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Supplemental Information

Brassinosteroids Regulate Circadian Oscillation via the BES1/TPL-CCA1/LHY Module in *Arabidopsisthaliana*

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Transparent Methods

Plant Materials and Growth Conditions

Arabidopsis thaliana ecotypes Columbia (Col-0), Wassilewskija (Ws-2), and Enkheim (En-2) were used as wild-type controls. Plants were grown in a growth chamber (Vision Scientific) under neutral day (ND) conditions (12-h light/12-h dark cycles) using cool white fluorescent lamps (120 μmol photons m^{-2} s^{-1}) at 22-23°C, otherwise specified.

The *bri1-5* (Ws-2), *bes1-D* (En-2 background), *bzr1-1D* (Col-0 background), *bes1-ko* (Col-0 background), *bzr1-2* (Col-0 background), *pBES1::bes1-D-HA* (Col-0 background), and *pTPL-TPL-HA* (Col-0 background) plants were previously described (Noguchi et al., 1999; Wang et al., 2002; Ryu et al., 2014; Espinosa-Ruiz et al., 2017; Galstyan and Nemhauser, 2019). The T-DNA insertion mutant *tpl-8* (SALK_036566), *bzr1-2* mutant (GK-857E04), and *cca1-1 lhy-21* (Ws-2 background, CS9809) were obtained from the *Arabidopsis* Biological Resource Center (ABRC), Ohio, USA. The *tpl-8* mutant (Col-0 background) had a T-DNA insertion at 10th exon of *TPL* gene, which resulted in a complete null allele (Long, 2006). *bri1-5* x *pCCA1:LUC* plants were generated by crossing *bri1-5* with *pCCA1:LUC* transgenic plant and further backcrossed to *pCCA1:LUC* plants (Col-0 background) (Salome and McClung, 2005), which was obtained from Dr. C Robertson McClung (Dartmouth College, USA).

Short-Term BL Treatment

Seedlings were grown on 1/2 MS-solid medium supplemented with 0.8% (w/v) agar and 1% (w/v) sucrose under ND conditions for 2 weeks. Plants were transferred to 1/2 MS-liquid medium (without sucrose) supplemented with 1 μM epi-BL (E1641; Sigma, St Louis, MO,

USA) or 0.1% (v/v) DMSO (mock control) at the indicated time points and incubated for 4 h. The concentration was determined based on the previous report (Ren et al., 2009).

Quantitative Real-Time RT-PCR Analysis

To extract total RNA, the harvested samples were ground in liquid nitrogen, and 700 μL TRI reagent (TAKARA Bio, Singa, Japan) was mixed thoroughly with the ground tissue. The samples were centrifuged at 4℃ for 10 min. Then, 250 μL chloroform was added to the supernatant, and RNA in the aqueous phase was precipitated with isopropanol. The RNA pellet was washed with 70% ethanol and dissolved in RNase-free deionized water. Subsequently, 2 μg RNA pretreated with DNAse I (New England Biolabs, Beverly, MA. USA) was reverse transcribed using the Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Dr. Protein, Seoul, Korea).

Then, qPCR was performed in 96-well blocks on the Step-One Plus Real-Time PCR System (Applied Biosystems) using sequence-specific primers (Table S1). The expression of each gene was normalized relative to the *EUKARYOTIC TRANSLATION INITIATION FACTOR 4A1* (*eIF4A*) gene (At3g13920). All RT-qPCR reactions were performed using total RNA extracted from independent replicates. The comparative threshold cycle (CT) method was employed to evaluate relative quantities of each amplified product. The CT value of each reaction was determined automatically by the system using default parameters. The specificity of RT-qPCR reactions was determined by melt curve analysis of the amplified products using the standard method installed in the system.

Bioluminescence Assays

pCCA1:LUC (Salome and McClung, 2005) or *pCCA1:LUC* x *bri1-5* seeds (backcrossed to

Col-0 background) were sown in 1/2 MS-solid medium, supplemented with 0.72% (w/v) agar and 3% (w/v) sucrose, and stratified at 4℃ for 3 days (Davis et al., 2018; Chen et al., 2020). Germinated seedlings grown and entrained for 7 days under ND conditions at 22-23℃ were transferred to 96-well microplates containing 1/2 MS-liquid medium supplemented with 3% sucrose, 0.24 mM D-luciferin (BioThema, Handen, Sweden), and either 100 μM epi-BL or 40 μM bikinin. For epi-BL treatment, 4 mM epi-BL stock solution dissolved in EtOH were used for final concentration of 100 μM epi-BL and 2.5% (v/v) EtOH was used as a mock treatment. Long-term treatment with BL required higher concentrations possibly due to signaling attenuation by intrinsic feedback regulation (Tanaka et al., 2005). DMSO was used as a vehicle for bikinin (SML0094; Sigma, St Louis, MO, USA) treatment, 10 mM bikinin stock solution was diluted to make the final concentration of 40 μM (Uehara et al., 2019) and 0.4% (v/v) DMSO was used as a mock treatment.

Luminescence rhythms were monitored using the Tristar^2 LB 942 Multimode Microplate Reader (Berthold Technologies, Wildbad, Germany) and analyzed as previously described (Lee et al., 2016). The circadian period was estimated using the Fast Fourier Transform-Nonlinear Least Squares (FFT-NLLS) suite of programs available in the Biodare2 software (Zielinski et al., 2014).

To examine circadian oscillations in protoplasts in the presence of epi-BL, mesophyll protoplasts were isolated from the lower epidermal layer of leaves of 3-week-old *Arabidopsis* seedlings. The detached leaves were rinsed twice using sterile water. Leaf surfaces were soaked in 10 mL of an enzyme solution (400 mM mannitol, 20 mM KCl, 20 mM MES-KOH [pH 5.7], 10 mM $CaCl_2$, 1% Cellulase R10, 0.5% Macerozyme R10, and 0.1% bovine serum albumin) in a petri dish for 4 h in the dark. The released protoplasts were filtered through sterile 100 mm stainless mesh, and resuspended in W5 solution as previously described (Kim

and Somers, 2010; Hansen and van Ooijen, 2016). The *pCCA1:LUC* plasmids were prepared by CsCl gradient purification. Then, the *pCCA1:LUC* recombinant construct was transiently introduced into *Arabidopsis* protoplasts via polyethylene glycol (PEG)-mediated transformation (Kim and Somers, 2010; Hansen and van Ooijen, 2016).

