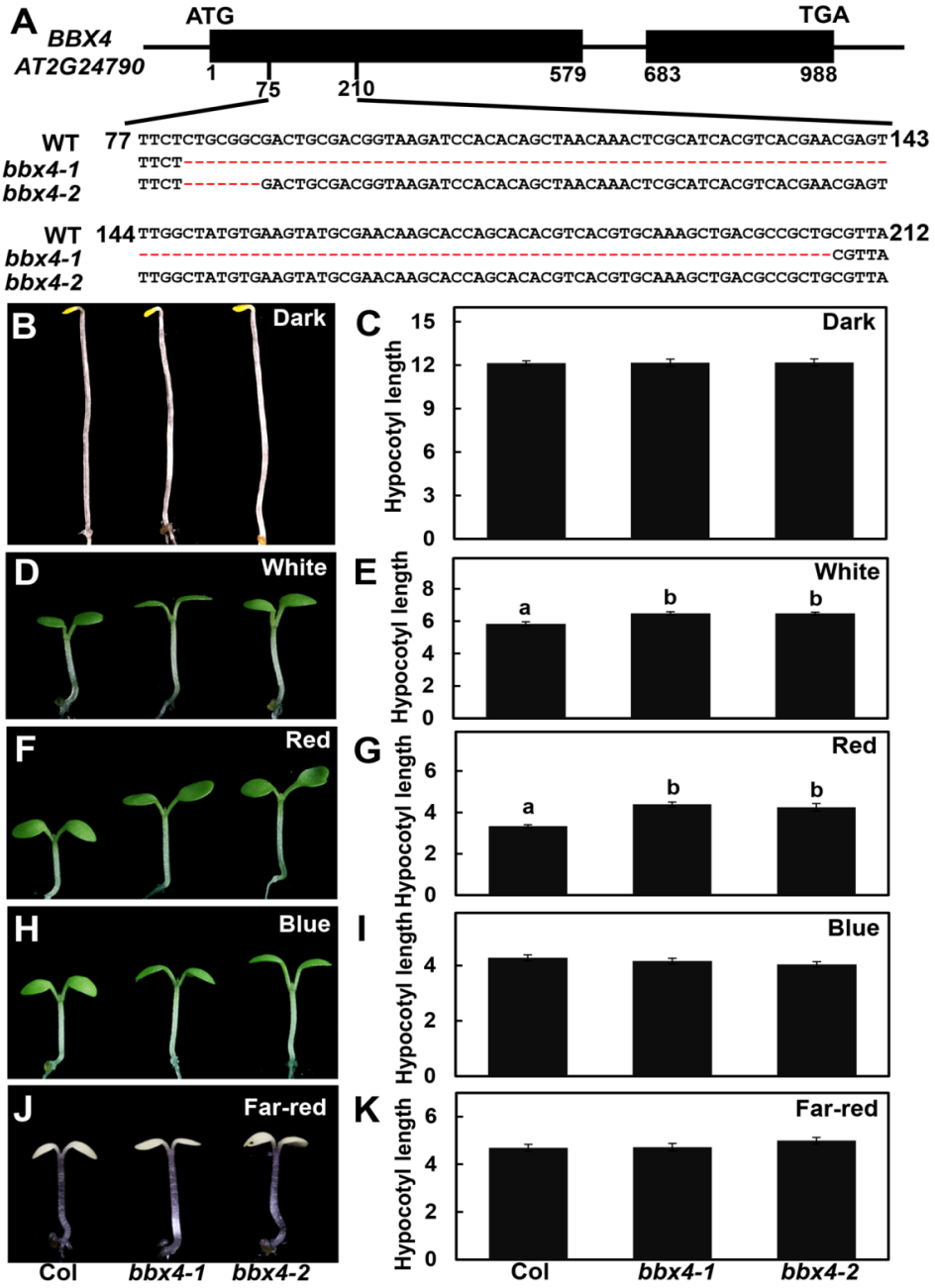

Fig. S1. The *bbx4* mutant seedlings are hyposensitive to red light.

