1 **Short title:** Highly Organ-specific Rhythms in Sugarcane

2 **Carlos Takeshi Hotta (hotta@iq.usp.br)**

3

4 **Article title: Rhythms of Transcription in Field-Grown Sugarcane Are Highly**
5 **Organ Specific**

6 Luíza Lane de Barros Dantas[1,2], Felipe Marcelo Almeida-Jesus[1], Natalia Oliveira de
7 Lima[1], Cícero Alves-Lima[1], Milton Yutaka Nishiyama-Jr[3], Monalisa Sampaio Carneiro[4],
8 Glaucia Mendes Souza[1], Carlos Takeshi Hotta[1*]

9 1 Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São
10 Paulo, SP, 05508-000, Brazil

11 2 Present address: Max Planck Institute for Molecular Plant Physiology, Potsdam-
12 Golm, 14476, Germany

13 3 Laboratório Especial de Toxicologia Aplicada, Instituto Butantan, São Paulo, SP,
14 05503-900, Brazil

15 4 Departamento de Biotecnologia e Produção Vegetal e Animal, Centro de Ciências
16 Agrárias, Universidade Federal de São Carlos, São Carlos, SP, 13600-970, Brazil.

17 **One sentence summary:** The rhythmic transcriptome of field-grown sugarcane is
18 highly organ-specific.

19 **Short title:** Highly Organ-specific Rhythms in Sugarcane

20

21 **Author Contributions**

22 Conceptualization, L.L.B.D., M.S.C., G.M.S., C.T.H; Methodology, L.L.B.D., C.T.H;
23 Software, M.Y.N., C.T.H; Validation, L.L.B.D., N.O.L., F.M.A.J.; Investigation, L.L.B.D.,
24 N.O.L., C.A.L., C.T.H; Resources, M.S.C., G.M.S.; Data Curation, M.Y.N., C.T.H;
25 Writing – Original Draft, L.L.B.D., C.T.H; Writing – Review & Editing, L.L.B.D., C.A.L.,
26 C.T.H; Visualization; C. T. H.; Project Administration, C. T. H.; Funding Acquisition,
27 C.T.H

28

29 **Competing Interests**

30 The authors have no competing interests to declare.

31

32 **Acknowledgements:** This work was supported by the São Paulo Research
33 Foundation (FAPESP) (grant nos. 11/00818-8 and 15/06260-0; BIOEN Program) and
34 by the Serrapilheira Institute (grant no. Serra-1708-16001). L.L.B.D, C. A. L., and N. O.
35 L. were supported by FAPESP scholarships (grants 11/08897-4, 13/05301-9, and
36 16/06740-4, respectively). F.M.A.J was supported by CAPES scholarships (Finance
37 Code 001).

38

39 ***Correspondence:** Carlos Takeshi Hotta (hotta@iq.usp.br)

40

## Abstract

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

## Introduction

The circadian clock is an endogenous signaling network that allows organisms to adapt to rhythmically changing environments. Plants with a circadian clock synchronized with environmental rhythms accumulate more biomass and have better fitness than plants with defective or no circadian clocks[1,2]. In crops, changes in the circadian clock have been indirectly selected through traditional breeding to change photoperiodic responses, such as the transition to flowering. For example, the circadian clocks of European tomatoes have longer periods than those of native American tomatoes, as such periods allow these crops to adapt better to the long summer days occurring at the high latitudes of much of Europe[3]. Similarly, some genotypes of *Hordeum vulgare* L. (barley) and *Triticum aestivum* L. (wheat) carry mutations in their circadian clock genes that reduce flowering induced by photoperiodic triggers, allowing cultivation in higher latitudes in Europe[4,5].

The circadian clock is conceptually divided into three associated parts: the *Input Pathways*, the *Central Oscillator*, and the *Output Pathways*. The *Input Pathways* detect entraining cues that keep the circadian clock continuously synchronized to the environment. In plants, these cues include light, temperature, and sugar levels[6–8]. The *Central Oscillator* is a series of interlocking transcriptional-translational feedback loops that can generate 24-h rhythms independently of the environment. In *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis), one loop, called the morning loop, starts with the light induction of *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*) at dawn. Next, *PSEUDO-RESPONSE REGULATOR7* (*PRR7*) and *PRR9* are activated by CCA1 and LHY. In turn, *CCA1* and *LHY* are repressed by PRR7 and PRR9. In the core loop, CCA1 and LHY are repressed, and this represses *TIME FOR CAB EXPRESSION1* (*TOC1*), also known as *PRR1*. During the night, TOC1 forms an interaction known as the evening loop with the EVENING COMPLEX (EC). The EC is a protein complex formed by EARLY FLOWERING3 (ELF3), ELF4, and LUX ARRHYTHMO (LUX) that also inhibits the expression of *PRR7* and *PRR9* the next morning. Other essential components of the oscillator include GIGANTEA (GI), REVEILLE8 (RVE8), and CCA1 HIKING EXPEDITION (CHE)[8–11]. The *Output Pathways* transduce the temporal information generated by the interaction between the *Central Oscillator* and the *Input Pathways* to a plethora of biochemical pathways. The circadian clock thus has a broad impact throughout the plant, regulating processes such as photosynthesis, cell elongation, stomata opening, and flowering[12].

Even though the plant circadian clock is highly conserved, there are a few differences between the circadian clocks of Arabidopsis and grasses (Poales). For instance, there is only copy of the paralogs *CCA1*/*LHY*, usually assigned as *LHY*[13]. The grass PRRs consist of *TOC1*, *PRR37*, *PRR73*, *PRR59*, and *PRR95*, and it is not clear whether they have the same functions as their Arabidopsis counterparts, even though they are capable of complementing Arabidopsis mutations[13,14]. In sugarcane, a highly polyploid crop that accumulates sucrose in the culm, the circadian clock has high-amplitude rhythms and regulates a large proportion of the leaf transcriptome (>30%)[15,16].

Most research to date on plant circadian rhythms has been done in controlled conditions, inside a growth room or growth chamber. Under such circumstances, plants can be grown either under circadian conditions, in which they are kept under constant abiotic conditions as a means to separate endogenous rhythms from rhythms driven by the environment, or under diel conditions, in which they are subjected to abiotic rhythms such as light/dark and warm/cold. Abiotic changes in controlled conditions are

usually stepwise, in contrast to the gradients found in natural or field conditions, which can lead to significant changes in plant physiology[17–19]. For example, different patterns of metabolite rhythms are observed if plants are grown under white fluorescent tubes, light-emitting diodes that simulate the sunlight spectrum, or naturally illuminated greenhouse[18]. In another study, the period and phase of the circadian clock affected shoot and rosette branch numbers in multiple Arabidopsis mutants in natural, but not controlled, conditions[20]. Finally, the rice mutant *osgi*, which has a late-flowering phenotype in controlled conditions, flowered at the same time as the wild type in field conditions[21].

Only two plant species have had their rhythmic transcripts identified in field conditions: rice and pineapple[21–24]. However, these studies focused on the leaves. To better understand how the plant circadian clock regulates transcription under natural conditions in 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.

## Results

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

We planted a field of commercial sugarcane (*Saccharum* hybrid SP80-3280) in autumn 2012 in Araras (Brazil, 22°18'41.0"S, 47°23'05.0"W). Nine months later (summer 2013), after a dry winter and spring (Fig. S1), we did a time course experiment in which the leaf +1 (L1), internodes 1 and 2 (I1), and internode 5 (I5) were harvested every 2 h for 26 h, starting 2 h before dawn. On the day of harvest, the stalks were 76 ± 0.16 cm, with 11 ± 2 internodes, and their sugar content was 12.0 ± 1.4°Bx (mean ± SD; $n$ = 20). The temperature varied throughout the day from 17°C to 30°C, with the maximum

141 occurring at 11 h after dawn (ZT11); the maximum light intensity was 2.67 MJ/m$^2$ at
142 ZT07, and dusk occurred 13.25 h after dawn (ZT13.25) (Fig. S1B and D).

143 RNA extracted from each organ was hybridized in 44k custom oligoarrays[15,25]. The
144 data from the time course experiment generated 14,521 time series with 14 time points.
145 After the selection of time points that had a signal above the background noise (Figure
146 S3A), we had 12,501 transcripts considered to be expressed in at least one organ (Fig.
147 1). L1 had 9,822 expressed transcripts, 94.3% of them were also expressed in a
148 previous circadian experiment[15] (Fig. 1B). I1 had the highest number of expressed
149 transcripts (12,053), followed by I5 (10,448). A total of 9,380 transcripts were
150 expressed in all three organs (75.0%, Fig. 1E). I1 and I5 shared the most substantial
151 proportion of the expressed transcripts (89.3%), and I1 had the most substantial
152 proportion of unique expressed transcripts (7.5%).

153 We identified rhythmic transcripts by combining a weighted correlation network analysis
154 (WGCNA) that grouped expressed transcripts in coexpression modules[26] with
155 JTK_CYCLE, which identified which of the modules contained rhythmic transcripts[27]
156 (Figure S3B). This method identified 6,705 rhythmic transcripts in L1 (68.3%), 3,755 in
157 I1 (31.2%), and 3,242 in I5 (28.8%) (Fig. 1A and Fig. S6). As a comparison, 32.1% of
158 the transcripts were rhythmic in L1 under circadian conditions[15]. The overlap between
159 circadian transcripts and rhythmic transcripts in the field (in diel conditions) was 2,623,
160 representing 76.4% of circadian transcripts and 60.1% of rhythmic transcripts (Fig. 1C).

161

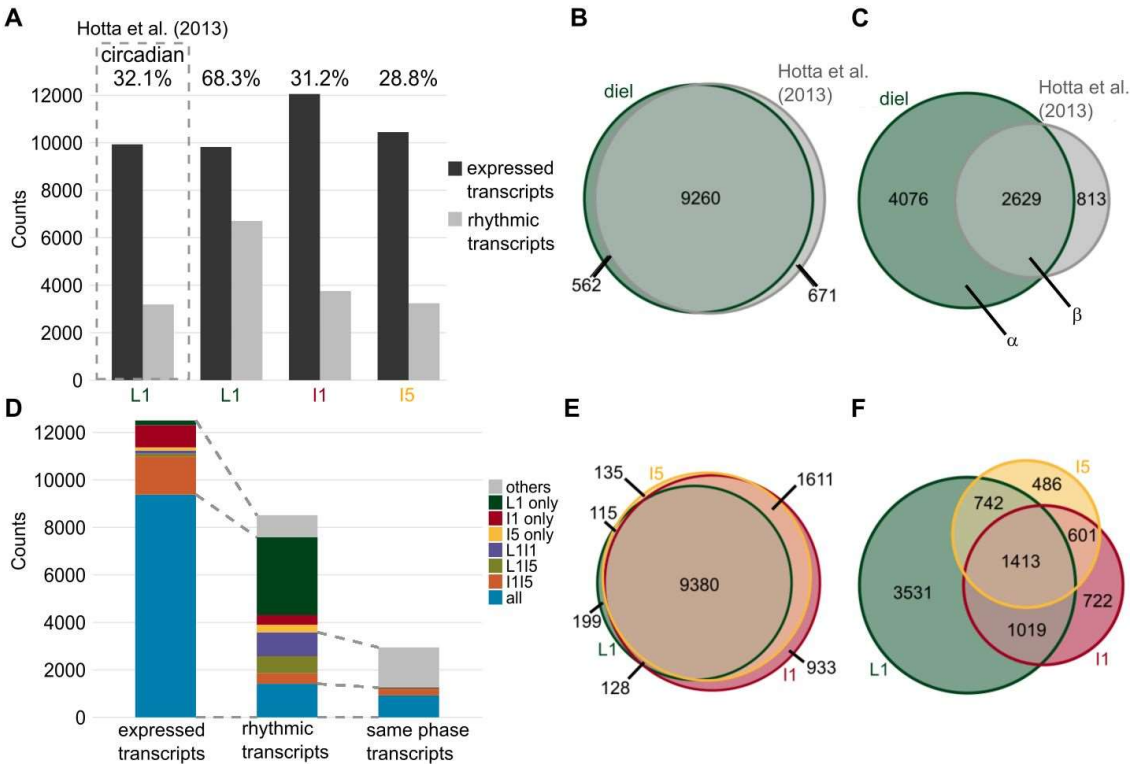



**Figure 1 – Different organs have specific sets of rhythmic transcripts in sugarcane. (A)** The numbers of expressed and rhythmic transcripts detected in leaf +1 (L1), internodes 1 and 2 (I1), and internode 5 (I5) in field-grown (diel) conditions, and in leaf +1 in circadian conditions published in Hotta et al. (2013)[15]. **(B, C)** Euler diagrams of expressed transcripts **(B)** and rhythmic transcripts **(C)** in L1 in sugarcane in diel (green) and circadian (gray) conditions. **(D)** Number of expressed transcripts, rhythmic transcripts, and rhythmic transcripts with the same phase that were found specifically in L1, I1, or I5; in both L1 and I1 (L1I1, purple); in both L1 and I5 (L1I5, light green); in both I1 and I5 (I1I5, orange); and in all three organs (L1I1I5, blue). In the second bar, the gray area corresponds to rhythmic transcripts that are expressed in only one or two organs. In the third bar, the gray area corresponds to rhythmic transcripts in only two organs that have the same phase. The gray dashed lines show the associations among bars. **(E, F)** Euler diagram of expressed and rhythmic transcripts in L1, I1, and I5 in field-grown sugarcane in diel conditions.

