

1 **Rhythms of Transcription in Field-Grown Sugarcane Are Highly Organ Specific**  
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## 25 Summary

- 26 • We investigated whether different specialized organs in field-grown sugarcane  
27 follow the same temporal rhythms in transcription.
- 28 • We assayed the transcriptomes of three organs during the day: leaf, a source  
29 organ; internodes 1 and 2, sink organs focused on cell division and elongation;  
30 and internode 5, a sink organ focused on sucrose storage.
- 31 • The leaf had twice as many rhythmic transcripts (>68%) as internodes, and the  
32 rhythmic transcriptomes of the two types of internodes were more similar to  
33 each other than to those of the leaves. More transcripts were rhythmic under  
34 field conditions than under circadian conditions and most of their peaks were  
35 during the day. Among the transcripts that were considered expressed in all  
36 three organs, only 7.4% showed the same rhythmic time course pattern. The  
37 central oscillators of these three organs — the networks that generate circadian  
38 rhythms — had similar dynamics with different amplitudes.
- 39 • The differences between the rhythmic transcriptomes in circadian conditions  
40 and field conditions highlight the importance of field experiments to understand  
41 how the plant circadian clock works *in natura*. The highly specialized nature of  
42 the rhythmic transcriptomes in sugarcane organs probably arises from  
43 amplitude differences in the circadian clock and different sensitivities to  
44 environmental cues.

45

46 **Keywords:** circadian clock, field conditions, natural conditions, organ-specific,  
47 rhythms, *Saccharum*, sugarcane, tissue-specific

48

## 49 Introduction

50 The circadian clock is an endogenous signaling network that allows organisms to adapt  
 51 to rhythmically changing environments. Plants with a circadian clock synchronized with  
 52 environmental rhythms accumulate more biomass and have better fitness than plants  
 53 with defective or no circadian clocks (Green *et al.*, 2002; Dodd *et al.*, 2005). In crops,  
 54 changes in the circadian clock have been indirectly selected through traditional  
 55 breeding to change photoperiodic responses, such as the transition to flowering. For  
 56 example, the circadian clocks of European tomatoes have longer periods than those of  
 57 native American tomatoes, as such periods allow these crops to adapt better to the  
 58 long summer days occurring at the high latitudes of much of Europe (Muller *et al.*,  
 59 2016). Similarly, some genotypes of *Hordeum vulgare* L. (barley) and *Triticum*  
 60 *aestivum* L. (wheat) carry mutations in their circadian clock genes that reduce flowering  
 61 induced by photoperiodic triggers, allowing cultivation in higher latitudes in Europe  
 62 (Turner *et al.*, 2005; Gawroński *et al.*, 2014).

63 The circadian clock is conceptually divided into three associated parts: the *Input*  
 64 *Pathways*, the *Central Oscillator*, and the *Output Pathways*. The *Input Pathways* detect  
 65 entraining cues that keep the circadian clock continuously synchronized to the  
 66 environment. In plants, these cues include light, temperature, and sugar levels  
 67 (Oakenfull & Davis, 2017; Frank *et al.*, 2018; Webb *et al.*, 2019). The *Central Oscillator*  
 68 is a series of interlocking transcriptional-translational feedback loops that can generate  
 69 24-h rhythms independently of the environment. In *Arabidopsis thaliana* (L.) Heynh.  
 70 (*Arabidopsis*), one loop, called the morning loop, starts with the light induction of  
 71 *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL*  
 72 (*LHY*) at dawn. Next, *PSEUDO-RESPONSE REGULATOR7* (*PRR7*) and *PRR9* are  
 73 activated by *CCA1* and *LHY*. In turn, *CCA1* and *LHY* are repressed by *PRR7* and  
 74 *PRR9*. In the core loop, *CCA1* and *LHY* are repressed, and this represses *TIME FOR*  
 75 *CAB EXPRESSION1* (*TOC1*), also known as *PRR1*. During the night, *TOC1* forms an  
 76 interaction known as the evening loop with the EVENING COMPLEX (EC). The EC is a  
 77 protein complex formed by EARLY FLOWERING3 (ELF3), ELF4, and LUX  
 78 ARRHYTHMO (LUX) that also inhibits the expression of *PRR7* and *PRR9* the next  
 79 morning. Other essential components of the oscillator include GIGANTEA (GI),  
 80 REVEILLE8 (RVE8), and CCA1 HIKING EXPEDITION (CHE) (Hsu *et al.*, 2013; Millar,  
 81 2016; Henriques *et al.*, 2018; Webb *et al.*, 2019). The *Output Pathways* transduce the  
 82 temporal information generated by the interaction between the *Central Oscillator* and  
 83 the *Input Pathways* to a plethora of biochemical pathways. The circadian clock thus

84 has a broad impact throughout the plant, regulating processes such as photosynthesis,  
85 cell elongation, stomata opening, and flowering (Hotta *et al.*, 2007).

86 Even though the plant circadian clock is highly conserved, there are a few differences  
87 between the circadian clocks of Arabidopsis and grasses (Poales). For instance, *CCA1*  
88 is absent from grasses, as it was created by a duplication exclusive to Brassicaceae.  
89 The grass PRRs consist of TOC1, PRR37, PRR73, PRR59, and PRR95, and it is not  
90 clear whether they have the same functions as their Arabidopsis counterparts, even  
91 though they are capable of complementing Arabidopsis mutations (Kusakina *et al.*,  
92 2015; Calixto *et al.*, 2015). In sugarcane, a highly polyploid crop that accumulates  
93 sucrose in the culm, the circadian clock has high-amplitude rhythms and regulates a  
94 large proportion of the leaf transcriptome (>30%) (Hotta *et al.*, 2013; Glassop & Rae,  
95 2019).

96 Most research to date on plant circadian rhythms has been done in controlled  
97 conditions, inside a growth room or growth chamber. Under such circumstances, plants  
98 can be grown either under circadian conditions, in which they are kept under constant  
99 abiotic conditions as a means to separate endogenous rhythms from rhythms driven by  
100 the environment, or under diel conditions, in which they are subjected to abiotic  
101 rhythms such as light/dark and warm/cold. Abiotic changes in controlled conditions are  
102 usually stepwise, in contrast to the gradients found in natural or field conditions, which  
103 can lead to significant changes in plant physiology (Shalit-Kaneh *et al.*, 2018;  
104 Annunziata *et al.*, 2018; Song *et al.*, 2018). For example, simulations of natural  
105 conditions in growth chambers showed that the clock-controlled gene *FLOWERING*  
106 *LOCUS T (FT)* has a different phase under such conditions than it does under  
107 controlled conditions (Song *et al.*, 2018), highlighting the need for adjustments in the  
108 current models to reflect events in natural conditions. In another study, the period and  
109 phase of the circadian clock affected shoot and rosette branch numbers in multiple  
110 Arabidopsis mutants in natural, but not controlled, conditions (Rubin *et al.*, 2017).  
111 Finally, the rice mutant *osgi*, which has a late-flowering phenotype in controlled  
112 conditions, flowered at the same time as the wild type in field conditions (Izawa *et al.*,  
113 2011).

114 Only two plant species have had their rhythmic transcripts identified in field conditions:  
115 rice and pineapple (Izawa *et al.*, 2011; Nagano *et al.*, 2012; Matsuzaki *et al.*, 2015;  
116 Ming *et al.*, 2015). However, these studies focused on the leaves. To better understand  
117 how the plant circadian clock regulates transcription under natural conditions in  
118 different organs, we measured transcription in three organs of field-grown sugarcane

grown during the day. We harvested leaf +1 (L1), a source organ, and two sink organs: internodes 1 and 2 (I1), organs focused on cell division and cell elongation that includes the shoot apical meristem; and internode 5 (I5), an organ focused on sucrose accumulation. We describe in detail one cycle (24 h) with 14 time points, starting 2 h before dawn. This approach allowed us to obtain a better resolution to describe transcripts with fast dynamics. We found that the rhythmic transcripts of the L1, I1, and I5 are widely specialized and likely to respond differently to environmental cues.

126

## 127 **Materials and Methods**

128

### 129 *Plant growth and harvesting*

Commercial sugarcane (*Saccharum* hybrid SP80-3280) was planted in a field in Araras, Brazil (22°18'41.0"S, 47°23'05.0"W, at an altitude of 611 m), in April 2012 (autumn) (Fig. S1). The soil on the site was a Typic Eutroferic Red Latosol. Plants were harvested 9 months later, in January 2013 (summer), after an unusually dry winter and spring. Harvest started 2 h before dawn and continued every 2 h until the next dawn (so that each expression time course had 14 time points in total). Dawn was at 5:45, and dusk was at 19:00 (13.25 h light/10.75 h dark) (Fig. S1). At each time point, leaf +1 (the first leaf from the top with clearly visible dewlap), internodes 1 and 2, and internode 5 of nine individuals were harvested (Fig. S2), frozen in liquid N<sub>2</sub>, and stored in three pools of three individuals each. Two pools were used as biological replicates for oligoarrays, and one pool was used for validation using the reverse-transcription quantitative PCR (RT-qPCR).

142

### 143 *Oligoarray hybridizations*

All frozen samples were pulverized in dry ice using a coffee grinder (Model DCG-20, Cuisinart, China). One hundred milligrams of each pulverized sample was used for extraction of total RNA using Trizol (Life Technologies), following the supplier's instructions. The RNA was treated with 2 U DNase I (Life Technologies) for 30 min at 37°C and cleaned using the RNeasy Plant Mini kit (Qiagen). The quality and quantity of RNA were assayed using an Agilent RNA 6000 Nano Kit Bioanalyzer chip (Agilent Technologies). Sample labeling was done following the Low Input Quick Amp Labelling protocol of the Two-Color Microarray-Based Gene Expression Analysis system (Agilent

Technologies). Hybridizations were done using a custom 4x44 k oligoarray (Agilent Technologies) that was previously described (Lembke *et al.*, 2012; Hotta *et al.*, 2013). Two hybridizations were done for each time point against an equimolar pool of all samples of each organ. Each duplicate was prepared independently using dye swaps. Data were extracted using the Feature Extraction software (Agilent Technologies). Background correction was applied to each dataset. A nonlinear LOWESS normalization was also applied to the datasets to minimize variations due to experimental manipulation. Signals that were distinguishable from the local background signal were taken as an indication that the corresponding transcript was expressed. The GenBank ID and Sugarcane Assembled Sequences (SAS) numbers for sugarcane genes are listed in Table S1. The complete dataset can be found at the Gene Expression Omnibus public database under the accession number GSE129543.

164

# *Data analysis*

For the purposes of further analysis, only transcripts that were found to be expressed in more than 7 of the 14 time points were considered to be expressed. All of the time courses were grouped in coexpressed modules using the R package weighted correlation network analysis (WGCNA) to identify rhythmic transcripts (Langfelder & Horvath, 2008). Network adjacency was calculated using a soft thresholding power of 18 for all organs. Modules that had a dissimilarity value of  $\leq 0.25$  were merged. Final modules were generated using a 0.175 adjacency threshold. As WGCNA groups together time courses that have a positive or a negative correlation, we normalized each time course using a Z-score, separated these time courses into two new modules, and generated a typical time course for each module by finding the median of all transcripts. Then, each representative time course was classified as rhythmic or nonrhythmic using JTK-CYCLE (Hughes *et al.*, 2010). Modules that had an adjusted *P*-value of  $< 0.75$  were considered rhythmic. Finally, we filtered out noisy time courses, defined as those that had a Spearman correlation of  $< 0.3$  when compared against the representative time course. Phase was assigned using the phase estimated by JTK-CYCLE corrected against a dendrogram with the representative time courses of all modules of all organs. Modules that clustered together in the dendrogram were considered to have the same phase. The phase of a time course is defined as the time between dawn and the peak of the time course. Euler diagrams were done using the R package *eulerr*. Chi-squared ( $\chi^2$ ) tests were used to compare Euler diagrams. Heatmaps were created using the R packages *circlize* (Gu *et al.*, 2014) and

187 *ComplexHeatmap* (Gu *et al.*, 2016). To evaluate if a group of transcripts were under- or  
188 overrepresented, we used a hypergeometric test (*phyper* function in R). With this test,  
189 a *P*-value < 0.05 suggests that the analyzed group is overrepresented in the dataset,  
190 while a *P*-value > 0.95 suggests that the analyzed group is underrepresented in the  
191 dataset. Code to fully reproduce our analysis is available on GitHub  
192 ([https://github.com/LabHotta/sugarcane\\_field\\_rhythms](https://github.com/LabHotta/sugarcane_field_rhythms)) and archived on Zenodo  
193 (<http://doi.org/10.5281/zenodo.2636813>).

194

## 195 *RT-qPCR analysis*

196

197 As described for the oligoarray hybridizations, 100 mg of the pulverized frozen samples  
198 for all three organs was used for total RNA extractions following the same Trizol (Life  
199 Technologies) protocol and then were treated with DNase I (Life Technologies) and  
200 cleansed using the RNeasy Plant Mini Kit (Qiagen). RNA quality and concentration of  
201 each sample were checked using an Agilent RNA 6000 Nano Kit Bioanalyzer chip  
202 (Agilent Technologies). Five micrograms of total purified RNA was enough for the  
203 reverse transcription reactions using the SuperScript III First-Strand Synthesis System  
204 for RT-PCR (Life Technologies). The RT-qPCR reactions for all samples were done  
205 using Power SYBR Green PCR Master Mix (Applied Biosystems), 10x diluted cDNA,  
206 and specific primers described by Hotta *et al.* (2013) (Figure S8). Reactions were  
207 placed in 96-well plates and read with the Fast 7500/7500 Real-Time PCR System  
208 (Applied Biosystems). Data analysis was performed using the Fast 7500/7500 Real-  
209 Time PCR System built-in software (Applied Biosystems).

