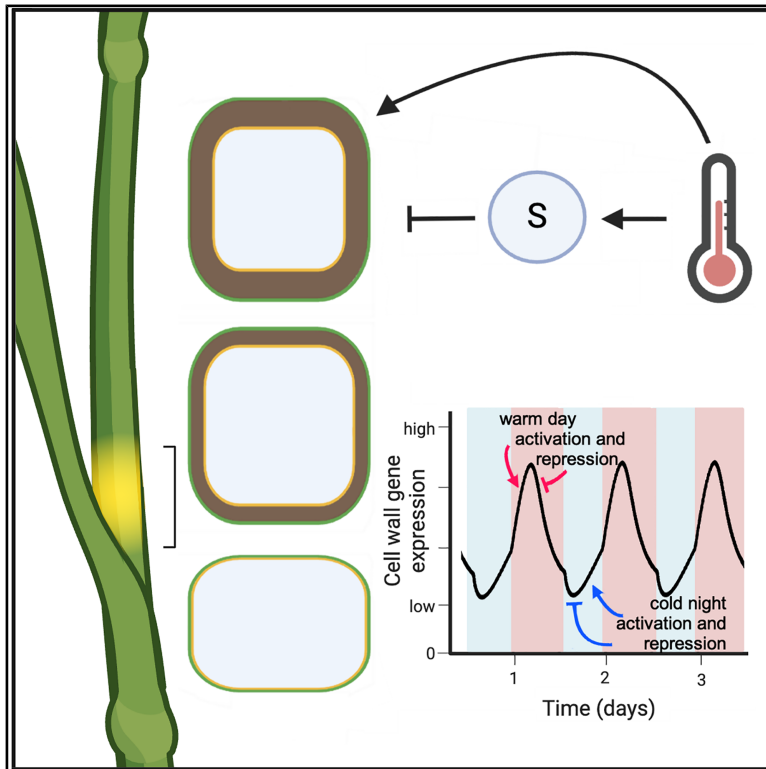


Current Biology

Temperature signals drive grass secondary cell wall thickening

Graphical abstract



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In brief

Gregory et al. show that secondary wall-associated CESA8 expression in grasses is driven by temperature cycles rather than by light or the circadian clock. Temperature-responsive transcription is tightly coordinated with stem elongation and can be explained by an incoherent feedforward loop integrating short- and long-term temperature responses.

Highlights

- Thermocycles, not light or the circadian clock, drive *CESA8* rhythms
- *CESA8* expression increases during cool nights and declines during warm days
- Secondary-wall gene expression is coordinated with stem elongation
- Warm and cold pulses trigger opposing responses, explained by an incoherent feedforward loop model



Article

Temperature signals drive grass secondary cell wall thickening

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SUMMARY

Secondary cell wall thickening is essential for plant structural development, providing the mechanical strength and rigidity required for upright growth. However, direct observation of this process in its endogenous developmental context within living plants has remained limited. Cellulose, the predominant component of secondary walls and the most abundant biopolymer on Earth, is synthesized at the plasma membrane by complexes containing CELLULOSE SYNTHASE A (CESA) proteins. Despite its central role, the precise timing and regulation of cellulose deposition during plant development remain unclear. To address this gap, we developed a real-time bioluminescence imaging system in the model grass *Brachypodium distachyon* using a luciferase transcriptional reporter driven by the CESA8 cis-regulatory region. Bioluminescence imaging revealed a consistent spatial pattern of CESA8 expression within elongating internodes, coinciding with regions undergoing secondary wall deposition and progressive increases in cellulose crystallinity. Time-lapse imaging showed that expression follows a robust daily rhythm driven by temperature cycles, independent of light or endogenous circadian signals. Temperature-pulse experiments uncovered rapid, transient inverse responses that were accurately predicted by a mathematical model based on an incoherent feedforward loop. CESA8 expression correlated strongly with stem elongation, linking structural reinforcement with temperature-driven shoot growth in grasses.

INTRODUCTION

In grasses, the growth of above-ground vegetative tissues occurs through repeated iterations of phytomers, each comprising a leaf blade, a sheath, a stem internode, a node, and an axillary bud. Intercalary meristems at the node-internode junction initiate internode growth. As elongation begins, cells within intercalary meristems divide, elongate, and mature in distinct spatial zones defined by molecular and cellular changes.¹ Growth at the base of each phytomer pushes older cells upward, with growth rates varying throughout development and within a single day, reflecting shifts in cell division and elongation of primary-walled cells.² The transition from elongation to secondary wall thickening is marked by changes in cell-wall-related gene expression and subsequent changes in wall composition.³ Primary walls are thin, flexible, and lack lignin, whereas secondary walls are substantially thicker, composed mostly of crystalline cellulose, hemicelluloses, and lignin, making them rigid and less permeable.⁴ Plants owe their upright stature and water transport to these thickened walls that reinforce specific cell types.

When plants experience regular daily cycles of light and darkness (photocycles) and warm and cooler temperatures (thermocycles), growth rate can peak at dawn, dusk, during the day, or at

night, depending on species, tissue, and environment.^{2,5} These growth responses are often mediated indirectly through changes in water status, evaporative demand, and cellular turgor pressure, which can influence rates of cell expansion and wall stress. These peaks can persist under constant temperatures when photocycles are present or under constant light conditions with thermocycles.^{6–9} Once established, these rhythms continue even without external cues.^{2,5} In contrast, grass leaf growth rhythms are regulated by temperature, independent of photocycles or the circadian clock.^{7,9} Beyond elongation growth, plant secondary walls constitute a major portion of global biomass,¹⁰ yet daily regulation of their formation remains poorly understood, largely because it is impractical to track wall thickening in living tissues over the course of a day. Secondary wall growth is hidden from direct observation and occurs at a microscopic scale; for example, mature interfascicular fiber walls measure just a few micrometers in *B. distachyon* and rice.^{11,12} To overcome this hurdle, we developed a luciferase reporter system to monitor CELLULOSE SYNTHASE A8 (CESA8) expression in real time.

We anticipated that this reporter would serve as an indicator of active secondary wall thickening orchestrated by a network of mostly MYB and NAC family transcription factors.⁴ A large suite of downstream genes associated with secondary wall synthesis,



including *CESA4*, *CESA7*, *CESA8*, *COBRA-like*, and *KORRIGAN*, along with hemicellulose and lignin biosynthesis genes, such as *4CL*, *CAD*, and *COMT*, all exhibit remarkably correlated expression patterns.^{13–16} By capturing whole-plant luminescent images, we could observe the effects of light, temperature, and the circadian clock on *CESA8* expression and, by proxy, secondary wall thickening.

RESULTS

To develop a real-time readout of secondary wall-associated transcriptional activity in live plants, we quantified bioluminescence in *B. distachyon* plants stably transformed with a *CESA8* promoter-driven luciferase reporter. The *CESA8* cis-regulatory region driving the *CESA8* coding sequence is sufficient to rescue the stunted growth and reduced cellulose phenotype of the *cesa8-1* mutant.¹⁷ Strong bioluminescence signals were confined to three tissues: stem nodes, discrete internode regions, and the inflorescence (Figures 1A–1C), while leaves and sheaths showed minimal activity (Figures S1A–1D). Within elongating internodes, *CESA8pro:LUC* expression displayed a consistent spatial pattern: a ~3 mm “dark zone” of low-luminescence above the node, a “bright zone” peaking ~6 mm above the node, and a second low-luminescence region around 12 mm above the node (Figures 1D, S1E, and S1F). This pattern persisted until the internode reached full maturity and the signal ceased. These results are consistent with cells just above the node undergoing division and elongation with minimal secondary wall deposition, while peak *CESA8* expression in the bright zone marks the onset of pronounced wall thickening.

To further evaluate *CESA8pro:LUC* as a proxy for secondary wall formation, we stained stem sections harvested from dark, bright, and upper zones of elongating internodes. Basic Fuchsin staining revealed increasing wall thickness in interfascicular fibers across these regions, matching luminescence intensity until wall thickening was complete (Figures 1E and 1F). X-ray diffraction measurements were consistent with increasing cellulose crystallinity as cells progressed from the dark to the bright zone (Figure S1G). These measurements do not imply that secondary walls are fully mature in the bright zone, but rather indicate that wall thickening is accompanied by progressive increases in cellulose crystallinity as cells transition out of the elongation zone. *CESA8* mRNA levels mirrored reporter activity, confirming that *CESA8pro:LUC* reliably reflects *CESA8* transcription (Figure 1G). Notably, developing nodes maintained high expression, consistent with thickened vascular and interfascicular walls in mature nodes (Figure S2). Similarly, strong signals in maturing spikelets corresponded to heavily thickened cell walls in the lemma and palea, but not in seeds (Figure S3). Together, these data demonstrate that *CESA8* promoter-driven luciferase activity faithfully reports native *CESA8* transcription and marks tissues undergoing secondary wall thickening and progressive increases in cellulose crystallinity.

In contrast to eudicots, daily shoot elongation rhythms in grasses, such as maize, rice, and *B. distachyon*, are driven by thermocycles rather than photocycles or the circadian clock.^{2,7,9} We next sought to determine when grasses most actively thicken their secondary walls and what cues modulate this thickening. Using time-lapse imaging of *CESA8pro:LUC* under

combinations of photocycles and thermocycles, we found that rhythmic expression was driven by thermocycles alone (Figures 2A–2D and S4A–S4D). We tested an additional independent *CESA8pro:LUC* transformation event under thermocycles alone and obtained results indistinguishable from those shown in Figure 2C, demonstrating that the observed rhythmicity is not due to transgene insertion position effects (Figure S4E). Most plants showed a significant daily rhythm (relative amplitude error [RAE] < 0.6) with a period of ~25 h (Figures 2F and 2G). Peak expression occurred early in the subjective day, rising during cool 17°C nights and falling during warm 25°C days, with all tissues synchronized (Figures 2E and 2H). Infrequently, *CESA8pro:LUC* showed a brief end-of-day deviation near the day-night temperature transition, a variable feature that was not consistently observed across cycles. These results suggest that *CESA8* expression is not anticipatory of daily changes in light or temperature, indicating a lack of circadian clock regulation. While these findings suggest potential consequences for secondary wall biosynthesis within a single day, the long-term effects on phase transitions, photosynthesis, and metabolism under perpetual laboratory conditions, such as constant light or temperature, make the outcome difficult to predict.

