Circadian expression of the circadian clock genes in a commercial sugarcane variety

<u>Hotta C.T.</u>[1], Nishiyama-Jr[1], M. Y., Souza G. M[1]

[1] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil

carlos.hotta@cantab.net

**INTRODUCTION: ** Sugarcane is a C4 grass that is able to accumulate great amounts of sucrose in its shoot. The circadian clock modulates major pathways associated to sucrose synthesis, such as photosynthesis, sucrose synthesis and sugar transport. The circadian clock can be divided into three parts: the central oscillator, that generates circadian rhythms; the input pathways, that brings information from the environment to the central oscillator; and the output pathways that take the temporal information generated by the central oscillator to the rest of the plant. The most known input pathways are associated with light perception, even though temperature is also involved. There are at least three families of photoreceptors that regulate the circadian clock: the red/far-red light-receptor PHYTOCHROMES (PHY) and the blue light-receptors CRYPTOCHROME (CRY) and ZEITLUPE (ZTL). The central oscillator is mainly a series of interlocked negative feedback loops based on the regulation of gene expression. One loop consists of CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), LATE ELONGATED HYPOCOTYL (LHY), CCA1 HIKING EXPEDITION (CHE) and TIMING OF CAB EXPRESSION 1(TOC1). CCA1 and LHY are transcription factors of the same family and have redundant function. Both proteins repress <i>*TOC1*</i> and <i>*CHE*</i> expression. In turn, TOC1 activates <i>*CCA1*</i> and <i>*LHY*</i> expression and also inactivates CHE, which acts as a repressor of <i>*CCA1*</i> and <i>*LHY*</i> expression. Another loop, called “morning loop” is composed of PSEUDO RESPONSE REGULATOR 5 (PRR5), PRR7 and PRR9 that interact with CCA1 and LHY. A final loop, called “evening loop” is composed of GIGANTEA (GI), PRR3 and ZTL, which interact with each other in order to regulate TOC1 degradation. **AIM: ** The aim of this study is to identify transcripts associated with the generation of circadian rhythms in sugarcane and test if their pattern of expression under constant light is consistent with a role in the circadian clock. **METHODS: ** Putative circadian genes were identified in sugarcane from the SUCEST, a sugarcane EST database. Each SAS (sugarcane assembled sequence) was selected using tBLASTx with an <i>*Arabidopsis thaliana*</i> circadian gene as an input. SAS with highest identity were selected (usually e-value < e-30) and used as input in a tBLASTx query against an <i>*Arabidopsis*</i> database. If the original <i>Arabidopsis</i>