210

## 211 **Results**

212

### 213 *A significant proportion of the sugarcane transcriptome is rhythmic in diel conditions*

214 We planted a field of commercial sugarcane (*Saccharum* hybrid SP80-3280) in autumn  
215 2012 in Araras (Brazil, 22°18'41.0"S, 47°23'05.0"W). Nine months later (summer 2013),  
216 after a dry winter and spring (Fig. S1), we harvested leaf +1 (L1), internodes 1 and 2  
217 (I1), and internode 5 (I5) every 2 h for 26 h, starting 2 h before dawn. Thus, a time  
218 course had 14 time points. On the day of harvest, the stalks were 76 ± 0.16 cm, with 11

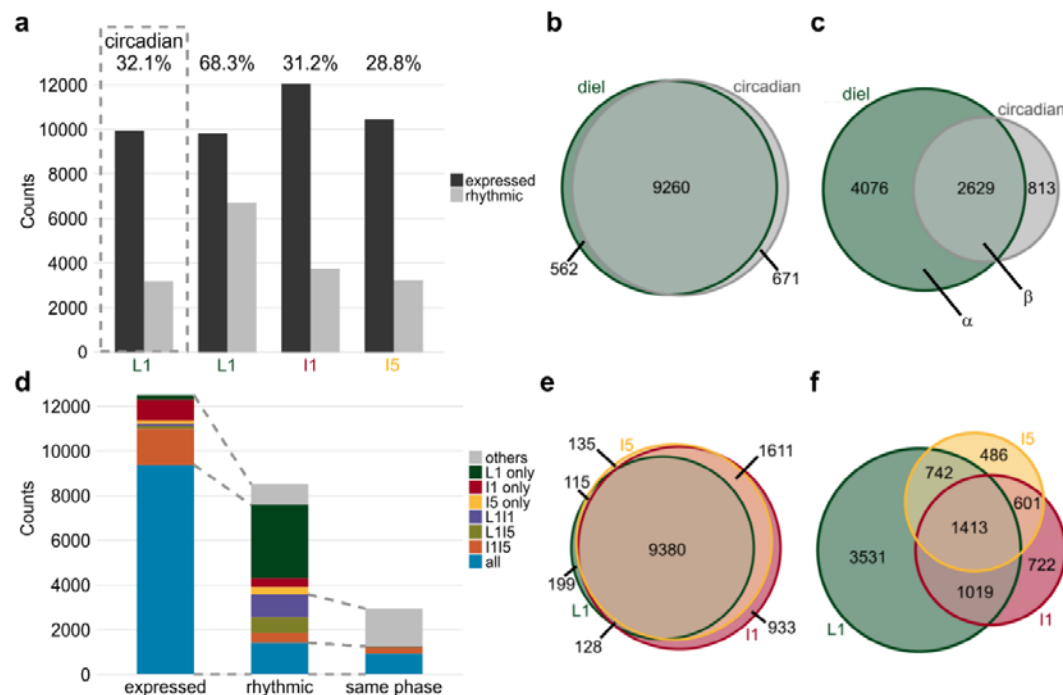
219  $\pm 2$  internodes, and their sugar content was  $12.0 \pm 1.4^\circ\text{Bx}$  (mean  $\pm$  SD;  $n = 20$ ). The  
220 temperature varied throughout the day from  $17^\circ\text{C}$  to  $30^\circ\text{C}$ , with the maximum occurring  
221 at 11 h after dawn (ZT11); the maximum light intensity was  $2.67 \text{ MJ/m}^2$  at ZT07, and  
222 dusk occurred 13.25 h after dawn (ZT13.25) (Fig. S1B and D).

223 RNA extracted from each organ was hybridized in 44k custom oligoarrays (Lembke *et*  
224 *al.*, 2012; Hotta *et al.*, 2013). Of the 14,521 probes in the array, 12,501 resulted in time  
225 courses considered to be expressed in at least one organ (Fig. 1). L1 had 9,822  
226 expressed time courses, which was similar to a result obtained in a previous circadian  
227 experiment from our group, in which we measured transcripts from 3-month-old leaves  
228 (9,932) (Hotta *et al.*, 2013). These datasets shared 94.3% of the expressed time  
229 courses (Fig. 1b). I1 had the highest number of expressed time courses (12,053),  
230 followed by I5 (10,448). A total of 9,380 time courses were expressed in all three  
231 organs (75.0%, Fig. 1e). I1 and I5 shared the most substantial proportion of the time  
232 courses considered to be expressed (89.3%), and I1 had the most substantial  
233 proportion of unique expressed time courses (7.5%).

234 We identified rhythmic time courses by combining a weighted correlation network  
235 analysis (WGCNA) that grouped time courses in coexpression modules (Langfelder &  
236 Horvath, 2008) with JTK\_CYCLE, which identified which of the modules contained  
237 rhythmic time courses (Hughes *et al.*, 2010). This method identified 6,705 rhythmic  
238 time courses in L1 (68.3%), 3,755 in I1 (31.2%), and 3,242 in I5 (28.8%) (Fig. 1a and  
239 Fig. S3). As a comparison, 32.1% of the time courses were rhythmic in L1 under  
240 circadian conditions (Hotta *et al.*, 2013). The overlap between circadian time courses  
241 and rhythmic time courses in the field (in diel conditions) was 2,623, representing  
242 76.4% of circadian time courses and 60.1% of diel time courses (Fig. 1c).

243





244

## 245 Figure 1 – Different organs have specific sets of rhythmic transcripts in

246 **sugarcane. (a)** The numbers of expressed and rhythmic time courses detected in leaf  
247 +1 (L1), internodes 1 and 2 (I1), and internode 5 (I5) in field-grown (diel) conditions,  
248 and in leaf +1 in circadian conditions (Hotta *et al.*, 2013). **(b,c)** Euler diagrams of  
249 expressed time courses **(b)** and rhythmic time courses **(c)** in L1 in sugarcane in diel  
250 (green) and circadian (gray) conditions. **(d)** Number of expressed time courses,  
251 rhythmic time courses, and rhythmic time courses with the same phase that were found  
252 specifically in L1, I1, or I5; in both L1 and I1 (L1I1, purple); in both L1 and I5 (L1I5, light  
253 green); in both I1 and I5 (I1I5, orange); and in all three organs (L1I1I5, blue). In the  
254 second bar, the gray area corresponds to rhythmic time courses that are expressed in  
255 only one or two organs. In the third bar, the gray area corresponds to rhythmic time  
256 courses in only two organs that have the same phase. The gray dashed lines show the  
257 associations among bars. **(e,f)** Euler diagram of expressed and rhythmic time courses  
258 in L1, I1, and I5 in field-grown sugarcane in diel conditions.

259

## 260 Different sets of transcripts are rhythmic in different sugarcane organs

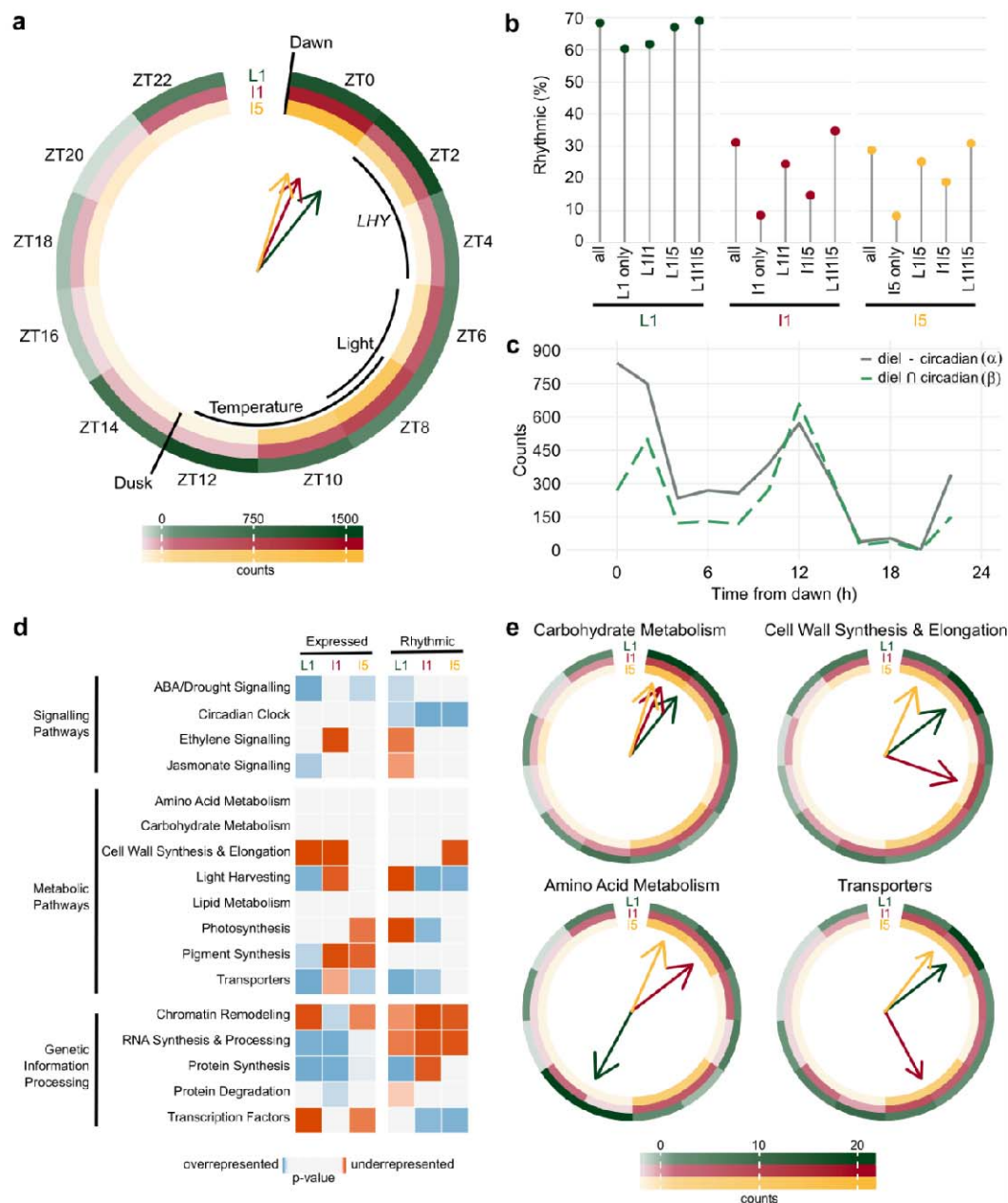
261 Although most expressed time courses were found in all three organs, only 1,413 of the  
262 expressed time courses were rhythmic in all three organs (16.6%) (Fig. 1d,f). L1 had  
263 the largest proportion of unique rhythmic time courses (41.5%), followed by I1 (8.5%)

264 and then I5 (5.7%) (Fig. 1f). Time courses that were expressed only in one organ were  
265 less likely to be rhythmic (60.3% for L1, 8.6% for I1, and 8.4% for I5) (Fig. 1h).

266 We estimated the phase of the transcripts by combining the phase calculated using  
267 JTK\_CYCLE with a dendrogram of the representative time course of each module.  
268 Among the time courses that were rhythmic in more than one organ, 27% had rhythms  
269 with phase differences >2 h (Fig. 1d). Overall, among the 12,501 unique expressed  
270 time courses in the three organs, only 7.4% (923) showed rhythms with the same  
271 phase in the three organs. Most of the time courses peaked during the day: this was  
272 true of 80.3% in L1, 90.4% in I1, and 96.3% in I5 (the photoperiod was 13.25 h, or  
273 56.3% of a cycle) (Fig. 1g). In L1, 2,363 time courses peaked between dawn (ZT00)  
274 and 2 h after dawn (ZT02) (35.2%), and 1,232 time courses peaked at ZT12 (18.4%)  
275 (Fig. 2a). When we separated rhythmic L1 time courses into those that were also  
276 rhythmic in circadian conditions (Fig. 2b,  $\alpha$ ) and those that were not (Fig. 2b,  $\beta$ ), two  
277 different phase distributions could be observed (Fig. 2c). The  $\alpha$  group had most time  
278 courses peaking at ZT00-02 (39.1%), followed by ZT12 (14.0%), while the  $\beta$  group  
279 peaked at ZT12 (25.1%), followed by ZT02 (19.1%). In I1, 1,201 time courses peaked  
280 at ZT0 (32.0%) and 716 peaked at ZT8 (19.1%). In I5, 1,373 time courses peaked at  
281 ZT0 (42.4%) and 894 peaked at ZT8 (27.6%) (Fig. 2a).

282 The majority of time courses from L1 (65.8%) grown in diel conditions had the same  
283 phase ( $\pm 2$  h) in leaves grown under circadian conditions (Fig. S4a). More time courses  
284 showed a delayed peak (19.6%) rather than an advanced peak (13.9%) under diel  
285 conditions than under circadian conditions. When we compared L1 and I1 time  
286 courses, 65.8% had the same phase, with the remainder divided roughly evenly  
287 between delayed and advanced phases (16.1% and 14.9%, respectively) (Fig. S4b).  
288 Similarly, 67.1% of the L1 time courses had the same phase as I5, 14.2% had a  
289 delayed phase, and 14.8% had an advanced phase (Fig. S4c). The phases were most  
290 similar between I1 and I5 time courses: 93.8% had the same phases, 2.8% a phase  
291 delay, and 3.1% a phase advance (Fig. S4d).

292



293

294 **Figure 2 – Transcripts have unique phases in different sugarcane organs. (a)**

295 Circular heatmap of the rhythmic transcript peak time (ZT0 = 0 h after dawn)

296 distribution in leaf +1 (L1), internodes 1 and 2 (I1), and internode 5 (I5). The colored

297 arrows show the times at which the most transcripts are found in each organ. The

298 times of dawn, dusk, *LHY* transcription peak, maximum light intensity, and maximum

299 temperatures are indicated by black arcs. **(b)** Proportions of transcripts that were

300 rhythmic in L1, I1, and I5 among all expressed transcripts in each organ (All), among

301 the transcripts expressed only in one organ (L1 only, I1 only, or I5 only), among the

302 transcripts expressed in two organs (L1I1, L1I5, or I1I5), and among transcripts

expressed in all three organs (L1I1I5). **(c)** Distribution of rhythmic transcript peak time in time courses that were rhythmic in L1 but not in circadian conditions ( $\alpha$  in Fig. 1c) and rhythmic transcripts in time courses that were rhythmic in L1 and circadian conditions ( $\beta$ ). **(d)** Heatmap of functional categories that are overrepresented (shades of blue) or underrepresented (shades of red) among the expressed and rhythmic transcripts of L1, I1, and I5. The *P*-value was calculated using a hypergeometric test. **(e)** Circular heatmap with the distribution of the peak times of rhythmic transcripts associated with the pathways *Carbohydrate Metabolism*, *Cell Wall Synthesis & Elongation*, *Amino Acid Metabolism*, and *Transporters*.