To investigate whether warm temperatures repress or cold temperatures activate *CESA8*, we conducted temperature-pulse experiments. Plants grown under thermocycles were subjected to a 3-h daytime cold pulse at 17°C. Expression dropped sharply, returning to levels seen under uninterrupted thermocycles within 2.5 h (Figures 3A and S4F). In contrast, a 3-h nighttime warm pulse of 25°C elevated *CESA8* expression to typical morning levels, followed by a decline once the temperature returned to 17°C (Figures 3B and S4G). These findings indicate that *CESA8* expression responds rapidly to temperature fluctuations, with warm temperatures promoting expression and cold temperatures suppressing it, an outcome that contrasts with the pattern observed under diurnal thermocycles.

To determine when *CESA8* expression shifts from an acute temperature response to a thermocycle-driven pattern, we extended temperature pulses into the next cycle to create short- or very long-day conditions. Under short days, plants exhibited a pattern similar to long-day (16 h 25°C:8 h 17°C) conditions, expression initially decreased after transitioning to nighttime temperatures, then gradually rose during the cool night, and declined during the warm day (Figures 3C and S4H). Under extended long days, warm temperatures started 5 h early, and expression quickly returned to morning levels before resuming a typical daytime decline (Figures 3D and S4I). Given that grass leaf elongation is temperature-driven, we next asked whether stem elongation correlates with *CESA8* expression. Measurements revealed a positive correlation, with Pearson coefficients of 0.82 under standard thermocycles and 0.58 with a cold pulse, indicating that both processes are positively coupled under these conditions (Figures 3E and 3F). These findings suggest that *CESA8* follows a diurnal thermocycle, rising at night and falling during the day, with immediate but inverse responses to temperature shifts.

To resolve transcriptional dynamics around rapid temperature transitions and to further test whether the *CESA8pro:LUC* reporter faithfully reflects endogenous *CESA8* transcription, we performed RNA sequencing (RNA-seq) of the bright zone of

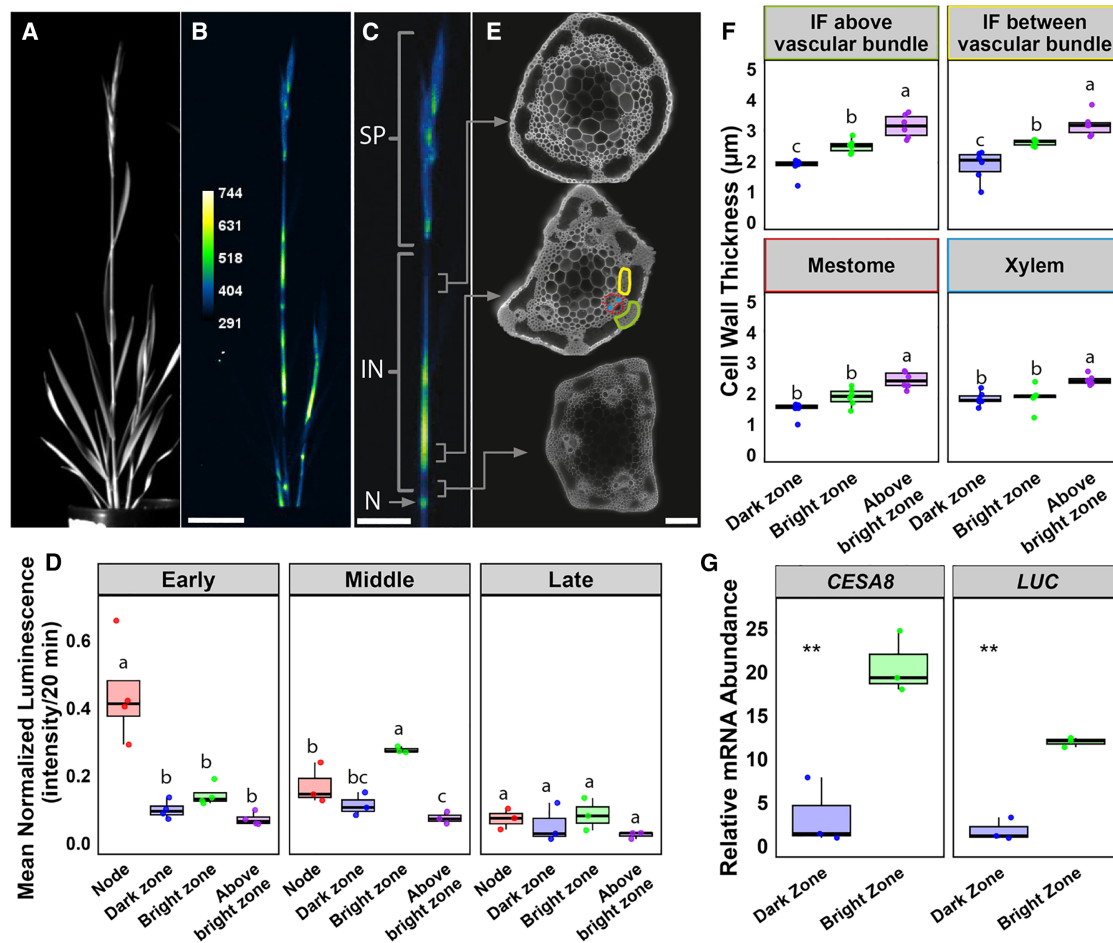


Figure 1. Spatial distribution and intensity of *CESA8pro:LUC* during stem elongation

(A and B) (A) Bright-field and (B) luminescence image of a *CESA8pro:LUC* *Brachypodium distachyon* plant captured with a CCD camera. The heatmap represents luminescence intensity in arbitrary units, ranging from low (blue) to high (yellow). Scale bar, 2 cm.

(C) In the expanded view of the top internode (IN), luminescence is brightest in the inflorescence (SP), node (N), and near the bottom of the IN. Scale bar, 1 cm.

(D) Mean luminescence was quantified in each region, at three stages of development determined by peduncle length (early: IN length < 2.5 cm, middle: IN length > 2.5 cm and < 4.5 cm, late: IN length > 4.5 cm). Regions are designated by distance from the peduncle node: node < 1.5 mm, 1.5 mm < dark zone < 5 mm, 5 mm < bright zone < 12 mm, and above bright zone > 12 mm. $n = 3$ or 4 plants.

(E) Fluorescence micrographs of Basic fuchsin-stained transverse IN sections from above the bright zone (top), within the bright zone (middle), and in the dark zone (bottom). In the bright-zone image, interfascicular cells located above the vascular bundles are circled in green, those between the vascular bundles are circled in yellow, mestome cells are highlighted in red, and xylem cells are highlighted in blue. Scale bar, 100 μm .

(F) Quantification of interfascicular fiber (IF), mestome, and xylem wall thickness. Groups that do not share a letter are significantly different using Tukey's HSD test, $p \leq 0.05$.

(G) Relative expression of *CESA8* and *LUC* from three biological replicates using reverse-transcription quantitative PCR and normalized relative to three control genes, *Bradi1g25170*, *Bradi4g24887*, and *Bradi3g49600*. Student's t test, $**p \leq 0.01$. Box edges indicate the interquartile range. Whiskers extend to the most extreme data points within 1.5 times the interquartile range from the 25th and 75th percentiles. Each point represents the mean of one plant. See also Figures S1–S4.

elongation internodes across a single diurnal cycle under thermocycles alone, with increased sampling density at thermocycle boundaries. Endogenous *CESA8* transcript abundance closely mirrored the temporal pattern observed with the *CESA8pro:LUC* reporter, and expression of the *LUC* transgene itself followed a nearly identical trajectory, further supporting the conclusion that the reporter accurately reflects endogenous transcriptional dynamics (Figure 3G). Consistent with this, *CESA8* and *LUC* expression were significantly positively correlated across the sampled time course (Pearson's $r = 0.83$, $p = 0.031$). Using

weighted gene co-expression network analysis to identify modules of genes with similar expression patterns across time points, we next asked whether other genes within the *CESA8* co-expression module exhibit coordinated, temperature-responsive expression in the internode (Table S1). This module, defined by shared expression dynamics with *CESA8*, encompassed multiple functional classes associated with secondary wall formation. These included *CESA7*; genes associated with hemicellulose backbone synthesis and modification (*GT43B2*, *GUT1*, and *DUF579*, *GAUTL*, and *GXM* family members); lignin