*Different sets of transcripts are rhythmic in different sugarcane organs*

Although most expressed transcripts were found in all three organs, only 1,413 of the expressed transcripts were rhythmic in all three organs (16.6%) (Fig. 1D, F). L1 had the largest proportion of unique rhythmic transcripts (41.5%), followed by I1 (8.5%) and

then I5 (5.7%) (Fig. 1F). Transcripts that were expressed only in one organ were less likely to be rhythmic (60.3% for L1, 8.6% for I1, and 8.4% for I5) (Fig. 1H).

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

The majority of transcripts from L1 (65.8%) grown in diel conditions had the same phase (± 2 h) in leaves grown under circadian conditions (Fig. S7A). More transcripts showed a delayed peak (19.6%) rather than an advanced peak (13.9%) under diel conditions than under circadian conditions. When we compared L1 and I1 transcripts, 65.8% had the same phase, with the remainder divided roughly evenly between delayed and advanced phases (16.1% and 14.9%, respectively) (Fig. S7B). Similarly, 67.1% of the L1 transcripts had the same phase as I5, 14.2% had a delayed phase, and 14.8% had an advanced phase (Fig. S7C). The phases were most similar between I1 and I5 transcripts: 93.8% had the same phases, 2.8% a phase delay, and 3.1% a phase advance (Fig. S7D).

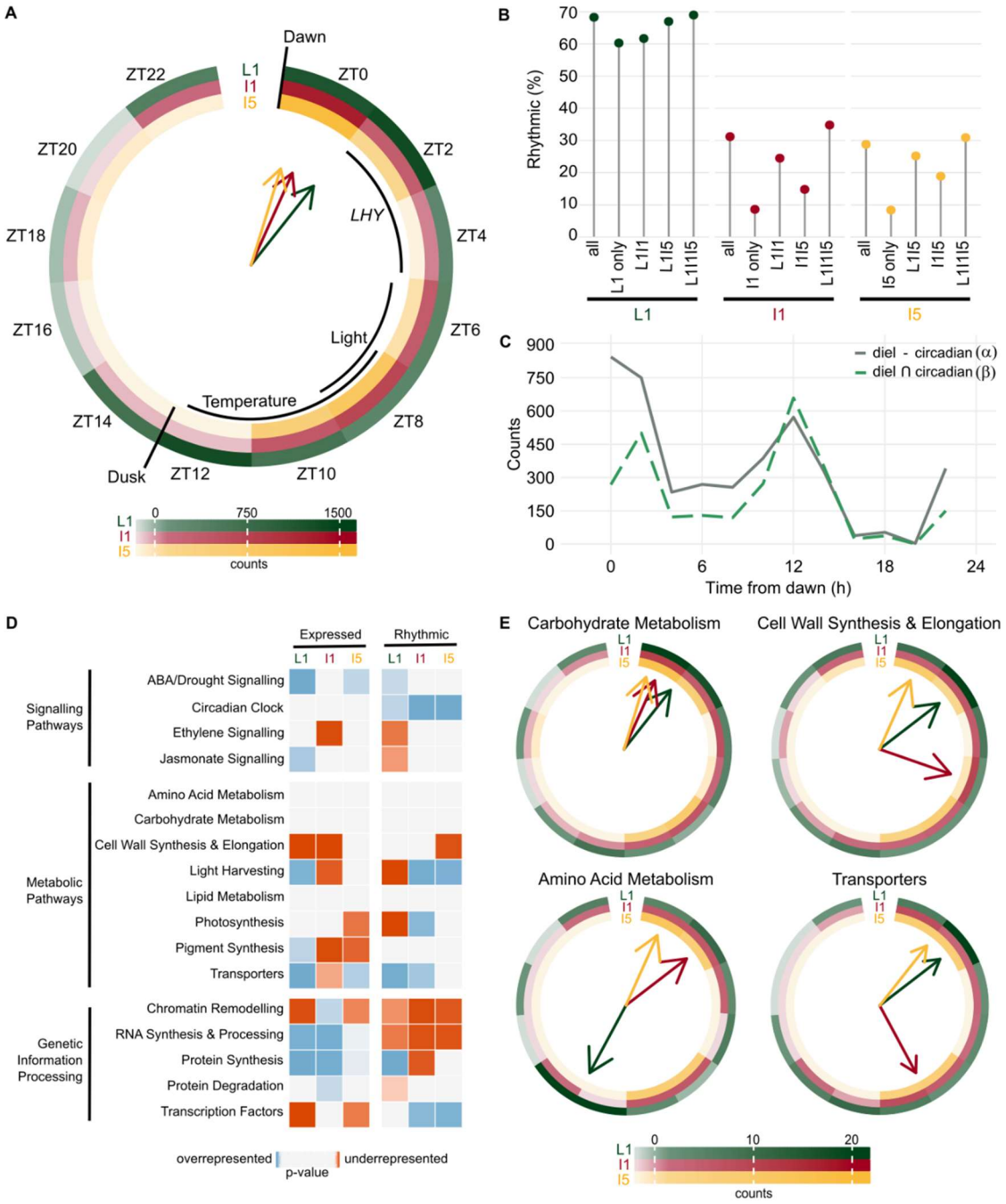



**Figure 2 – Transcripts have unique phases in different sugarcane organs. (A)** Circular heatmap of the rhythmic transcript peak time (ZT0 = 0 h after dawn) distribution in leaf +1 (L1), internodes 1 and 2 (I1), and internode 5 (I5). The colored arrows show the times at which the most transcripts are found in each organ. The times of dawn, dusk, *LHY* transcription peak, maximum light intensity, and maximum temperatures are indicated by black arcs. **(B)** Proportions of transcripts that were rhythmic in L1, I1, and I5 among all expressed transcripts in each organ (All), among the transcripts expressed only in one organ (L1 only, I1 only, or I5 only), among the 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 transcripts that were rhythmic in L1 but not in circadian conditions ($\alpha$ in Fig. 1C) and rhythmic transcripts in transcripts 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 transcripts and all the unique transcripts in the oligoarray (Fig. 2D and Fig. ). We used the same test comparing the frequency of transcripts associated with a Biochemical Pathway among the rhythmic transcripts and the expressed transcripts (Fig. 2D and Fig. S8). The transcript annotations were based on the SUCEST database annotation (http://sucest-fun.org).

Among expressed transcripts, 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 transcripts (Fig. 2D). Among rhythmic transcripts, *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 transcripts in L1 and I1 (Fig. 2D).

When we analyzed transcripts associated with important pathways for sugarcane growth, we found further organ-specific patterns; these differences could be seen in both expressed and rhythmic transcripts, as well as the phase of the rhythmic transcripts (Fig. 2E and Fig. S9). Transcripts associated with *Carbohydrate Metabolism*

tended to peak in the morning. Almost half (48.0%) of the transcripts had a peak at ZT00 in L1, while the majority peaked between ZT00 and ZT04 in both I1 (53.2%) and I5 (58%) (Fig. 2E). Amongst the individual transcripts, a putative ortholog of *SUCROSE SYNTHASE4* (*SuSy4*) had a similar rhythmic pattern in all three organs. A putative ortholog of *SUCROSE-PHOSPHATE SYNTHASE II* (*SPSII*) was rhythmic only in L1, while a putative ortholog of a *CELL WALL INVERTASE* (*CWI*) exhibited a sharp peak at ZT04 in L1 but a very broad peak at ZT08 in I1 and I5 (Fig. S9I, M, and Q).

Transcripts associated with *Cell Wall Synthesis & Elongation* had a more diverse phase distribution: in L1, 55% had a peak between ZT00 and ZT04; in I1, 73.4% had a peak between ZT00 and ZT08; and in I5, 45.8% had a peak between ZT08 and ZT10 and 37.8% had one at ZT00 (Fig. 2E). There was also a higher proportion of transcripts associated with *Cell Wall Synthesis & Elongation* that are expressed only in I1 and I5 (Fig. S9). Transcripts associated with *Amino Acid Metabolism* peaked between ZT12 and ZT14 in L1 (50%). In I1 and I5, they had two peaks: between ZT00 and ZT02 (37.5% and 57.1%) and between ZT08 and ZT10 (37.5% and 42.9%) (Fig. 2B). Transcripts associated with *Transporters* peaked at ZT02 (35.7%) and ZT12 (15.7%) in L1. In I1, most of the transcripts peaked 2 h earlier, at ZT00 (24.2%) and ZT10 (24.2%). I5 displayed a similar pattern to I1, with 53.6% peaking between ZT00 and ZT02 and 46.4% between ZT08 and ZT10 (Fig. 2B). This tendency for L1 to have later phases than I1 and I5 can be seen in the putative ortholog *SWEET2*, which peaked at ZT02 in L1 and at ZT18-20 in I1 and I5 (Fig. S9L).

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

The differences in the rhythmic transcripts of the three organs could be explained by the presence of organ-specific circadian clocks that could generate different patterns of rhythmic transcription. For this reason, we looked at rhythms in the *Input Pathways, Central Oscillator*, and *Output* Pathways of the circadian clock. Most of the known *Input Pathways* to the circadian clock are associated with *Light Signaling*[6]. *Light Signaling* is underrepresented among the transcripts expressed in I5 and the rhythmic transcripts in I1 (Fig. S8). Among the red light receptor genes, *PHYTOCHROME A.1* (*PHYA.*1) was rhythmic in L1, with a peak at ZT23, while *PHYB* was not rhythmic in any organ (Fig. S10A, B). In I1 and I5, both *PHYs* had two peaks: one near dawn (ZT00-02) and another at night (ZT18-20). Among the blue light receptors, *CRYPTOCHROME1.1* (*CRY1.1*) was rhythmic in L1, peaking at ZT03. *CRY2.1* was rhythmic in I5, peaking at

ZT19. *ZEITLUPE* (*ZTL.1*) was rhythmic in L1 and I5, peaking at ZT01 and ZT21, respectively (Fig. S10D-F).

The transcripts associated with the *Central Oscillator* displayed rhythms with similar dynamics (Fig. 3). *LHY* peaked early in the morning, between ZT02 and ZT04, with overlapping dynamics in all three organs (Fig. 3a). Similarly, *TOC1* peaked around dusk, between ZT10 and ZT12, in all three organs (Fig. 3D). The normalizations used to analyze the oligoarray data do not allow the comparison of expression levels, so we used RT-qPCR to show that *LHY* varied during the day by 750× in L1 and 150× in I1 and I5 (Fig. S11S). In contrast, *TOC1* differed 30× in L1 and 18× in I1 and I5 (Fig. S11B). The other PRR genes, *PRR59*, *PRR73*, and *PRR95* (referred to as *ScPRR3*, *ScPRR7*, and *ScPRR59*, respectively, in Hotta *et al.*, 2011), peaked between ZT06 and ZT10 (Fig. 3B, C, and E). *GI* peaked between ZT08 and ZT10 in all three organs (Fig. 3F). Finally, *ELF3* was rhythmic only in L1, with a peak at ZT14. In the internodes, *ELF3* had a similar pattern, but it was not regarded as rhythmic due to high noise (Fig. S10C).