(A) Mutations in two independent *bbx4* alleles created by the CRISPR/Cas9 method. The DNA sequence alignment shows altered bases in two *bbx4* mutants. "-" in red indicates nucleic acid deletion.

(B) to (K) Hypocotyl phenotype and length of 4-d-old Col and two independent *bbx4* single mutant seedlings grown in darkness (B and C),W (13.24 μmol/m^2/s) (D and E), R (115.8 μmol/m^2/s) (F and G), B (3.88 μmol/m^2/s) (H and I) and FR (4.3 μmol/m^2/s) (J and K) light conditions. The unit of hypocotyl length is millimeters. Error bars represent SE ($n \geq 20$). Letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Tukey's post-hoc analysis. The experiments were performed three times with similar results. The graphs depict the results of one of three experiments.

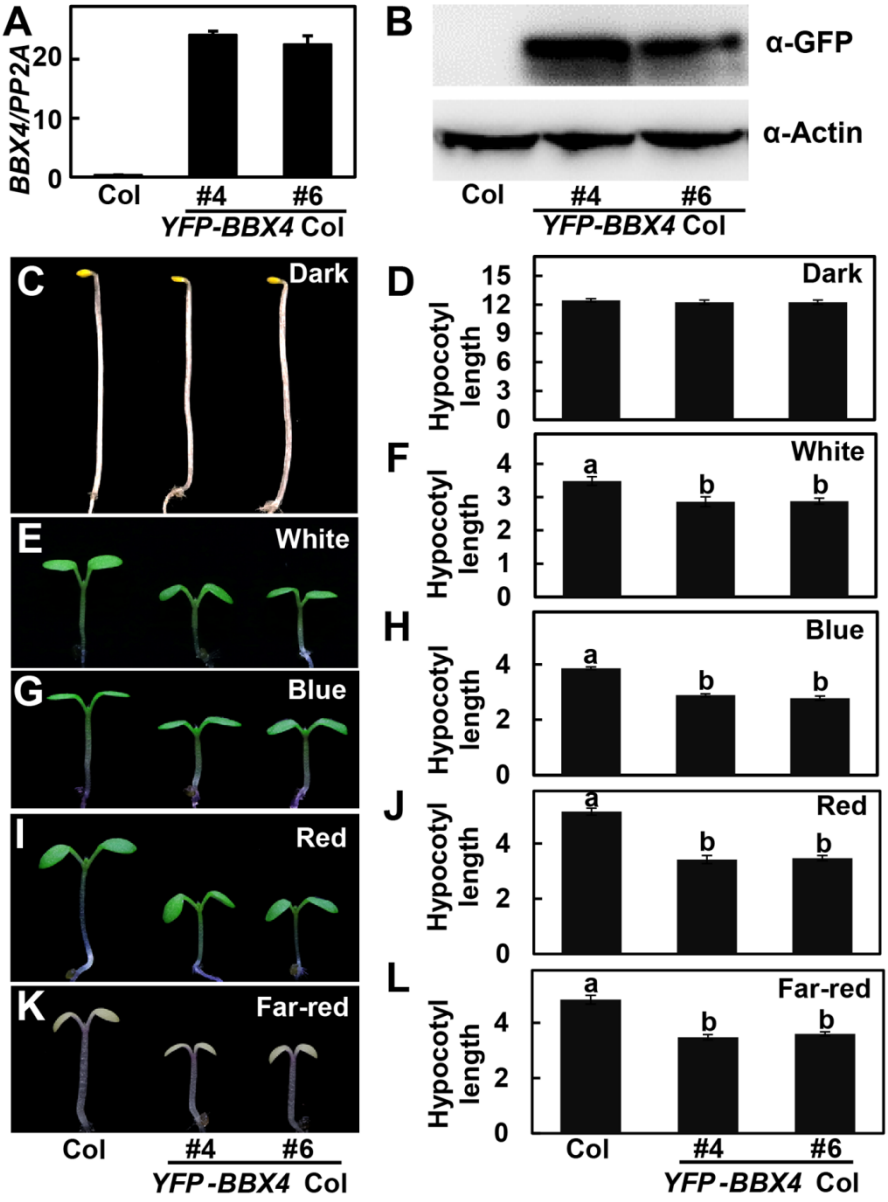

Fig. S2. *BBX4* transgenic seedlings are hypersensitive to light.