### Biochemical pathways have different rhythms in sugarcane organs

We used a hypergeometric test to detect if a pathway was over- or underrepresented by comparing the frequency of transcripts associated with a Biochemical Pathway among the expressed time courses and all the unique transcripts in the oligoarray (Fig. 2d and Fig. S5). We used the same test comparing the frequency of transcripts associated with a Biochemical Pathway among the rhythmic time courses and the expressed time courses (Fig. 2d and Fig. S5). The transcript annotations were based on the SUCEST database annotation (<http://sucest-fun.org>).

Among expressed time courses, each organ has a distinct profile. For example, L1 was the only organ that had the *Pigment Synthesis*, *Light Harvesting*, and *Jasmonate Signaling* pathways considered to be overrepresented. I1 had *Chromatin Remodeling* and *Protein Synthesis* pathways overrepresented and *Ethylene Signaling* underrepresented. *Transcription Factors* was underrepresented and *ABA/Drought Signaling* and *Transporters* were overrepresented in L1 and I5, but not in I1. I5 is the only organ in which *Cell Wall Synthesis & Elongation* was not underrepresented among the expressed time courses (Fig. 2d). Among rhythmic time courses, *Circadian Clock* was overrepresented, while *Chromatin Remodeling* and *RNA Synthesis & Processing* were underrepresented in all organs. *Protein Synthesis* was overrepresented in L1. *Transcription Factors* was overrepresented in I1 and I5, and *Transporters* was overrepresented among rhythmic time courses in L1 and I1 (Fig. 2d).

When we analyzed time courses associated with important pathways for sugarcane growth, we found further organ-specific patterns; these differences could be seen in both expressed and rhythmic time courses, as well as the phase of the rhythmic time courses (Fig. 2e and Fig. S6). Time courses associated with *Carbohydrate Metabolism*

337 tended to peak in the morning. Almost half (48.0%) of the time courses had a peak at  
338 ZT00 in L1, while the majority peaked between ZT00 and ZT04 in both I1 (53.2%) and  
339 I5 (58%) (Fig. 2e). Amongst the individual time courses, a putative ortholog of  
340 *SUCROSE SYNTHASE4 (SuSy4)* had a similar rhythmic pattern in all three organs. A  
341 putative ortholog of *SUCROSE-PHOSPHATE SYNTHASE II (SPSII)* was rhythmic only  
342 in L1, while a putative ortholog of a *CELL WALL INVERTASE (CWI)* exhibited a sharp  
343 peak at ZT04 in L1 but a very broad peak at ZT08 in I1 and I5 (Fig. 6i, m and q).

344 Time courses associated with *Cell Wall Synthesis & Elongation* had a more diverse  
345 phase distribution: in L1, 55% had a peak between ZT00 and ZT04 in L1; in I1, 73.4%  
346 had a peak between ZT00 and ZT08; and in I5, 45.8% had a peak between ZT08 and  
347 ZT10 and 37.8% had one at ZT00 (Fig. 2e). There was also a higher proportion of time  
348 courses associated with *Cell Wall Synthesis & Elongation* that are expressed only in I1  
349 and I5 (Fig. S6). Time courses associated with *Amino Acid Metabolism* peaked  
350 between ZT12 and ZT14 in L1 (50%). In I1 and I5, they had two peaks: between ZT00  
351 and ZT02 (37.5% and 57.1%) and between ZT08 and ZT10 (37.5% and 42.9%) (Fig.  
352 2b). Time courses associated with *Transporters* peaked at ZT02 (35.7%) and ZT12  
353 (15.7%) in L1. In I1, most of the time courses peaked 2 h earlier, at ZT00 (24.2%) and  
354 ZT10 (24.2%). I5 displayed a similar pattern to I1, with 53.6% peaking between ZT00  
355 and ZT02 and 46.4% between ZT08 and ZT10 (Fig. 2b). This tendency for L1 to have  
356 later phases than I1 and I5 can be seen in the putative ortholog *SWEET2*, which  
357 peaked at ZT02 in L1 and at ZT18-20 in I1 and I5 (Fig. 6l).

358

### 359 *Circadian clock transcripts have similar dynamics in different sugarcane organs*

360 The differences in the rhythmic time courses of the three organs could be explained by  
361 the presence of organ-specific circadian clocks that could generate different patterns of  
362 rhythmic transcription. The circadian clocks can be divided into three parts: *Input*  
363 *Pathways*, *Central Oscillator*, and *Output Pathways* (Henriques *et al.*, 2018).

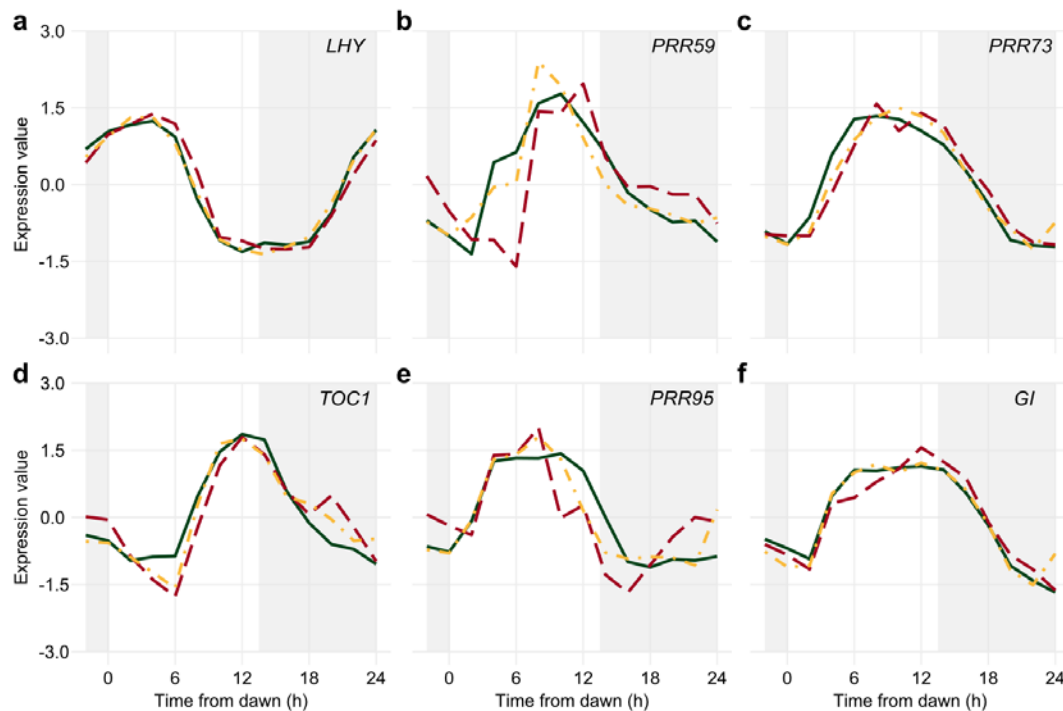
364 Most of the known *Input Pathways* to the circadian clock are associated with *Light*  
365 *Signaling* (Oakenfull & Davis, 2017). *Light Signaling* is underrepresented among the  
366 transcripts expressed in I5 and the rhythmic time courses in I1 (Fig. S5). Among the  
367 red light receptor genes, *PHYTOCHROME A.1 (PHYA.1)* was rhythmic in L1, with a  
368 peak at ZT23, while *PHYB* was not rhythmic in any organ (Fig. S7a,b). In I1 and I5,  
369 both *PHYs* had two peaks: one near dawn (ZT00-02) and another at night (ZT18-20).  
370 Among the blue light receptors, *CRYPTOCHROME1.1 (CRY1.1)* was rhythmic in L1,

371 peaking at ZT03. *CRY2.1* was rhythmic in I5, peaking at ZT19. *ZEITLUPE* (*ZTL.1*) was  
372 rhythmic in L1 and I5, peaking at ZT01 and ZT21, respectively (Fig. S7d-f).

373 The *Central Oscillator* generates rhythms that can run independently from  
374 environmental rhythms. In general, the time courses associated with the *Central*  
375 *Oscillator* displayed rhythms with similar dynamics (Fig. 3). *LHY* peaked early in the  
376 morning, between ZT02 and ZT04, with overlapping dynamics in all three organs (Fig.  
377 3a). Similarly, *TOC1* peaked around dusk, between ZT10 and ZT12, in all three organs  
378 (Fig. 3d). The normalizations used to analyze the oligoarray data do not allow the  
379 comparison of expression levels, so we used RT-qPCR to show that *LHY* varied during  
380 the day by 750× in L1 and 150× in I1 and I5 (Fig. S8a). In contrast, *TOC1* differed 30×  
381 in L1 and 18× in I1 and I5 (Fig. S8b). The other PRR genes, *PRR59*, *PRR73*, and  
382 *PRR95* (referred to as *ScPRR3*, *ScPRR7*, and *ScPRR59*, respectively, in Hotta *et al.*,  
383 2011), peaked between ZT06 and ZT10 (Fig. 3b, c, and e). *GI* peaked between ZT08  
384 and ZT10 in all three organs (Fig. 3f). Finally, *ELF3* was rhythmic only in L1, with a  
385 peak at ZT14. In the internodes, *ELF3* had a similar pattern, but it was not regarded as  
386 rhythmic due to high noise (Fig. S7c).

387 Among the possible pathways that can be recruited by the circadian clock that are  
388 considered part of the *Output Pathways* are those associated with *Chromatin*  
389 *Remodeling*, *Transcription Factors*, and *Protein Synthesis* (Fig. 4). Time courses  
390 associated with *Chromatin Remodeling* peaked at ZT00-02 and ZT10-12 in L1 (32.5%  
391 and 36.5%, respectively). In I1 and I5, they peaked at ZT00 (40.7% and 40.8%,  
392 respectively) and ZT08-10 (33.99% and 51.2%, respectively) (Fig. 4a). Time courses  
393 associated with *Transcription Factors* tended to peak near dawn, at ZT00-02, in all  
394 three organs (57.5% in L1, 46.4% in I1, and 50.3% in I5). A higher proportion (22.6%)  
395 of time courses associated with *Transcription Factors* were rhythmic when compared to  
396 all rhythmic (16.6%) time courses,  $\chi^2(6, n = 341) = 15.1$ ,  $P = 0.02$  (chi-square test, Fig.  
397 4e). These time courses also peaked similarly in all organs: 79.3% peaked in the same  
398 interval in L1 as in I1, 72.2% peaked in the same interval in L1 and I5, and 93.1% in I1  
399 and I5.





400

401 **Figure 3 – Diel rhythms of *Central Oscillator* transcripts in sugarcane organs.**

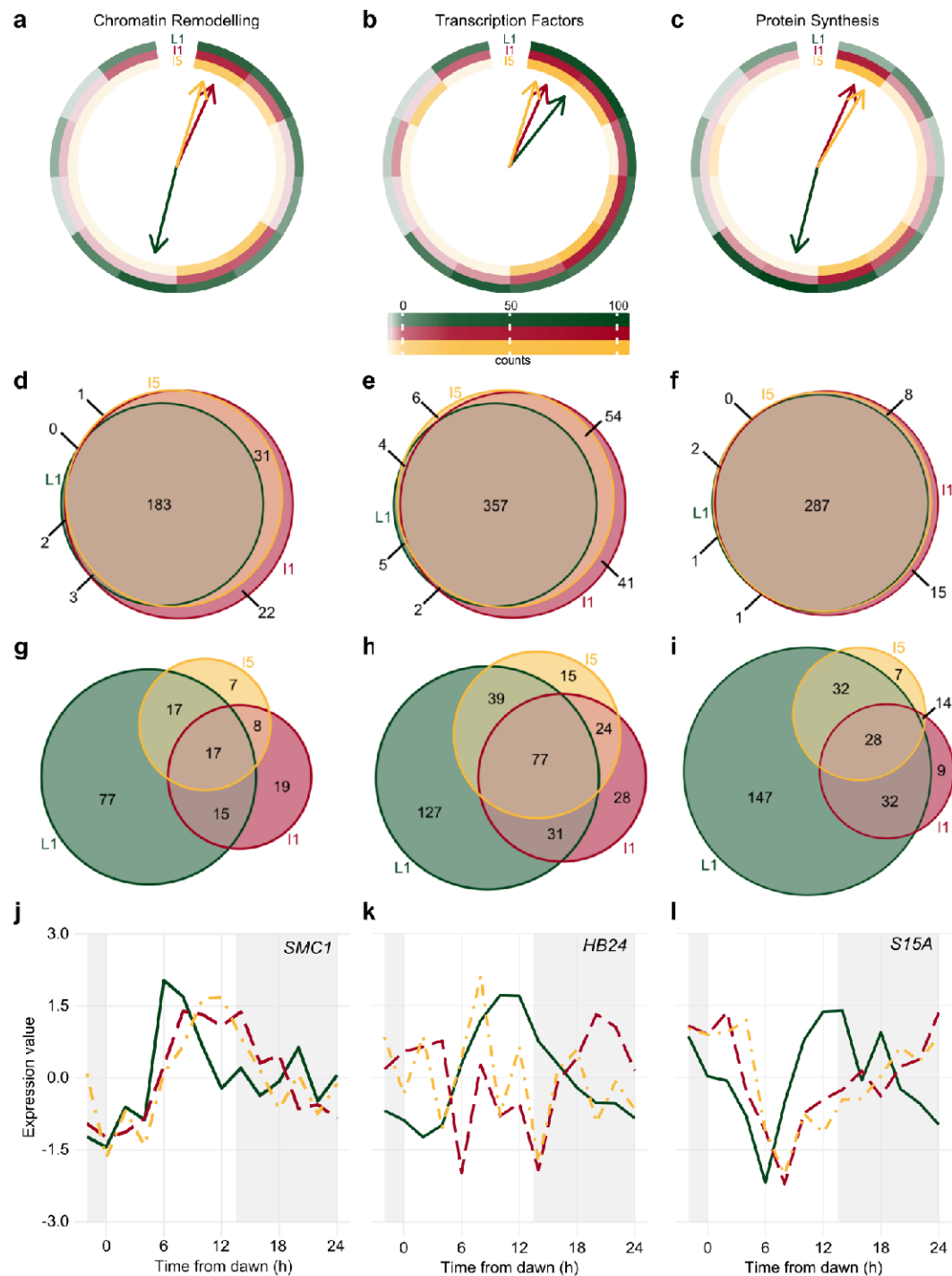
402 *LHY* (a), *PRR59* (b), *PRR73* (c), *TOC1* (d), *PRR95* (e), and *GI* (f) rhythms were  
 403 measured in leaf +1 (L1, green continuous line), internodes 1 and 2 (I1, red dashed  
 404 line), and internode 5 (I5, yellow dash-dotted line) of field-grown sugarcane using  
 405 oligoarrays. Time courses were normalized using Z-score. The light-gray boxes  
 406 represent the night periods.