Among the possible pathways that can be recruited by the circadian clock that are considered part of the *Output Pathways* are those associated with *Chromatin Remodeling*, *Transcription Factors*, and *Protein Synthesis* (Fig. 4). Transcripts associated with *Chromatin Remodeling* peaked at ZT00-02 and ZT10-12 in L1 (32.5% and 36.5%, respectively). In I1 and I5, they peaked at ZT00 (40.7% and 40.8%, respectively) and ZT08-10 (33.99% and 51.2%, respectively) (Fig. 4A). Transcripts associated with *Transcription Factors* tended to peak near dawn, at ZT00-02, in all three organs (57.5% in L1, 46.4% in i1, and 50.3% in I5). A higher proportion (22.6%) of transcripts associated with *Transcription Factors* were rhythmic when compared to all rhythmic (16.6%) transcripts, $\chi^2(6, n = 341) = 15.1$, $P = 0.02$ (chi-square test, Fig. 4E). These transcripts also peaked similarly in all organs: 79.3% peaked in the same interval in L1 as in I1, 72.2% peaked in the same interval in L1 and I5, and 93.1% in I1 and I5.

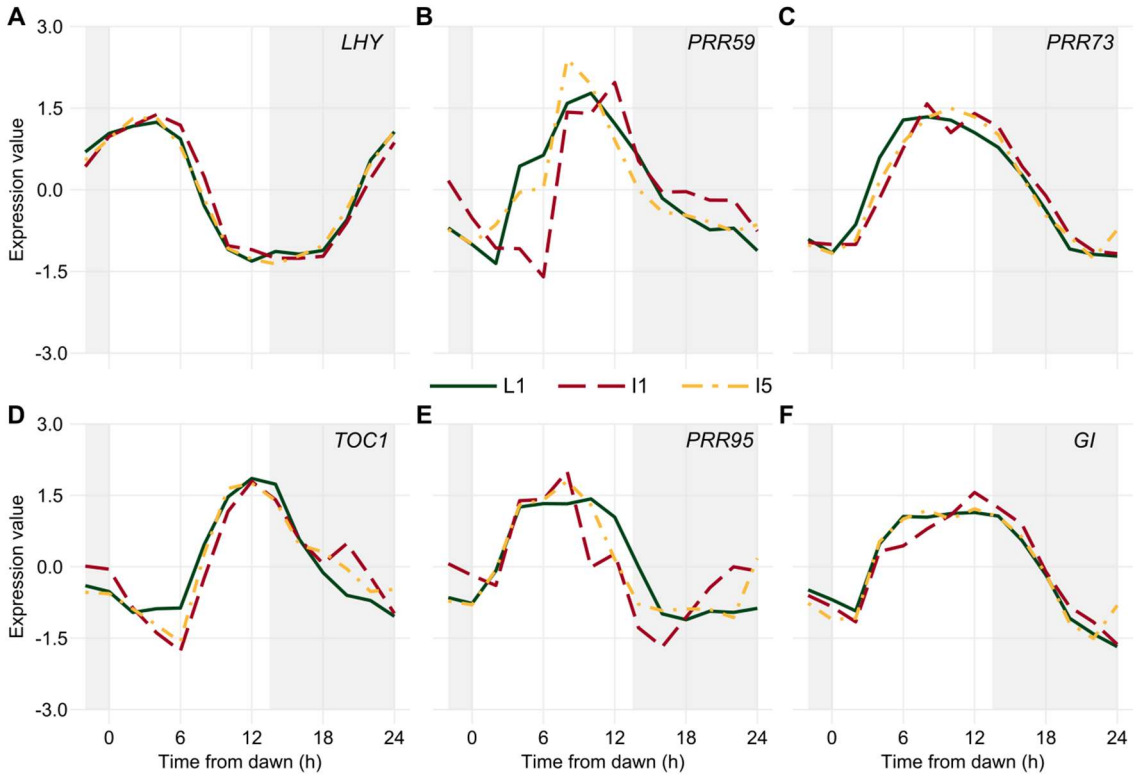



**Figure 3 – Diel rhythms of *Central Oscillator* transcripts in sugarcane organs.** *LHY* **(A)**, *PRR59* **(B)**, *PRR73* **(C)**, *TOC1* **(D)**, *PRR95* **(E)**, and *GI* **(F)** rhythms were measured in leaf +1 (L1, green continuous line), internodes 1 and 2 (I1, red dashed line), and internode 5 (I5, yellow dash-dotted line) of field-grown sugarcane using oligoarrays. Time series were normalized using *Z*-score. The light-gray boxes represent the night periods.

Transcripts associated with *Protein Synthesis* tended to peak at dusk in L1 (ZT12, 49.0%), at dawn and afternoon in I1 (ZT00, 36.1%; ZT10, 32.5%), and at dawn in I5 (ZT00, 61.7%) (Fig. 4C). A high proportion of transcripts associated with *Protein Synthesis* were expressed in all three organs (91.4%) (Fig. 4F). In contrast, more than half of the transcripts (54.6%) were rhythmic only in L1, whereas a lower frequency (41.5%) of total rhythmic transcripts were seen only in L1, $\chi^2(6, n = 269) = 34.8$, $P < 0.001$ (Fig. 4I).

Other transcripts showed a wide variety of oscillations amongst the three organs (Fig. 4J-L, and S11). The putative *STRUCTURAL MAINTENANCE OF CHROMOSOMES1* (*SMC1*), associated with *Chromatin Remodeling*, peaked at ZT06 in L1 and ZT11 in I1 and I5 (Fig. 4J). Two putative *JUMONJI-C (JMJC) DOMAIN-CONTAINING PROTEIN5* (*JMJD5*) genes, encoding proteins that can act as histone demethylases, were found in

337 sugarcane. *JMJD5.1* is expressed only in I1 and I5 and has a phase at ZT10 (Fig.
338 S12A, D); *JMJD5.2* is expressed in all organs with similar rhythmic patterns (Fig.
339 S12A). The transcription factor gene *HOMEOBOX PROTEIN24* (*HB24*) is rhythmic
340 only in L1, with a peak at ZT10 (Fig. 4K). Another rhythmic gene, *40S RIBOSOMAL*
341 *PROTEIN S15* (*S15A*), associated with *Protein Synthesis*, has a peak at ZT14 in L1
342 and at ZT00 in I1 and I5 (Fig. 4L).

343

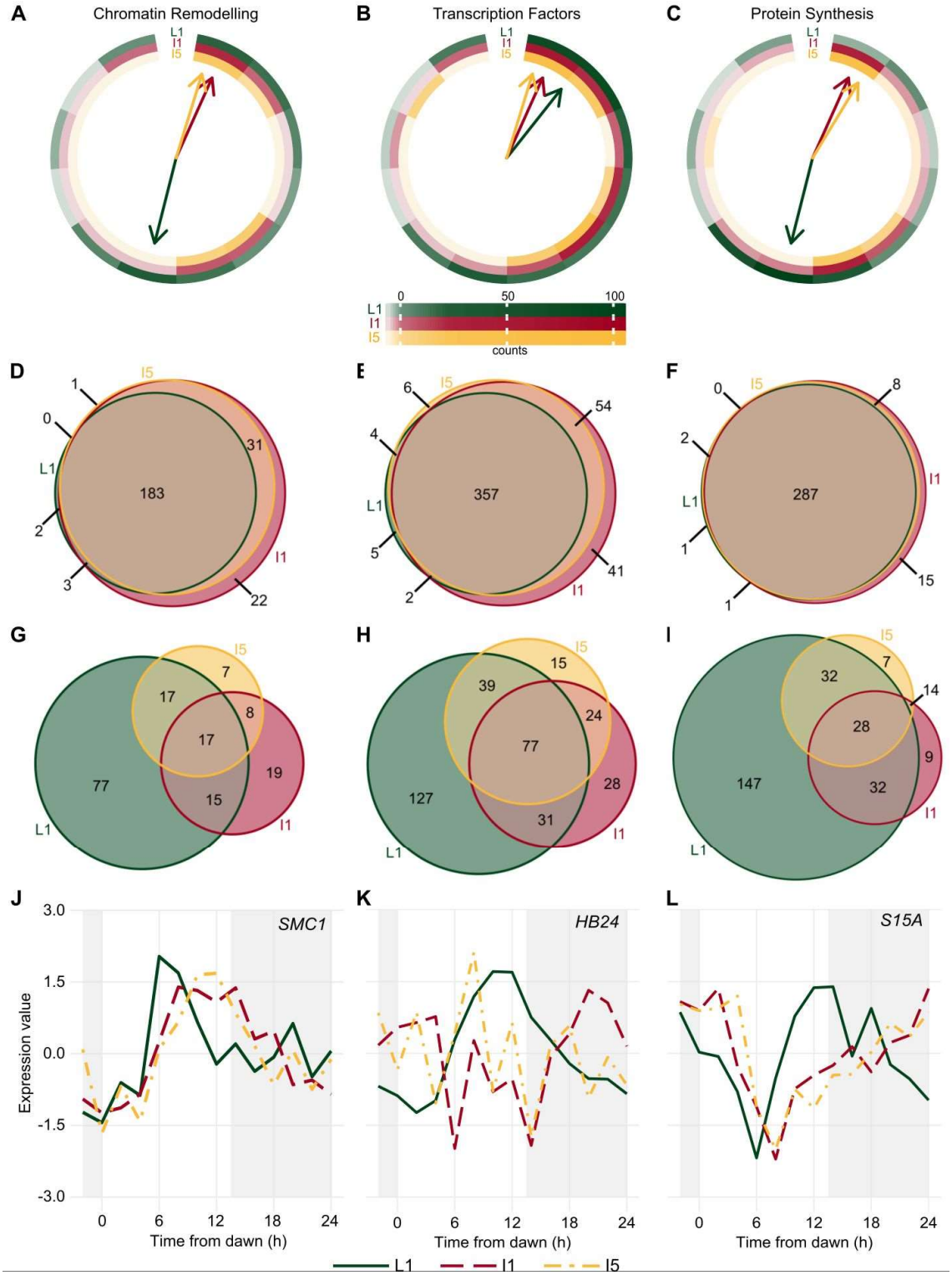



**Figure 4 – Transcripts associated with *Genetic Information Processing* have different rhythms in sugarcane organs. (A-C)** Circular heatmap of the distribution of the peak time of rhythmic transcripts related to *Chromatin Remodeling* **(A)**, *Transcription Factors* **(B)**, and *Protein Synthesis* **(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 series were normalized using *Z*-score. The light-gray boxes represent the night periods.

## Discussion

Organ-specific rhythms of transcription can be found in highly productive and intensively selected commercial sugarcane. The specialization of the rhythmic transcriptome may help the plant cells to adapt to local environmental rhythms, as well as to generate rhythms that are compatible with their specialized needs. Specialized rhythms may also be essential to rhythmic processes that require organ-to-organ coordination, such as sucrose transport from the leaves to the internodes[28].

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

Sugarcane leaves in field conditions had twice as many transcripts identified as rhythmic than plants assayed under circadian conditions. This difference is expected because some rhythms are driven by environmental oscillations, such as light and temperature. Also, some circadian-clock-driven rhythms may undergo amplitude increases due to a general increase in the amplitude of the *Central Oscillator*. In L1, the transcriptional rhythms of *LHY* vary by up to 60× in a day in circadian conditions and 750× in field conditions, while those of *TOC1* vary up to 5× in a day in circadian conditions and 40× in field conditions (Hotta et al., 2013, and Fig. S11).