(A) *BBX4* transcript levels in Col and *YFP-BBX4* transgenic seedlings grown in white light for four days, as determined by RT-qPCR. Error bars represent SD (n=3).

(B) YFP-BBX4 protein levels in *YFP*-tagged *BBX4* transgenic seedlings grown in white light for four days, as determined by immunoblot analysis.

(C) to **(L)** Hypocotyl phenotype and length of four-d-old Col and *YFP-BBX4* transgenic seedlings grown in darkness (C and D), W (13.24 μmol/m^2/s) (E and F), B (3.88 μmol/m^2/s) (G and H), R (115.8 μmol/m^2/s) (I and J) and FR (4.3 μmol/m^2/s) (K and L) light conditions. The unit of hypocotyl length is millimeters. Error bars represent SE ($n \geq 20$). Letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Tukey's post-hoc analysis. The experiments were performed three times with similar results. The graphs depict the results of one of three experiments.

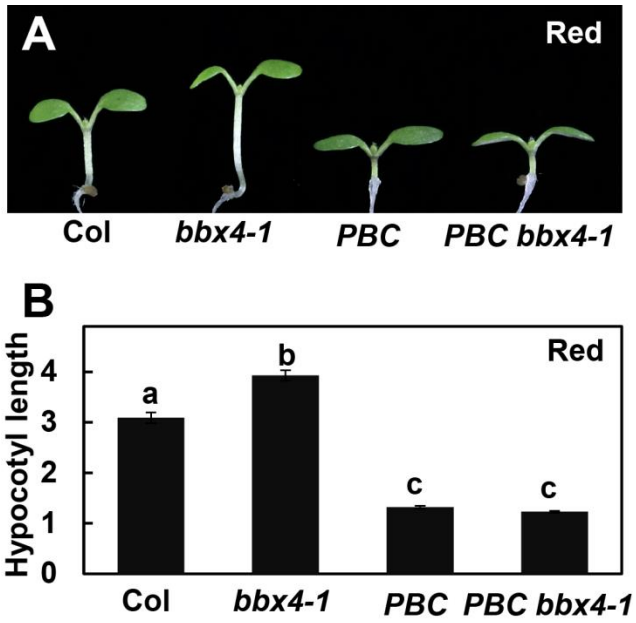

Fig. S3. ***PBC bbx4-1*** **shows similar hypocotyl phenotype with** ***PBC*** **grown in red light.**

(A) and (B) The hypocotyl phenotype (A) and length (B) of four-d-old Col, *bbx4-1*, *PBC(PHYB-CFP* Col*), PBC bbx4-1* seedlings grown in R (115.8 μmol/m^2/s) light. Error bars represent SE (n≥20). Letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Tukey's post-hoc analysis. The experiments were performed three times with similar results. The graphs depict the results of one of three experiments.

