

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

Temporal changes in the expression of genes involved in carbon partitioning in apical culms of contrasting sugarcane genotypes

Guilherme Kenichi Hosaka

Thesis presented to obtain the degree of Doctor in Science. Area: Bioenergy

**Piracicaba
2021**

Guilherme Kenichi Hosaka
Licentiate in Biological Science

Temporal changes in the expression of genes involved in carbon partitioning in apical culms of contrasting sugarcane genotypes

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:

Prof. PhD. **GABRIEL RODRIGUES ALVES MARGARIDO**

Thesis presented to obtain the degree of Doctor in Science. Area: Bioenergy

Piracicaba
2021

**Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA - DIBD/ESALQ/USP**

Hosaka, Guilherme Kenichi

Temporal changes in the expression of genes involved in carbon partitioning in apical culms of contrasting sugarcane genotypes / Guilherme Kenichi Hosaka. -- versão revisada de acordo com a resolução CoPGr 6018 de 2011 -- Piracicaba, 2021 .
41 p.

Tese (Doutorado) -- USP / Escola Superior de Agricultura "Luiz de Queiroz". Universidade Estadual de Campinas. Universidade Estadual Paulista "Julio de Mesquita Filho"

1. *Saccharum* 2. Sólidos solúveis 3. RNA-Seq 4. Expressão diferencial
5. Rede de co-expressão . I. Título.

ACKNOWLEDGMENTS

I wish to express my deepest gratitude to my advisor, Prof. Ph.D Gabriel Rodrigues Alves Margarido for the patience, teachings and trust expended to carry out this work.

I would like to thank CAPES for the opportunity of the scholarship in these four years of study, including the CAPES-PrInt.

I would like to thank Professor Ph.D Nicholas Provart for the support given during the sandwich doctorate period.

I owe my deepest gratitude to my parents (Tetsuya Hosaka and Marina J. M. Hosaka) and my sister (Cintia M. Hosaka) for support and encourage me in critical times.

I wish to show my appreciation to my girlfriend Gladys Angélica Apaza Castillo for the support and patience in every hard time that I faced with.

I also thank all the members of the Bioinformatics applied to Bioenergy Laboratory: Fernando Henrique Correr, Ana Letycia Basso Garcia, Victor Hugo de Mello Pessoa, Amanda Avelar de Oliveira, Amanda Ghelfi Dumit, Lorena Guimarães Batista, André Luis Patrício and Guilherme Bovi Ambrosano. Thanks for all the help, friendship, talks, parties and jokes.

To my friends Geraldo Felipe Ferreira e Silva, Paulo Henrique Ceciliato and Fausto Andres Ortiz Morea for the support and friendship.

Finally, to all those who have not been mentioned, but who directly or indirectly influenced the development of this work.

SUMMARY

Resumo	5
Abstract	6
1 Introduction	7
2 Conclusions	8
References	9

RESUMO

Alterações temporais na expressão dos genes envolvidos na partição do carbono em colmos apicais de genótipos contrastantes de cana-de-açúcar

Múltiplos genes na cana-de-açúcar controlam o acúmulo de sacarose e a biossíntese dos componentes da parede celular; contudo, não está claro como estes genes são expressos nos caules apicais. Para melhor compreender este processo, sequenciamos o mRNA do entrenó +1 de colmos coletados de quatro genótipos com diferentes concentrações de sólidos solúveis. Os colmos foram coletados em quatro tempos diferentes, variando de plantas com seis a 12 meses de idade. Neste trabalho, apresentamos genes diferentemente expressos relacionados ao metabolismo da sacarose e à biossíntese da parede celular, incluindo genes que codificam invertases, sacarose sintase e celulose sintase. Nos nossos resultados mostraram um aumento da expressão de invertases na IN84-58, genótipo com menor teor de açúcar e maior teor de fibras, bem como a expressão tardia da celulose synthase relacionada com a parede celular secundária para os outros genótipos. Curiosamente, os genes envolvidos no metabolismo de hormônios foram diferencialmente expressos ao longo do tempo nos três genótipos com maior conteúdo de sólidos solúveis. Um resultado semelhante foi observado para genes que controlam a maturação e a transição para as fases reprodutivas, possivelmente um resultado da seleção contra a floração em programas de melhoramento de cana-de-açúcar. Estes resultados indicam que a partição de carbono em caules apicais de genótipos contrastantes está principalmente associada na alteração da biossíntese de parede celular, podendo incluir modificações precoces para posterior acúmulo de sacarose. A análise da rede de coexpressão identificou factores de transcrição relacionados com o crescimento e desenvolvimento, mostrando uma provável mudança de tempo para a partição de carbono ocorrida em plantas com 10 meses de idade.