In circadian conditions, most transcripts peaked at subjective dusk (ZT12, 29.0%), which resulted in 60.5% of the transcripts peaking during subjective night. By contrast, in field conditions, most transcripts peaked near dawn (ZT00-02, 35.2%) in L1, which resulted in 80.3% of the transcripts 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 transcripts that peaked during the subjective night in circadian conditions showed phase changes that made their peak happen 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

384 during the subjective night under circadian conditions[29]. Most of the transcripts
385 associated with the *Central Oscillator* maintained their core phases, except *LHY*, which
386 had a later peak (ZT01 in circadian conditions; ZT04 in field conditions). As a
387 comparison, *LHY* is induced by light in Arabidopsis and is mostly insensitive to
388 temperature in rice[22,30]. In sugarcane, alternative splicing of LHY correlates with
389 environmental temperature[31]. The differences between the rhythmic transcriptomes in
390 circadian conditions and field conditions highlight the importance of experiments done
391 under field conditions to understanding how the circadian clock can affect the plant
392 transcriptome *in natura*. For example, simulations of natural conditions in growth
393 chambers showed that the flowering signal *FLOWERING LOCUS T* (*FT*) has a different
394 phase under such conditions than it does under controlled conditions in Arabidopsis[19].
395 This discovery will require adjustments to the current flowering models to reflect events
396 in natural conditions.

397 In recent years, the productivity gains of sugarcane crop through classical breeding has
398 been decreasing[32,33]. A possible strategy to increase productivity gains is the use of
399 molecular markers[33–35]. However, the association between genotype and phenotype
400 remains a challenge, despite many attempts[36,37]. Several studies have identified
401 drought-induced genes in order to identify targets for molecular breeding[25,38–40].
402 However, as most of these studies only harvest at one timepoint, it is possible that
403 important rhythmic drought-induced genes are missed[41]. In addition, delays in the
404 harvesting of plant material, or changes in phase or period of rhythmic genes, can lead
405 to the genes to be incorrectly considered differentially expressed[42]. Thus, the
406 identification of rhythmic genes in the field can both increase the identification of genes
407 of interest and help to reduce the number of false positive, aiding the identification of
408 targets for molecular breeding.

409

410 *Rhythmic transcripts are organ-specific*

411 The transcripts in L1, I1, and I5 have very different rhythmic patterns, even though
412 most of the expressed transcripts were found in all three organs. Rhythms in I1 and I5
413 were more like each other than to those in L1, and only 7.4% of the transcripts
414 expressed in all three organs showed the same rhythms. Thus, we conclude that these
415 three organs have vastly different and specialized circadian clocks. These specialized
416 circadian clocks could be the result of multiple organ sensitivities to environmental
417 cues, of organ-specific *Core Oscillators*, and of organ-specific interactions of *Output*
418 *Pathways* with environmental signals[43,44].

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[43]. In sugarcane, most L1 transcripts peak at ZT00-02 and ZT12, following dawn and dusk, while most I1 and I5 transcripts 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[22]. In sugarcane, rhythmic L1 transcripts that were also rhythmic in circadian conditions had peaks that follow *LHY* or *TOC1* expression. On the other hand, rhythmic L1 transcripts that were not rhythmic in circadian conditions peaked at dawn and dusk. In internodes, transcripts peaked at dawn and at the light and temperature maxima. Such organ-specific sensitivity to environmental cues was previously described in the vasculature and leaf epidermis[43].

The *Central Oscillators* of mesophyll and vasculature in Arabidopsis have similar components but with different amplitudes. *AtELF4* rhythms have an amplitude 10× higher in the vasculature, *AtPRR7* and *AtPRR9* amplitudes are 2× higher in the mesophyll, and *AtTOC1* amplitude is analogous in both tissues[45]. In sugarcane, *LHY* amplitude is 6× higher and *TOC1* amplitude is 2× higher in L1 compared to I1 and I5. As leaves are exposed to direct sunlight, whereas internodes are protected by layers of leaf sheaths, it is probable that sunlight is responsible for these amplitude differences. The dynamics of *LHY*, *TOC1*, and *GI* during the day were very similar in the three organs. Indeed, they were considered to be coexpressed when analyzed together (data not shown). As the three organs have different levels of exposure to the environment, the existence of a common environmental signal is unlikely. Alternatively, the oscillators of the three organs could be coupled. There is evidence in Arabidopsis of root oscillators being regulated by the oscillators of the aerial parts of the plants, either the leaves or the shoot apical meristem (SAM)[46,47]. As the leaves are a source signal to both internodes, it is possible that synchronizing signals are transported with sucrose and other sugars. In Arabidopsis, sugars can also act as an entrainment signal[7,48].

Even though there is much evidence for tissue-specific circadian clocks in Arabidopsis[29,45,49–51], less is known about their effect on the rhythmic regulation of transcripts. In contrast, tissue-specific rhythms have been widely studied in mammals[52–55]. Sampling of 12 different mouse organs over time showed that 43% (~8,500) of all transcripts had circadian rhythms in at least one organ, but only 10

transcripts were rhythmic in all organs[54]. As in sugarcane leaves, the rhythmic transcripts in mammalian organs tended to peak at dawn and dusk. In general, the only transcripts that had similar phases across all organs were the ones associated with the mammalian *Core Oscillator*[54].

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. S13). 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, and chromatin interactions[56].

In our datasets, transcripts 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. Transcripts associated with *Transcription Factors* were more likely to be rhythmic in all three organs, and these transcripts 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[57]. 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[56,58]. 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[59–62]. In sugarcane, rhythms in transcripts associated with *Chromatin Remodeling* were underrepresented among the rhythmic transcripts. However, chromatin remodeling tends to be regulated post-transcriptionally through histone modifications. Transcription can also be regulated at the chromatin level through topologically associating domains (TADs). TADs are domains of DNA that self-interact, generating regulatory compartments within the chromosomes[63]. An enhancer only interacts with a gene if they share the same TAD. In consequence, it is possible to change the enhancers that interact with a gene by changing the boundaries of a TAD, which are maintained by cohesins and CCCTC-binding factors (CTCF) in mammals[63]. TADs can be regulated to generate tissue-specific transcription and even rhythms[64–66]. In plants, TADs are maintained by cohesins, but there are still no known CTCF

490 counterparts[67,68]. In sugarcane, the cohesin subunit *SMC1* has different phases in
491 leaves and internodes (Figure 4J).

492

493 *The role of organ-specific rhythms in sugarcane*

494 Organ-specific rhythms may affect sugarcane productivity as they allow the different
495 tissues to be more efficient according to their function and local environmental
496 rhythms[56]. In mammals, rhythms in fibroblasts allow wound healing to occur faster
497 during the active phase than the rest phase[69]. Rhythms in the liver lead to larger cell
498 sizes and protein levels during the active phase and after feeding, making
499 detoxification more efficient during the active and post-feeding periods[70].

500 Nutrient and photoassimilate transportation inside the plant is essential for rhythmic
501 processes and may also be part of organ-to-organ coordination and C partitioning[28,71–
502 73]. In sugarcane, expressed transcripts associated with *Transporters* were
503 overrepresented in L1 and I5, while rhythmic transcripts associated with *Transporters*
504 were overrepresented in L1 and I1. Furthermore, transcripts associated with
505 *Transporters* tended to peak 2 h later in L1 than in the internodes, which may indicate
506 that the latter is the driving force of this process. The phloem and xylem are also
507 important organs for the integration of multiple rhythmic information generated by
508 specialized circadian clocks, such as flowering[74].

509 Sugarcane have rhythms of sucrose and starch in the leaves but not in the
510 internodes[75]. In this crop, sucrose is synthesized in the leaves and is degraded in the
511 apoplast or cytosol of internodes to be re-synthetized in their vacuoles[28]. Organ-
512 specific regulation of transcripts may regulate sucrose storage in sugarcane.
513 Differences in the rhythms of transcripts associated with *Carbohydrate metabolism* may
514 be a way to regulate C partitioning to different organs. In our experiments, transcripts
515 associated with *Carbohydrate metabolism* peaked later in internodes than in the leaves
516 (Figure 2E). *CWI* had a peak at ZT04 in L1, and at ZT08 in I1 and I5 (Figure S9Q). In
517 sugarcane, higher activities of cell wall invertases are associated with higher sucrose
518 content, possibly by enhancing sucrose unloading in the internodes [28,76–78]. *SPSII*, one
519 of the enzymes that synthesize sucrose, was only rhythmic in L1, with a morning peak
520 (Figure S9M). Interestingly, two rhythmic *SuSy* had the same dynamics in all organs
521 (Figure S9I) but the other four were only expressed in the internodes. Sucrose
522 synthases can function either degrading or synthesizing sucrose. In sugarcane, *SuSys*

mainly work in the degradative direction, and their pattern of expression are associated with the regulation of sucrose uptake in the internodes [28,79,80].

## Conclusions

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.

## Materials and Methods

### *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 Eutroferric Red Latosol. Plants were harvested 9 months later, in January 2013 (summer), after an unusually dry winter and spring. The time course experiment started 2 h before dawn and continued every 2 h until the next dawn, generating time series with 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 $N_2$, 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).

### *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 4×44 k oligoarray (Agilent Technologies) that was previously described[15,25]. 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) (Figure S3A). 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. We have validated 10 transcripts (30 time series) using RT-qPCR (Figures S4 and S5). Among the time series identified as rhythmic (n = 23), 91% were also rhythmic using data from RT-qPCR (Table S2), and 77% were considered correlated using Spearman's rank correlation coefficient. Among the time series identified as not rhythmic (n = 7), 86% were also not rhythmic using data from RT-qPCR, and 36.7% were considered correlated using Spearman's rank correlation coefficient. 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.

*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 expressed transcripts time series were grouped in coexpressed modules using the R package weighted correlation network analysis (WGCNA) to identify rhythmic transcripts[26] (Figure S3B). Network adjacency was calculated using a soft thresholding power of 18 for all organs. Modules that had a dissimilarity value of ≤ 0.25 were merged. Final modules were generated using a 0.175 adjacency threshold. As

WGCNA groups together time series that have a positive or a negative correlation, we normalized each time series using a *Z*-score, separated these time series into two new modules, and generated a typical time series for each module by finding the median of all time series. Then, each representative time series was classified as rhythmic or non-rhythmic using JTK-CYCLE[27]. Modules that had an adjusted *P*-value of < 0.75 were considered rhythmic. Finally, we filtered out noisy time series, defined as those that had a Spearman's rank correlation coefficient of < 0.3 when compared against the representative time series. Phase was assigned using the phase estimated by JTK-CYCLE corrected against a dendrogram with the representative time series 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 series 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*[81] and *ComplexHeatmap*[82]. To evaluate if a group of transcripts were under- or overrepresented, we used a hypergeometric test (*phyper* function in R). With this test, a *P*-value < 0.05 suggests that the analyzed group is overrepresented in the dataset, while a *P*-value > 0.95 suggests that the analyzed group is underrepresented in the dataset. Code to fully reproduce our analysis is available on GitHub (https://github.com/LabHotta/sugarcane_field_rhytms) and archived on Zenodo (http://doi.org/10.5281/zenodo.2636813).

*RT-qPCR analysis*

As described for the oligoarray hybridizations, 100 mg of the pulverized frozen samples for all three organs was used for total RNA extractions following the same Trizol (Life Technologies) protocol and then were treated with DNase I (Life Technologies) and cleansed using the RNeasy Plant Mini Kit (Qiagen). RNA quality and concentration of each sample were checked using an Agilent RNA 6000 Nano Kit Bioanalyzer chip (Agilent Technologies). Five micrograms of total purified RNA was enough for the reverse transcription reactions using the SuperScript III First-Strand Synthesis System for RT-PCR (Life Technologies). The RT-qPCR reactions for all samples were done using Power SYBR Green PCR Master Mix (Applied Biosystems), 10× diluted cDNA, and specific primers described by Hotta et al. (2013) (Figure S11). Reactions were placed in 96-well plates and read with the Fast 7500/7500 Real-Time PCR System

623 (Applied Biosystems). Data analysis was performed using the Fast 7500/7500 Real-
624 Time PCR System built-in software (Applied Biosystems).