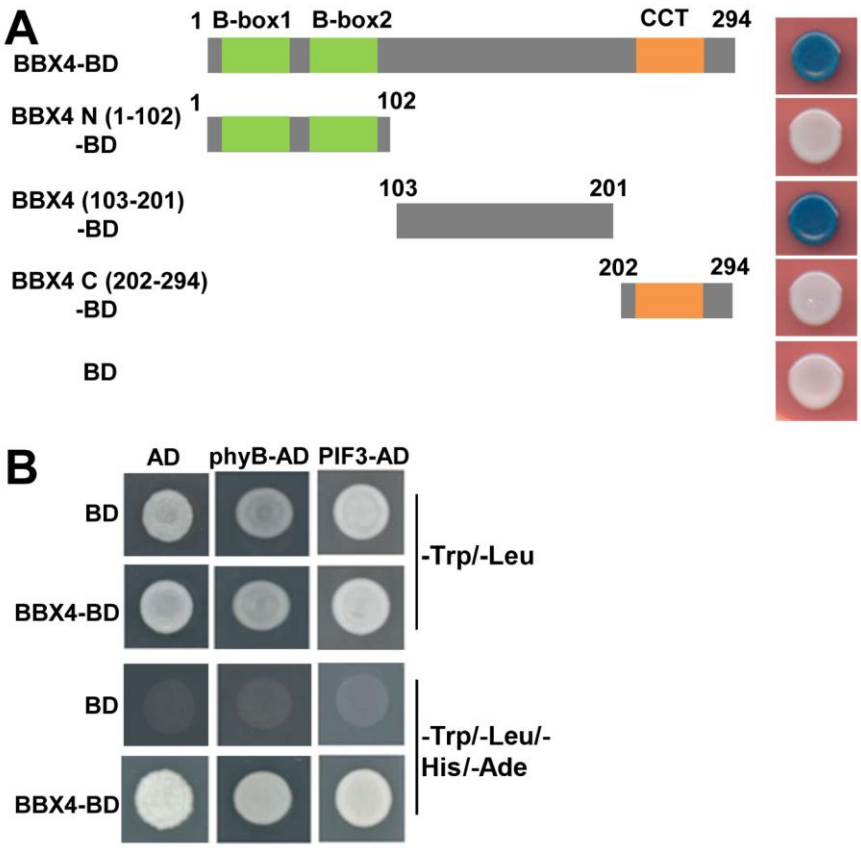

Fig. S4. BBX4 has intrinsic transcriptional activation activity.

(A) Transactivation activity analysis of full-length and various truncated BBX4 proteins in LexA yeast system. BD indicates LexA DNA-binding domain. Empty vector was used as the negative control.

(B) The GAL4 yeast two-hybrid assay showing that full-length BBX4 has self-activation on -Trp/-Leu/-His/-Ade plate.

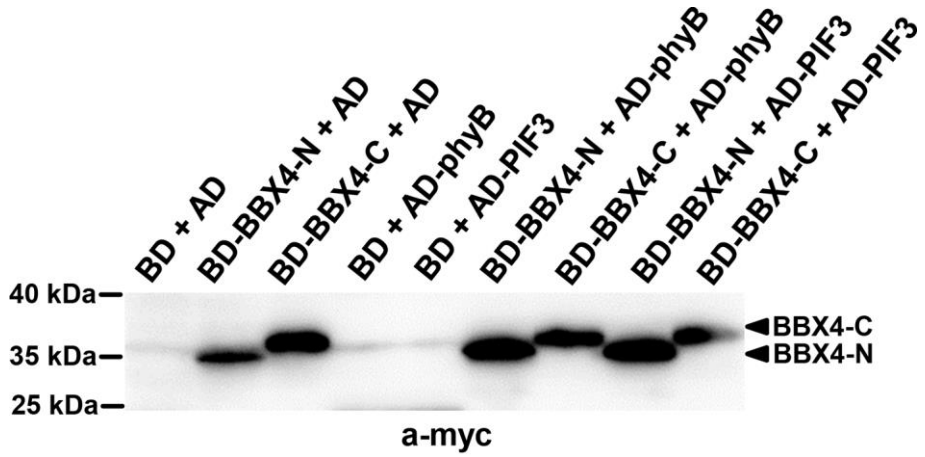

Fig. S5. Immunoblot analysis showing the similar levels of BBX4-N and BBX4-C proteins in yeast cells.

Proteins were extracted from yeast cells transformed with various pairs of plasmids as indicated, and then immmunobloted with monoclonal myc antibodies.