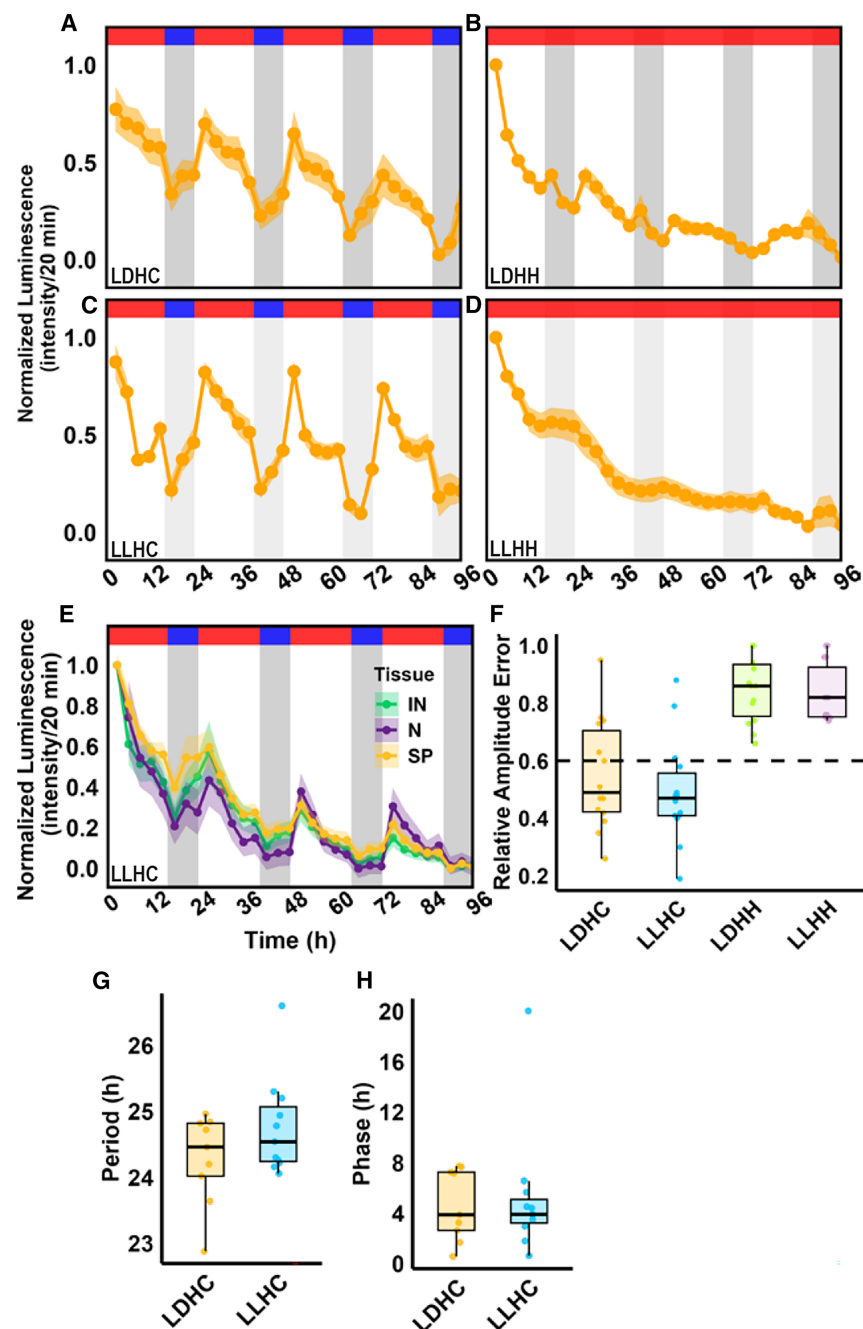


Figure 2. Diurnal thermocycles regulate the morning-phase expression of *CELLULOSE SYNTHASE A8*

CESA8pro:LUC plants were entrained under photo and thermocycles and then imaged under (A and E) photo and thermocycles, (B) constant light with thermocycles, (C) constant 25°C with photoperiods, or (D) constant light and 25°C. (E) Luminescence of inflorescence (SP), node (N), and near the bottom of the internode (IN). Circadian (F) rhythmic amplitude (measured as RAE), (G) period length, and (H) phase were determined using BioDare. Luminescence data represent mean $\pm$ SEM, $n = 7$. Dark gray shading represents night, light gray shading represents subjective night, red shading indicates 25°C, and blue shading indicates 17°C. The experiments were performed at least twice with similar results (Figures S4A–S4D).

LDHC, photo and thermocycles; LDHH, photoperiods and constant temperature; LLHC, thermocycles and constant light; LLHH, constant light and constant warm temperature. Box edges indicate the interquartile range. Whiskers extend to the most extreme data points within 1.5 times the interquartile range from the 25th and 75th percentiles. Each point represents the mean of one plant. See also Figure S4.

of cytokinin biosynthesis and signaling. Across functional categories, module members displayed highly similar expression profiles, with transcript abundance increasing during cool nighttime temperatures and decreasing during the warm day, placing *CESA8* within an integrated, thermocycle-responsive secondary-wall gene program active in grass internodes.

To explain the biphasic *CESA8* response, we developed a mathematical model based on biological hypotheses. The model posits that temperature (T) has a dual effect: direct activation and indirect inhibition mediated by a temperature-sensitive factor (variable S), while accounting for synthesis and degradation of *CESA8* mRNA (variable X) (Figures 4A and 4B). This incoherent

biosynthesis (*PAL2*, *4CL3*, *CCR1*, *CAD1*, *COMT6*, *LAC10*, *LAC11*, and *LAC28*); β -1,3-glucan modification (GH17 family); and additional cell-wall-structure-associated components (*FLA2*, *FLA23*, and *TBL* and *STRUBBELIG* family members) (Figure 3H). The module also contained multiple transcriptional regulators previously implicated in secondary wall formation, including NAC, MYB, KNOX, and BLH family members. Notably, the module also included GTF2, a SWI/SNF chromatin remodeling subunit, suggesting a potential role for chromatin-level control in coordinating thermoresponsive secondary-wall gene expression. In addition, hormone-associated genes were present, including auxin-responsive SAUR genes and components

feedforward loop^{18,19} generates a transient pulse of *CESA8* expression at the onset of the warm period. As S accumulates, expression is subsequently repressed. The model reproduces the experimentally observed dynamics under thermocycles, including the 3-h phase shift relative to the onset of the warm period and the apparent inverse relationship when a warm pulse is applied during the cold night or a cold pulse during the warm day (Figures 4C–4H). To assess robustness to parameter variation, we conducted 20 simulations using independently generated parameter sets, each with kinetic parameters randomly varied by up to 10%. These results indicate that while variability primarily affects the amplitude and, to a lesser extent,

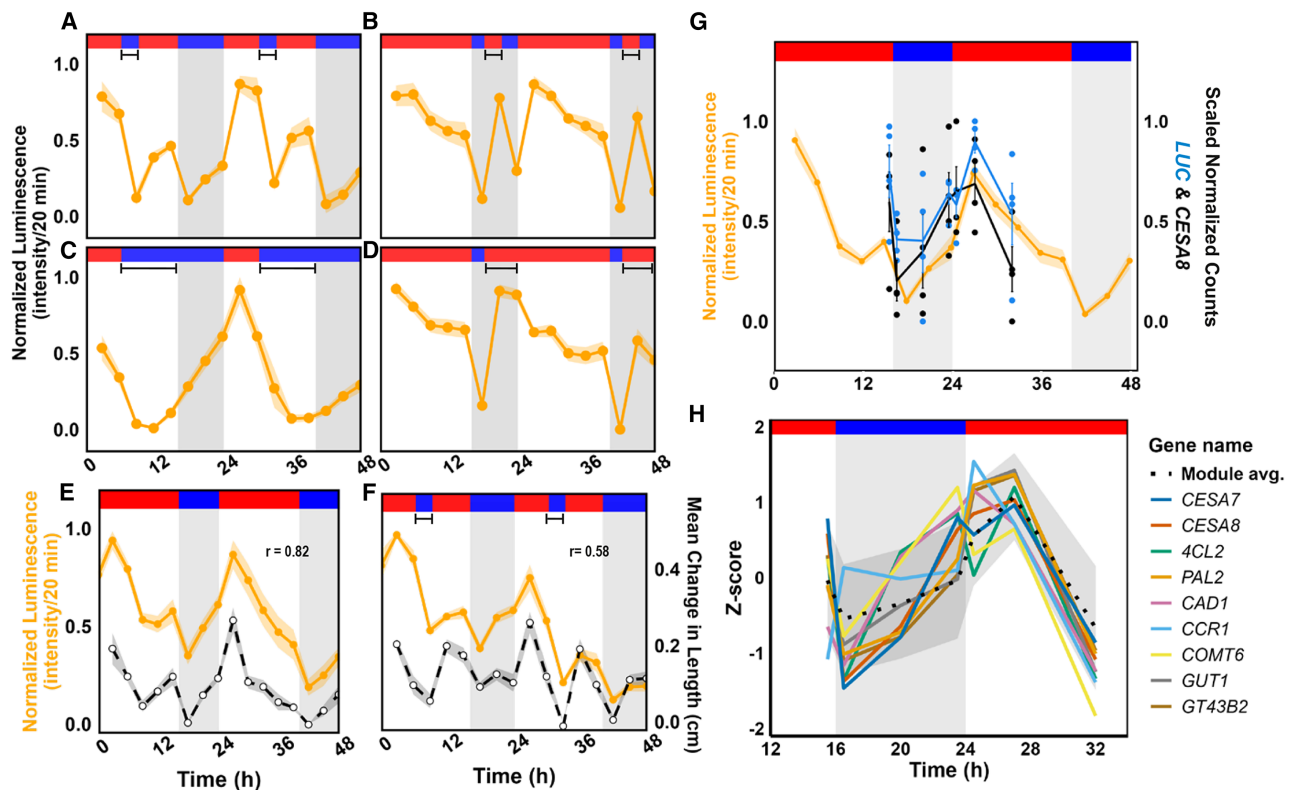


Figure 3. *CELLULOSE SYNTHASE A8* expression responds immediately to temperature changes, increasing with warmth and decreasing with cold

(A–D) *CESA8pro:LUC* plants were entrained under photo and thermocycles, transferred to thermocycles alone, and then given a (A) 17°C cold pulse for 3 h at midday or a (B) 25°C warm pulse for 3 h at night. Long-day entrained plants encountered (C) early nighttime temperature, equivalent to a short-day thermocycle (6 h 25°C:18 h 17°C) or (D) early daytime temperature, equivalent to a very long-day thermocycle (21 h 25°C:3 h 17°C). The experiments were performed at least twice with similar results (Figures S4F–S4I).

(E and F) (E) Luminescence and elongation of the primary stem were measured for each time point of plants grown with a thermocycle, as in Figure 2C, or (F) daytime cold pulse as in (A). Brackets indicate deviations from standard long-day conditions. Luminescence data means $\pm$ SEM, $n = 7$. The experiments were performed at least twice with similar results.