gene was the top hit, the SAS was confirmed as a putative circadian gene. The expression levels of putative genes associated with the circadian clock was estimated using custom oligoarrays. A commercial sugarcane variety (RB855453), which still has a functional flowering apparatus, was grown in soil for three months in a growth room in a 12 h light/ 12 h dark photoperiod and constant temperature. The plants were kept in constant light and temperature for 24 h then leaves from nine individuals were harvested every 4 h for 48 h. RNA from the samples was extracted and samples from each time points were hybridized against a RNA pool containing equimolar amounts of each sample. The oligoarray contains 21902 elements of which 7380 are specific for the antisense sequences sugarcane ESTs. **RESULTS AND CONCLUSION: ** We have identified a total of 2354 circadian transcripts (~16%). <i>*A. thaliana*</i> has five versions of PHY (AtPHYA, AtPHYB,At PHYC, AtPHYD and AtPHYE) while grasses seem to have only three (PHYA, PHYB and PHYC). Five SAS were found to have high identity to <i>*AtPHY*s</i> but they could be grouped in three isoforms, like found in grasses: <i>*ScPHYA*</i>-1, <i>*ScPHYA*</i>-2, *ScPHYB*, <i>*ScPHYC*</i>-1 and <i>*ScPHYC*</i>-2. Of these SAS, only <i>*ScPHYB*</i> is not circadian regulated. <i>*ScPHYA*</i>-1 and <i>*ScPHYA*</i>-2 peaked at ZT16 (16 h after subjective dawn) and <i>*ScPHYC*</i>-1 and <i>*ScPHYC*</i>-2 peaked at ZT12. In *Arabidopsis*, only <i>*AtPHYA*</i> - peaking at ZT0 and <i>At*PHYE*</i> - peaking at ZT20 – were circadian regulated. Sugarcane has five SAS with high identity to <i>*AtCRY1*</i> (ZT8): <i>*ScCRY1*</i>-1 to <i>*ScCRY1*</i>-5. Of these, only two are circadian regulated: <i>*ScCRY1*</i>-2 (ZT4) and <i>*ScCRY1*</i>-3 (ZT12). In contrast, only one SAS had high identity to <i>*AtCRY2*</i> (ZT12) but it was not circadian regulated. Three sugarcane <i>*ZTL*</i> were found, one with a peak at ZT4 (<i>*ScZTL*</i>-1) and one with a peak at ZT20 (<i>*ScZTL*</i>-2) while <i>*AtZTL*</i> is not circadian regulated. In the central oscillator, only one SAS (<i>*ScLHY*</i>, ZT0) had high identity to <i>*AtCCA1*</i> (ZT0) and <i>*AtLHY*</i> (ZT0). In contrast, two SAS were found with high identity to <i>*AtTOC1*</i>, <i>*ScTOC1*</i>-1 and <i>*ScTOC1*</i>-2, both with a peak at ZT12. <i>*ScPRR3*</i>, <i>*ScPRR59*</i> and <i>*ScPRR7*</i> had all peaks at ZT8. Finally, there were four SAS with high identity to <i>*AtGI*</i>. Of those, <i>*ScGI*</i>-1 was not circadian-regulated, <i>*ScGI*</i>-2 had a peak at ZT12 and <i>*ScGI*</i>-3 had a peak at ZT8. <i>*ScGI*</i>-4 was not represented in the oligoarrays but real-time PCR showed that it was also circadian-regulated with a peak at ZT8. <i>*AtGI*</i> is found to have a peak that varies between ZT8 and ZT12 depending on the growth conditions. The circadian clock is thought to be conserved among the plants. Sugarcane has robust circadian rhythms, despite its aneuploidy and polyploidy. Sugarcane photoreceptors associated to the input pathways to the circadian clock are also circadian regulated but their regulation differs to their <i>*Arabidopsis*</i> counterpart, as seen by their time of peak. At the same time, genes associated to the central oscillator of the circadian clock all peak at the similar times in sugarcane and <i>*Arabidopsis*</i>, probably because they are essential to the maintenance of circadian function. Finally, the number of components of the central oscillator seems to vary in sugarcane in comparison to *Arabidopsis*: sugarcane has only one <i>*CCA1*/*LHY*</i>, two <i>*TOC1*</i>, one <i>*PRR5*/*PRR9*</i> and four <i>*GI*</i>. In conclusion, the sugarcane circadian clock has small differences in regulation and composition when compared to the <i>*Arabidopsis*</i> circadian clock but it is uncertain how those differences impact the function of sugarcane circadian clock and whether they impact sugarcane capacity to accumulate sucrose.

<i>*Supported by FAPESP*</i>

Author publications

Hotta, C.T.; Lembke, C.G.; Domingues, D.S.; Ochoa, E.A.; Cruz, G.M.Q. ; Melotto-Passarin, D.M.; Marconi, T.G.; Santos, M.O.; Mollinari, M.; Margarido, G.R.A.; Crivellari, A.C.; Santos, W.D.; Souza, A.P.; Hoshino, A.A.; Carrer, H.; Souza, A.P.; Garcia, A.A.F.; Buckeridge, M.S.; Menossi, M.; Sluys, M.A.; Souza, G.M. (2010) The Biotechnology Roadmap for Sugarcane Improvement. Tropical Plant Biology,. 3, 75-87

Dalchau, N.; Hubbard, K.E.; Robertson, F.C.; Hotta, C.T.; Briggs, H.M.; Stan, G.-B.; Goncalves, J.M.; Webb, A.A.R. (2010) Correct biological timing in Arabidopsis requires multiple light-signaling pathways. Proceedings of the National Academy of Sciences of the United States of America, 107, 13171-13176.

Dodd, AN; Gardner, MJ; Hotta, C.T.; Hubbard, K.E.; Dalchau, N.; Robertson, F.C.; Love, J.; Sanders, D.; Webb, A.A.R. (2009) Response to Comment on “The Arabidopsis Circadian Clock Incorporates a cADPR-Based Feedback Loop”. Science, 326, 230-230.

This document was created with Win2PDF available at http://www.win2pdf.com.
The unregistered version of Win2PDF is for evaluation or non-commercial use only.
This page will not be added after purchasing Win2PDF.