Chromatin Immunoprecipitation (ChIP) Assays

ChIP assays were conducted using *pBES1:bes1-D-HA*, *pBZR1:bzr1-1D-HA*, and *pTPL:TPL-HA* transgenic plants. Seeds sown on 1/2 MS-solid medium supplemented with 1% (w/v) sucrose, 0.75% (w/v) agar were stratified for 3 days, then grown and entrained in a growth chamber for 2 weeks under ND conditions at 22-23ºC. Whole plants were harvested, fixed in 1% formaldehyde, and ground in liquid nitrogen. Chromatin solubilized by the nuclei lysis buffer (50 mM Tris-HCl [pH 8.0], 10 mM ethylene diamine tetraacetic acid [EDTA; pH 8.0], 1% sodium dodecyl sulfate [SDS], 1 mM phenylmethylsulfonyl fluoride [PMSF], and 1X PIs) was sonicated at 4°C to approximately 500 bp fragments using a Bioruptor. After fragmentation, the extract was precleared with 100 μL protein A/G agarose beads (sc2003; Santa Cruz, CA, USA) for 2 h. The precleared supernatant was incubated overnight with 1 μg corresponding antibodies, including anti-HA antibody (ab9110; Abcam, Cambridge, UK), and the supernatant was mixed with 100 μL protein A/G agarose beads for 16 h at 4°C on a rotator. Then, the DNA fragments were purified using the DNA elution kit and quantified by quantitative real-time PCR (qRT-PCR) using sequence-specific primers (Table S2). The amplified PCR products were normalized relative to the input DNA.

Y2H Assays

Y2H assays were performed using the BD Matchmaker system (Clontech, Mountain View,

CA, USA). PCR products were subcloned into the pGBKT7 and pGADT7 vectors to generate GAL4 activation domain (AD) and GAL4 DNA-binding domain (BD) fusion constructs, respectively. The GAL4 AD and BD constructs were cotransformed into the yeast strain pJG69-4A harboring the *LacZ* and *His* reporter genes, and the transformed cells were selected by growth on synthetic defined (SD) medium lacking leucine and tryptophan (SD/-Leu/-Trp), and SD medium lacking Leu, Trp, histidine, and adenine (SD/-Leu/-Trp/-His/-Ade). Protein–protein interactions were analyzed by measuring β-galactosidase activity using o-nitrophenyl-β-D-galactopyranoside as the substrate.

Hypocotyl Length Measurement

35S:*CCA1α-MYC* (Col-0 background) (Seo et al., 2012) and *cca1-1 lhy-21* (Ws-2 background) seeds were sown on 1/2 MS medium containing 1 μM epi-BL or 0.1% DMSO (mock treatment). After stratification at 4℃ for 3 days, plants were vertically grown under ND conditions at 22-23℃. Seedlings were photographed at the indicated time and hypocotyl lengths were measured using the Image J software (http://rsb.info.nih.gov/ij).

Transient Gene Expression Assays

Transient gene expression assays were conducted in Arabidopsis protoplasts using reporter and effector plasmids. The core element in *CCA1* and *LHY* promoters was subcloned into the reporter plasmid containing a minimal 35S (Min 35S) promoter sequence and the *GUS* gene. To construct p35S:bes1-D, p35S:bzr1-1D, and p35S:TPL effector plasmids, coding sequences of *bes1-D*, *bzr1-1D*, and *TPL* genes were cloned downstream of the cauliflower mosaic virus (CaMV) 35S promoter. Recombinant reporter and effector plasmids were cotransformed into *Arabidopsis* protoplasts along with the p35S:LUC construct (internal control) via PEG-

mediated transformation. GUS activity was measured using a fluorometric method, and LUC activity was measured using the Luciferase Assay System Kit (Promega, Madison, WI, USA).

REFERENCES

Chen, W.W., Takahashi, N., Hirata, Y., Ronald, J., Porco, S., Davis, S.J., Nusinow, D.A., Kay, S.A., and Mas, P. (2020). A mobile ELF4 delivers circadian temperature information from shoots to roots. Nature Plants **6**:416-426.

Davis, A.M., Ronald, J., Ma, Z., Wilkinson, A.J., Philippou, K., Shindo, T., Queitsch, C., and Davis, S.J. (2018). HSP90 contributes to entrainment of the Arabidopsis circadian clock via the morning loop. Genetics **210**:1383-1390.

Espinosa-Ruiz, A., Martinez, C., de Lucas, M., Fabregas, N., Bosch, N., Cano-Delgado, A.I., and Prat, S. (2017). TOPLESS mediates brassinosteroid control of shoot boundaries and root meristem development in Arabidopsis thaliana. Development **144**:1619-1628.

Galstyan, A., and Nemhauser, J.L. (2019). Auxin promotion of seedling growth via ARF5 is dependent on the brassinosteroid-regulated transcription factors BES1 and BEH4. Plant Direct **3**:e00166.

Hansen, L.L., and van Ooijen, G. (2016). Rapid analysis of circadian phenotypes in Arabidopsis protoplasts transfected with a luminescent clock reporter. J. Vis. Exp. doi: 10.3791/54586.

Kim, J., and Somers, D.E. (2010). Rapid assessment of gene function in the circadian clock using artificial microRNA in Arabidopsis mesophyll protoplasts. Plant Physiol. **154**:611-621.

Lee, H.G., Mas, P., and Seo, P.J. (2016). MYB96 shapes the circadian gating of ABA

signaling in Arabidopsis. Sci Rep **6**:17754.

Long, J.A. (2006). TOPLESS Regulates Apical Embryonic Fate in Arabidopsis. Science **312**:1520-1523.

Noguchi, T., Fujioka, S., Choe, S., Takatsuto, S., Yoshida, S., Yuan, H., Feldmann, K.A., and Tax, F.E. (1999). Brassinosteroid-insensitive dwarf mutants of Arabidopsis accumulate brassinosteroids. Plant Physiol. **121**:743-752.

Ren, C., Han, C., Peng, W., Huang, Y., Peng, Z., Xiong, X., Zhu, Q., Gao, B., and Xie, D. (2009). A leaky mutation in *DWARF4* reveals an antagonistic role of brassinosteroid in the inhibition of root growth by jasmonate in Arabidopsis. Plant Physiol. **151**:1412-1420.