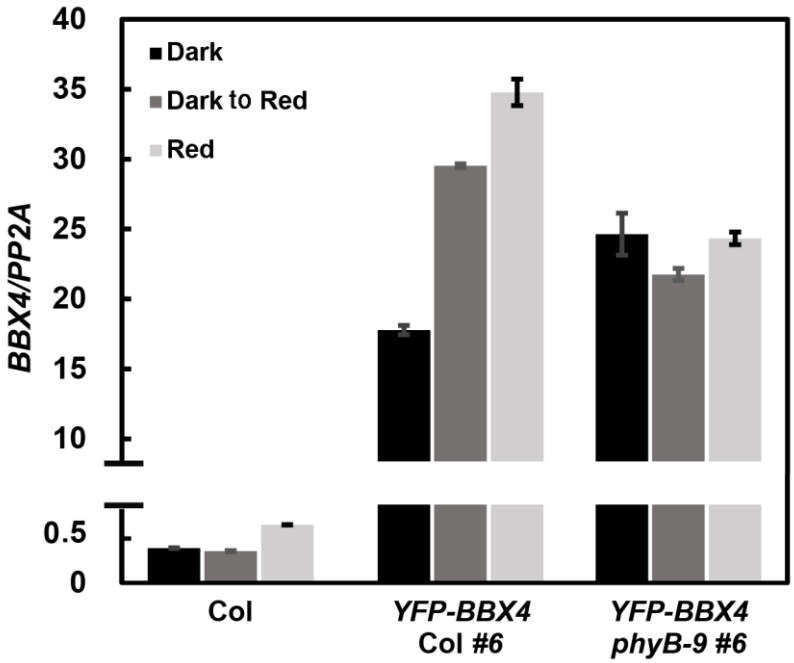

Fig. S6. The transcript levels of *BBX4* in Col, *YFP-BBX4* Col and *YFP-BBX4 phyB-9*.

Seedlings were grown in the dark for 4d (Dark), then transferred into red light for 3h (Dark to Red), or grown in constant red light for 4d (Red). The expression levels were normalized to those of *PP2A*. Error bars represent SD (n = 3).

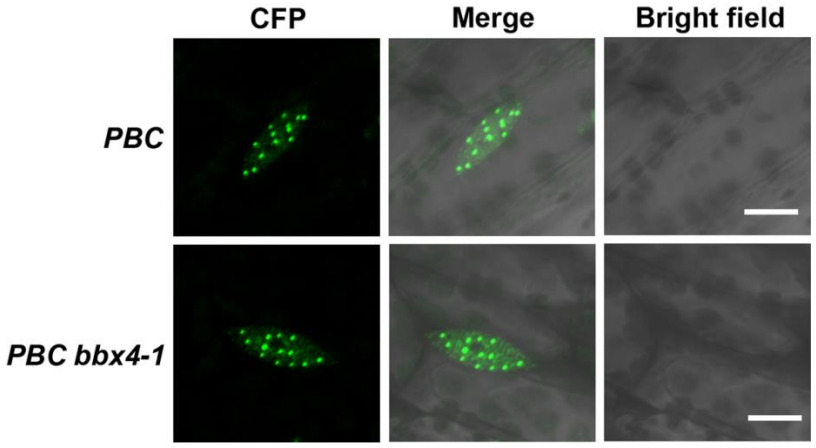

Fig. S7. BBX4 does not affect phyB nuclear bodies.

Subnuclear localisation of PHYB-CFP in hypocotyl cells of 4-d-old *PBC* and *PBC bbx4-1* seedlings grown in R (115.8 μmol/m^2/s) light.