407

408 Time courses associated with *Protein Synthesis* tended to peak at dusk in L1 (ZT12,  
 409 49.0%), at dawn and afternoon in I1 (ZT00, 36.1%; ZT10, 32.5%), and at dawn in I5  
 410 (ZT00, 61.7%) (Fig. 4c). A higher proportion of time courses associated with *Protein*  
 411 *Synthesis* were expressed in all three organs (91.4%) than for all time courses (75.0%),  
 412  $\chi^2(6, n = 314) = 48.7, P < 0.001$  (Fig. 4f). In contrast, more than half of the time courses  
 413 (54.6%) were rhythmic only in L1, whereas a lower frequency (41.5%) of total rhythmic  
 414 time courses were seen only in L1,  $\chi^2(6, n = 269) = 34.8, P < 0.001$  (Fig. 4i).

415 Other time courses showed a wide variety of oscillations amongst the three organs  
 416 (Fig. 4j-l, and S9). The putative *STRUCTURAL MAINTENANCE OF*  
 417 *CHROMOSOMES1* (*SMC1*), associated with *Chromatin Remodeling*, peaked at ZT06  
 418 in L1 and ZT11 in I1 and I5 (Fig. 4j). Two putative *JUMONJI-C* (*JMJC*) *DOMAIN-*  
 419 *CONTAINING PROTEIN5* (*JMJD5*) genes, encoding proteins that can act as histone

420 demethylases, were found in sugarcane. *JMJD5.1* is expressed only in L1 and I5 and  
 421 has a phase at ZT10 (Fig. S9a,d); *JMJD5.2* is expressed in all organs with similar  
 422 rhythmic patterns (Fig. S9a). The transcription factor gene *HOMEBOX PROTEIN24*  
 423 (*HB24*) is rhythmic only in L1, with a peak at ZT10 (Fig. 4k). Another rhythmic gene,  
 424 *40S RIBOSOMAL PROTEIN S15* (*S15A*), associated with *Protein Synthesis*, has a  
 425 peak at ZT14 in L1 and at ZT00 in I1 and I5 (Fig. 4l).





**Figure 4 – Transcripts associated with *Genetic Information Processing* have different rhythms in sugarcane organs. (a-c)** Circular heatmap of the distribution of the peak time of rhythmic transcripts related to *Chromatin Remodeling* (a), *Transcription Factors* (b), and *Protein Synthesis* (c) in leaf +1 (L1, green), internodes 1 and 2 (I1, red), and internode 5 (I5, yellow). The colored arrows show the time at which the most transcripts are found in each organ. **(d-i)** Euler diagrams of all expressed transcripts (d-f) and rhythmic transcripts (g-i) in L1, I1, and I5 in field-grown sugarcane in diel conditions. **(j-l)** *SMC1* (j), *HB24* (k), and *S15A* (l) rhythms measured in L1 (continuous green line), I1 (red dashed line), and I5 (yellow dash-dotted line) of field-grown sugarcane using oligoarrays. Time courses were normalized using Z-score. The light-gray boxes represent the night periods.

438

## 439 Discussion

440 Organ-specific rhythms of transcription can be found in highly productive and  
441 intensively selected commercial sugarcane. The specialization of the rhythmic  
442 transcriptome may help the plant cells to adapt to local environmental rhythms, as well  
443 as to generate rhythms that are compatible with their specialized needs. Specialized  
444 rhythms may also be essential to rhythmic processes that require organ-to-organ  
445 coordination, such as sucrose transport from the leaves to the internodes (Wang *et al.*,  
446 2013).

447

### 448 *Rhythms in field conditions are different from those in controlled conditions*

449 Sugarcane leaves in field conditions had twice as many time courses identified as  
450 rhythmic than plants assayed under circadian conditions. This difference is expected  
451 because some rhythms are driven by environmental oscillations, such as light and  
452 temperature. Also, some circadian-clock-driven rhythms may undergo amplitude  
453 increases due to a general increase in the amplitude of the *Central Oscillator*. In L1, the  
454 transcriptional rhythms of *LHY* vary by up to 60× in a day in circadian conditions and  
455 750× in field conditions, while those of *TOC1* vary up to 5× in a day in circadian  
456 conditions and 40× in field conditions (Hotta *et al.*, 2013, and Fig. S8). In circadian  
457 conditions, most time courses peaked at subjective dusk (ZT12, 29.0%), which resulted  
458 in 60.5% of the time courses peaking during subjective night.

By contrast, in field conditions, most time courses peaked near dawn (ZT00-02, 35.2%) in L1, which resulted in 80.3% of the time courses peaking during the day. This reinforces the role of the light/dark transition as the driving force of rhythms in leaves in field conditions. A high proportion (64.1%) of the time courses that peaked during the subjective night in circadian conditions showed phase changes that resulted in higher expression during the day in field conditions. This might suggest the existence of dampening mechanisms that actively decrease nocturnal peaks. A similar mechanism keeps cytoplasmic calcium concentration lower during the night under diel conditions (day/night) than during the subjective night under circadian conditions (Xu *et al.*, 2007). Most of the time courses associated with the *Central Oscillator* maintained their core phases, except *LHY*, which had a later peak (ZT01 in circadian conditions; ZT04 in field conditions). As a comparison, *LHY* is induced by light in *Arabidopsis* and is mostly insensitive to temperature in rice (Kim *et al.*, 2003; Nagano *et al.*, 2012). The differences between the rhythmic transcriptomes in circadian conditions and field conditions highlight the importance of experiments done under field conditions to understanding how the circadian clock can affect the plant transcriptome *in natura*.

475

#### 476 *Rhythmic transcripts are organ-specific*

The time courses in L1, I1, and I5 have very different rhythmic patterns, even though most of the expressed time courses were found in all three organs. Rhythms in I1 and I5 were more similar to each other than to those in L1, and only 7.4% of the time courses expressed in all three organs showed the same rhythms. Thus, we conclude that these three organs have vastly different and specialized circadian clocks. These specialized circadian clocks could be the result of multiple organ sensitivities to environmental cues, of organ-specific *Core Oscillators*, and of organ-specific interactions of *Output Pathways* with environmental signals.

In *Arabidopsis*, different sensitivities to environmental cues are found in the vascular phloem companion cells, which are more sensitive to photoperiodism, and the epidermis cells, which are more sensitive to temperature (Shimizu *et al.*, 2015). In sugarcane, most L1 time courses peak at ZT00-02 and ZT12, following dawn and dusk, while most I1 and I5 time courses peak at ZT00 and ZT08, following dawn and the daily light and temperature maxima. Thus, the circadian clocks of these organs respond differently to environmental cues such as photoperiod, light/dark transition, or temperature, as in *Arabidopsis*. In rice, a significant proportion of rhythmic transcripts are regulated either by the circadian clock or by temperature oscillations (Nagano *et*

494 *al.*, 2012). In sugarcane, rhythmic L1 time courses that were also rhythmic in circadian  
495 conditions had peaks that follow *LHY* or *TOC1* expression. On the other hand, rhythmic  
496 L1 time courses that were not rhythmic in circadian conditions peaked at dawn and  
497 dusk. In internodes, time courses peaked at dawn and at the light and temperature  
498 maximum. Such organ-specific sensitivity to environmental cues was previously  
499 described in the vasculature and epidermis (Shimizu *et al.*, 2015).

500 The *Central Oscillators* of mesophyll and vasculature in Arabidopsis have similar  
501 components but with different amplitudes. *AtELF4* rhythms have an amplitude 10×  
502 higher in the vasculature, *AtPRR7* and *AtPRR9* amplitudes are 2× higher in the  
503 mesophyll, and *AtTOC1* amplitude is similar in both tissues (Endo *et al.*, 2014). In  
504 sugarcane, *LHY* amplitude is 6× higher and *TOC1* amplitude is 2× higher in L1  
505 compared to I1 and I5. As leaves are exposed to direct sunlight, whereas internodes  
506 are protected by layers of leaf sheaths, it is probable that sunlight is responsible for  
507 these amplitude differences. The dynamics of *LHY*, *TOC1*, and *GI* during the day were  
508 very similar in the three organs. Indeed, they were considered to be coexpressed when  
509 analyzed together (data not shown). As the three organs have different levels of  
510 exposure to the environment, the existence of a common environmental signal is  
511 unlikely. Alternatively, the oscillators of the three organs could be coupled. There is  
512 evidence in Arabidopsis of root oscillators being regulated by the oscillators of the  
513 aerial parts of the plants, either the leaves or the shoot apical meristem (SAM) (James  
514 *et al.*, 2008; Takahashi *et al.*, 2015). As the leaves are a source signal to both  
515 internodes, it is possible that synchronizing signals are transported with sucrose and  
516 other sugars. In Arabidopsis, sugars can also act as an entrainment signal (Haydon *et al.*,  
517 2013; Frank *et al.*, 2018).

518 Even though there is much evidence for tissue-specific circadian clocks in Arabidopsis  
519 (Para *et al.*, 2007; Xu *et al.*, 2007; Kim *et al.*, 2011; Endo *et al.*, 2014; Endo, 2015), less  
520 is known about their effect on the rhythmic regulation of transcripts. In contrast, tissue-  
521 specific rhythms have been widely studied in mammals (Storch *et al.*, 2002; Panda *et al.*,  
522 2002; Zhang *et al.*, 2014; Ruben *et al.*, 2018). Sampling of 12 different mouse  
523 organs over time showed that 43% (~8,500) of all transcripts had circadian rhythms in  
524 at least one organ, but only 10 transcripts were rhythmic in all organs (Zhang *et al.*,  
525 2014). As in sugarcane leaves, the rhythmic transcripts in mammalian organs tended to  
526 peak at dawn and dusk. In general, the only transcripts that had similar phases across  
527 all organs were the ones associated with the mammalian *Core Oscillator* (Zhang *et al.*,  
528 2014).

At least two regulatory pathways are required to generate tissue-specific sets of rhythmic transcripts: one that confers organ specificity and one that confers rhythmicity. These pathways can be organized in different nonexclusive ways: they could act on a gene independently, the tissue specificity pathways could regulate the rhythmicity pathways, or the rhythmicity pathways could regulate the organ specificity pathways (Fig. S10). The rhythmicity pathways can be dependent on the circadian clock, on environmental rhythms, or both. The tissue specificity pathways can include transcription factors, protein-protein interactions, alternative promoter usage, chromatin interactions, and topologically associating domains (TADs) (Yeung & Naef, 2018).

In our datasets, time courses that were expressed only in one organ or only in the internodes were less likely to be considered rhythmic (Fig. 2b). Thus, it is possible that rhythmic pathways regulate only a small proportion of organ-specific pathways. Time courses associated with *Transcription Factors* were more likely to be rhythmic in all three organs, and these time courses had a higher probability of having the same phase. However, just a few tissue-specific rhythms in transcription factors can have a sizeable cascading effect (Yeung *et al.*, 2018). Tissue-specific transcription factors, even if nonrhythmic, could also change the phase of rhythmic transcripts through protein-protein interactions or by changing the promoter usage (Smieszek *et al.*, 2014; Yeung & Naef, 2018). Finally, chromatin remodeling could be a significant regulatory pathway in the generation of the tissue-specific rhythmic transcriptome. In Arabidopsis, chromatin remodeling can regulate the *Central Oscillator*, but little is known about how the plant circadian clock can use chromatin remodeling to generate rhythms (Lu *et al.*, 2011; Malapeira *et al.*, 2012; Lee *et al.*, 2015; Yang *et al.*, 2018). In sugarcane, rhythms in time courses associated with *Chromatin Remodeling* were underrepresented among the rhythmic time courses. However, chromatin remodeling tends to be regulated post-transcriptionally through histone modifications. Transcription can also be regulated at the chromatin level through TADs. In mammals, TADs can be regulated to generate tissue-specific transcription and even rhythms (Aguilar-Arnal *et al.*, 2013; Kim *et al.*, 2018; Mermet *et al.*, 2018). In plants, TADs are maintained by cohesins, but there are still no known CCCTC-binding factors (CTCF) counterparts (Schubert *et al.*, 2013; Liu *et al.*, 2017). In sugarcane, the cohesin subunit *SMC1* has different phases in leaves and internodes.

Specialized tissue-specific circadian clocks allow the different tissues to be more efficient according to their function and local environmental rhythms (Yeung & Naef, 2018). In mammals, rhythms in fibroblasts allow wound healing to occur faster during the active phase than the rest phase (Hoyle *et al.*, 2017). Furthermore, rhythms in the

liver lead to larger cell sizes and protein levels during the active phase and after feeding, making detoxification more efficient during the active and post-feeding periods (Sinturel *et al.*, 2017). In sugarcane, expressed time courses associated with *Transporters* were overrepresented in L1 and I5, while rhythmic time courses associated with *Transporters* were overrepresented in L1 and I1. Nutrient and photosynthate transportation inside the plant are essential for rhythmic processes and may also be part of organ-to-organ coordination (Wang *et al.*, 2013; Xu, 2018; López-Salmerón *et al.*, 2019). Sucrose accumulation in the internodes inhibits leaf photosynthesis in sugarcane (Ribeiro *et al.*, 2017). Furthermore, time courses associated with *Transporters* tended to peak 2 h later in L1 than in the internodes, which may indicate that the latter is the driving force of this process. Other processes, such as *Pigment Synthesis* and *Light Harvesting* in L1, *Chromatin Remodeling* and *Protein Synthesis* in I1, and *Cell Wall Synthesis & Elongation* in I5, also suggest a functional specialization to the organ-specific circadian clock.

The vast differences found in the rhythmic transcriptomes of different plant organs provide important clues to understanding the way that tissue-specific circadian clocks are generated and their impact on plant physiology. However, little is still known about the molecular mechanisms that control this specialization. The combination of organ- or tissue-specific studies with the observation of rhythms in the field, where conditions are fluctuating and variable as is normal in natural environments, is essential to understanding the nuances of how the plant circadian clock increases the fitness of plants and, in turn, crop productivity.