Ryu, H., Cho, H., Bae, W., and Hwang, I. (2014). Control of early seedling development by BES1/TPL/HDA19-mediated epigenetic regulation of ABI3. Nat. Commun. **5**:4138.

Salome, P.A. and McClung, C.R. (2005). PSEUDO-RESPONSE REGULATOR 7 and 9 are partially redundant genes essential for the temperature responsiveness of the Arabidopsis circadian clock. **17**:791-803.

Seo, P.J., Park, M.-J., Lim, M.-H., Kim, S.-G., Lee, M., Baldwin, I.T., and Park, C.-M. (2012). A self-regulatory circuit of CIRCADIAN CLOCK-ASSOCIATED1 underlies the circadian clock regulation of temperature responses in Arabidopsis. The Plant Cell **24**:2427-2442.

Tanaka, K., Asami, T., Yoshida, S., Nakamura, Y., Matsuo, T., and Okamoto, S. (2005). Brassinosteroid homeostasis in Arabidopsis is ensured by feedback expressions of multiple genes involved in its metabolism. Plant Physiol. **138**:1117-1125.

Uehara, T.N., Mizutani, Y., Kuwata, K., Hirota, T., Sato, A., Mizoi, J., Takao, S., Matsuo, H., Suzuki, T., Ito, S., *et al.* (2019). Casein kinase 1 family regulates PRR5 and TOC1 in the Arabidopsis circadian clock. Proc. Natl. Acad. Sci. U S A **116**:11528-11536.

Wang, Z.Y., Nakano, T., Gendron, J., He, J., Chen, M., Vafeados, D., Yang, Y., Fujioka, S., Yoshida, S., Asami, T., *et al.* (2002). Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. Dev Cell **2**:505-513.

Zielinski, T., Moore, A.M., Troup, E., Halliday, K.J., and Millar, A.J. (2014). Strengths and limitations of period estimation methods for circadian data. PLoS One **9**:e96462.

Supplemental Figure

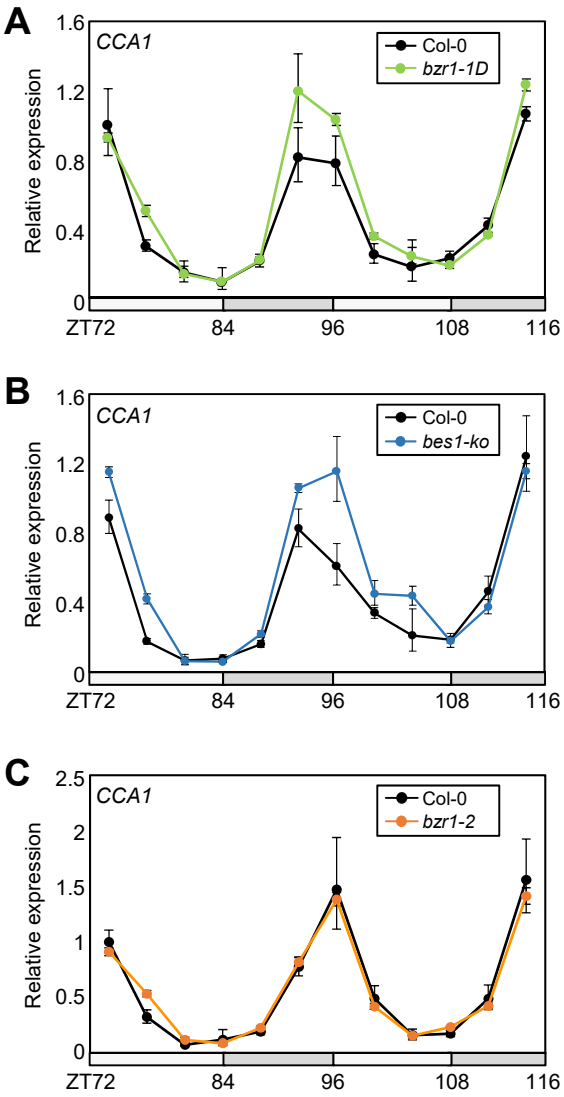

Figure S1. Circadian expression of *CCA1* in *bes1-ko*, *bzr1-1D*, and *bzr1-2*, Related to Figure 1.
Two-week-old seedlings grown under neutral day (ND) conditions were transferred to continuous light (LL) conditions at ZT0. Whole seedlings were harvested from ZT72 to ZT116 to analyze transcript accumulation. Bars indicate the standard error of the mean. The white and grey boxes indicate the subjective day and night, respectively.

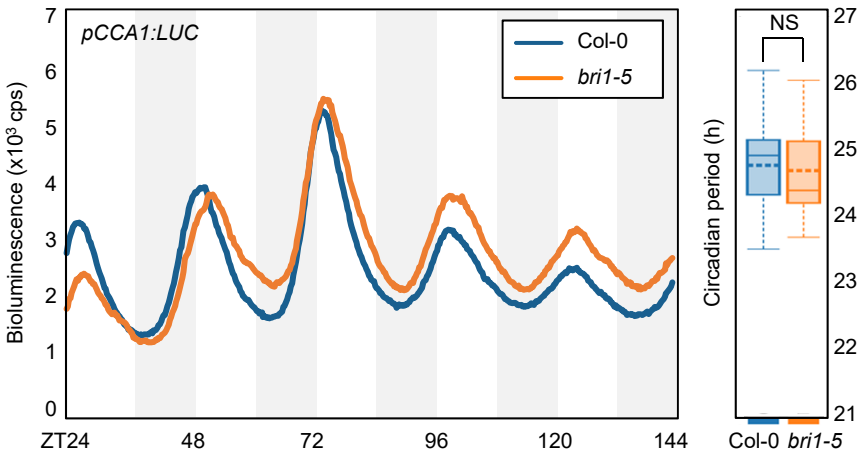

Figure S2. Circadian expression of *CCA1* in *bri1-5* under normal conditions, Related to Figure 1. Seven-day-old seedlings grown under neutral day (ND) conditions were transferred to continuous dark (DD) conditions at ZT0 (left panel). Circadian period estimates of *pCCA1:LUC* activity were shown (right panel). Data represented as mean ± SEM were analyzed with using the Fast Fourier Transform Non-linear Least Squares (FFT-NLLS) suite of programs available in the Biodare2 software ($n > 14$). NS, not significant.