(G) RNA-seq time course under thermocycles showing endogenous *CESA8* and *LUC* transcript abundance in the bright zone of elongating INs, alongside the corresponding *CESA8pro:LUC* reporter signal from the same conditions.

(H) Average expression profiles of representative secondary wall-associated genes from the same RNA-seq experiment. All genes shown belong to the same weighted gene co-expression network module as *CESA8*, and their accession numbers are included in the main text. RNA-seq data represent mean $\pm$ SEM, $n = 4$. See also Figure S4 and Table S1.

the phase of the response, the overall dynamics remain consistent. The model provides a potential explanation for how daytime temperatures initially activate, then repress, *CESA8* expression, while nighttime temperatures have the opposite effect, first repressing, then activating it.

DISCUSSION

In grasses such as maize, rice, and *B. distachyon*, daily shoot elongation is driven primarily by diurnal temperature cycles rather than by photoperiods or circadian regulation.^{7–9} Here, we show that secondary wall-associated gene expression follows the same thermocycle dependence. Time-lapse imaging of *CESA8pro:LUC* under combined and isolated photoperiods and thermocycles revealed robust rhythmic expression driven exclusively by temperature, with peak activity early in the subjective day. Expression increased during cool nights and

declined during warm days, with most plants exhibiting synchronized rhythms of approximately 25 h across all tissues examined. In contrast, no rhythmicity was detected under photoperiods alone or under constant light and temperature, indicating that *CESA8* daily rhythm is neither light- nor clock-regulated. Although luciferase enzyme activity can be temperature sensitive under some *in vitro* conditions, the temperature-dependent bioluminescence patterns observed here are opposite to those expected from enzyme kinetics and are recapitulated by endogenous *CESA8* transcript abundance. These observations rule out temperature-dependent reporter activity as an explanation for our results and indicate that the observed dynamics arise from regulation of *CESA8* expression. *CESA8* expression closely tracked stem elongation rates, suggesting tight coupling between temperature-driven elongation and secondary wall-associated transcription in grass internodes.

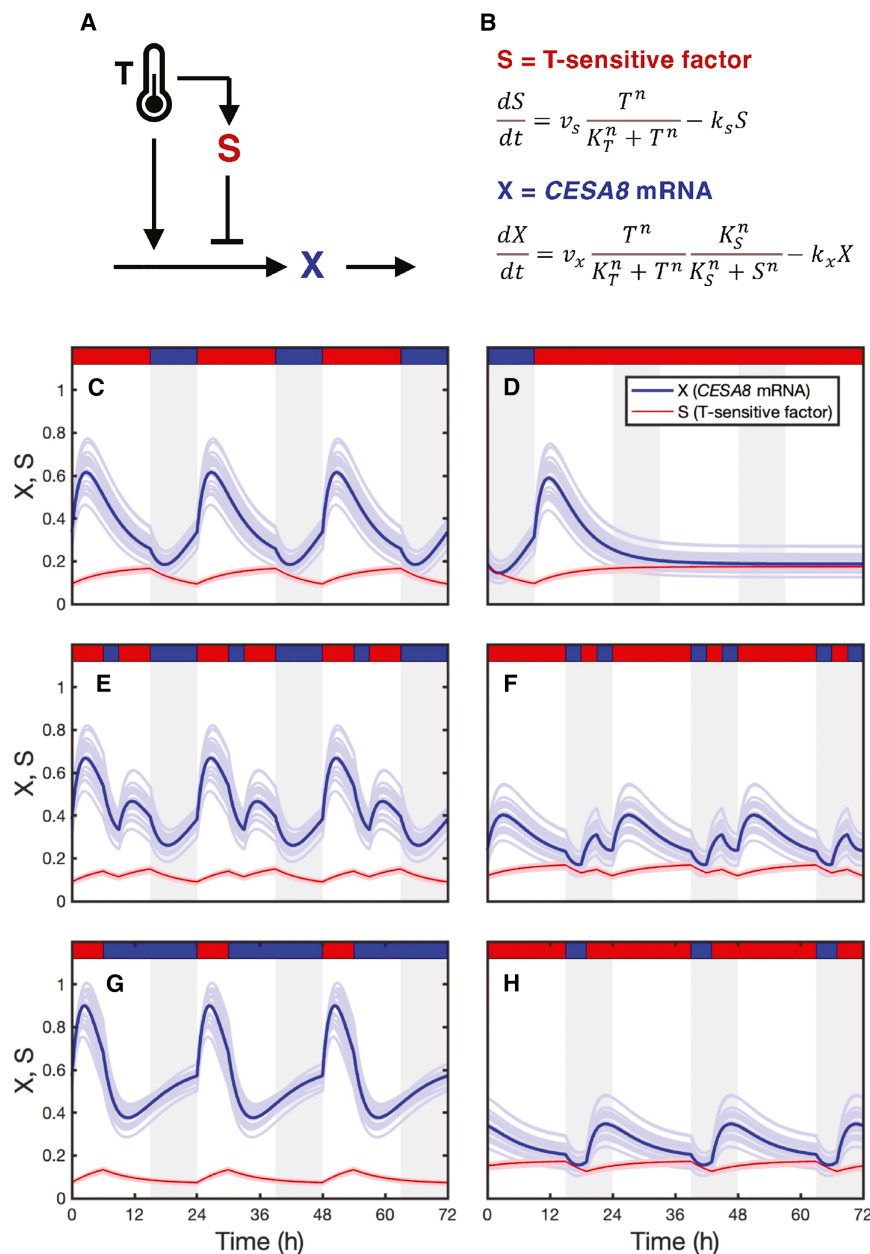


Figure 4. The dynamics of CESA8 can be reproduced by an incoherent feedforward loop model

(A) In this model, temperature (T) directly induces the expression of CESA8 (variable X) while also indirectly suppressing it through a hypothetical temperature-sensitive factor (variable S).

(B) The activation of S and X expression by T, along with the inhibition of X expression by S, follows Hill function dynamics.

(C–H) Numerical simulations of S (red) and X (blue) over time under (C) thermocycles, (D) release to continuous daytime conditions, (E) daytime cold pulse, (F) nighttime warm pulse, (G) early nighttime temperature, and (H) early daytime temperature show qualitative agreement with the experimental observations. Parameter values: $v_x = 1.2 \text{ h}^{-1}$, $K_T = 23^\circ\text{C}$, $K_S = 0.1$, $n = 4$, $k_x = 0.36 \text{ h}^{-1}$, $v_s = 0.045 \text{ h}^{-1}$, $k_s = 0.15 \text{ h}^{-1}$, $T_{\text{warm}} = 25^\circ\text{C}$, $T_{\text{cold}} = 17^\circ\text{C}$. The light blue and red curves represent results obtained by introducing variability in parameter values. Specifically, the values of v_x , K_T , K_S , k_x , v_s , and k_s were randomly increased or decreased by up to 10% from their default values. A total of 20 simulations were performed, each using a new randomly generated set of randomized parameter values.

Although CESA8 followed a consistent thermocycle-driven rhythm, acute temperature perturbations revealed an apparently opposing response. Warm temperature pulses applied during the cool night rapidly increased CESA8 expression, whereas cold pulses during the warm day sharply reduced expression, with both responses reversing upon return to baseline conditions. In *A. thaliana*, circadian regulation of cell expansion can be uncoupled from cellulose biosynthesis, which is instead governed by metabolic status rather than clock inputs.²⁰ Our findings extend this distinction to grasses, where secondary wall-associated CESA8 expression is likewise independent of circadian regulation but is instead driven by temperature cues. Importantly, the direction of the transcriptional response

depends on the duration of temperature exposure. To reconcile these short- and long-term behaviors, we developed a mathematical model incorporating an incoherent feedforward loop, in which temperature exerts both direct activation and delayed repression of CESA8 expression. The model successfully recapitulates both the transient pulse responses and the sustained dynamics observed under thermocycles. In this framework, the temperature-sensitive variable S represents a delayed regulatory influence that counteracts direct temperature activation of CESA8; rather than corresponding to a discrete molecular regulator, this variable could plausibly reflect integrated physiological states, potentially encompassing metabolic feedback, hydraulic status, or turgor-related signaling. Rapid temperature shifts inevitably alter evaporative demand and water fluxes, but the opposing short- and long-term responses of CESA8 expression are difficult to reconcile with a simple, monotonic effect of turgor alone. Instead, the model suggests that CESA8 transcription integrates temperature information over time, enabling coordinated regulation across daily cycles.

Our RNA-seq analysis extends these findings by placing CESA8 within a coordinated, temperature-responsive secondary-wall transcriptional network and by confirming that endogenous CESA8 mRNA closely mirrors reporter dynamics across thermocycles. Co-expression analysis revealed that CESA8 resides in a module enriched for genes associated with cellulose,

hemicellulose, and lignin biosynthesis, together with established secondary wall regulators. The shared thermocycle-dependent expression profiles across these functional classes indicate that temperature cues act through coordinated transcriptional control of wall biosynthesis rather than on individual pathways. The presence of chromatin-associated and hormone-responsive genes further suggests multi-layered regulation linking temperature sensing, growth, and secondary wall reinforcement. Together, these results indicate that *CESA8* functions within an integrated transcriptional program that synchronizes wall deposition with temperature-driven stem growth.