587

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593

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598 Original Draft, L.L.B.D., C.T.H; Writing – Review & Editing, L.L.B.D., C.A.L., C.T.H;  
599 Visualization; C. T. H.; Project Administration, C. T. H.; Funding Acquisition, C.T.H

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## 601 References

- 602 **Aguilar-Arnal L, Hakim O, Patel VR, Baldi P, Hager GL, Sassone-Corsi P. 2013.** Cycles in spatial  
603 and temporal chromosomal organization driven by the circadian clock. *Nature Structural &*  
604 *Molecular Biology* **20**: 1206–1213.
- 605 **Annunziata MG, Apelt F, Carillo P, Krause U, Feil R, Koehl K, Lunn JE, Stitt M. 2018.** Response  
606 of Arabidopsis primary metabolism and circadian clock to low night temperature in a natural  
607 light environment. *Journal of Experimental Botany* **69**: 4881–4895.
- 608 **Calixto CPG, Waugh R, Brown JWS. 2015.** Evolutionary relationships among barley and  
609 Arabidopsis core circadian clock and clock-associated genes. *Journal of Molecular Evolution* **80**:  
610 108–119.
- 611 **Dodd AN, Salathia N, Hall A, Kevei E, Toth R, Nagy F, Hibberd JM, Millar AJ, Webb AA. 2005.**  
612 Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage.  
613 *Science* **309**: 630–3.
- 614 **Endo M. 2015.** Tissue-specific circadian clocks in plants. *Current Opinion in Plant Biology* **29**:  
615 44–49.
- 616 **Endo M, Shimizu H, Nohales MA, Araki T, Kay SA. 2014.** Tissue-specific clocks in Arabidopsis  
617 show asymmetric coupling. *Nature* **515**: 419–22.
- 618 **Frank A, Mantioli CC, Viana AJC, Hearn TJ, Kusakina J, Belbin FE, Wells Newman D, Yochikawa**  
619 **A, Cano-Ramirez DL, Chembath A, et al. 2018.** Circadian entrainment in Arabidopsis by the  
620 sugar-responsive transcription factor bZIP63. *Current Biology* **28**: 2597-2606.e6.
- 621 **Gawroński P, Ariyadasa R, Himmelbach A, Poursarebani N, Kilian B, Stein N, Steuernagel B,**  
622 **Hensel G, Kumlehn J, Sehgal SK, et al. 2014.** A distorted circadian clock causes early flowering  
623 and temperature-dependent variation in spike development in the Eps-3Am mutant of einkorn  
624 wheat. *Genetics* **196**: 1253–1261.
- 625 **Glassop D, Rae AL. 2019.** Expression of sugarcane genes associated with perception of  
626 photoperiod and floral induction reveals cycling over a 24-hour period. *Functional Plant*  
627 *Biology* **46**: 314–327.
- 628 **Green RM, Tingay S, Wang Z-Y, Tobin EM. 2002.** Circadian rhythms confer a higher level of  
629 fitness to Arabidopsis plants. *Plant Physiology* **129**: 576–584.
- 630 **Gu Z, Eils R, Schlesner M. 2016.** Complex heatmaps reveal patterns and correlations in  
631 multidimensional genomic data. *Bioinformatics* **32**: 2847–2849.
- 632 **Gu Z, Gu L, Eils R, Schlesner M, Brors B. 2014.** circlize Implements and enhances circular  
633 visualization in R. *Bioinformatics (Oxford, England)* **30**: 2811–2812.

634 **Haydon MJ, Mielczarek O, Robertson FC, Hubbard KE, Webb AA. 2013.** Photosynthetic  
635 entrainment of the *Arabidopsis thaliana* circadian clock. *Nature* **502**: 689–92.

636 **Henriques R, Papdi C, Ahmad Z, Bögre L. 2018.** Circadian regulation of plant growth. In: Annual  
637 Plant Reviews online. American Cancer Society, 1–29.

638 **Hotta CT, Gardner MJ, Hubbard KE, Baek SJ, Dalchau N, Suhita D, Dodd AN, Webb AA. 2007.**  
639 Modulation of environmental responses of plants by circadian clocks. *Plant Cell Environ* **30**:  
640 333–49.

641 **Hotta CT, Nishiyama MY, Souza GM. 2013.** Circadian rhythms of sense and antisense  
642 transcription in sugarcane, a highly polyploid crop. *PLoS One* **8**: e71847.

643 **Hoyle NP, Seinkmane E, Putker M, Feeney KA, Krogager TP, Chesham JE, Bray LK, Thomas JM,  
644 Dunn K, Blaikley J, et al. 2017.** Circadian actin dynamics drive rhythmic fibroblast mobilization  
645 during wound healing. *Science Translational Medicine* **9**.

646 **Hsu PY, Devisetty UK, Harmer SL. 2013.** Accurate timekeeping is controlled by a cycling  
647 activator in *Arabidopsis*. *eLife* **2**: e00473.

648 **Hughes ME, Hogenesch JB, Kornacker K. 2010.** JTK\_CYCLE: an efficient nonparametric  
649 algorithm for detecting rhythmic components in genome-scale data sets. *Journal of Biological  
650 Rhythms* **25**: 372–380.

651 **Izawa T, Mihara M, Suzuki Y, Gupta M, Itoh H, Nagano AJ, Motoyama R, Sawada Y, Yano M,  
652 Hirai MY, et al. 2011.** Os-GIGANTEA confers robust diurnal rhythms on the global  
653 transcriptome of rice in the field. *The Plant Cell* **23**: 1741–1755.

654 **James AB, Monreal JA, Nimmo GA, Kelly CL, Herzyk P, Jenkins GI, Nimmo HG. 2008.** The  
655 circadian clock in *Arabidopsis* roots is a simplified slave version of the clock in shoots. *Science*  
656 **322**: 1832–5.

657 **Kim YH, Marhon SA, Zhang Y, Steger DJ, Won K-J, Lazar MA. 2018.** Rev-erba dynamically  
658 modulates chromatin looping to control circadian gene transcription. *Science (New York, N.Y.)*  
659 **359**: 1274–1277.

660 **Kim J-Y, Song H-R, Taylor BL, Carré IA. 2003.** Light-regulated translation mediates gated  
661 induction of the *Arabidopsis* clock protein LHY. *The EMBO Journal* **22**: 935–944.

662 **Kim S-G, Yon F, Gaquerel E, Gulati J, Baldwin IT. 2011.** Tissue specific diurnal rhythms of  
663 metabolites and their regulation during herbivore attack in a native tobacco, *Nicotiana  
664 attenuata*. *PLoS One* **6**: e26214.

665 **Kusakina J, Rutterford Z, Cotter S, Martí MC, Laurie DA, Greenland AJ, Hall A, Webb AAR.  
666 2015.** Barley Hv CIRCADIANT CLOCK ASSOCIATED 1 and Hv PHOTOPERIOD H1 are circadian  
667 regulators that can affect circadian rhythms in *Arabidopsis*. *PLoS One* **10**: e0127449.

668 **Langfelder P, Horvath S. 2008.** WGCNA: an R package for weighted correlation network  
669 analysis. *BMC bioinformatics* **9**: 559.

670 **Lee HG, Lee K, Jang K, Seo PJ. 2015.** Circadian expression profiles of chromatin ing factor genes  
671 in *Arabidopsis*. *Journal of Plant Research* **128**: 187–199.

672 **Lembke CG, Nishiyama MY, Sato PM, de Andrade RF, Souza GM. 2012.** Identification of sense  
673 and antisense transcripts regulated by drought in sugarcane. *Plant Molecular Biology* **79**: 461–  
674 477.

675 **Liu C, Cheng Y-J, Wang J-W, Weigel D. 2017.** Prominent topologically associated domains  
676 differentiate global chromatin packing in rice from Arabidopsis. *Nature Plants* **3**: 742–748.

677 **López-Salmerón V, Cho H, Tonn N, Greb T. 2019.** The phloem as a mediator of plant growth  
678 plasticity. *Current biology: CB* **29**: R173–R181.

679 **Lu SX, Knowles SM, Webb CJ, Celaya RB, Cha C, Siu JP, Tobin EM. 2011.** The Jumonji C  
680 Domain-Containing Protein JMJ30 regulates period length in the Arabidopsis circadian clock.  
681 *Plant Physiology* **155**: 906–915.

682 **Malapeira J, Khaitova LC, Mas P. 2012.** Ordered changes in histone modifications at the core  
683 of the Arabidopsis circadian clock. *Proc Natl Acad Sci U S A* **109**: 21540–5.

684 **Matsuzaki J, Kawahara Y, Izawa T. 2015.** Punctual transcriptional regulation by the rice  
685 circadian clock under fluctuating field conditions. *The Plant Cell* **27**: 633–648.

686 **Mermet J, Yeung J, Hurni C, Mauvoisin D, Gustafson K, Jouffe C, Nicolas D, Emmenegger Y,  
687 Gobet C, Franken P, et al. 2018.** Clock-dependent chromatin topology modulates circadian  
688 transcription and behavior. *Genes & Development* **32**: 347–358.

689 **Millar AJ. 2016.** The intracellular dynamics of circadian clocks reach for the light of ecology and  
690 evolution. *Annual Review of Plant Biology* **67**: 595–618.

691 **Ming R, VanBuren R, Wai CM, Tang H, Schatz MC, Bowers JE, Lyons E, Wang M-L, Chen J,  
692 Biggers E, et al. 2015.** The pineapple genome and the evolution of CAM photosynthesis.  
693 *Nature Genetics* **47**: 1435–1442.

694 **Muller NA, Wijnen CL, Srinivasan A, Ryngajlo M, Ofner I, Lin T, Ranjan A, West D, Maloof JN,  
695 Sinha NR, et al. 2016.** Domestication selected for deceleration of the circadian clock in  
696 cultivated tomato. *Nature genetics* **48**: 89–93.

697 **Nagano AJ, Sato Y, Mihara M, Antonio BA, Motoyama R, Itoh H, Nagamura Y, Izawa T. 2012.**  
698 Deciphering and prediction of transcriptome dynamics under fluctuating field conditions. *Cell*  
699 **151**: 1358–1369.

700 **Oakenfull RJ, Davis SJ. 2017.** Shining a light on the Arabidopsis circadian clock. *Plant, Cell &  
701 Environment* **40**: 2571–2585.

702 **Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi  
703 JS, Hogenesch JB. 2002.** Coordinated transcription of key pathways in the mouse by the  
704 circadian clock. *Cell* **109**: 307–320.

705 **Para A, Farré EM, Imaizumi T, Pruneda-Paz JL, Harmon FG, Kay SA. 2007.** PRR3 Is a vascular  
706 regulator of TOC1 stability in the Arabidopsis circadian clock. *The Plant Cell* **19**: 3462–3473.

707 **Ribeiro RV, Machado EC, Magalhães Filho JR, Lobo AKM, Martins MO, Silveira JAG, Yin X,  
708 Struik PC. 2017.** Increased sink strength offsets the inhibitory effect of sucrose on sugarcane  
709 photosynthesis. *Journal of Plant Physiology* **208**: 61–69.



710 **Ruben MD, Wu G, Smith DF, Schmidt RE, Francey LJ, Lee YY, Anafi RC, Hogenesch JB. 2018.** A  
711 database of tissue-specific rhythmically expressed human genes has potential applications in  
712 circadian medicine. *Science Translational Medicine* **10**.

713 **Rubin MJ, Brock MT, Davis AM, German ZM, Knapp M, Welch SM, Harmer SL, Maloof JN,  
714 Davis SJ, Weinig C. 2017.** Circadian rhythms vary over the growing season and correlate with  
715 fitness components. *Molecular Ecology* **26**: 5528–5540.

716 **Schubert V, Lermontova I, Schubert I. 2013.** The Arabidopsis CAP-D proteins are required for  
717 correct chromatin organisation, growth and fertility. *Chromosoma* **122**: 517–533.

718 **Shalit-Kaneh A, Kumimoto RW, Filkov V, Harmer SL. 2018.** Multiple feedback loops of the  
719 Arabidopsis circadian clock provide rhythmic robustness across environmental conditions.  
720 *Proceedings of the National Academy of Sciences of the United States of America* **115**: 7147–  
721 7152.

722 **Sharma A, Wai CM, Ming R, Yu Q. 2017.** Diurnal cycling transcription factors of pineapple  
723 revealed by genome-wide annotation and global transcriptomic analysis. *Genome Biology and  
724 Evolution* **9**: 2170–2190.

725 **Shimizu H, Katayama K, Koto T, Torii K, Araki T, Endo M. 2015.** Decentralized circadian clocks  
726 process thermal and photoperiodic cues in specific tissues. *Nature Plants* **1**: 15163.

727 **Sinturel F, Gerber A, Mauvoisin D, Wang J, Gatfield D, Stubblefield JJ, Green CB, Gachon F,  
728 Schibler U. 2017.** Diurnal oscillations in liver Mass and cell size accompany ribosome assembly  
729 cycles. *Cell* **169**: 651–663.e14.

730 **Smieszek SP, Yang H, Paccanaro A, Devlin PF. 2014.** Progressive promoter element  
731 combinations classify conserved orthogonal plant circadian gene expression modules. *Journal  
732 of the Royal Society, Interface* **11**.

733 **Song YH, Kubota A, Kwon MS, Covington MF, Lee N, Taagen ER, Laboy Cintrón D, Hwang DY,  
734 Akiyama R, Hodge SK, et al. 2018.** Molecular basis of flowering under natural long-day  
735 conditions in Arabidopsis. *Nature Plants* **4**: 824–835.

736 **Storch K-F, Lipan O, Leykin I, Viswanathan N, Davis FC, Wong WH, Weitz CJ. 2002.** Extensive  
737 and divergent circadian gene expression in liver and heart. *Nature* **417**: 78–83.

738 **Takahashi N, Hirata Y, Aihara K, Mas P. 2015.** A hierarchical multi-oscillator network  
739 orchestrates the Arabidopsis circadian system. *Cell* **163**: 148–159.

740 **Turner A, Beales J, Faure S, Dunford RP, Laurie DA. 2005.** The pseudo-response regulator Ppd-  
741 H1 provides adaptation to photoperiod in barley. *Science (New York, N.Y.)* **310**: 1031–1034.

742 **Wang J, Nayak S, Koch K, Ming R. 2013.** Carbon partitioning in sugarcane (*Saccharum* species).  
743 *Frontiers in Plant Science* **4**.

744 **Webb AAR, Seki M, Satake A, Caldana C. 2019.** Continuous dynamic adjustment of the plant  
745 circadian oscillator. *Nature Communications* **10**: 550.

746 **Xu G. 2018.** Sensing and transport of nutrients in plants. *Seminars in Cell & Developmental  
747 Biology* **74**: 78–79.

748 **Xu X, Hotta CT, Dodd AN, Love J, Sharrock R, Lee YW, Xie Q, Johnson CH, Webb AA. 2007.**  
749 Distinct light and clock modulation of cytosolic free Ca<sup>2+</sup> oscillations and rhythmic  
750 CHLOROPHYLL A/B BINDING PROTEIN2 promoter activity in Arabidopsis. *Plant Cell* **19**: 3474–  
751 90.