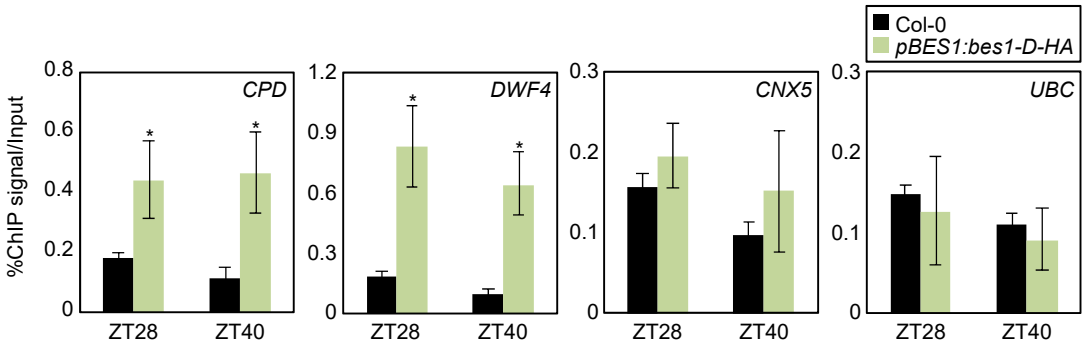

Figure S3. Positive and negative controls for BES1 binding, Related to Figure 2.

Two-week-old seedlings entrained under ND cycles were subjected to LL conditions. Plants were harvested for ChIP analysis with anti-HA antibody. Enrichment of fragmented genomic regions of positive control genes (*CPD* and *DWF4*) and negative control genes (*CNX5* and *UBC*) was analyzed by ChIP-qPCR (Huang et al., 2013; Wang et al., 2018). Biological triplicates were averaged, and statistical significance was determined by Student's *t*-test ($P < 0.05$). Bars indicate standard error of the mean.

- **Huang, H.Y., Jiang, W.B., Hu, Y.W., Wu, P., Zhu, J.Y., Liang, W.Q., Wang, Z.Y., and Lin, W.H.** (2013). BR signal influences Arabidopsis ovule and seed number through regulating related genes expression by BZR1. Mol. Plant **6**:456-469.
- **Wang, W., Lu, X., Li, L., Lian, H., Mao, Z., Xu, P., Guo, T., Xu, F., Du, S., Cao, X., et al.** (2018). Photoexcited CRYPTOCHROME1 interacts with dephosphorylated BES1 to regulate brassinosteroid signaling and photomorphogenesis in Arabidopsis. Plant Cell **30**:1989-2005.

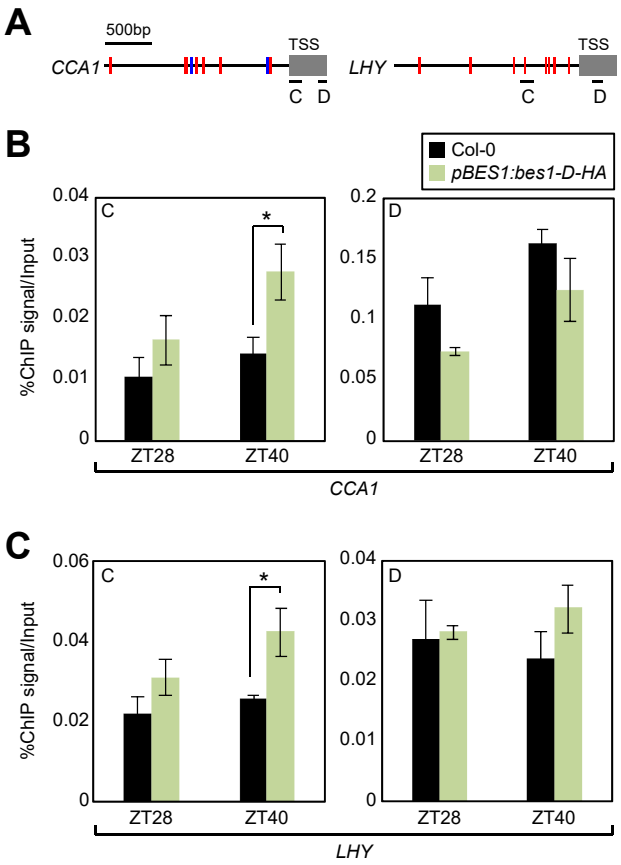

Figure S4. Binding of BES1 to coding regions of *CCA1* and *LHY*, Related to Figure 2.
Two-week-old plants entrained with ND cycles were subjected to LL conditions. Plants were harvested at ZT28 and ZT40 for ChIP analysis with anti-HA antibody. Enrichment of fragmented genomic regions was analyzed by ChIP-qPCR. Biological triplicates were averaged, and statistical significance was determined by Student's *t*-test (*$P < 0.05$). Bars indicate standard error of the mean. TSS, transcriptional start site.