625

626 **Figure Legends**

627

628 **Figure 1 – Different organs have specific sets of rhythmic transcripts in**
629 **sugarcane. (A)** The numbers of expressed and rhythmic transcripts detected in leaf +1
630 (L1), internodes 1 and 2 (I1), and internode 5 (I5) in field-grown (diel) conditions, and in
631 leaf +1 in circadian conditions published in Hotta et al. (2013)[15]. **(B, C)** Euler diagrams
632 of expressed transcripts **(B)** and rhythmic transcripts **(C)** in L1 in sugarcane in diel
633 (green) and circadian (gray) conditions. **(D)** Number of expressed transcripts, rhythmic
634 transcripts, and rhythmic transcripts with the same phase that were found specifically in
635 L1, I1, or I5; in both L1 and I1 (L1I1, purple); in both L1 and I5 (L1I5, light green); in
636 both I1 and I5 (I1I5, orange); and in all three organs (L1I1I5, blue). In the second bar,
637 the gray area corresponds to rhythmic transcripts that are expressed in only one or two
638 organs. In the third bar, the gray area corresponds to rhythmic transcripts in only two
639 organs that have the same phase. The gray dashed lines show the associations among
640 bars. **(E, F)** Euler diagram of expressed and rhythmic transcripts in L1, I1, and I5 in
641 field-grown sugarcane in diel conditions.

642

643 **Figure 2 – Transcripts have unique phases in different sugarcane organs. (A)**
644 Circular heatmap of the rhythmic transcript peak time (ZT0 = 0 h after dawn)
645 distribution in leaf +1 (L1), internodes 1 and 2 (I1), and internode 5 (I5). The colored
646 arrows show the times at which the most transcripts are found in each organ. The
647 times of dawn, dusk, *LHY* transcription peak, maximum light intensity, and maximum
648 temperatures are indicated by black arcs. **(B)** Proportions of transcripts that were
649 rhythmic in L1, I1, and I5 among all expressed transcripts in each organ (All), among
650 the transcripts expressed only in one organ (L1 only, I1 only, or I5 only), among the
651 transcripts expressed in two organs (L1I1, L1I5, or I1I5), and among transcripts
652 expressed in all three organs (L1I1I5). **(C)** Distribution of rhythmic transcript peak time
653 in transcripts that were rhythmic in L1 but not in circadian conditions ($\alpha$ in Fig. 1C) and
654 rhythmic transcripts in transcripts that were rhythmic in L1 and circadian conditions ($\beta$).
655 **(D)** Heatmap of functional categories that are overrepresented (shades of blue) or
656 underrepresented (shades of red) among the expressed and rhythmic transcripts of L1,
657 I1, and I5. The *P*-value was calculated using a hypergeometric test. **(E)** Circular
658 heatmap with the distribution of the peak times of rhythmic transcripts associated with

659 the pathways *Carbohydrate Metabolism*, *Cell Wall Synthesis & Elongation*, *Amino Acid*
660 *Metabolism*, and *Transporters*.

661

662 **Figure 3 – Diel rhythms of *Central Oscillator* transcripts in sugarcane organs.**
663 *LHY* **(A)**, *PRR59* **(B)**, *PRR73* **(C)**, *TOC1* **(D)**, *PRR95* **(E)**, and *GI* **(F)** rhythms were
664 measured in leaf +1 (L1, green continuous line), internodes 1 and 2 (I1, red dashed
665 line), and internode 5 (I5, yellow dash-dotted line) of field-grown sugarcane using
666 oligoarrays. Time series were normalized using *Z*-score. The light-gray boxes
667 represent the night periods.

668

669 **Figure 4 – Transcripts associated with *Genetic Information Processing* have**
670 **different rhythms in sugarcane organs. (A-C)** Circular heatmap of the distribution of
671 the peak time of rhythmic transcripts related to *Chromatin Remodeling* **(A)**,
672 *Transcription Factors* **(B)**, and *Protein Synthesis* **(C)** in leaf +1 (L1, green), internodes 1
673 and 2 (I1, red), and internode 5 (I5, yellow). The colored arrows show the time at which
674 the most transcripts are found in each organ. **(D-I)** Euler diagrams of all expressed
675 transcripts **(D-F)** and rhythmic transcripts **(G-I)** in L1, I1, and I5 in field-grown
676 sugarcane in diel conditions. **(J-L)** *SMC1* **(J)**, *HB24* **(K)**, and *S15A* **(L)** rhythms
677 measured in L1 (continuous green line), I1 (red dashed line), and I5 (yellow dash-
678 dotted line) of field-grown sugarcane using oligoarrays. Time series were normalized
679 using *Z*-score. The light-gray boxes represent the night periods.

680

681 **Literature Cited**

682 1. Green, R. M., Tingay, S., Wang, Z.-Y. & Tobin, E. M. Circadian rhythms confer a higher level
683 of fitness to Arabidopsis plants. *Plant Physiol.* **129**, 576–584 (2002).
684 2. Dodd, A. N. *et al.* Plant circadian clocks increase photosynthesis, growth, survival, and
685 competitive advantage. *Science (New York, N.Y.)* **309**, 630–3 (2005).
686 3. Muller, N. A. *et al.* Domestication selected for deceleration of the circadian clock in
687 cultivated tomato. *Nat Genet* **48**, 89–93 (2016).

4. Turner, A., Beales, J., Faure, S., Dunford, R. P. & Laurie, D. A. The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. *Science* **310**, 1031–1034 (2005).
5. Gawroński, P. *et al.* A distorted circadian clock causes early flowering and temperature-dependent variation in spike development in the Eps-3Am mutant of einkorn wheat. *Genetics* **196**, 1253–1261 (2014).
6. Oakenfull, R. J. & Davis, S. J. Shining a light on the Arabidopsis circadian clock. *Plant Cell Environ.* **40**, 2571–2585 (2017).
7. Frank, A. *et al.* Circadian entrainment in Arabidopsis by the sugar-responsive transcription factor bZIP63. *Current Biology* **28**, 2597-2606.e6 (2018).
8. Webb, A. A. R., Seki, M., Satake, A. & Caldana, C. Continuous dynamic adjustment of the plant circadian oscillator. *Nature Communications* **10**, 550 (2019).
9. Hsu, P. Y., Devisetty, U. K. & Harmer, S. L. Accurate timekeeping is controlled by a cycling activator in Arabidopsis. *Elife* **2**, e00473 (2013).
10. Millar, A. J. The intracellular dynamics of circadian clocks reach for the light of ecology and evolution. *Annu Rev Plant Biol* **67**, 595–618 (2016).
11. Henriques, R., Papdi, C., Ahmad, Z. & Bögre, L. Circadian regulation of plant growth. in *Annual Plant Reviews online* 1–29 (American Cancer Society, 2018). doi:10.1002/9781119312994.apr0655.
12. Hotta, C. T. *et al.* Modulation of environmental responses of plants by circadian clocks. *Plant, cell & environment* **30**, 333–49 (2007).
13. Calixto, C. P. G., Waugh, R. & Brown, J. W. S. Evolutionary relationships among barley and Arabidopsis core circadian clock and clock-associated genes. *J. Mol. Evol.* **80**, 108–119 (2015).

14. Kusakina, J. *et al.* Barley Hv CIRCADIAN CLOCK ASSOCIATED 1 and Hv PHOTOPERIOD H1 are circadian regulators that can affect circadian rhythms in Arabidopsis. *PLoS ONE* **10**, e0127449 (2015).
15. Hotta, C. T., Nishiyama, M. Y. & Souza, G. M. Circadian rhythms of sense and antisense transcription in sugarcane, a highly polyploid crop. *PloS one* **8**, e71847 (2013).
16. Glassop, D. & Rae, A. L. Expression of sugarcane genes associated with perception of photoperiod and floral induction reveals cycling over a 24-hour period. *Functional Plant Biol.* **46**, 314–327 (2019).
17. Shalit-Kaneh, A., Kumimoto, R. W., Filkov, V. & Harmer, S. L. Multiple feedback loops of the Arabidopsis circadian clock provide rhythmic robustness across environmental conditions. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 7147–7152 (2018).
18. Annunziata, M. G. *et al.* Response of Arabidopsis primary metabolism and circadian clock to low night temperature in a natural light environment. *J. Exp. Bot.* **69**, 4881–4895 (2018).
19. Song, Y. H. *et al.* Molecular basis of flowering under natural long-day conditions in Arabidopsis. *Nat Plants* **4**, 824–835 (2018).
20. Rubin, M. J. *et al.* Circadian rhythms vary over the growing season and correlate with fitness components. *Mol. Ecol.* **26**, 5528–5540 (2017).
21. Izawa, T. *et al.* Os-GIGANTEA confers robust diurnal rhythms on the global transcriptome of rice in the field. *Plant Cell* **23**, 1741–1755 (2011).
22. Nagano, A. J. *et al.* Deciphering and prediction of transcriptome dynamics under fluctuating field conditions. *Cell* **151**, 1358–1369 (2012).
23. Matsuzaki, J., Kawahara, Y. & Izawa, T. Punctual transcriptional regulation by the rice circadian clock under fluctuating field conditions. *Plant Cell* **27**, 633–648 (2015).
24. Ming, R. *et al.* The pineapple genome and the evolution of CAM photosynthesis. *Nat. Genet.* **47**, 1435–1442 (2015).

737 25. Lembke, C. G., Nishiyama, M. Y., Sato, P. M., de Andrade, R. F. & Souza, G. M.
738 Identification of sense and antisense transcripts regulated by drought in sugarcane. *Plant*
739 *Mol Biol* **79**, 461–477 (2012).
740 26. Langfelder, P. & Horvath, S. WGCNA: an R package for weighted correlation network
741 analysis. *BMC Bioinformatics* **9**, 559 (2008).
742 27. Hughes, M. E., Hogenesch, J. B. & Kornacker, K. JTK_CYCLE: an efficient nonparametric
743 algorithm for detecting rhythmic components in genome-scale data sets. *J Biol Rhythms*
744 **25**, 372–380 (2010).
745 28. Wang, J., Nayak, S., Koch, K. & Ming, R. Carbon partitioning in sugarcane (Saccharum
746 species). *Front. Plant Sci.* **4**, (2013).
747 29. Xu, X. *et al.* Distinct light and clock modulation of cytosolic free Ca2+ oscillations and
748 rhythmic CHLOROPHYLL A/B BINDING PROTEIN2 promoter activity in Arabidopsis. *The*
749 *Plant cell* **19**, 3474–90 (2007).
750 30. Kim, J.-Y., Song, H.-R., Taylor, B. L. & Carré, I. A. Light-regulated translation mediates gated
751 induction of the Arabidopsis clock protein LHY. *The EMBO Journal* **22**, 935–944 (2003).
752 31. Dantas, L. L. B. *et al.* Alternative Splicing of Circadian Clock Genes Correlates With
753 Temperature in Field-Grown Sugarcane. *Front. Plant Sci.* **10**, (2019).
754 32. Waclawovsky, A. J., Sato, P. M., Lembke, C. G., Moore, P. H. & Souza, G. M. Sugarcane for
755 bioenergy production: an assessment of yield and regulation of sucrose content. *Plant*
756 *Biotechnology Journal* **8**, 263–276 (2010).
757 33. Barreto, F. Z. *et al.* A genome-wide association study identified loci for yield component
758 traits in sugarcane (Saccharum spp.). *PLoS One* **14**, (2019).
759 34. Dal-Bianco, M. *et al.* Sugarcane improvement: how far can we go? *Current opinion in*
760 *biotechnology* **23**, 265–70 (2012).
761 35. Hotta, C. T. *et al.* The Biotechnology Roadmap for Sugarcane Improvement. *Tropical Plant*
762 *Biol.* **3**, 75–87 (2010).