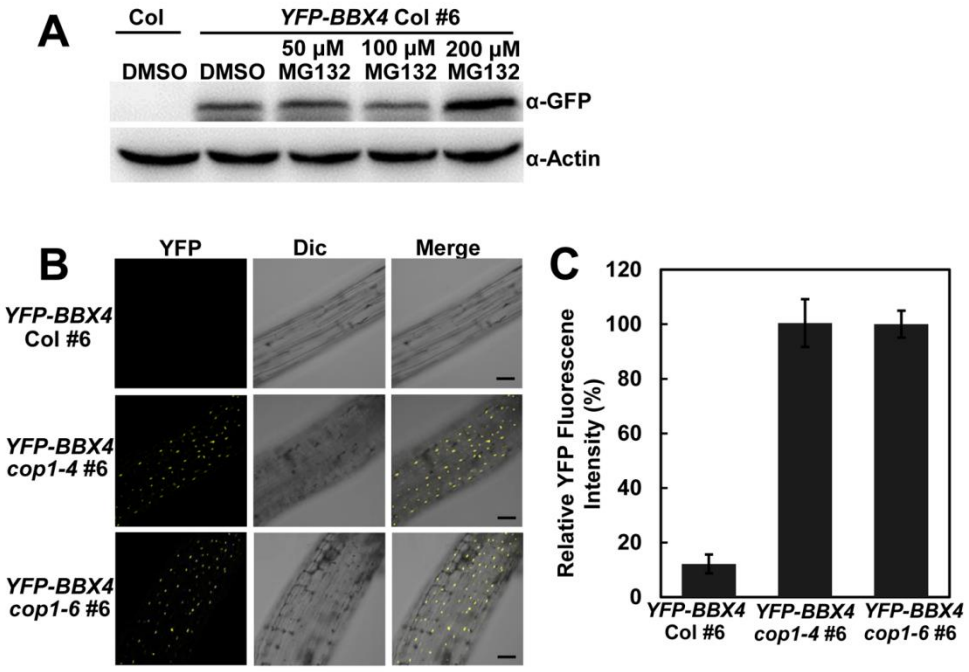

Fig. S8. BBX4 is subjected to COP1-mediated degradation in darkness.

(A) Immunoblot analysis of YFP-BBX4 protein levels in 4-d-old dark-grown *YFP-BBX4* Col #6 transgenic seedlings treated with various concentrations (0, 50, 100, and 200 μM) of MG132. Col treated with DMSO served as a negative control, and anti-actin served as a loading control.

(B) Analysis of YFP fluorescence signals in hypocotyls of *YFP-BBX4* Col #6, *YFP-BBX4 cop1-4* #6 and *YFP-BBX4 cop1-6* #6 seedlings grown in darkness for 4d. Bar = 100 μm.

(C) Relative YFP fluorescence intensity in hypocotyls of *YFP-BBX4* Col #6, *YFP-BBX4 cop1-4* #6 and *YFP-BBX4 cop1-6* #6 seedlings grown in darkness for 4d. The corresponding fluorescence intensity was measured using Image J software and was compared between the overall signals from the images, as shown in (F). Error bars represent SE (n≥10).