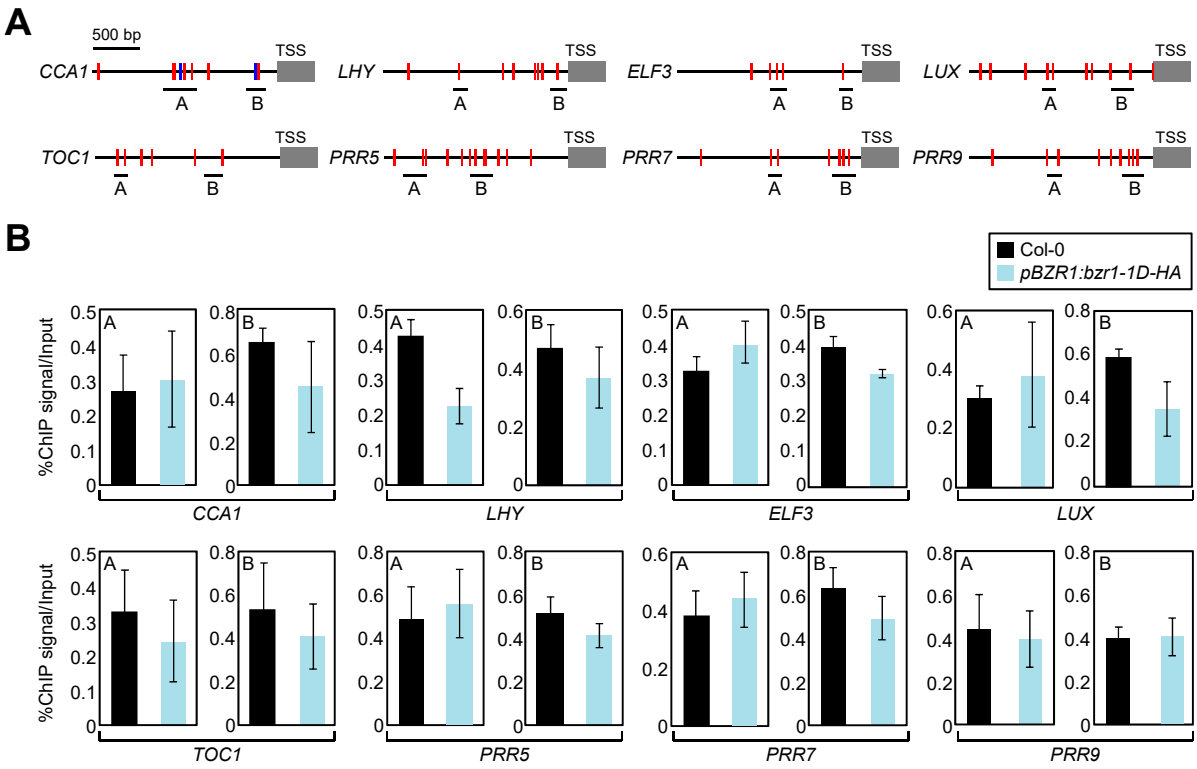

Figure S5. Binding of BZR1 to core clock genes, Related to Figure 2.

(A) Genomic structures of core clock genes. Underbars indicate the regions amplified by quantitative PCR (qPCR) following chromatin immunoprecipitation (ChIP). Red lines indicate E-boxes, and blue lines indicate BR-response elements (BRREs). TSS, transcription start site.

(B) Binding of BZR1 to clock gene promoters at ZT40. Two-week-old seedlings entrained under ND cycles were subjected to LL conditions. Plants were harvested at ZT40 for ChIP analysis with anti-HA antibody. Biological triplicates were averaged. Data represent mean ± SEM.

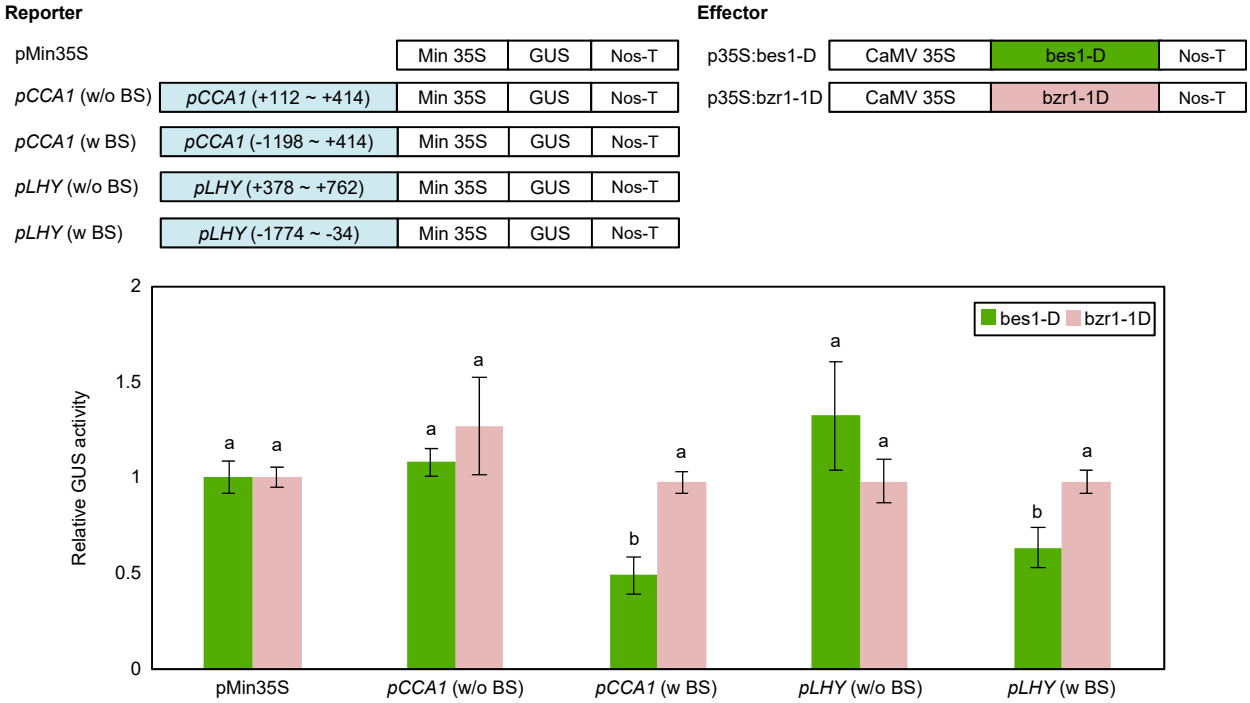

Figure S6. Transient expression assays using *Arabidopsis* protoplasts, Related to Figure 2.

The recombinant reporter and effector constructs were coexpressed transiently in *Arabidopsis* protoplasts, and GUS activity was determined. Luciferase gene expression was used to normalize GUS activity. The normalized values in control protoplasts were set to 1 and represented as relative values. Three independent measurements were averaged. Different letters represent a significant difference at $P < 0.05$ (one-way ANOVA with Fisher's *post hoc* test). Bars indicate the standard error of the mean. Note that two promoter constructs for each gene were designed to examine the relevance of BES1-binding sites (BS) (see Figures 2A and 2B) in gene regulation. w/o BS, without binding site; w BS, with binding site.