While our findings, together with prior studies, support a relationship between *CESA8* transcript abundance and secondary-wall cellulose synthesis,^{13–16,21–23} post-translational regulation of cellulose synthase complex (CSC) activity must also be considered. While turgor pressure is a central driver of cell expansion in primary-walled tissues, secondary wall deposition occurs after elongation has largely ceased and cell dimensions are fixed. This distinction highlights that secondary wall biosynthesis is decoupled from the rapid, turgor-driven processes that dominate primary growth and instead reflects longer-term regulatory inputs. Accordingly, transcriptional regulation of the wall biosynthetic machinery is likely to respond to developmental and environmental cues distinct from those governing acute changes in turgor. Consistent with this separation, cellulose synthesis is subject to extensive post-translational control that complicates direct inference from transcript levels alone. The CSC responsible for primary wall synthesis persists in the Golgi for over 48 h, with longevity inversely correlated with temperature, yet catalytic activity occurs only at the plasma membrane.^{24,25} In *A. thaliana*, primary wall CSCs remain active at the plasma membrane for only minutes before clathrin-mediated endocytosis, and in cotton fibers the *CESA8* ortholog has a half-life of less than 30 min.^{26,27} These observations, together with the paucity of data from grasses, underscore the challenge of directly assessing *CESA* protein activity *in vivo*. Accordingly, our analysis focuses on transcriptional regulation of *CESA8* rather than on cellulose synthase protein abundance or enzymatic activity, both of which are subject to extensive post-translational control. Given the multiple layers of regulation separating transcription and cellulose polymerization, the strength and consistency of the correlation between *CESA8* transcriptional output and regions of active secondary wall thickening was not necessarily expected, highlighting transcription as a major and informative control point for coordinating secondary wall deposition with temperature-driven growth in grasses.

Grass stem development differs fundamentally from that of eudicots, relying on stacked intercalary meristems at the base of internodes rather than elongation driven by apical and lateral meristems. This growth pattern enables multiple zones within the stem to simultaneously undergo cell division and elongation before being structurally reinforced through secondary wall thickening. A potential consequence of this organization is that intercalary meristems experience greater mechanical stress than cells derived from apical growth. The seemingly paradoxical effects of diurnal temperature cycles, where short-term responses oppose long-term patterns, may reflect a mechanism that prepares the stem for the mechanical demands of the approaching warm period. By coupling elongation with secondary

wall thickening, grasses maintain stem integrity during rapid internode elongation. These findings lay the groundwork for uncovering molecular links among temperature sensing, transcriptional regulation, and biomass accumulation, with implications for improving stem strength and yield in cereal crops under variable climate conditions.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Samuel P. Hazen (hazensam@umass.edu).

Materials availability

Transgenic *CESA8pro:LUC B. distachyon* seeds generated in this study are available from the lead contact upon request. The plasmid generated in this study has been deposited at addgene: <https://www.addgene.org/256446/>.

Data and code availability

- All data reported in this study are available within the paper and its supplemental information and have been deposited at Zenodo: <https://doi.org/10.5281/zenodo.19633777>.
- Raw RNA-seq data have been deposited in the European Nucleotide Archive under accession number ENS: E-MTAB-16515.
- All original code and analysis pipelines have been deposited at Zenodo and are publicly available at Zenodo: <https://doi.org/10.5281/zenodo.19633777> as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

G.A.G. and S.P.H. conceived and designed the study. G.A.G., J.H.C., K.A.G., D.G., and D.F. developed techniques and reagents. G.A.G., B.K., S.O., K.A.M., K.R.C., and C.E.S. acquired the data. G.A.G., B.K., D.G., and S.P.H. analyzed and interpreted the data. G.A.G. and S.P.H. drafted the manuscript. S.P.H. agrees to serve as the author responsible for contact and ensures communication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
<i>Brachypodium distachyon</i> Bd21-3	Laboratory stock	Bd21-3
<i>CESA8pro:LUC</i> transgenic <i>B. distachyon</i> lines	This study	Generated in this study
Bacterial and virus strains		
<i>Agrobacterium tumefaciens</i> strain AGL1	Laboratory stock	AGL1
Chemicals, peptides, and recombinant proteins		
D-luciferin	Gold Biotechnology	Cat# LUCK-1G
Hygromycin B	Thermo	Used at 40 mg/L
Basic Fuchsin	Various suppliers	Histological stain
Phloroglucinol-HCl	Various suppliers	Histological stain
Critical commercial assays		
Plant RNeasy Mini Kit	Qiagen	Plant RNeasy Kit
QuantiTect Reverse Transcription Kit	Qiagen	Reverse transcription kit
Deposited data		
RNA-seq data	This study	ENA: E-MTAB-16515
Processed datasets, imaging data, and scripts	This study	Zenodo: 10.5281/zenodo.19633777
Experimental models: Organisms/strains		
<i>B. distachyon</i> Bd21-3	Laboratory stock	Wild type
<i>CESA8pro:LUC</i> reporter line	This study	Stable transgenic line
Oligonucleotides		
LUC2_F	This study	TTTGTTTCATCGGTGTGGCTG
LUC2_R	This study	CAAGCGTTCTATGTGGCAA
CESA8-LUC_F	This study	TCTTCCTCCCATCCCATGCCCG
CESA8-LUC_R	This study	CGAATGAACGACCGCCCAACCA
LUC2_Cloning_F	This study	CACCATGGAAGATGCTAAGAATATTAAGAAG
LUC2_Cloning_R	This study	ACTGCAGCTGGGAGCTCTC
LUC-F	This study	CTCTCACGCAAGAGATCCTATTT
LUC-R	This study	ATCCGAGTGTAGTGAACATTCC
CESA8-F	This study	CAAAGCACAAGTTCCGCTGTG
CESA8-R	This study	TGGCTCGTATGCATCTGTCAAATC
Bradi1g25170-F	This study	GAATGTGGGTGTGTAAGGTCATA
Bradi1g25170-R	This study	GGGTCCTTTCGAAGGATTGT
Bradi4g24887-F	This study	CCATGATTGTCTCGAGGAAGTTGC
Bradi4g24887-R	This study	TCGCTGCAGTTTCCACATTTT
Bradi3g49600-F	This study	CACCCAAATGTTCACTTCAAGACG
Bradi3g49600-R	This study	GTTAAGTGGGCTGTCTGCTTCTG
Software and algorithms		
Fiji	Fiji/ImageJ	RRID: SCR_002285
BioDare2	BioDare2	Rhythmicity analysis
HISAT2 v2.2.1	HISAT2	RNA-seq alignment
StringTie v1.3.4	StringTie	Transcript quantification
DESeq2	DESeq2	Differential expression
WGCNA	WGCNA	Co-expression analysis
FastP v0.24.0	fastp	FASTQ preprocessing
SAMtools	SAMtools	BAM/SAM processing

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
MATLAB	MATLAB	Crystallinity analysis
R	R Project	Statistical analysis
NIS-Elements D v5.30.04	Nikon NIS-Elements	Imaging acquisition
CLICK Programming Software v3.40	AutomationDirect CLICK PLC Software	Environmental control
Other		
Andor iKon-M 934 CCD camera	Oxford Instruments	Bioluminescence imaging
Nikkor 50 mm f/1.2 lens	Nikon	Imaging lens
MistKing v5.0 Starter Misting System	MistKing	Automated luciferin delivery
X'Pert Pro powder X-ray diffractometer	Malvern Panalytical	XRD measurements
BZ-X800 fluorescence microscope	Keyence	Fluorescence imaging
Eclipse E200MV microscope	Nikon	Brightfield microscopy
PGC-105 growth chamber	Percival Scientific	Plant growth chamber

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS**Plant materials and growth conditions**

The *CESA8pro:LUC* reporter plasmid was developed by recombining the codon optimized *LUC2* luciferase (*LUC*) coding sequence into the *B. distachyon* *CESA8* Utility Promoter Vector (Genbank KT962835).^{17,28} The *CESA8* promoter fragment includes the 1,888 nucleotides of sequence upstream from the translational start site of the *CESA8* gene of accession Bd21-3, BdiBd21-3.2G0638500. While not used here, the Bd21 reference genome gene identifier is Bradi2g49912.²³ The *CESA8pro:LUC* plasmid was transformed into *Agrobacterium tumefaciens* strain AGL1, which was then used for transformation of Bd21-3 embryonic callus as previously described.²³ Plants harboring the *CESA8pro:LUC* reporter were regenerated from callus tissue and putative transgenic plants were initially confirmed by selection on 40 mg/L hygromycin. Genomic DNA was extracted from leaf tissue of putative transgenic plants and the *LUC* coding sequence was PCR-amplified using primers *Luc_F* and *Luc_R* (Table S3). Plants were also confirmed as transgenic by PCR and screening for bioluminescence.

Wildtype Bd21-3 and *CESA8pro:LUC* transgenic seeds were imbibed for 21 days at 4°C and then planted in Pro-Mix BX Mycorrhizae potting mix. The seeds were planted 1.5 cm deep in 22 cm tall Cone-tainer pots with a 3.8 cm diameter (Ray Leach SC10R, Stuewe & Sons, Inc.). Plants were first grown in a PGC-105 growth chamber (Percival Scientific, Perry, IA) with a $\sim 450 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ day period of 16 h at 25°C and a dark night period of 8 h at 17°C and watered daily. At the beginning of peduncle elongation, approximately BBCH stage 5.3,²⁹ plants were moved to the imaging chamber at the same long day thermo and photocycles with a daytime light intensity of $\sim 150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

METHOD DETAILS**Development of bioluminescent imaging chamber**

The bioluminescence imaging chamber consists of an inner and outer shell (Figure S4J). The inner shell of a 61 x 122 x 183 cm grow tent (Gorilla Grow Tent, Santa Rosa, CA) placed on its side on a 183 x 61 x 214 cm metal wire shelving unit (H-2948-86, ULINE) with two wire shelves at a height of 80 and 205 cm. An outer shell of Black Out Heavy 6 Mil Plastic (B & H Foto & Electronics Corp., New York NY) covers the entire shelving unit. A 45 x 19 cm section was removed from the top shelf to mount the custom light-emitting diodes (LED). The light array consists of royal blue (448 nm), green (530 nm), red (627 nm), deep red (655 nm), far red (720 nm), and 6500 K and 5650 K cool white (Table S2). Diodes were mounted onto a grade 6063 aluminum heat sink from a Makers LED Grow Light Kit (LEDSupply, Randolph, VT). The lights are mounted into the lining of the inner and outer shell. This design allowed the heat sink to be exposed outside the chamber to reduce heat transfer from the LEDs into the chamber.