763 36. Margarido, G. R. A., Pastina, M. M., Souza, A. P. & Garcia, A. A. F. Multi-trait multi-
764 environment quantitative trait loci mapping for a sugarcane commercial cross provides
765 insights on the inheritance of important traits. *Mol Breed* **35**, (2015).
766 37. Balsalobre, T. W. A. *et al.* GBS-based single dosage markers for linkage and QTL mapping
767 allow gene mining for yield-related traits in sugarcane. *BMC Genomics* **18**, (2017).
768 38. Vantini, J. S. *et al.* Differential gene expression in drought-tolerant sugarcane roots. *Genet.*
769 *Mol. Res.* **14**, 7196–7207 (2015).
770 39. Gentile, A., Dias, L. I., Mattos, R. S., Ferreira, T. H. & Menossi, M. MicroRNAs and drought
771 responses in sugarcane. *Front Plant Sci* **6**, 58 (2015).
772 40. Li, C. *et al.* Differential expression profiles and pathways of genes in sugarcane leaf at
773 elongation stage in response to drought stress. *Sci Rep* **6**, 25698 (2016).
774 41. Dubois, M., Claeys, H., Broeck, L. V. den & Inzé, D. Time of day determines Arabidopsis
775 transcriptome and growth dynamics under mild drought. *Plant, Cell & Environment* **40**,
776 180–189 (2017).
777 42. Hsu, P. Y. & Harmer, S. L. Circadian Phase Has Profound Effects on Differential Expression
778 Analysis. *PLOS ONE* **7**, e49853 (2012).
779 43. Shimizu, H. *et al.* Decentralized circadian clocks process thermal and photoperiodic cues in
780 specific tissues. *Nat Plants* **1**, 15163 (2015).
781 44. Bordage, S., Sullivan, S., Laird, J., Millar, A. J. & Nimmo, H. G. Organ specificity in the plant
782 circadian system is explained by different light inputs to the shoot and root clocks. *New*
783 *Phytol.* **212**, 136–149 (2016).
784 45. Endo, M., Shimizu, H., Nohales, M. A., Araki, T. & Kay, S. A. Tissue-specific clocks in
785 Arabidopsis show asymmetric coupling. *Nature* **515**, 419–22 (2014).
786 46. James, A. B. *et al.* The circadian clock in Arabidopsis roots is a simplified slave version of
787 the clock in shoots. *Science (New York, N.Y.)* **322**, 1832–5 (2008).

788 47. Takahashi, N., Hirata, Y., Aihara, K. & Mas, P. A hierarchical multi-oscillator network
789 orchestrates the Arabidopsis circadian system. *Cell* **163**, 148–159 (2015).
790 48. Haydon, M. J., Mielczarek, O., Robertson, F. C., Hubbard, K. E. & Webb, A. A.
791 Photosynthetic entrainment of the Arabidopsis thaliana circadian clock. *Nature* **502**, 689–
792 92 (2013).
793 49. Para, A. *et al.* PRR3 Is a vascular regulator of TOC1 stability in the Arabidopsis circadian
794 clock. *Plant Cell* **19**, 3462–3473 (2007).
795 50. Kim, S.-G., Yon, F., Gaquerel, E., Gulati, J. & Baldwin, I. T. Tissue specific diurnal rhythms of
796 metabolites and their regulation during herbivore attack in a native tobacco, Nicotiana
797 attenuata. *PLoS ONE* **6**, e26214 (2011).
798 51. Endo, M. Tissue-specific circadian clocks in plants. *Curr. Opin. Plant Biol.* **29**, 44–49 (2015).
799 52. Storch, K.-F. *et al.* Extensive and divergent circadian gene expression in liver and heart.
800 *Nature* **417**, 78–83 (2002).
801 53. Panda, S. *et al.* Coordinated transcription of key pathways in the mouse by the circadian
802 clock. *Cell* **109**, 307–320 (2002).
803 54. Zhang, R., Lahens, N. F., Ballance, H. I., Hughes, M. E. & Hogenesch, J. B. A circadian gene
804 expression atlas in mammals: implications for biology and medicine. *Proc. Natl. Acad. Sci.*
805 *U.S.A.* **111**, 16219–16224 (2014).
806 55. Ruben, M. D. *et al.* A database of tissue-specific rhythmically expressed human genes has
807 potential applications in circadian medicine. *Sci Transl Med* **10**, (2018).
808 56. Yeung, J. & Naef, F. Rhythms of the genome: circadian dynamics from chromatin topology,
809 tissue-specific gene expression, to behavior. *Trends in Genetics* **34**, 915–926 (2018).
810 57. Yeung, J. *et al.* Transcription factor activity rhythms and tissue-specific chromatin
811 interactions explain circadian gene expression across organs. *Genome Res.* **28**, 182–191
812 (2018).

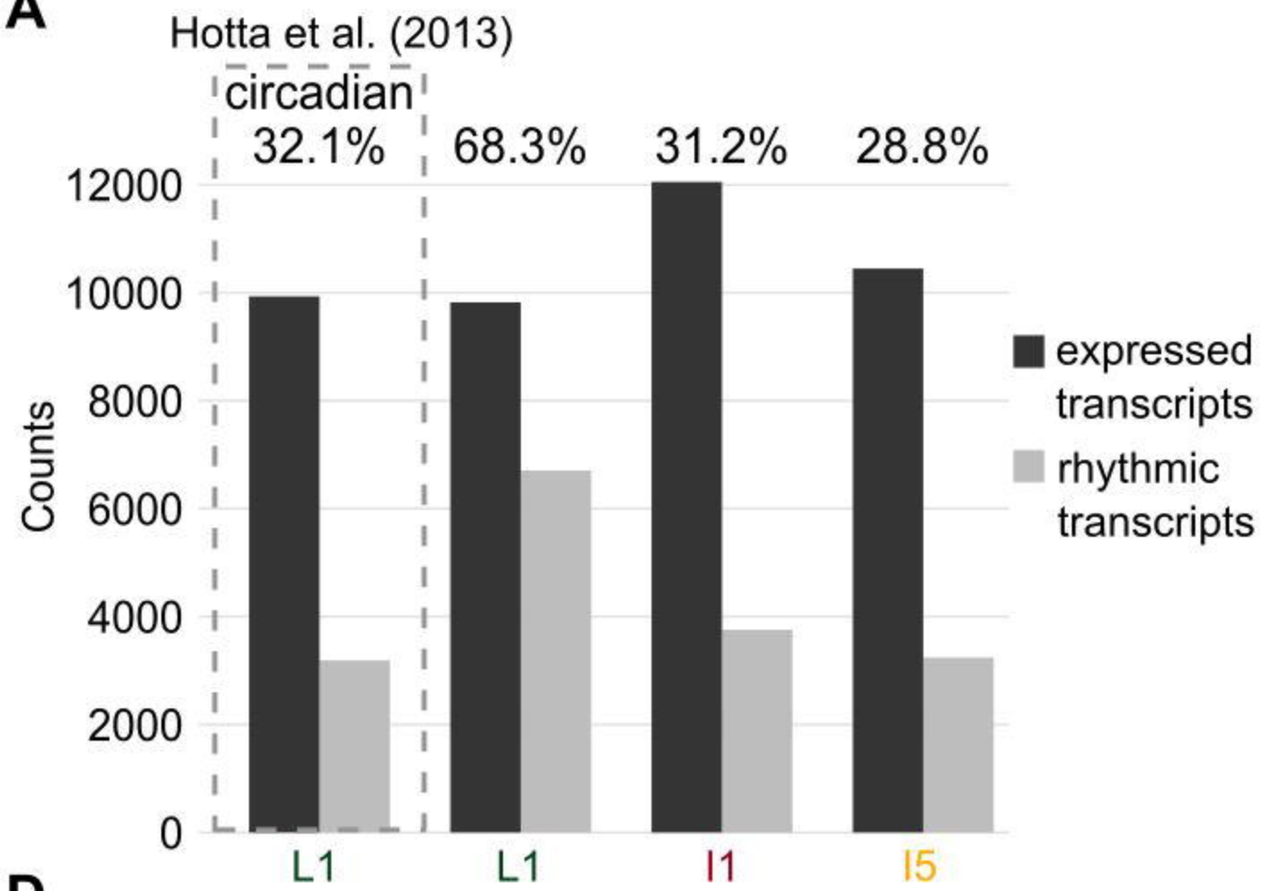
813 58. Smieszek, S. P., Yang, H., Paccanaro, A. & Devlin, P. F. Progressive promoter element
814 combinations classify conserved orthogonal plant circadian gene expression modules. *J R*
815 *Soc Interface* **11**, (2014).
816 59. Lu, S. X. *et al.* The Jumonji C Domain-Containing Protein JMJ30 regulates period length in
817 the Arabidopsis circadian clock. *Plant Physiology* **155**, 906–915 (2011).
818 60. Malapeira, J., Khaitova, L. C. & Mas, P. Ordered changes in histone modifications at the
819 core of the Arabidopsis circadian clock. *Proceedings of the National Academy of Sciences*
820 *of the United States of America* **109**, 21540–5 (2012).
821 61. Lee, H. G., Lee, K., Jang, K. & Seo, P. J. Circadian expression profiles of chromatin
822 remodeling factor genes in Arabidopsis. *J. Plant Res.* **128**, 187–199 (2015).
823 62. Yang, P., Wang, J., Huang, F.-Y., Yang, S. & Wu, K. The plant circadian clock and chromatin
824 modifications. *Genes (Basel)* **9**, (2018).
825 63. Long, H. K., Prescott, S. L. & Wysocka, J. Ever-changing landscapes: transcriptional
826 enhancers in development and evolution. *Cell* **167**, 1170–1187 (2016).
827 64. Aguilar-Arnal, L. *et al.* Cycles in spatial and temporal chromosomal organization driven by
828 the circadian clock. *Nature Structural & Molecular Biology* **20**, 1206–1213 (2013).
829 65. Kim, Y. H. *et al.* Rev-erbα dynamically modulates chromatin looping to control circadian
830 gene transcription. *Science* **359**, 1274–1277 (2018).
831 66. Mermet, J. *et al.* Clock-dependent chromatin topology modulates circadian transcription
832 and behavior. *Genes Dev.* **32**, 347–358 (2018).
833 67. Schubert, V., Lermontova, I. & Schubert, I. The Arabidopsis CAP-D proteins are required for
834 correct chromatin organisation, growth and fertility. *Chromosoma* **122**, 517–533 (2013).
835 68. Liu, C., Cheng, Y.-J., Wang, J.-W. & Weigel, D. Prominent topologically associated domains
836 differentiate global chromatin packing in rice from Arabidopsis. *Nat Plants* **3**, 742–748
837 (2017).

838 69. Hoyle, N. P. *et al.* Circadian actin dynamics drive rhythmic fibroblast mobilization during
839 wound healing. *Sci Transl Med* **9**, (2017).
840 70. Sinturel, F. *et al.* Diurnal oscillations in liver Mass and cell size accompany ribosome
841 assembly cycles. *Cell* **169**, 651-663.e14 (2017).
842 71. Xu, G. Sensing and transport of nutrients in plants. *Semin. Cell Dev. Biol.* **74**, 78–79 (2018).
843 72. López-Salmerón, V., Cho, H., Tonn, N. & Greb, T. The phloem as a mediator of plant growth
844 plasticity. *Curr. Biol.* **29**, R173–R181 (2019).
845 73. Osorio, S., Ruan, Y.-L. & Fernie, A. R. An update on source-to-sink carbon partitioning in
846 tomato. *Front. Plant Sci.* **5**, (2014).
847 74. Inoue, K., Araki, T. & Endo, M. Oscillator networks with tissue-specific circadian clocks in
848 plants. *Seminars in Cell & Developmental Biology* **83**, 78–85 (2018).
849 75. Souza, A. P. D., Grandis, A., Arenque-Musa, B. C. & Buckeridge, M. S. Diurnal variation in
850 gas exchange and nonstructural carbohydrates throughout sugarcane development.
851 *Functional Plant Biol.* **45**, 865–876 (2018).
852 76. Chandra, A., Jain, R. & Solomon, S. Complexities of invertases controlling sucrose
853 accumulation and retention in sugarcane. *Current Science* **102**, 857–866 (2012).
854 77. Lingle, S. E. Evidence for the Uptake of Sucrose Intact into Sugarcane Internodes. *Plant*
855 *Physiol* **90**, 6–8 (1989).
856 78. Moore, P. H. Temporal and Spatial Regulation of Sucrose Accumulation in the Sugarcane
857 Stem. *Functional Plant Biol.* **22**, 661–679 (1995).
858 79. Buczynski, S. R., Thom, M., Chourey, P. & Maretzki, A. Tissue Distribution and
859 Characterization of Sucrose Synthase Isozymes in Sugarcane. *Journal of Plant Physiology*
860 **142**, 641–646 (1993).
861 80. Thirugnanasambandam, P. P. *et al.* Analysis of the diversity and tissue specificity of
862 sucrose synthase genes in the long read transcriptome of sugarcane. *BMC Plant Biology*
863 **19**, 160 (2019).