Palavras-chave: *Saccharum*, Sólidos solúveis, RNA-Seq, Expressão diferencial, Rede de co-expressão

ABSTRACT

Temporal changes in the expression of genes involved in carbon partitioning in apical culms of contrasting sugarcane genotypes

Multiple genes in sugarcane control sucrose accumulation and the biosynthesis of cell wall components; however, it is unclear how these genes are expressed in its apical culms. To better understand this process, we sequenced mRNA from +1 stem internodes collected from four genotypes with different concentrations of soluble solids. Culms were collected at four different time points, ranging from six to 12-month-old plants. Here we show differentially expressed genes related to sucrose metabolism and cell wall biosynthesis, including genes encoding invertases, sucrose synthase and cellulose synthase. Our results showed increased expression of invertases in IN84-58, the genotype with lower sugar and higher fiber content, as well as delayed expression of secondary cell wall-related cellulose synthase for the other genotypes. Interestingly, genes involved with hormone metabolism were differentially expressed across time points in the three genotypes with higher soluble solids content. A similar result was observed for genes controlling maturation and transition to reproductive stages, possibly a result of selection against flowering in sugarcane breeding programs. These results indicate that carbon partitioning in apical culms of contrasting genotypes is mainly associated with differential cell wall biosynthesis, and may include early modifications for subsequent sucrose accumulation. Co-expression network analysis identified transcription factors related to growth and development, showing a probable time shift for carbon partitioning occurred in 10-month-old plants.

Keywords: *Saccharum*, Soluble solids, RNA-Seq, Differential Expression, Co-expression network

1 INTRODUCTION

Brazil is the main sugarcane producer in the world, followed by India, Thailand and China (FOOD AND AGRICULTURE ORGANIZATION, 2021). Sugarcane is the most efficient plant at accumulating sucrose (WACLAWOVSKY *ET AL.*, 2010) and is widely used as raw material for sugar and ethanol production. The bioethanol produced from sugarcane can reduce dependence on fossil fuels, which are limited resources responsible for increasing greenhouse gas emissions (FLUGGE *ET AL.*, 2017).

Currently used commercial sugarcane genotypes are hybrids, mainly between the species *S. officinarum* and *S. spontaneum*. The former was used because of its higher sugar content in its culms, and the latter for its robustness, resistance to diseases (CAMPOS and CALIGARI, 2017) and abiotic stress tolerance (ZHANG *ET AL.*, 2018). Following hybridization, successive backcrossing with *S. officinarum* was used to increase sugar content yield, a process called “nobilization” (PIPERIDIS and D’HONT, 2020). These noble canes exhibit lower fiber content, thicker culms and recalcitrant flowering when compared to *S. spontaneum* (KAFFKA and GRANTZ, 2014). Also, modern sugarcane cultivars (*Saccharum X officinarum*) are aneuploid and autopolyploid, with a basic ploidy level of 10x to 12x (PIPERIDIS and D’HONT, 2020), resulting in a total genome size of roughly 10 Gbp (D’HONT, 2005). Interspecific crossing has led to genotypes with chromosome numbers varying from 100 to 120, of which 80% come from *S. officinarum*, 10% from *S. spontaneum* and 10% from interspecific rearrangements (PIPERIDIS *ET AL.*, 2010).