Temperature control is accomplished with a GE 6100-BTU Portable Air Conditioner (Part number 2759118, Lowe's), wiring in a new thermostat (Stego Smart Sensor temperature/humidity sensor, part number 014202-00, AutomationDirect.com) fixed to the inner shell adjacent to where plants are staged. Control of the light and temperature cycles is achieved through a CLICK Programmable Logic Controller (ClickPLUS CPU, C2-01CPU). Temperature and light cycles are programmed on CLICK Programming Software version 3.40. An Andor iKon-M 934 CCD camera (Oxford Instruments, Concord MA) with a Nikkor 50 mm f/1.2 lens was used to capture plant bioluminescence as described below. NIS-Elements D 5.30.04 was used to control capture settings and manage time-lapse imaging.

Bioluminescence imaging and quantification

Luciferin substrate was delivered using an automated fine-droplet misting system (v5.0 Starter Misting System, MistKing, Ontario, Canada) that sprayed the plants with a 1 mM D-luciferin (Gold Biotechnology, St. Louis, MO) solution for 3 s, every 3 h (Video S1). Plants were allowed to acclimate to the imaging chamber and dissipate the effects of luciferase build-up by spraying with luciferin and delaying image acquisition 1 d.

Images were then captured every 3 h for 4 d. To avoid the effects of delayed fluorescence, image capture began 5 min after the lights were turned off. Raw imaging data was processed using Fiji³⁰ to generate masks for each image, overlay the mask on the images, and apply a minimum threshold of 350 AU to eliminate background noise.

To measure luminescence, regions of interest were manually drawn using Fiji. To measure elongation, a segmented line with a width of 3 pixels was drawn on the main stem of each plant in each image. Elongation was calculated by subtracting the length at the previous time point, three hours earlier.

Stem histology and cell wall analysis

Peduncles of the tallest stem were excised when they reached 3.0–3.5 cm in length. Nodes and seeds were collected for sectioning after plants had fully senesced. Each internode was segmented into three regions: the bottom 5 mm (dark zone), a 5 mm region above the dark zone (bright zone), and the uppermost 5 mm. Segments were embedded and sectioned transversely at 60 μ m. Cell wall thickness and staining intensity following phloroglucinol-HCl or Basic Fuchsin staining were quantified as previously described.³¹ Node and seed tissues were embedded and sectioned transversely at 45 μ m and 60 μ m, respectively. Brightfield imaging of phloroglucinol-HCl stained sections was conducted using an Eclipse E200MV microscope (Nikon) equipped with a Pixelink 3 MP camera. For Basic Fuchsin stained sections, both fluorescence and brightfield imaging were captured using a BZ-X800 fluorescent microscope (Keyence) equipped with Plan Apochromat objectives (10X, 20X, and 40X). Fluorescence images were acquired using the TRITC filter cube (OP-87764; excitation 545/25 nm, emission 605/70 nm; dichroic mirror 565 nm). Images were captured using a cooled monochrome CCD detector with 14-bit dynamic range. Exposure times were 33 ms (10X), 3.3 ms (20X), and 40 ms (40X), with gain maintained at default settings. Imaging parameters were held constant within experiments to enable quantitative comparison. Five cross-sections from each of the three regions of five plants were analyzed using the line tool in Fiji, and mean cell wall thickness was calculated for each cross-section. Within each cross-section, walls from seven metostome, four metaxylem, and five interfascicular fiber cells were measured.

X-ray diffraction profiling

X-ray diffraction and calculation of a crystallinity index were done as described by²³ with slight modifications. Peduncle stem tissue from the dark zone, bright zone, and above bright zone were harvested at stage 5.3 on the *B. distachyon* BBCH-scale. For each pool of tissue > 100 plants were harvested to attain a sufficient quantity for analysis. Stems were then dried for 2 weeks at 60°C in a HERA THERM Oven (Thermo Scientific). Tissue was ground using 3.2 mm diameter stainless steel metal balls (Biospec Products, Bartlesville, OK) in a ball mill (Mixer Mill, MM400, Retsch, Newtown, PA). Diffraction was analyzed on an X'Pert Pro powder X-ray diffractometer (PANalytical BV, The Netherlands) operated at 45 kV and 45 mA using CuK α radiation at both K α 1 (λ = 1.5406 Å) and K α 2 (λ = 1.5444 Å). The diffraction profile was acquired from 5 to 40° in 0.0167 steps, with 66 s per step. The crystallinity index was calculated using the peak deconvolution method in MatLab, based on five Gaussian crystalline peaks. It was determined as the ratio of the combined area of all crystalline peaks to the total area under the curve.³²

Measurement of transcript abundance by quantitative PCR

Total RNA was extracted using a kit (Plant RNeasy, Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The peduncle internode of the main stem was harvested at stage 5.3 on the *B. distachyon* BBCH-scale.²⁹ The peduncle was then subdivided into dark zone (1.5 to 5 mm above the node), bright zone (5 to 12 mm above the node), and then frozen in liquid nitrogen. Five individual plants were pooled together for each replicate. First strand cDNA was synthesized from 1 μ g of total RNA using QuantiTect Reverse Transcription Kit (Qiagen). Three technical replicates of three biological replicates of real-time quantitative PCR were carried out using a QuantStudio 3 Real-Time PCR System. Expression values were normalized to three reference genes (Bradi1g25170, Bradi4g24887, and Bradi3g49600) selected empirically based on transcriptome-wide expression stability rather than from previously established standards. These genes were among those with the lowest coefficients of variation across developing stem tissues and exhibited minimal expression variability across nodes and the dark, bright, and maturing zones of elongating internodes. Additional selection criteria included moderate to high mean expression levels and complete data coverage with no missing values. This data-driven strategy was used to identify stably expressed reference genes under the experimental conditions examined and is consistent with recommended best practices for real-time quantitative PCR normalization.³³ All oligonucleotides are listed in Table S3.

RNA-seq and analysis

CESA8pro:LUC plants were grown as described above. At the onset of peduncle elongation, plants were transferred from long-day conditions with photocycles and thermocycles to the imaging chamber under constant light with thermocycles. The *CESA8* bright zone of the peduncle was harvested at seven time points over the course of one day, with four biological replicates per time point. Each replicate comprised three segments collected from the tallest stem of three individual plants. Sampling began near the end of

the warm daytime period, 30 min prior to the transition to nighttime temperature (ZT15.5), followed by three time points during the cooler night (ZT16.5, ZT20, and ZT23.5), and three time points after the onset of the warm daytime period, which began at ZT24 (ZT24.5, ZT27, ZT32). Total RNA was extracted from each time point using the Plant RNeasy mini kit (Qiagen) according to manufacturer instructions, including on-column treatment digestions with RNase-free DNase I.

RNA quality assessment, library preparation, and sequencing were performed by Plasmidsaurus using an Illumina platform with a 3' end counting approach. Quality control and filtering of sequencing reads were performed using FastP v0.24.0³⁴ with the following parameters: trim_poly_x, cut_tail, qualified_quality_phred 20 and length_required 50. Filtered reads were aligned to the Bd21 genome v3.2 using Hisat2 v2.2.1,³⁵ with the parameters `-no-unal` and `-dta`. SAM files were converted to BAM format using SAMtools with default settings.³⁶ Gene-level read counts were generated using StringTie v1.3.4³⁷ with the `-e` option, and normalization was performed using the DESeq2 R package.³⁸ Raw sequencing data were deposited in the European Nucleotide Archive for public access under accession number E-MTAB-16515.

Co-expression network construction

Weighted gene co-expression networks were constructed using the WGCNA R package following variance stabilizing transformation normalization with the DESeq2 R package.³⁹ Genes expressed across all time points were included in the construction of the co-expression network. Following standard WGCNA workflows, an appropriate soft-thresholding power was selected using the pickSoftThreshold function to approximate scale-free topology. An adjacency matrix was generated using the soft-thresholded Pearson correlations with a signed network type. The adjacency matrix was then transformed into a topological overlap matrix (TOM) to quantify gene co-expression similarity, and the TOM-based dissimilarity ($1 - \text{TOM}$) was used for hierarchical clustering. Gene modules were identified using the dynamic tree cut algorithm with a minimum module size of 30 genes. Modules with highly correlated eigen-genes (correlation > 0.95) were merged using the mergeCloseModules function. The following gene aliases are used for genes discussed in the co-expression network analysis: *4CL3* (Bradi1g31320), *CAD1* (Bradi3g06480), *CCR1* (Bradi3g36887), *CESA7* (Bradi4g30540), *CESA8* (Bradi2g49912), *COMT6* (Bradi3g16530), *GIF2* (Bradi4g13730), *GT43B2* (Bradi1g33320), *GUT1* (Bradi2g59410), *FLA2* (Bradi4g39640), *FLA23* (Bradi1g06290), *LAC10* (Bradi2g54680), *LAC11* (Bradi2g54690), *LAC28* (Bradi4g39330), *PAL2* (Bradi3g49260), *STRUBBELIG* (Bradi1g72430).

QUANTIFICATION AND STATISTICAL ANALYSIS

Histology and cell wall measurements

Statistical analyses were performed in R unless otherwise specified. Histological datasets were analyzed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test with a significance threshold of $p < 0.05$.

Luminescence normalization and rhythmicity analysis

Luminescence values were normalized by subtracting the moving mean and dividing by the moving standard deviation for each biological replicate prior to averaging across plants. Luminescence time-course rhythmicity analyses were performed using BioDare2 with the MESA algorithm and amplitude/baseline detrending.⁴⁰ Plants with relative amplitude error (RAE) values below 0.6 were considered rhythmic.⁴¹

Correlation analyses

Pearson correlation coefficients were calculated using `cor.test` in R.

Data presentation

Data are presented as mean $\pm$ SEM unless otherwise stated. Biological replicate numbers are indicated in the corresponding figure legends.