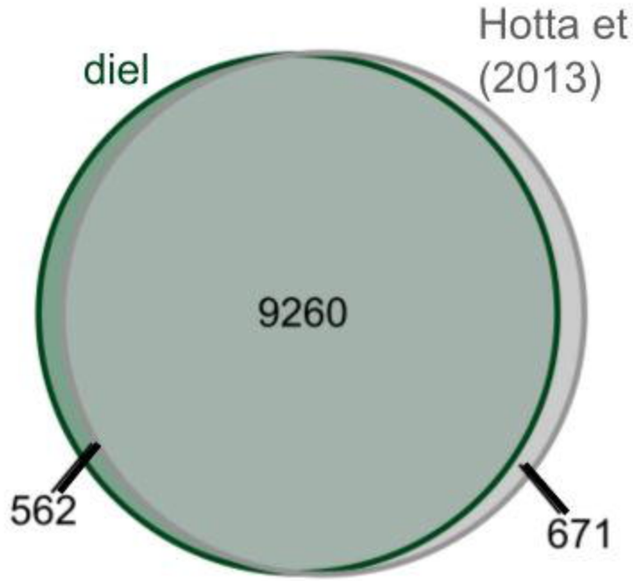
81. Gu, Z., Gu, L., Eils, R., Schlesner, M. & Brors, B. circlize Implements and enhances circular visualization in R. *Bioinformatics* **30**, 2811–2812 (2014).

82. Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**, 2847–2849 (2016).

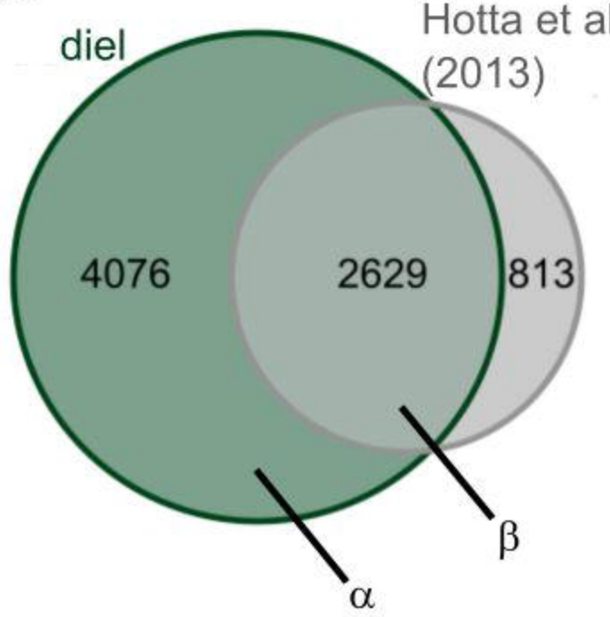
A


Hotta et al. (2013)
circadian
32.1%
68.3%
31.2%
28.8%
12000
10000
8000
6000
4000
2000
0
Counts
expressed transcripts
rhythmic transcripts
L1
L1
I1
I5

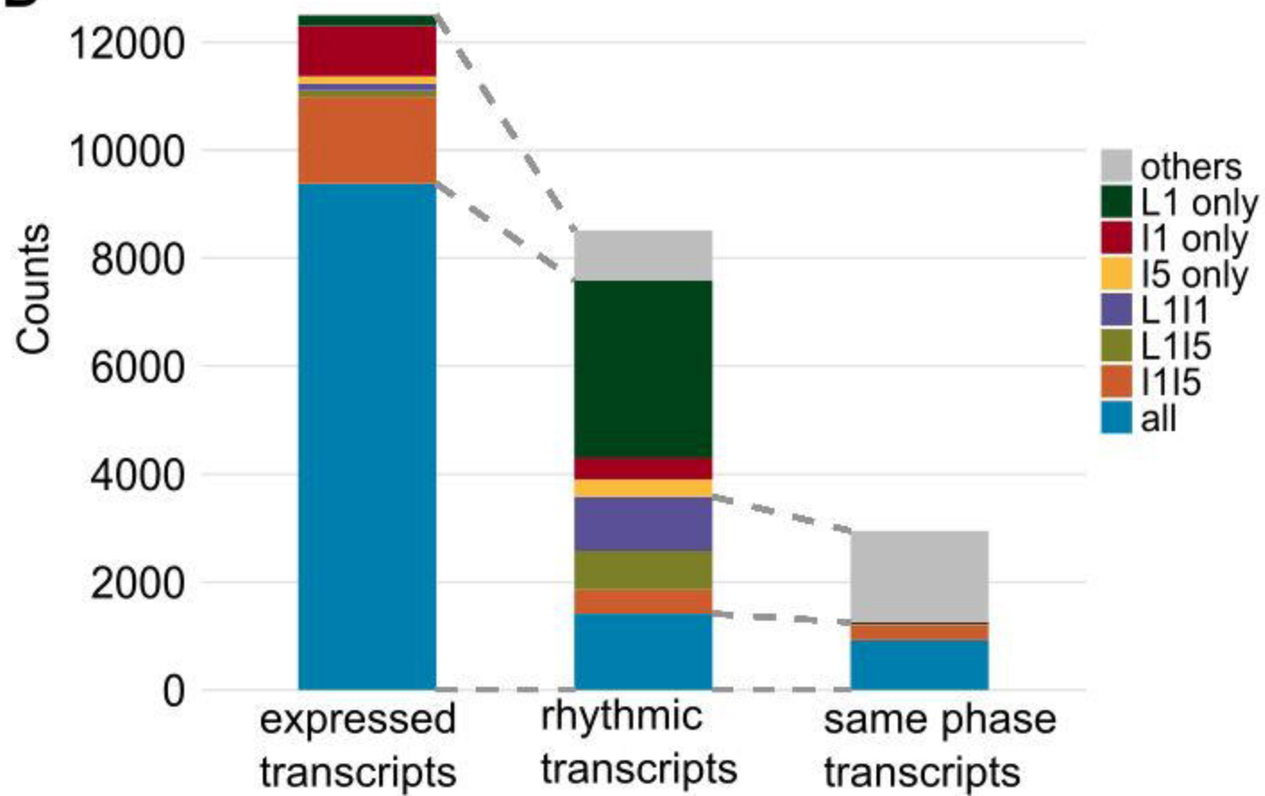
B


diel
Hotta et al. (2013)
9260
562
671

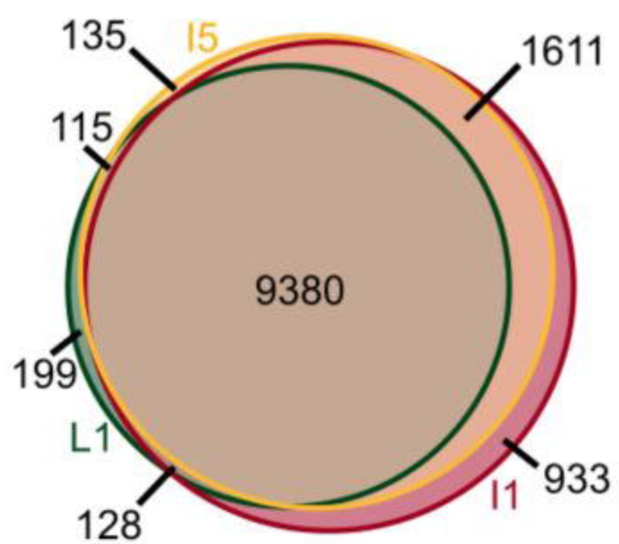
C


diel
Hotta et al. (2013)
4076
2629
813
α
β

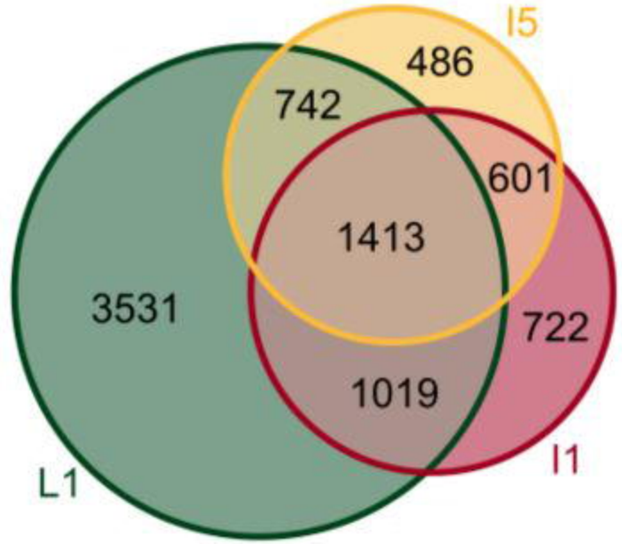
D

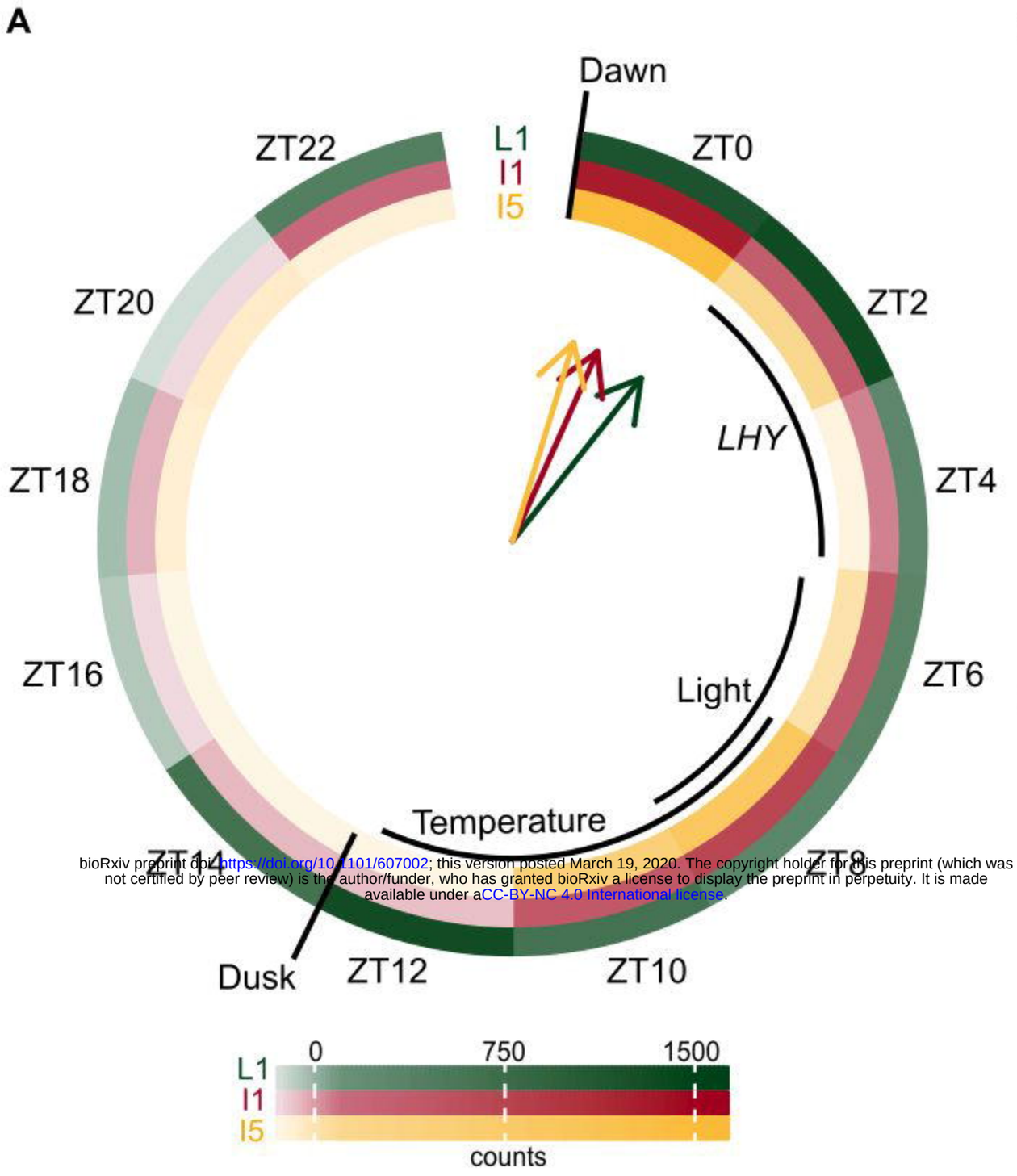

12000
10000
8000
6000
4000
2000
0
Counts
others
L1 only
I1 only
I5 only
L1I1
L1I5
I1I5
all
expressed transcripts
rhythmic transcripts
same phase transcripts