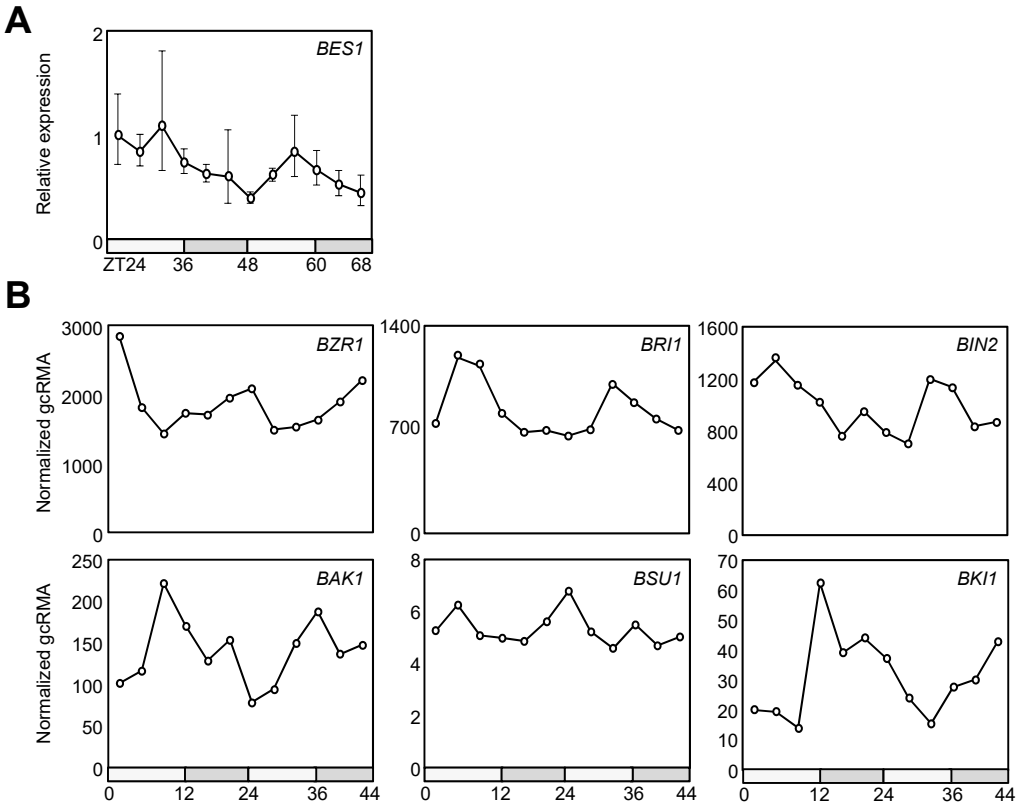

Figure S7. Expression of BR signaling components in circadian cycles, Related to Figure 2.
(A) *BES1* expression. Two-week-old seedlings grown under ND conditions were transferred to LL conditions at ZT0. Transcript levels were determined by RT-qPCR. Bars indicate the standard error of the mean. The white and grey boxes indicate the subjective day and night, respectively.
(B) Circadian expression of BR signaling genes. Data from the DIURNAL project (http://diurnal.mocklerlab.org) microarray repository were used to compare RNA levels. Raw data from the experimental set LL_LDHC were investigated.

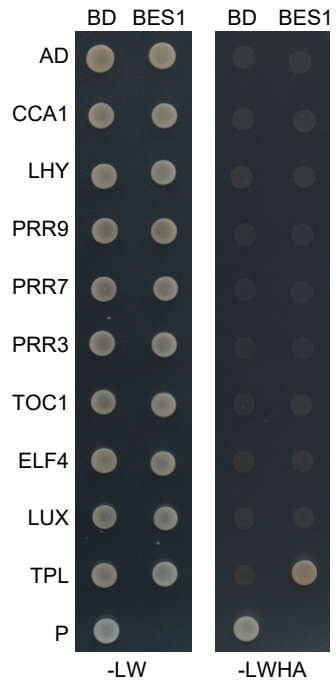

Figure S8. Yeast-two-hybrid (Y2H) assays, Related to Figure 5.
Y2H assays were performed with the BES1 protein fused with the DNA binding domain (BD) of GAL4 and circadian clock proteins fused with the transcriptional activation domain (AD) of GAL4 for analysis of their interactions. Interactions were examined by cell growth on selective media. -LWHA indicates Leu, Trp, His, and Ade drop-out plates. -LW indicates Leu and Trp drop-out plates. GAL4 was used as a positive control (P).

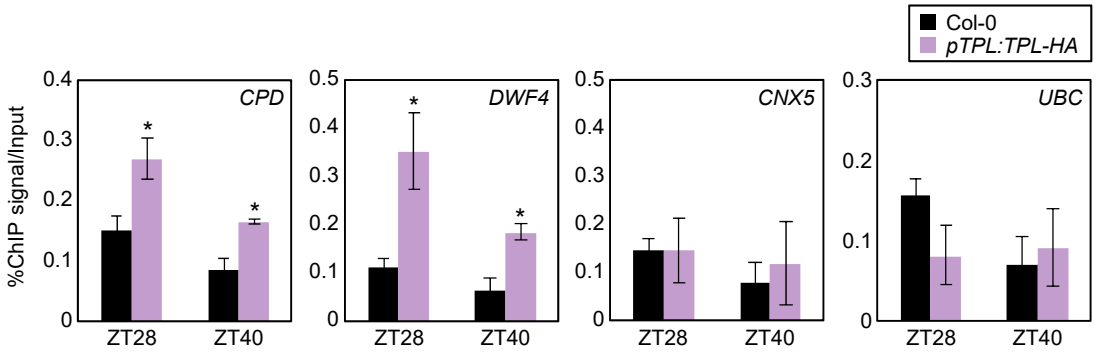

Figure S9. Positive and negative controls for TPL binding, Related to Figure 5.

Two-week-old seedlings entrained under ND cycles were subjected to LL conditions. Plants were harvested for ChIP analysis with anti-HA antibody. Enrichment of fragmented genomic regions of positive control genes (*CPD* and *DWF4*) and negative control genes (*CNX5* and *UBC*) was analyzed by ChIP-qPCR (Huang et al., 2013; Wang et al., 2018). Biological triplicates were averaged, and statistical significance was determined by Student's *t*-test ($P < 0.05$). Bars indicate standard error of the mean.