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Supplemental Information

Temperature signals drive grass secondary cell wall thickening

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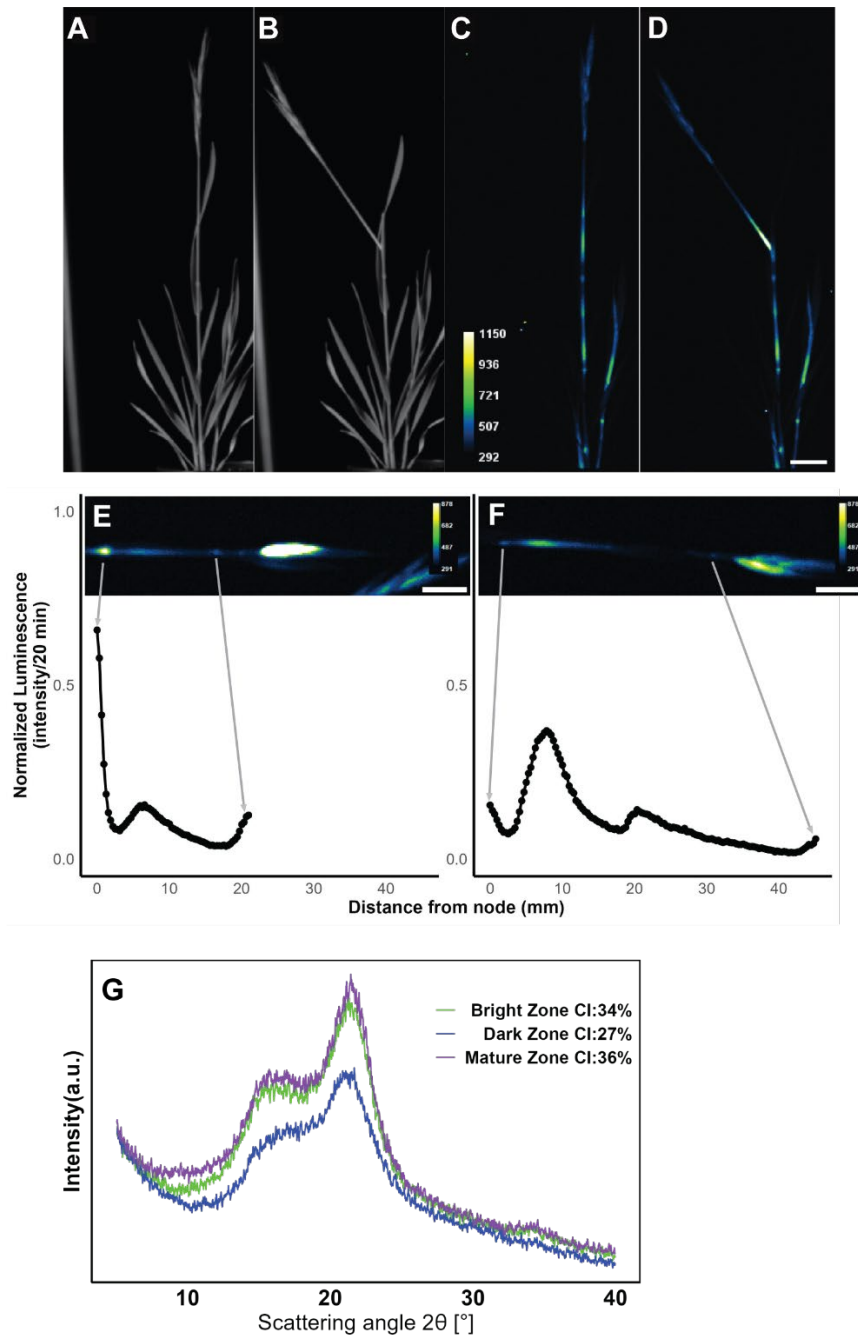


Figure S1. Spatial distribution of *CESA8pro:LUC* expression and cellulose crystallinity during internode elongation. Related to Figure 1. (A, B) Bright-field images and **(C, D)** corresponding luminescence images of elongating stems captured with a CCD camera. The heat map represents luminescence intensity in arbitrary units, ranging from low (blue) to high (white). Separation of the sheath from the stem internode reveals minimal detectable luminescence in the sheath (B, D). Scale bar, 1 cm. **(E, F)** Normalized luminescence intensity across a single peduncle two days after internode elongation began (E) and three days later (F), demonstrating that the peak position of the luminescence zone remains constant relative to the node below. Scale bar, 1 cm. **(G)** X-ray diffraction analysis of ground stem tissue collected from the dark zone, bright zone, and upper mature zone of elongating internodes. Diffractogram profiles were used to calculate the crystallinity index (CI). For each tissue pool, material from more than 100 plants was harvested to obtain sufficient biomass for analysis.

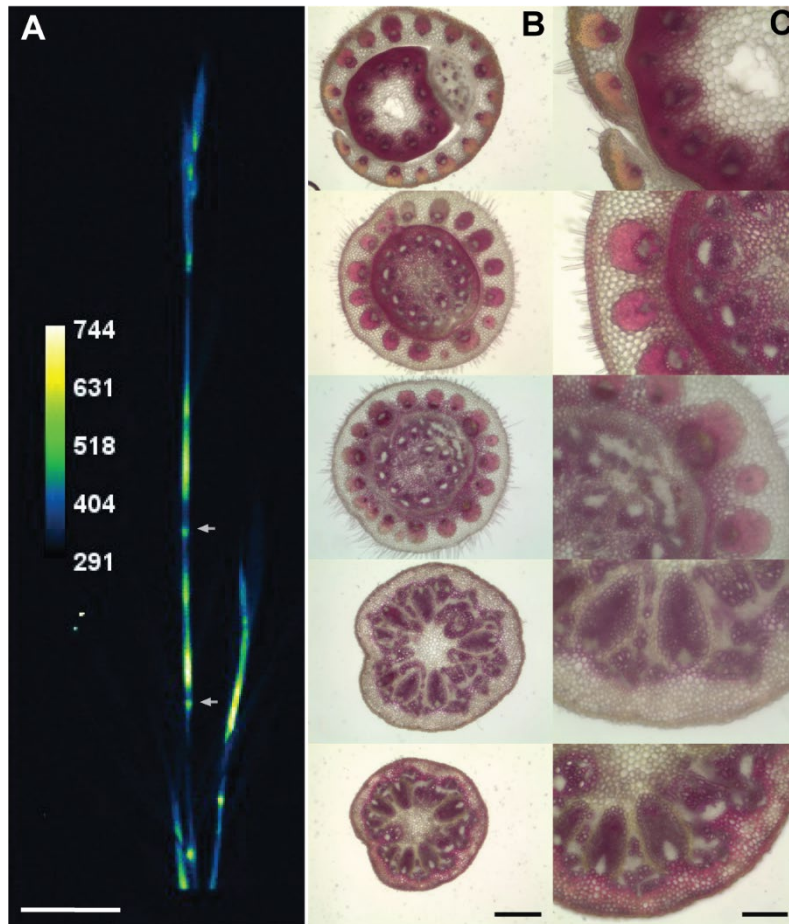


Figure S2. Histological characterization of the *Brachypodium distachyon* bottom peduncle node. Related to Figure 1. (A) Representative image of above ground *CESA8pro:LUC* luminescence with arrows indicating the stem nodes. Warmer colors indicate greater luminescence. The heat map represents luminescence intensity in arbitrary units, ranging from low (blue) to high (white). Scale bar, 2 cm. **(B, C)** Phloroglucinol-HCl stained transverse sections of a fully senesced stem node, progressing upward from the base of the node to the point where the sheath separates from the internode, shown at 4x (B) and 10x (C) magnification. Scale bars 75 μm (A) and 30 μm (B).

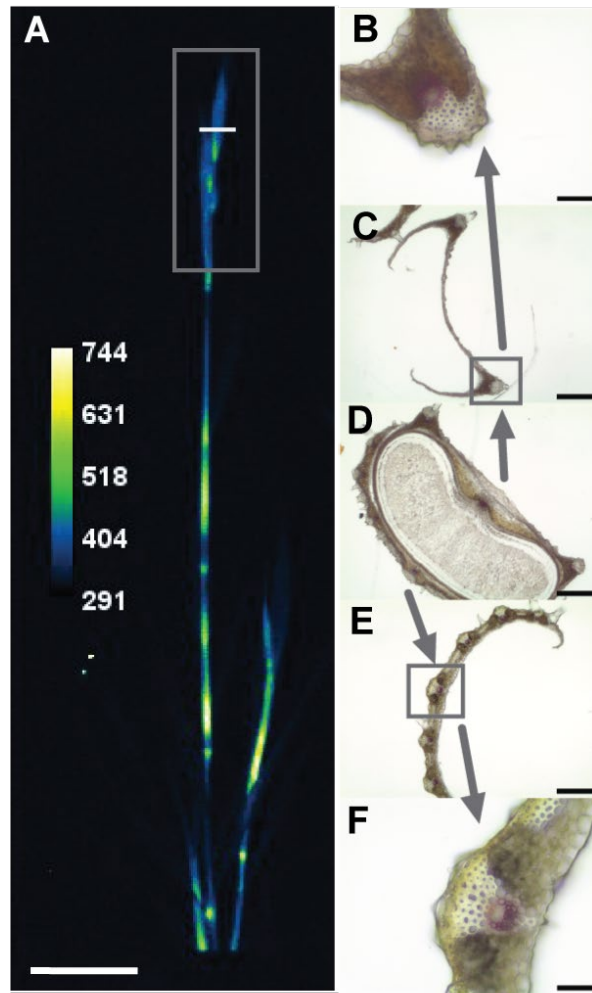


Figure S3. Histological analysis of *Brachypodium distachyon* seeds reveals extensive secondary cell wall thickening in the lemma and palea. Related to Figure 1. (A) Representative image showing above-ground *CESA8pro:LUC* luminescence; the white line indicates the position of transverse sections. The heat map represents luminescence intensity in arbitrary units, ranging from low (blue) to high (white). Scale bar, 2 cm. Seeds with the palea (**B, C**) and lemma (**E, F**) intact were sectioned and stained with phloroglucinol-HCl, shown at 20X (**B, F**) and 4X (**C–E**) magnification. (**D**) Whole seed with both lemma and palea intact. Scale bars: 50 μ m for 20X images, 250 μ m for 4X images.