Table S1. Primers used in this study

Primer name	Primer sequences (5'→3')	Construct name
Plasmid Constructs	**Underscored nucleotides indicate restriction sites for cloning**	
BBX4-attB1	GGGGACAAGTTTGTACAAAAAAG CAGGCTGGATGGCGTCGTCGTCA AGACTTTG	*pDONR223-BBX 4*
BBX4-attB2	GGGGACCACTTTGTACAAGAAAG CTGGGTGTCAGAAACTCGGAACA ACACCGAA	
phyB-attB1	GGGGACAAGTTTGTACAAAAAAG CAGGCTGGATGGTTTCCGGAGTC GGGGGTA	*pDONR223-phyB*
phyB-attB2	GGGGACCACTTTGTACAAGAAAG CTGGGTG CTAATATGGCATCATCAGCATCAT	
PIF3-attB1	GGGGACAAGTTTGTACAAAAAAG CAGGCTGGATGCCTCTGTTTGAGC TTTTCAG	*pDONR223-PIF3*
PIF3-attB2	GGGGACCACTTTGTACAAGAAAG CTGGGTG TCACGACGATCCACAAAACTGAT	
BBX4-EcoRI (F)	ATGGCCATGGAGGCCGAATTCAT GGCGTCGTCGTCAAGACTTTG	*pGBKT7-BBX4*
BBX4-BamHI (R)	CCGCTGCAGGTCGACGGATCCTC AGAAACTCGGAACAACACCGAA	
BBX4N-EcoRI (F)	ATGGCCATGGAGGCCGAATTCAT GGCGTCGTCGTCAAGACTTTG	*pGBKT7-BBX4N*
BBX4N-BamHI (R)	CCGCTGCAGGTCGACGGATCCTT ATGGACCTACAGCGTCGTAG	
BBX4C-EcoRI (F)	ATGGCCATGGAGGCCGAATTCAT GACGGAGACGCCAGCTGTGCA	*pGBKT7-BBX4C*
BBX4C-BamHI (R)	CCGCTGCAGGTCGACGGATCCTC AGAAACTCGGAACAACACCGAA	
phyB-EcoRI (F)	GCCATGGAGGCCAGTGAATTCATG GTTTCCGGAGTCGGGGGTA	*pGADT7-phyB*
phyB-BamHI (R)	CAGCTCGAGCTCGATGGATCCCTA ATATGGCATCATCAGCATCAT	
PIF3-EcoRI (F)	GCCATGGAGGCCAGTGAATTCATG CCTCTGTTTGAGCTTTTCAG	*pGADT7-PIF3*
PIF3-BamHI (R)	CAGCTCGAGCTCGATGGATCCTCA CGACGATCCACAAAACTGAT	

CRISPR primers		
BBX4-DT1-BsF	ATATATGGTCTCGATTGCAGACGCCGCGTTTCTCTGGTT	*pCambia1300-AtU6-26K-BBX4-crisp1-CAS9-AtU6-26K-BBX4-crisp2-CAS9*
BBX4-DT1-F0	TGCAGACGCCGCGTTTCTCTGGTTTTAGAGCTAGAAATAGC	
BBX4-DT2-R0	AACCTGCGTTATGCGTCACGTGCAATCTCTTAGTCGACTCTAC	
BBX4-DT2-BsR	ATTATTGGTCTCGAAACCTGCGTTATGCGTCACGTGC	
Genotyping primers		
Crispr bbx4 seq(F)	ATCGCCACTTCCATAACACC	
Crispr bbx4 seq(R)	AGCTAAAAGCCACGAAGCAG	
phyB-9- seq(F)	TGCTGTTCAATCGCAGAAAC	
phyB-9- seq(R)	TCGCAGTGTGAGATCGAAAC	
pif3-1-seq(LP)	GATGTGGAAGAAGAATCAGGAGA	
pif3-1-seq(RP)	GTCGTTGTTTGATCTTTAGAACCAG	
cop1-6-seq(F)	GGCCACATGAGAAGAACCAGATT	
cop1-6-seq(R)	CACAGATTGAAAATCTGCAAGGC	
Real-time qPCR		
PP2A(F)	TATCGGATGACGATTCTTCGTGCAG	
PP2A(R)	GCTTGGTCGACTATCGGAATGAGAG	
BBX4(F)	ATGTGTAGAGGGTTTGAGAAAGA	
BBX4(R)	TGCGTCTGCCTCACAATACA	
PIL1(F)	AAATTGCTCTCAGCCATTCGTGG	
PIL1(R)	TTCTAAGTTTGAGGCGGACGCAG	
PIL2(F)	CACCTTTCATTCCAACGGAAA	
PIL2(R)	GCACGATGGAGGGACAGATT	
BBX23(F)	TCCAAAGACATCACCGAGTCG	
BBX23(R)	GTACCCTTTTCTCTCCTGGCAG	
XTR7(F)	CGGCTTGCACAGCCTCTT	
XTR7(R)	TCGGTTGCCACTTGCAATT	
bHLH87(F)	GAGCCCGATGCTGAGGCGATTG	
bHLH87(R)	CCCTTCTCTGTCTCGCTGCAACC	
SNRK2.5(F)	GCCAAAGGAGCTTACAGAGCCTGC	
SNRK2.5(R)	AGACGGAGCTGGATTCCTGGC	