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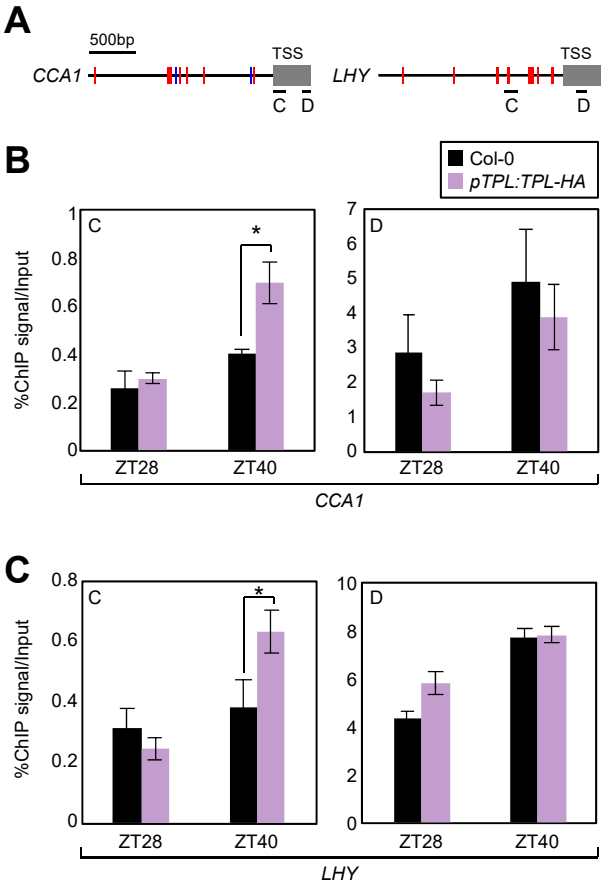

Figure S10. Binding of TPL to coding regions of *CCA1* and *LHY*, Related to Figure 5.
Two-week-old plants entrained with ND cycles were subjected to LL conditions. Plants were harvested at ZT28 and ZT40 for ChIP analysis with anti-HA antibody. Enrichment of fragmented genomic regions was analyzed by ChIP-qPCR. Biological triplicates were averaged, and statistical significance was determined by Student's *t*-test (*$P < 0.05$). Bars indicate standard error of the mean. TSS, transcriptional start site.

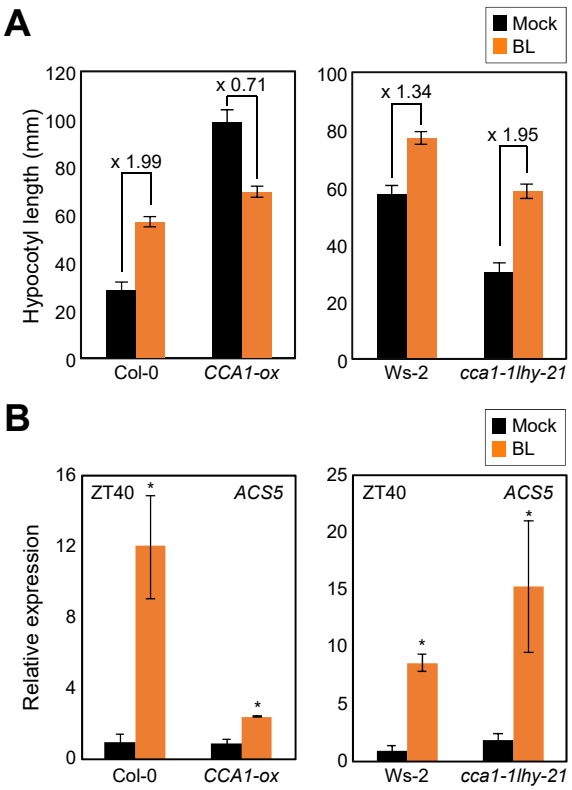

Figure S11. Reduced BR responses in *CCA1-ox* plants, Related to Figure 7.

(A) Hypocotyl length. Ten-day-old seedlings were grown on MS-medium supplemented with mock solution (0.1% DMSO) or 1 μM epi-BL under ND conditions. The numbers above the bars indicate the ratio of hypocotyl length in mock and BL-treated seedlings (BL/mock). Data represent mean ± SEM ($n > 8$).

(B) *ACS5* expression at ZT40. Two-week-old seedlings grown under ND conditions were used to analyze transcript accumulation of a BR-responsive marker gene (Xiong et al. 2019). The *eIF4a* gene was used as an internal control. Biological triplicates were averaged, and statistical significance was determined by Student's *t*-test ($P < 0.05$). Bars indicate standard error of the mean.

- **Xiong, F., Zhuo, F., Reiter, R.J., Wang, L., Wei, Z., Deng, K., Song, Y., Qanmber, G., Feng, L., Yang, Z., et al.** (2019). Hypocotyl elongation inhibition of melatonin is involved in repressing brassinosteroid biosynthesis in Arabidopsis. Front Plant Sci. **10**:1082.

Supplemental Table

Table S1. Primers used in this study, Related to Figure 1, 3 and 6.