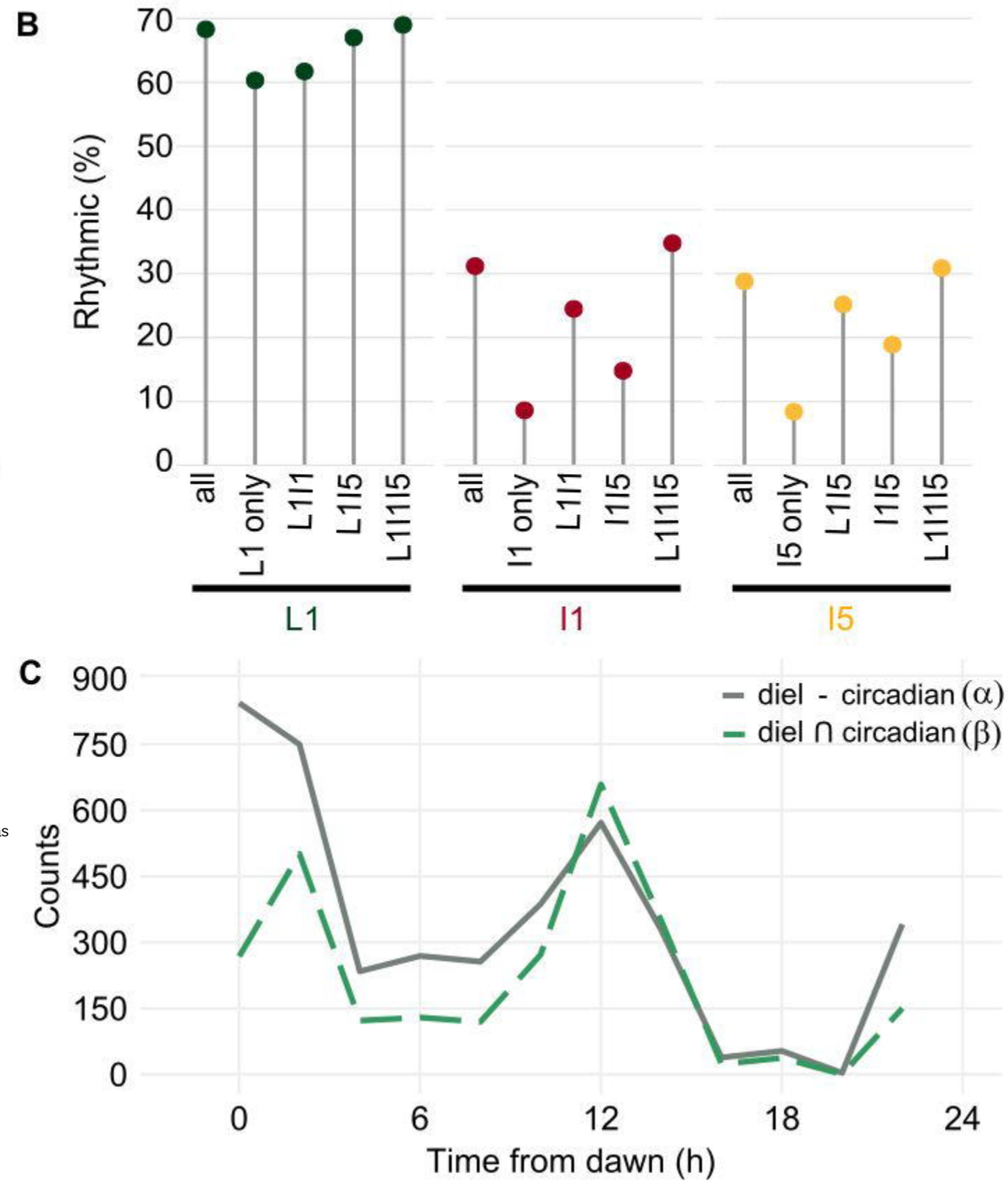
E

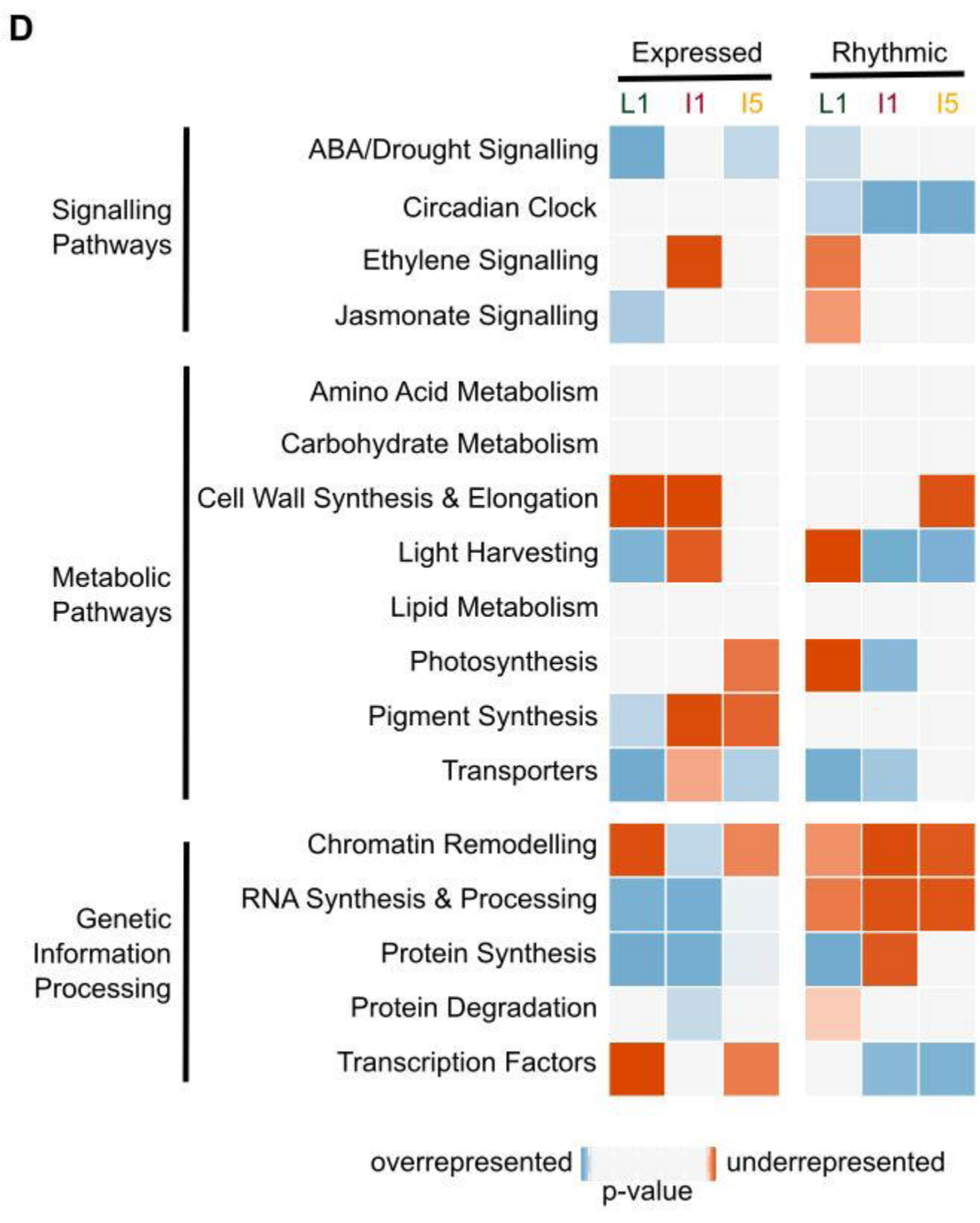

135
I5
1611
115
9380
199
L1
I1
933
128

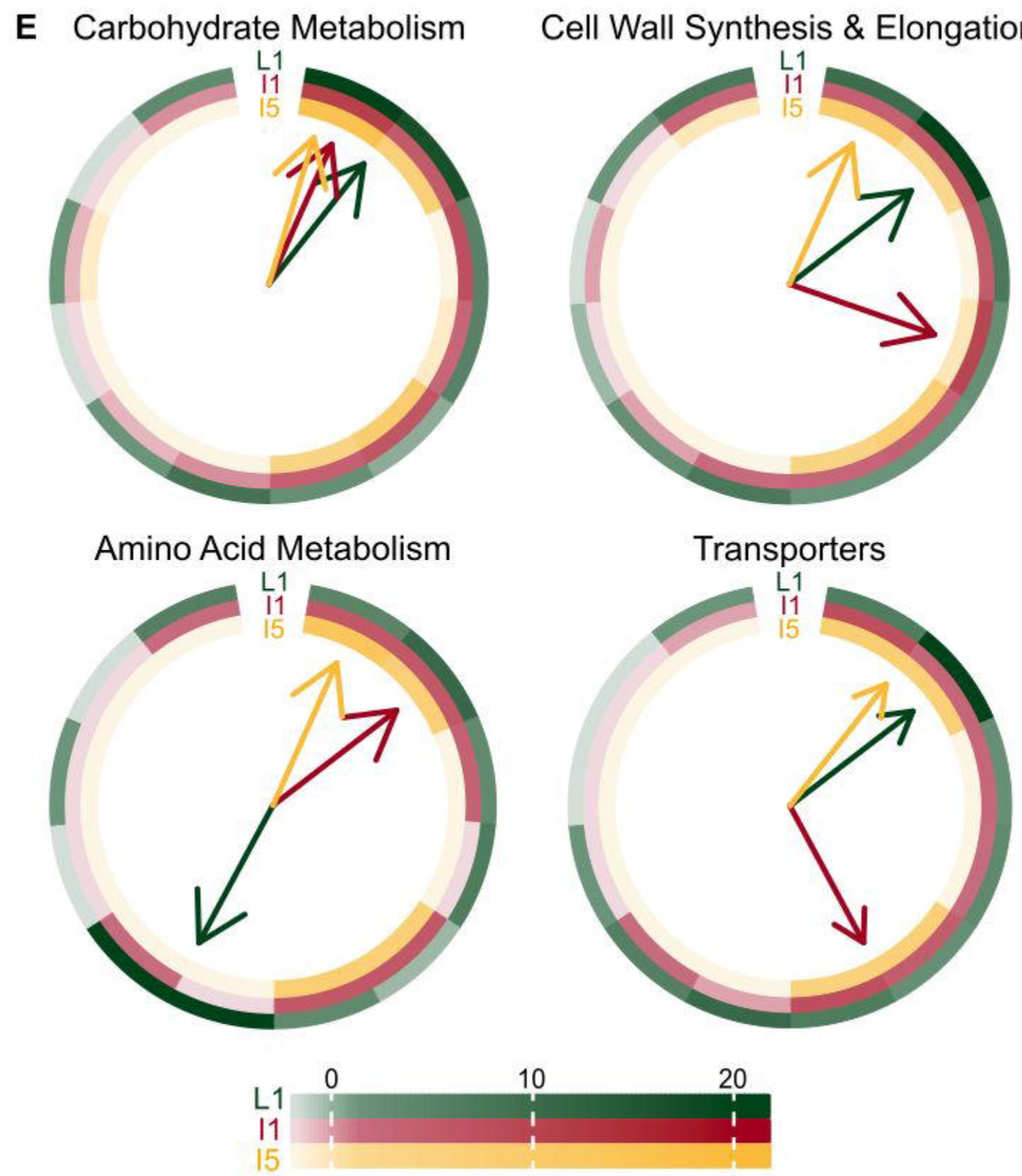
F


I5
486
742
601
1413
3531
722
1019
L1
I1



A
B
C
D
E
F
LHY
PRR59
PRR73
TOC1
PRR95
GI
3.0
1.5
0.0
-1.5
-3.0
Expression value
0
6
12
18
24
Time from dawn (h)
L1
I1
I5