752 **Yang P, Wang J, Huang F-Y, Yang S, Wu K. 2018.** The plant circadian clock and chromatin  
753 modifications. *Genes* **9**.

754 **Yeung J, Mermet J, Jouffe C, Marquis J, Charpagne A, Gachon F, Naef F. 2018.** Transcription  
755 factor activity rhythms and tissue-specific chromatin interactions explain circadian gene  
756 expression across organs. *Genome Research* **28**: 182–191.

757 **Yeung J, Naef F. 2018.** Rhythms of the genome: circadian dynamics from chromatin topology,  
758 tissue-specific gene expression, to behavior. *Trends in Genetics* **34**: 915–926.

759 **Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB. 2014.** A circadian gene  
760 expression atlas in mammals: implications for biology and medicine. *Proceedings of the*  
761 *National Academy of Sciences of the United States of America* **111**: 16219–16224.

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763

764

## 765 Figure Legends

766

767 **Figure 1 – Different organs have specific sets of rhythmic transcripts in**  
 768 **sugarcane. (a)** The numbers of expressed and rhythmic time courses detected in leaf  
 769 +1 (L1), internodes 1 and 2 (I1), and internode 5 (I5) in field-grown (diel) conditions,  
 770 and in leaf +1 in circadian conditions (Hotta *et al.*, 2013). **(b,c)** Euler diagrams of  
 771 expressed time courses **(b)** and rhythmic time courses **(c)** in L1 in sugarcane in diel  
 772 (green) and circadian (gray) conditions. **(d)** Number of expressed time courses,  
 773 rhythmic time courses, and rhythmic time courses with the same phase that were found  
 774 specifically in L1, I1, or I5; in both L1 and I1 (L1I1, purple); in both L1 and I5 (L1I5, light  
 775 green); in both I1 and I5 (I1I5, orange); and in all three organs (L1I1I5, blue). In the  
 776 second bar, the gray area corresponds to rhythmic time courses that are expressed in  
 777 only one or two organs. In the third bar, the gray area corresponds to rhythmic time  
 778 courses in only two organs that have the same phase. The gray dashed lines show the  
 779 associations among bars. **(e,f)** Euler diagram of expressed and rhythmic time courses  
 780 in L1, I1, and I5 in field-grown sugarcane in diel conditions.

781

782 **Figure 2 – Transcripts have unique phases in different sugarcane organs. (a)**  
 783 Circular heatmap of the rhythmic transcript peak time (ZT0 = 0 h after dawn)  
 784 distribution in leaf +1 (L1), internodes 1 and 2 (I1), and internode 5 (I5). The colored  
 785 arrows show the times at which the most transcripts are found in each organ. The  
 786 times of dawn, dusk, *LHY* transcription peak, maximum light intensity, and maximum  
 787 temperatures are indicated by black arcs. **(b)** Proportions of transcripts that were  
 788 rhythmic in L1, I1, and I5 among all expressed transcripts in each organ (All), among  
 789 the transcripts expressed only in one organ (L1 only, I1 only, or I5 only), among the  
 790 transcripts expressed in two organs (L1I1, L1I5, or I1I5), and among transcripts  
 791 expressed in all three organs (L1I1I5). **(c)** Distribution of rhythmic transcript peak time  
 792 in time courses that were rhythmic in L1 but not in circadian conditions ( $\alpha$  in Fig. 1c)  
 793 and rhythmic transcripts in time courses that were rhythmic in L1 and circadian  
 794 conditions ( $\beta$ ). **(d)** Heatmap of functional categories that are overrepresented (shades  
 795 of blue) or underrepresented (shades of red) among the expressed and rhythmic  
 796 transcripts of L1, I1, and I5. The *P*-value was calculated using a hypergeometric test.  
 797 **(e)** Circular heatmap with the distribution of the peak times of rhythmic transcripts

798 associated with the pathways *Carbohydrate Metabolism*, *Cell Wall Synthesis &*  
799 *Elongation*, *Amino Acid Metabolism*, and *Transporters*.

800

801 **Figure 3 – Diel rhythms of *Central Oscillator* transcripts in sugarcane organs.**

802 *LHY* (a), *PRR59* (b), *PRR73* (c), *TOC1* (d), *PRR95* (e), and *GI* (f) rhythms were  
803 measured in leaf +1 (L1, green continuous line), internodes 1 and 2 (I1, red dashed  
804 line), and internode 5 (I5, yellow dash-dotted line) of field-grown sugarcane using  
805 oligoarrays. Time courses were normalized using Z-score. The light-gray boxes  
806 represent the night periods.

807

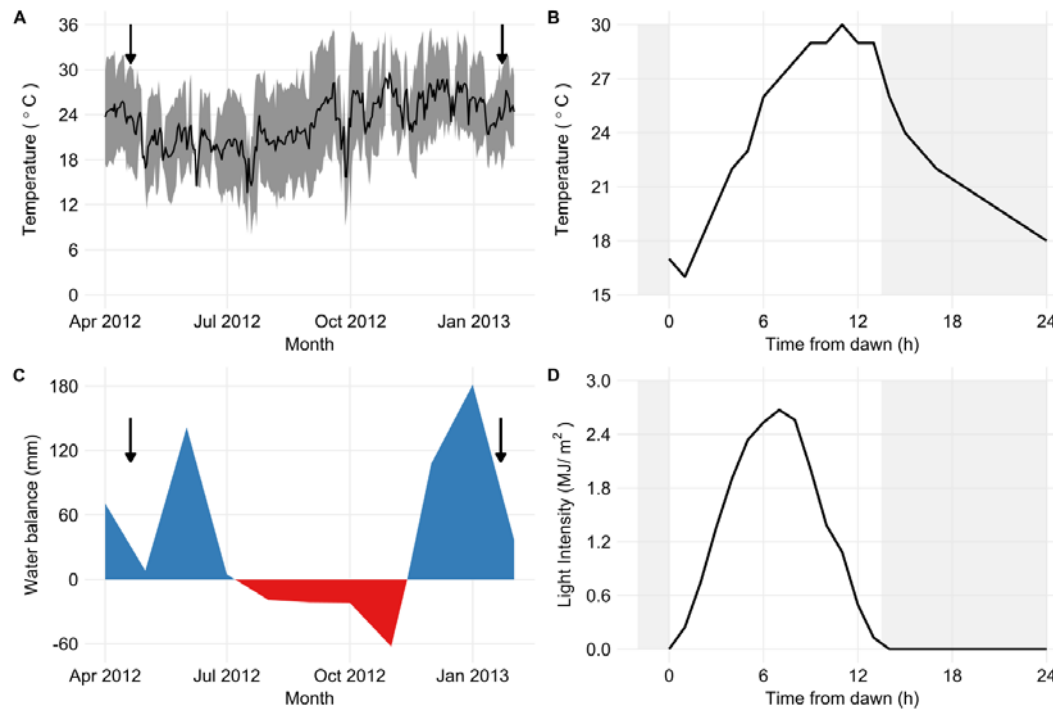
808 **Figure 4 – Transcripts associated with *Genetic Information Processing* have**  
809 **different rhythms in sugarcane organs. (a-c)** Circular heatmap of the distribution of  
810 the peak time of rhythmic transcripts related to *Chromatin Remodeling* (a),  
811 *Transcription Factors* (b), and *Protein Synthesis* (c) in leaf +1 (L1, green), internodes 1  
812 and 2 (I1, red), and internode 5 (I5, yellow). The colored arrows show the time at which  
813 the most transcripts are found in each organ. (d-i) Euler diagrams of all expressed  
814 transcripts (d-f) and rhythmic transcripts (g-i) in L1, I1, and I5 in field-grown sugarcane  
815 in diel conditions. (j-l) *SMC1* (j), *HB24* (k), and *S15A* (l) rhythms measured in L1  
816 (continuous green line), I1 (red dashed line), and I5 (yellow dash-dotted line) of field-  
817 grown sugarcane using oligoarrays. Time courses were normalized using Z-score. The  
818 light-gray boxes represent the night periods.

819

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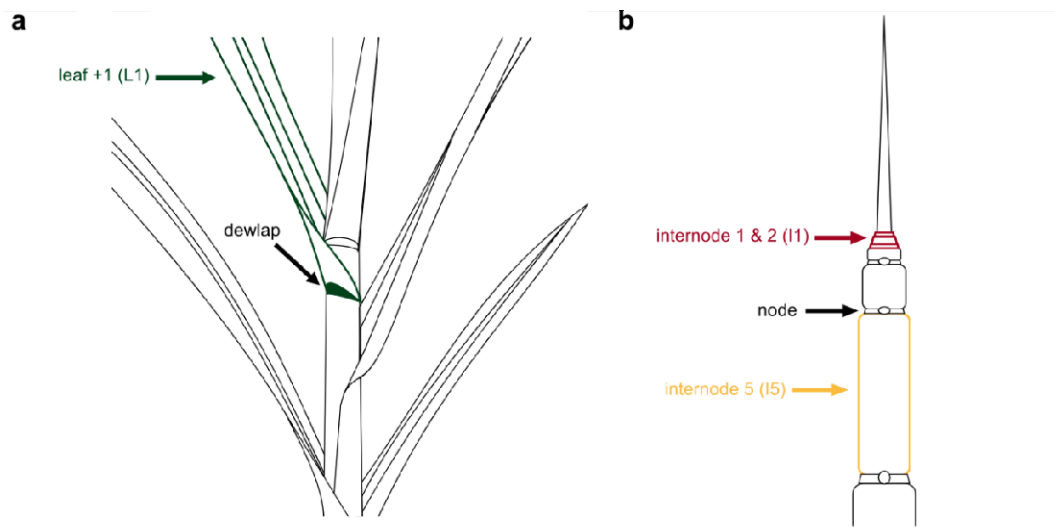
## Supporting Information (SI)

### Supplemental Figure 1



**Figure S1 – Environmental conditions in Araras (SP, Brazil).** Environmental conditions during the growth period and harvesting day. **(a)** Average temperature variation during the growth period (black line). The gray area shows the temperature variation (maximum and minimum) during each day. The arrows show the day of planting and the day of harvesting. **(b)** Temperature during the harvesting day. The light-gray boxes represent the night period. **(c)** Water balance during growth period. **(d)** Light intensity during the harvesting day.

## 833 Supplemental Figure 2

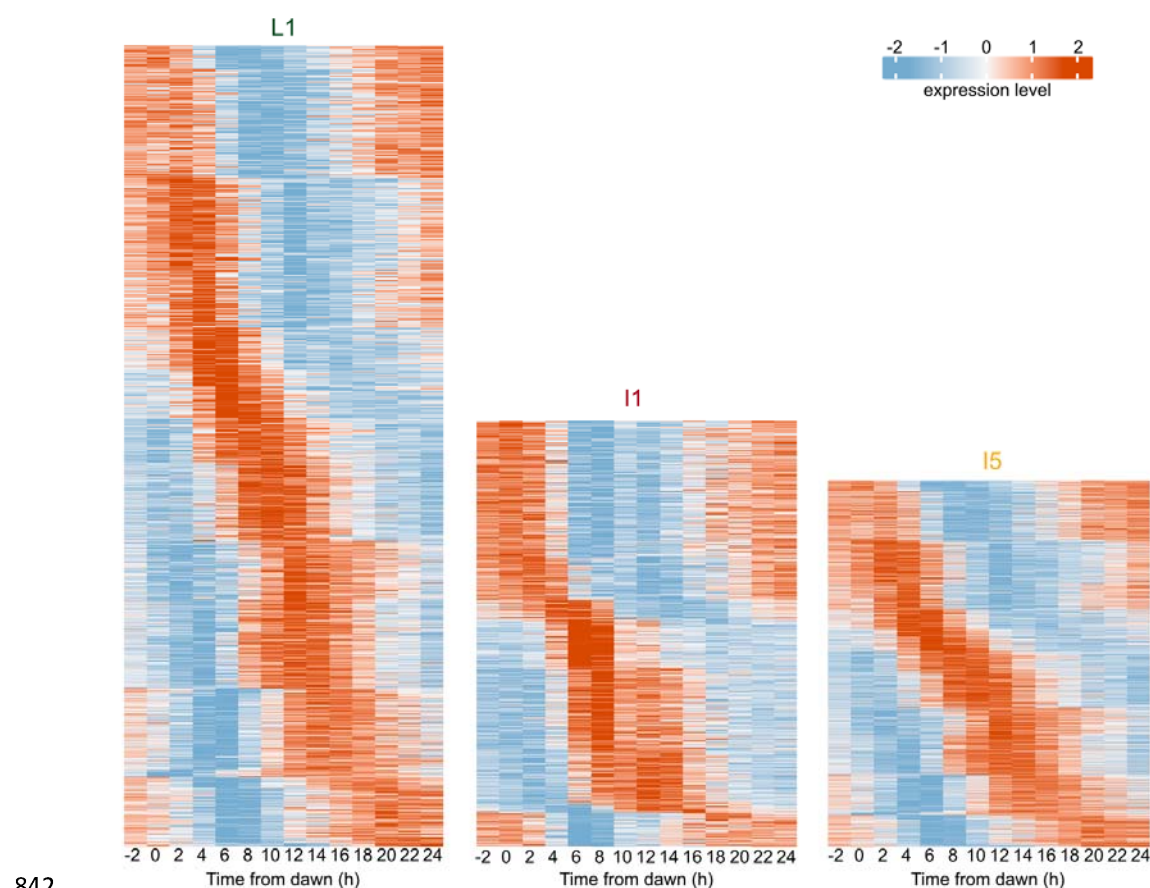


834

835 **Figure S2 – Three sugarcane organs were harvested. (a)** The leaf +1 (L1, green)  
836 harvested for this study is the first leaf from the top with a clearly visible dewlap. **(b)**  
837 After all of the leaves are peeled off and removed, the full stalk is exposed, so that all  
838 nodes and internodes can be seen. The top two internodes (internodes 1 and 2, I1,  
839 red) and the fifth internode (internode 5, I5, yellow) were also harvested.