Primer	Usage	Sequence
eIF4a-F	RT-qPCR	5′-TGACCACACAGTCTCTGCAA
eIF4a-R	RT-qPCR	5′-ACCAGGGAGACTTGTTGGAC
CAB2-F	RT-qPCR	5′-TTCCCAAGTAATCGAGCC
CAB2-R	RT-qPCR	3′-CCTTACCGGAGAGTTCCC
CCA1-F	RT-qPCR	5′-GATCTGGTTATTAAGACTCGGAAGCCATATAC
CCA1-R	RT-qPCR	5′-GCCTCTTTCTCTACCTTGGAGA
LHY-F	RT-qPCR	5′-CTTATCCTCGAAAGCCTGGG
LHY-R	RT-qPCR	5′-GAACATCTTGAACCGCGTTG
LUX-F	RT-qPCR	5′-TAACGTGGAGGAGGAAGATCGA
LUX-R	RT-qPCR	5′-TCCATCACCGTTTGATGTCTTT
ELF3-F	RT-qPCR	5′-AGAAAGAGCAGGCAAGGGAG
ELF3-R	RT-qPCR	5′-CCTCCCCCATCTCTTGTTGT
TOC1-F	RT-qPCR	5′-TCTTCGCAGAATCCCTGTGAT
TOC1-R	RT-qPCR	5′-GCTGCACCTAGCTTCAAGCA
PRR5-F	RT-qPCR	5′-GCTGCAGTACCTGATGGCTT
PRR5-R	RT-qPCR	5′-TCTTCTCCAGACATGCTGCC
PRR7-F	RT-qPCR	5′-TGGGCCATATGGAAGCAGTA
PRR7-R	RT-qPCR	5′-TTTACGCACAAATTGGCCTC
PRR9-F	RT-qPCR	5′-TTGGTCCTGAGCTTGGACTTT
ACS5-F	RT-qPCR	5′-GCTATGGCTGAGTTTATGG
ACS5-R	RT-qPCR	5′-TTGAGCAGTGAATGGGTAC
SAUR15-F	RT-qPCR	5′-TTGAGGAGTTTCTTGGGTGCT
SAUR15-R	RT-qPCR	5′-ACAAAGGCTGGTTTAAGTATG
bes1-D-F(pBA002)	cloning	5′-GAGGCGCGCCATGAAAAGATTCTTCTATAATTCCAGC
bes1-D-R(pBA002)	cloning	5′-GAACTAGTACTATGAGCTTTACCATTTCCA
bzr1-1D-F(pBA002)	cloning	5′-GAGGCGCGCCATGACTTCGGATGGAGCTACG
bzr1-1D-R(pBA002)	cloning	5′-GAACTAGTACGAGCCTTCCCATTTCCAA
TPL-F(myc-pBA)	cloning	5′-GATCTAGAATGTCTTCTCTTAGTAGAGAGCT
TPL-R(myc-pBA)	cloning	5′-GAGGCGCGCCAGCTCTCTACTAAGAGAAGACATC
pCCA1(-1198~+414)-F(pMin35S)	cloning	5′-GAGGATCCGAACTTGTAGGCATCGGTTACAC
pCCA1(-1198~+414)-R(pMin35S)	cloning	5′-GAAAGCTTCACTAAGCTCCTCTACACAACTT
pCCA1(+112~+414)-F(pMin35S)	cloning	5′-GAGGATCCGTAGCTTCTGGTCTCTTTTCTTTG
pCCA1(+112~+414)-R(pMin35S)	cloning	5′-GAAAGCTTCACTAAGCTCCTCTACACAACTT
pLHY(+378~+762)-F(pMin35S)	cloning	5′-GAGGATCCGCTCTAAAAGTTAGCATAAATGATACG
pLHY(+378~+762)-R(pMin35S)	cloning	5′-GAGGATCCTATTCGCTGCTTCAAATCCTCTCT
pLHY(-1774~-34)-F(pMin35S)	cloning	5′-GAGGATCCATTTAACCATAGGAACAGTGTTG
pLHY(-1774~-34)-R(pMin35S)	cloning	5′-GAGGATCCTTGAAAAGTTTATTTGAGGCTGGAAC

RT-qPCR primers were designed using the Primer Express Software installed into the Applied Biosystems PCR System. The sizes of PCR products ranged from 80 to 300 nucleotides in length. F, forward primer; R, reverse primer.

Table S2. Primers used in chromatin immunoprecipitation (ChIP) assays, Related to Figure 2 and 5 .

Primer	Sequence
CCA1(A)-F	CTTCTCTTTGTATCACTTGAACCAA
CCA1(A)-R	GAATTTGAGTCTTCCATTCTCAGTATTA
CCA1(B)-F	ATATAAAACTATGGCCCAAATAAGTTTAG
CCA1(B)-R	ATCTTGATCTAGTGGGACCTAC
CCA1(C)-F	CATTTCCGTAGCTTCTGGTCTCTT
CCA1(C)-R	ATCAGCTTGGATTCGATAAAGATTC
CCA1(D)-F	ACTCGGAAGCCATATACGATAAC
CCA1(D)-R	CAAAGCTTCAATGAATCTATTATG
LHY(A)-F	GTGTGATTGGATCACTAGTGAC
LHY(A)-R	ACTTAGAGTCGTTATCCTTATTAG
LHY(B)-F	ACCTACGTGAGCTTCACATTG
LHY(B)-R	TTGAAAAGTTTATTTGAGGCTGGAAC
LHY(C)-F	CTACATGCTTCGGTTAAGAC
LHY(C)-R	TCTTCATCTTTTCATATAATATCATGCAATG
LHY(D)-F	TCCTCCATGGCTACTCTCAAGG
LHY(D)-R	TCAGCAGCCAAACAGAGATCTTAG
ELF3(A)-F	TTTAGTAAATAAGAGTGTCCAAGTG
ELF3(A)-R	AGAAACATAGCAAAAGCTCTAG
ELF3(B)-F	AACCTCTAACATGGTAATATATCTATG
ELF3(B)-R	ATCATCCAATACATCACTTTTTG
LUX(A)-F	GAAAAAAGTTGTAATTTGCCTAATGTTC
LUX(A)-R	CTTGCTGATTGTTCTTCAATAG
LUX(B)-F	GATACATAATGTGAACTGATGACG
LUX(B)-R	AAGTTTGGTTTGTGAAGATGATGAGC
TOC1(A)-F	AAGAAACTATCCGAATAACTTCATGC
TOC1(A)-R	TTTGATGAAATTCCTCAGAGAAGATG
TOC1(B)-F	AACAGAAAAATAAAATTCTGATAATAG
TOC1(B)-R	AAACCAAATTTTAGGATTCG
PRR5(A)-F	ATTTAAAGGTGAAAGACTGTG
PRR5(A)-R	AATTTTAAAGTAATTTTCCGCGG
PRR5(B)-F	AAATATCCTTATTGTTTTTAAAATAAAC
PRR5(B)-R	AAAAAGTATCTATACAAATTTATCC
PRR7(A)-F	TTTGTCTTTTAGCACTATACGGTC
PRR7(A)-R	TTCTCCTTCAGTGTTCCTTC
PRR7(B)-F	CTCTTCCGCCAAAATCTATTCAACGGTC
PRR7(B)-R	GAAGTTCCACGTCAGAGCGGATATTTC
PRR9(A)-F	CTTCGGATAAGCTTAAAATCATTTC
PRR9(A)-R	TCCAGGTGAAAGTGATCGATG
PRR9(B)-F	CAAACTTAGCCACACAATTACAATAG
PRR9(B)-R	CACCTTAACACGTGTCATTAAC
CPD-F	CCATTGAAGAAGAAGATGATGATGA
CPD-R	CCCCCCGTGTGCCCACTC
DWF4-F	GGGTTTGACTGTCCAGTTCGGTAAT
DWF4-R	ACCCTTAGGATATGGGAAAAGGGTG
CNX5-F	TGACATCGTCTTCTTTGCTGCTTCT
CNX5-R	TGCCTTCTTGAGCTTTAACTCTTCC
UBC-F	CAAATCCAAAACCCTAGAAACCGAA
UBC-R	AACGACGAAGATCAAGAACTGGGAA

F, forward primer; R, reverse primer.