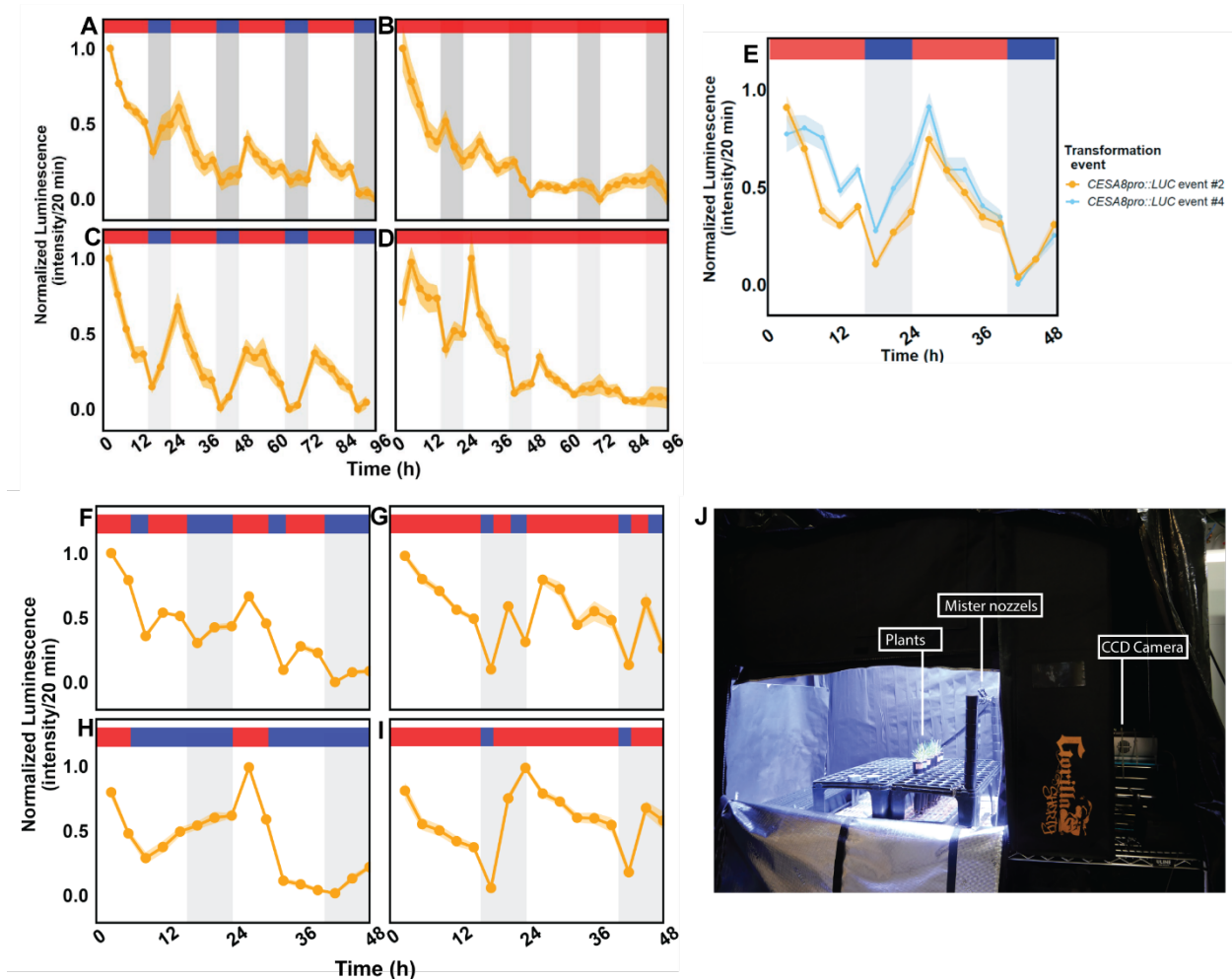


Figure S4. Replicate thermocycle and temperature-pulse experiments of *CESA8pro:LUC* expression dynamics. Related to Figures 1-3. (A–D) Replicate experiments of *CESA8pro:LUC* time-course analyses. Plants were entrained under photo- and thermocycles and subsequently imaged under (A) photo- and thermocycles, (B) constant light with thermocycles, (C) constant 25 °C with photocycles, or (D) constant light and constant 25 °C. **(E)** Independent *CESA8pro:LUC* transformation events exhibited similar thermocycle-dependent expression dynamics under constant light with thermocycles. **(F–I)** Replicate experiments of temperature-pulse treatments. *CESA8pro:LUC* plants were entrained under photo- and thermocycles, transferred to thermocycles alone, and then subjected to either a (F) 17 °C cold pulse for 3 h during the daytime or an (G) 25 °C warm pulse for 3 h at night. Long-day entrained plants were also exposed to (H) early nighttime temperatures, equivalent to a short-day thermocycle (6 h 25 °C:18 h 17 °C), or (I) early daytime temperatures, equivalent to a very long-day thermocycle (21 h 25 °C:3 h 17 °C). Brackets indicate deviations from standard long-day conditions. Luminescence data means $\pm$ SEM ($n = 7$). Dark gray shading represents night, light gray shading represents subjective night, red shading indicates 25 °C, and blue shading indicates 17 °C. LDHC, photo- and thermocycles; LDHH, photocycles and constant temperature; LLHC, thermocycles and constant light; LLHH, constant light and constant warm temperature. **(J)** Custom imaging chamber for long-term bioluminescence monitoring consists of a light sealed grow tent containing a rack for eight plants in containers. An overhead misting system delivers aerosolized luciferin to ensure continuous substrate availability throughout extended imaging periods. Luminescence emitted by the plants is captured by a cooled CCD camera positioned in an adjacent light-tight compartment. This enclosed configuration maintains stable environmental conditions and enables multi-day imaging experiments of the same plants.

Channel	Code	Description	Current (mA)	LED qty	Min Vf/Module	Max Vf/Module	String Qty	Total Min Vf	Total Max Vf	Link
1	D4	Far Red (720 nm)	350	3	5.4	7.2	5	27	36	https://www.luxeonstar.com/far-red-720nm-sinkpad-ii-20mm-tri-star-led-module-780mW
2	D2	Deep Red (655 nm)	350	3	6.3	8.4	5	31.5	42	https://www.luxeonstar.com/deep-red-655nm-sinkpad-ii-20mm-tri-star-led-module-1080mW
3	R2	Red (627 nm)	350	3	8.7	10.53	4	34.8	42.12	https://www.luxeonstar.com/red-627nm-sinkpad-ii-20mm-tri-star-led-module-138lm
4	G3	Green (530 nm)	700	3	8.7	10.53	4	34.8	42.12	https://www.luxeonstar.com/green-530nm-sinkpad-ii-20mm-tri-star-led-module-456lm
5	V2	Royal Blue (448 nm)	700	3	8.85	10.53	4	35.4	42.12	https://www.luxeonstar.com/royal-blue-448nm-sinkpad-ii-20mm-tri-star-led-module-2652mw
6	W3	Cool White 6500 K 540 lm	700	3	9	11.97	4	36	47.88	https://www.luxeonstar.com/cool-white-6500k-sinkpad-ii-20mm-tri-star-led-module-540lm
7	W4	Cool White 6500 K 660 lm	700	3	9	11.97	4	36	47.88	https://www.luxeonstar.com/cool-white-6500K-sinkpad-ii-20mm-tri-star-led-module-660lm
8	W5	Cool White 5650 K	700	3	8.7	9.75	4	34.8	39	https://www.luxeonstar.com/cool-white-5650K-sinkpad-ii-20mm-tri-star-led-module-705lm

Table S2. Description of light-emitting diodes in luciferase imaging chamber. Related to STAR Methods.

Primer name	Sequence	Application
<i>LUC2_F</i>	TTTGTTTCATCGGTGTGGCTG	Plant genotyping
<i>LUC2_R</i>	CAAGCGGTTCTATGTGGCAA	Plant genotyping
<i>CESA8-LUC_F</i>	TCTTCCTCCCATCCCATGCCCG	Plant genotyping
<i>CESA8-LUC_R</i>	CGAATGAACGACCGCCCAACCA	Plant genotyping
<i>LUC2_Cloning_F</i>	CACCATGGAAGATGCTAAGAATATTAAGAAG	Plasmid construction
<i>LUC2_Cloning_R</i>	ACTGCAGCTGGGAGCTCTC	Plasmid construction
<i>LUC-F</i>	CTCTCACGCAAGAGATCCTATTT	qPCR
<i>LUC-R</i>	ATCCGAGTGTAGTGAACATTCC	qPCR
<i>CESA8-F</i>	CAAAGCACAAGTTCCGCCTGTG	qPCR
<i>CESA8-R</i>	TGGCTCGTATGCATCTGTCAAATC	qPCR
<i>Bradi1g25170-F</i>	GAATGTGGGTGTGTAAGGTCATA	qPCR
<i>Bradi1g25170-R</i>	GGGTCCTTTCGAAGGATTGT	qPCR
<i>Bradi4g24887-F</i>	CCATGATTGTCTCGAGGAAGTTGC	qPCR
<i>Bradi4g24887-R</i>	TCGCTGCAGTTTCCCACATTTC	qPCR
<i>Bradi3g49600-F</i>	CACCCAAATGTTCACTTCAAGACG	qPCR
<i>Bradi3g49600-R</i>	GTTAAGTGGGCTGTCTGCTTCTG	qPCR

Table S3. Oligonucleotide primers used in this study. Related to STAR Methods.