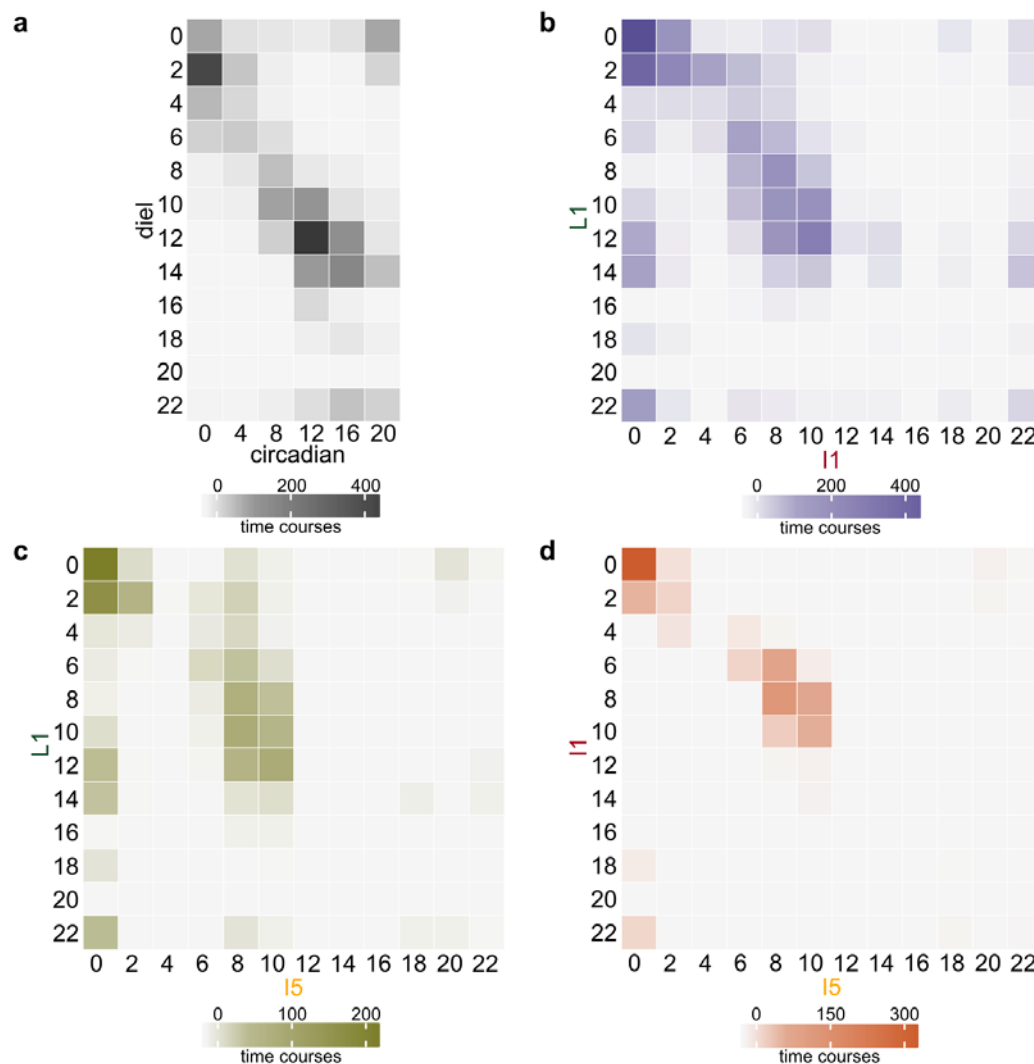
840

# Supplemental Figure 3



**Figure S3 – Heatmap of the expression levels of the rhythmic transcripts in sugarcane organs.** Each line represents the time course of each rhythmic transcript in leaf +1 (L1), internodes 1 and 2 (I1), and internode 5 (I5). The transcripts were separated by their phase. High transcription levels are in shades of red, and low transcription levels are in shades of blue. Transcription levels were normalized by Z-score.

# Supplemental Figure 4



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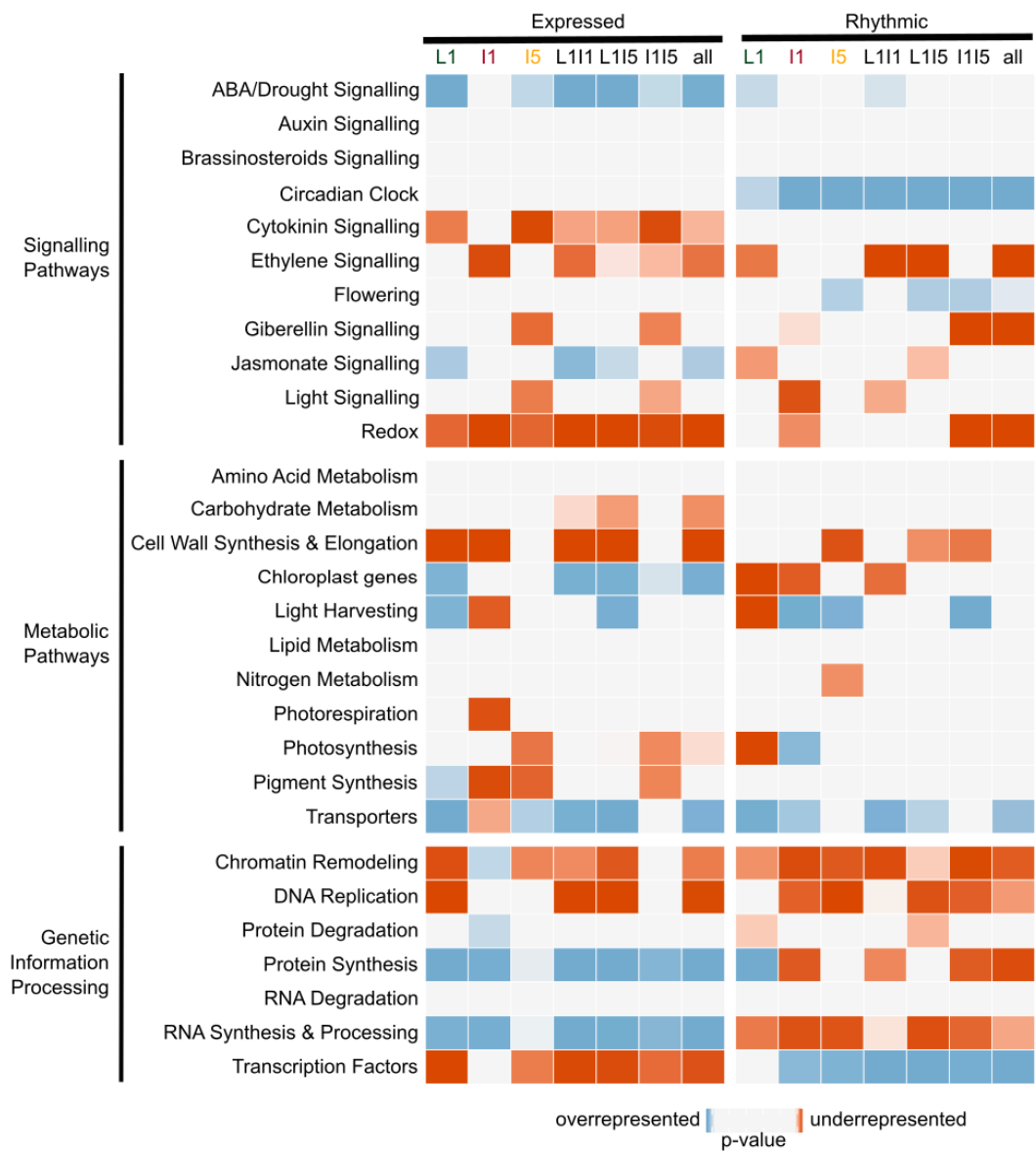
853 **Figure S4 – Heatmap of the phase distribution of rhythmic transcripts shared**  
 854 **between pairs of datasets. (a)** Phase distribution of transcripts found in leaf +1 (L1) of  
 855 field-grown sugarcane and L1 of sugarcane grown under circadian conditions (Hotta *et*  
 856 *al.*, 2013). **(b)** Phase distribution of transcripts found in L1 and internodes 1 and 2 (I1)  
 857 of field-grown sugarcane. **(c)** Phase distribution of transcripts found in L1 and internode  
 858 5 (I5) of field-grown sugarcane. **(d)** Phase distribution of transcripts found in I1 and I5  
 859 of field-grown sugarcane.

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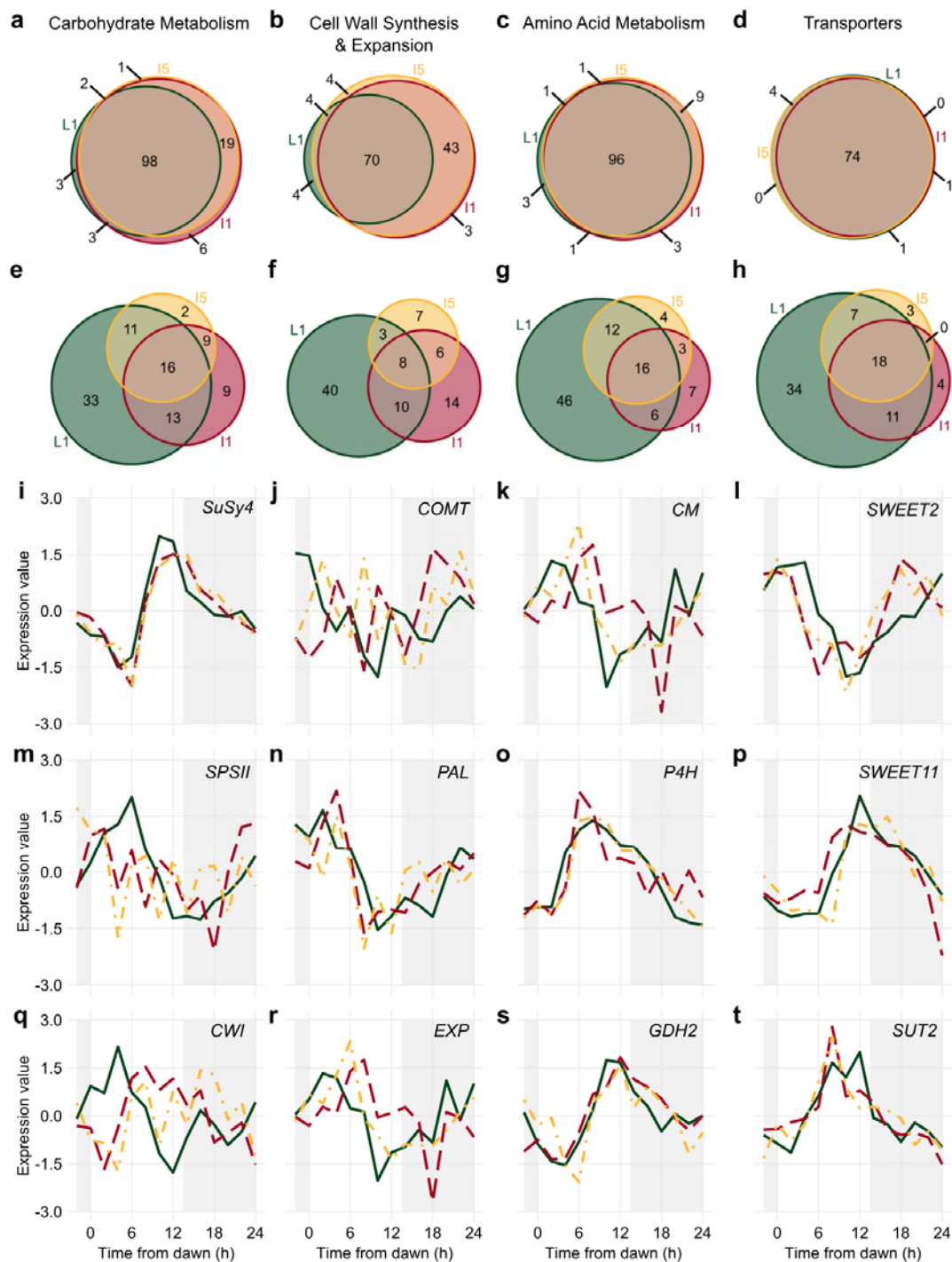
862 **Supplemental Figure 5**



863  
864 **Figure S5** – Heatmap of functional categories that are overrepresented (shades of  
865 blue) or underrepresented (shades of red) among the expressed and rhythmic  
866 transcripts of L1, I1, I5, L1I1, L1I5, I1I5, and all three organs. The *P*-values were  
867 calculated using a hypergeometric test.

868

869 **Supplemental Figure 6**



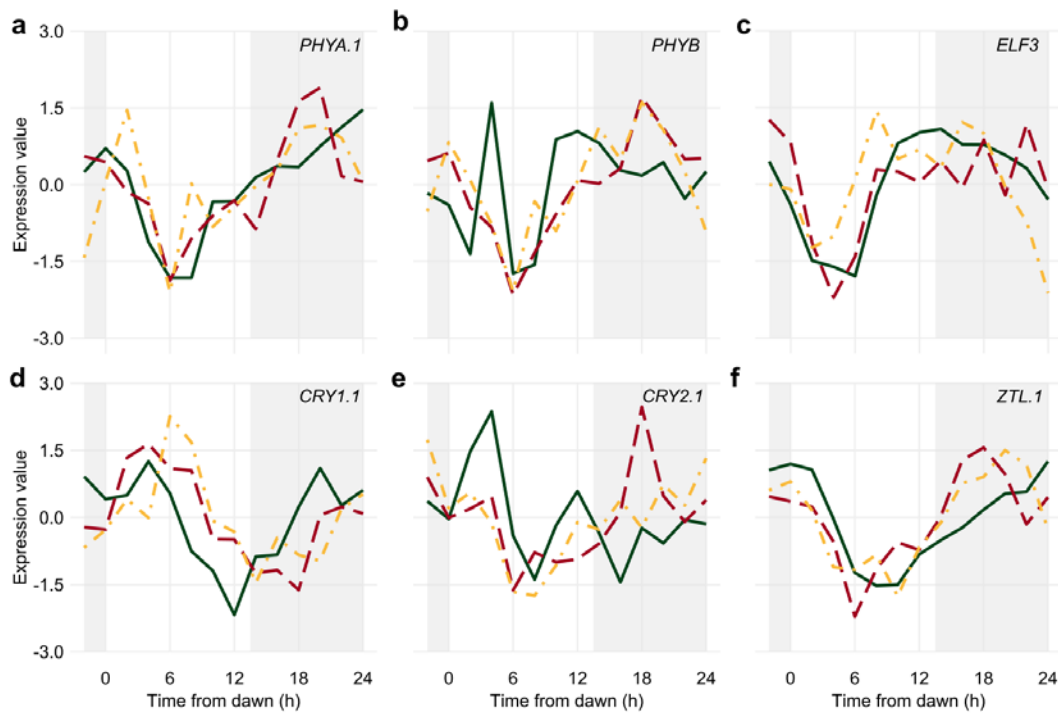
870

871 **Figure S6 – Transcripts associated with metabolic pathways have different**  
872 **rhythms in sugarcane organs. (a-h) Euler diagram of (a-d) expressed and (e-h)**  
873 **rhythmic transcripts in leaf +1 (L1, green), internodes 1 and 2 (I1, red), and internode 5**  
874 **(I5, yellow) in field-grown sugarcane in diel conditions. (i-t) Rhythms of (i) SUCROSE**

875 *SYNTHASE4 (SuSy4), (j) CATECHOL-O-METHYLTRANSFERASE (COMT), (k)*  
876 *CHORISMATE MUTASE (CM), (l) SWEET2, (m) SUCROSE-PHOSPHATE*  
877 *SYNTHASE II (SPSII), (n) PHENYLALANINE AMMONIA LYASE (PAL), (o) PROLYL*  
878 *4-HYDROXYLASE (P4H), (p) SWEET11, (q) CELL WALL INVERTASE (CWI), (r)*  
879 *EXPANSIN (EXP), (s) GLUTAMATE DEHYDROGENASE2 (GDH2), and (t) SUCROSE*  
880 *TRANSPORT PROTEIN2 (SUT2)* measured in the L1 (continuous green line), I1 (red  
881 dashed line), and I5 (yellow dash-dotted line) of field-grown sugarcane by oligoarrays.  
882 Time courses were normalized using Z-score. The light-gray boxes represent the night  
883 period.

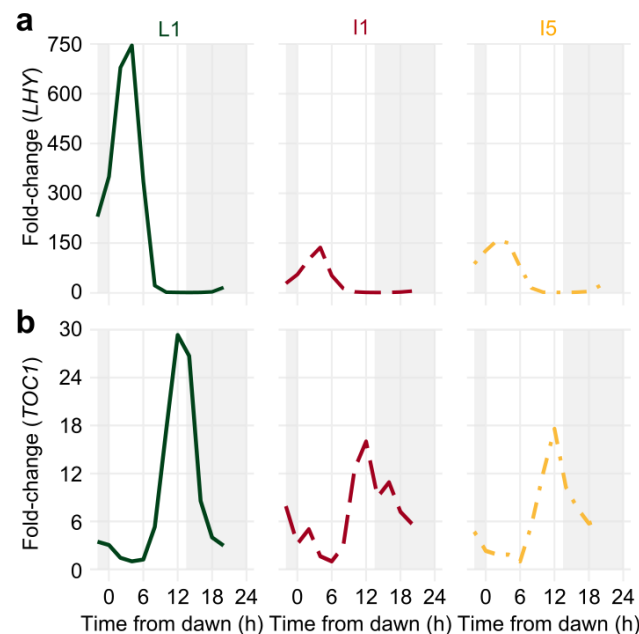
884

# Supplemental Figure 7



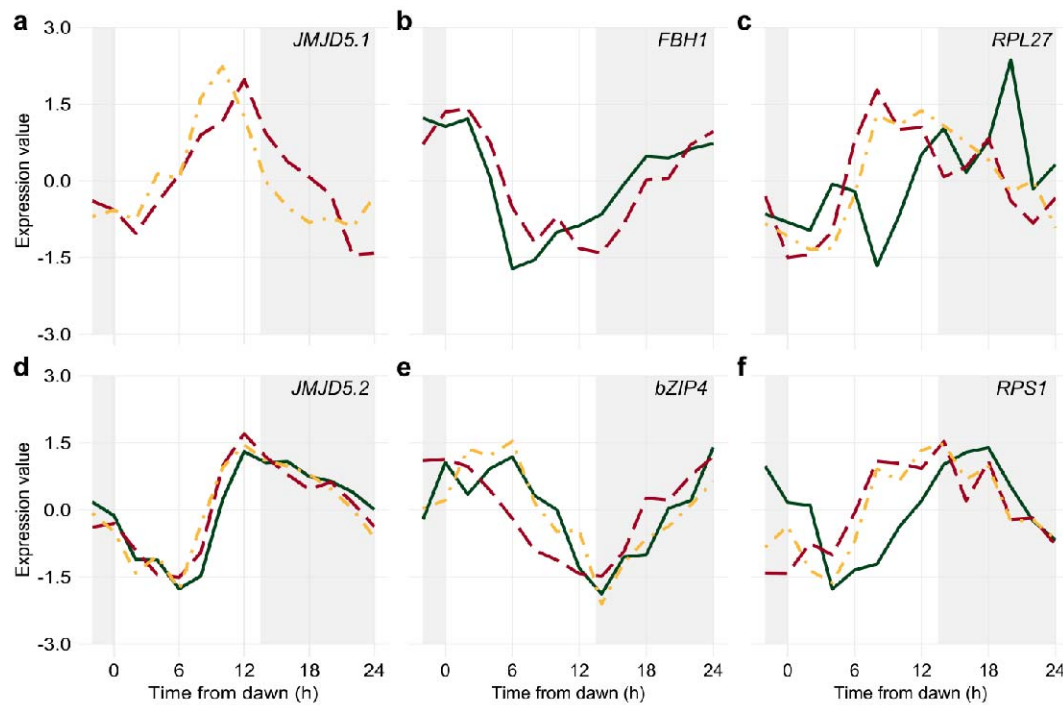
**Figure S7 – Diel rhythms of *Light Signalling* and *Central Oscillator* transcripts in sugarcane organs.** Rhythms of (a) *PHYTOCHROME A.1* (*PHYA.1*), (b) *PHYB*, (c) *EARLY FLOWERING3* (*ELF3*), (d) *CRYPTOCHROME 1.1* (*CRY1.1*), (e) *CRY2.1*, and (f) *ZEITLUPE* (*ZTL*) measured in leaf +1 (L1; continuous green line), internodes 1 and 2 (I1; red dashed line), and internode 5 (I5; yellow dash-dotted line) of field-grown sugarcane using oligoarrays. Time courses were normalized using Z-score. The light-gray boxes represent the night period.

# Supplemental Figure 8



**Figure S8 – Diel rhythms of transcripts associated with the circadian clock have different amplitudes in different sugarcane organs.** Rhythms of (a) *LATE ELONGATED HYPOCOTYL* (*LHY*) and (b) *TIME OF CAB EXPRESSION1* (*TOC1*) were measured in the leaf +1 (L1, continuous green line), internodes 1 and 2 (I1, red dashed line), and internode 5 (I5, yellow dash-dotted line) of field-grown sugarcane measured using RT-qPCR. *GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE* (*GAPDH*) was used as a reference gene. Time courses were normalized by their minimum value (minimum value = 1) using Z-score. The light-gray boxes represent the night period. *LHY* primers: FWD 5'-CCACCACGGCCTAAAAGAAA-3', RVS 5'-TGGTTTTGTTGACTTGTCATTTGG-3'; *TOC1* primers: FWD 5'-TTCTGCCTGAATTTGGCAAGTG-3', RVS 5'-GGCATCGAGCACACCAATGC-3'; *GAPDH* primers: FWD 5'-GGCATCGAGCACACCAATGC-3', RVS 5'-TCCTCAGGGTTCCTGATGC-3'.

# Supplemental Figure 9



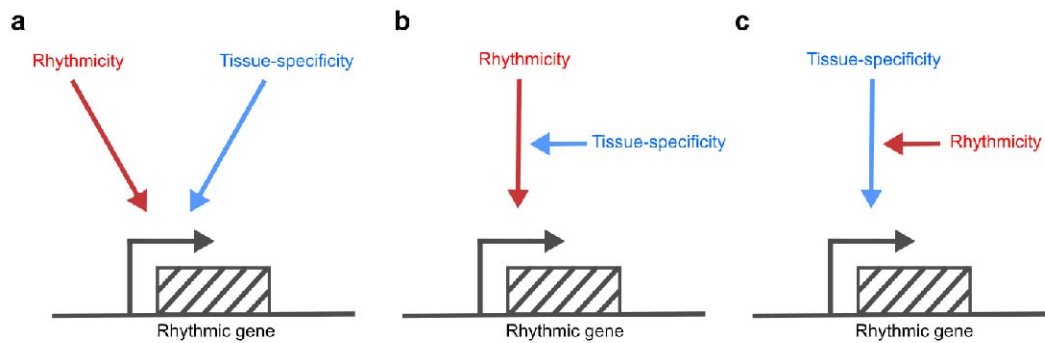
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## Figure S9 – Diel rhythms of *Output Pathways* transcripts in sugarcane organs.

Rhythms of (a) *JUMONJI-C (JMJC) DOMAIN-CONTAINING PROTEIN 5.1 (JMJD5.1)*, (b) *FLOWERING BASIC HELIX-LOOP-HELIX1 (FBH1)*, (c) *RIBOSOMAL PROTEIN L27 (RPL27)*, (d) *JMJD5.2*, (e) *BASIC LEUCINE ZIPPER4 (bZIP4)*, and (f) *30S RIBOSOMAL PROTEIN S1 (RPS1)* measured in the leaf +1 (L1, continuous green line), internodes 1 and 2 (I1, red dashed line), and internode 5 (I5, yellow dash-dotted line) of field-grown sugarcane by oligoarrays. Time courses were normalized using Z-score. The light-gray boxes represent the night period.

923

924 **Supplemental Figure 10**



925

926 **Figure S10 – At least two different pathways are necessary to regulate tissue-**  
 927 **specific rhythmic transcription.** One pathway confers rhythmicity to the transcript  
 928 levels (red), while the other pathway confers tissue specificity (blue). These two  
 929 pathways could regulate transcription independently (**a**), or one could regulate the  
 930 other (**b,c**).

931

932 **Table S1 – Sugarcane genes and GenBank IDs**

Gene Symbol	Gene Name	SAS	GenBank ID
<i>bZIP4</i>	<i>BASIC LEUCINE ZIPPER 4</i>	SCCCCL4005C09.g	CA094172.1
<i>CM</i>	<i>CHORISMATE MUTASE</i>	SCCCCL4006E08.g	CA094273.1
<i>CRY1.1</i>	<i>CRYPTOCHROME 1.1</i>	SCRUAD1133D10.b	CA217707.1
<i>CRY2.1</i>	<i>CRYPTOCHROME 2.1</i>	SCRFST1042F05.g	CA178248.1
<i>COMT</i>	<i>CATECHOL-O-METHYLTRANSFERASE</i>	SCEQLB2018G06.g	CA262225.1
<i>ELF3</i>	<i>EARLY FLOWERING 3</i>	SCEZLB1009F09.g	CA113166.1
<i>EXP</i>	<i>EXPANSIN</i>	SCCCCL5072C04.g	CA095540.1
<i>FBH1</i>	<i>FLOWERING BASIC HELIX-LOOP-HELIX 1</i>	SCCCLR1022C05.g	CA119653.1
<i>GAPDH</i>	<i>GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE</i>	SCQGAM2027G09.g	CA086777.1
<i>GDH2</i>	<i>GLUTAMATE DEHYDROGENASE 2</i>	SCJLRT1021C04.g	CA135753.1
<i>GI</i>	<i>GIGANTEA</i>	SCJFAD1014B07.b	CA067312.1
<i>HB24</i>	<i>HOMEODOMAIN PROTEIN 24</i>	SCEQRT2095E01.g	CA139249.1
<i>INV</i>	<i>INVERTASE</i>	SCVPRT3086D06.g	CA269857.1
<i>JMJD5.1</i>	<i>JUMONJI-C (JMJC) DOMAIN-CONTAINING PROTEIN 5.1</i>	SCEPRZ3086F09.g	CA156744.1
<i>JMJD5.2</i>	<i>JUMONJI-C (JMJC) DOMAIN-CONTAINING PROTEIN 5.2</i>	SCCCLB1002G12.g	CA110901.1
<i>LHY</i>	<i>LATE ELONGATED HYPOCOTYL</i>	SCCCLR1048E10.g	CA167119.1
<i>P4H</i>	<i>PROLYL 4-HYDROXYLASE</i>	SCCCST3006H07.g	CA180585.1
<i>PAL</i>	<i>PHENYLALANINE AMMONIA LYASE</i>	SCEQRT1024E12.g	CA132523.1
<i>PHYA.1</i>	<i>PHYTOCHROME A.1</i>	SCCCCL3080H06.g	CA093547.1
<i>PHYB</i>	<i>PHYTOCHROME B</i>	SCQSLR1040D12.g	CA124822.1
<i>PRR59</i>	<i>PSEUDO-RESPONSE REGULATOR 59</i>	SCACLR1057G02.g	CA116370.1
<i>PRR73</i>	<i>PSEUDO-RESPONSE REGULATOR 73</i>	SCACLR1057C07.g	CA116387.1
<i>PRR95</i>	<i>PSEUDO-RESPONSE REGULATOR 95</i>	SCCCLR1077F09.g	CA120437.1
<i>RPL27</i>	<i>RIBOSOMAL PROTEIN L27</i>	SCAGLR2026E10.g	CA128063.1
<i>RPS1</i>	<i>30S RIBOSOMAL PROTEIN S1</i>	SCBFST3135D12.g	CA181688.1
<i>S15A</i>	<i>40S RIBOSOMAL PROTEIN S15</i>	SCJFRZ2007B06.g	CA151179.1
<i>SMC1</i>	<i>STRUCTURAL MAINTENANCE OF CHROMOSOMES 1</i>	SCEZLB1008B03.g	CA113041.1
<i>SPSII</i>	<i>SUCROSE-PHOSPHATE SYNTHASE II</i>	SCAGRT2037G07.g	CA137278.1
<i>SuSy4</i>	<i>SUCROSE SYNTHASE 4</i>	SCEPCL6023F02.g	CA097247.1
<i>SUT2</i>	<i>SUCROSE TRANSPORT PROTEIN 2</i>	SCEPLR1008A12.g	CA120749.1
<i>SWEET11</i>	<i>SWEET 11</i>	SCCCTR2002G08.g	CA137196.1
<i>SWEET2</i>	<i>SWEET2</i>	SCSGRT2065C08.g	CA145445.1
<i>TOC1</i>	<i>TIME OF CAB EXPRESSION 1</i>	SCCCSB1002H04.g	CA167119.1
<i>ZTL.1</i>	<i>ZEITLUPE</i>	SCCCLR1C07F05.g	CA190027.1

933 \* Sugarcane Assembled Sequence.