Breeding for sugar accumulation has resulted in a sucrose content of 12-16% of the fresh weight and 50% in dry weight of mature culms (BINDON and BOTHA, 2002). The accumulation of sucrose in sugarcane culms occurs by stopping the active growth and elongation in some internodes (WHITTAKER and BOTHA, 1997). In mature culms, sucrose produced in mesophyll cells is unloaded to storage cells via the apoplast or symplast (RAE *ET AL.*, 2005), the latter being the most frequent type of transport. This symplastic pathway can follow two routes: translocation of sucrose into the cell mediated by sucrose transporters located in the plasma membrane (RAE *ET AL.*, 2005); or via sucrose hydrolyzation into fructose- 6-P and UDP-glucose by acid invertases. Sucrose phosphate synthase (SPS) subsequently converts these two products into sucrose-P, which is next converted into sucrose by the activity of sucrose phosphate phosphatase (SPP) (PARTIDA *ET AL.*, 2021). A peak in sucrose levels is achieved at the end of the vegetative cycle, when this carbohydrate is used during flowering and seed production (SLEWINSKI, 2012). For this reason, maturing and/or mature sugarcane culms are commonly used as material for the study of carbohydrate partitioning and metabolism related to sucrose accumulation (WHITTAKER and BOTHA, 1997; RAE *ET AL.*, 2005; VERMA *ET AL.*, 2011; THIRUGNANASAMBANDAM *ET AL.*, 2017, 2019). Immature culms arise from in apical meristems and, in turn, are responsible for increasing the height and width of sugarcane stalks by successive cell division (MOORE and BOTHA, 2014), providing a backbone for the plant. However, little is known about gene expression profiles early in the developmental process of sugarcane culms.

Immature (internode -1 to +3), intermediate (internode +5) and mature culms (internode +9 or more) were previously compared in terms of gene expression changes between different maturation stages (PAPINI-TERZI *ET AL.*, 2009; THIRUGNANASAMBANDAM *ET AL.*, 2017). PAPINI-TERZI *ET AL.* (2009) used cDNA microarrays to compare culms with low and high soluble solids content ($^{\circ}$ Brix), as well as mature culms vs. immature culms. Among the differentially expressed genes, the authors reported genes associated with hormone signaling, stress response, cell wall metabolism, calcium metabolism, protein kinases, protein phosphatases and transcription factors.

Gene co-expression networks can be used to provide complementary insights to differential expression analyses. These networks are based on pairwise correlations between the expression levels of genes across many samples, which are combined into a correlation matrix (LANGFELDER *ET AL.*, 2013).

From the correlations it is possible to identify expression profiles that can be related to certain biological processes (USADEL *ET AL.*, 2009). In this regard, a combination of gene expression profiles and co-expression analysis can provide more information about gene expression in apical culms of contrasting genotypes of sugarcane. In this study, we aimed to investigate the transcriptional profiles of immature sugarcane culms across time in one wild *S. spontaneum* accession and three sugarcane hybrids with varying contents of soluble solids.

2 CONCLUSIONS

The accumulation of sucrose in the culms of sugarcane is controlled by a complex network of genes involved in the hydrolysis, re-synthesis and transport of sucrose, cell wall biosynthesis and abiotic stress responses. Here we found that invertases, SWEET13, SuSy1, SuSy3, probable CAD1 and CAD6 are likely related to early processes associated with carbon partitioning in apical culms. Moreover, we found evidence of particular TFs as important switches in processes related to sugarcane growth and development in early stages of maturation, as well as a probable time shift for carbon partitioning at ten months. Additionally, the TFs ARF23-like, ARF25, EIN2-like and TS6 were upregulated only in hybrids, a possible reflection of selection in sugarcane breeding programs. Although multiple studies have been developed in sugarcane, there are still many gaps in completely understanding how it accumulates sucrose in its culms. In this regard, our work provides useful gene expression profiles in apical culms of different genotypes, which can be used as genetic biomarkers for further studies.

REFERENCES

- ALTSCHUL, S. F., W. GISH, W. MILLER, E. W. MYERS, and D. J. LIPMAN, 1990 Basic local alignment search tool. *Journal of Molecular Biology* **215**: 403–410.
- ANDREWS, S., 2010 FastQC: a quality control tool for high throughput sequence data. .
- ANSARI, M. I., A. YADAV, and R. LAL, 2013 An-Overview on invertase in sugarcane. *Bioinformatics* **9**: 464–465.
- APPENZELLER, L., M. DOBLIN, R. BARREIRO, H. WANG, X. NIU, K. KOLLIPARA, L. CARRIGAN, D. TOMES, M. CHAPMAN, and K. S. DHUGGA, 2004 Cellulose synthesis in maize: isolation and expression analysis of the cellulose synthase (CesA) gene family. *Cellulose* **11**: 287–299.
- BAO, Y., W. M. SONG, J. PAN, C. M. JIANG, R. SRIVASTAVA, B. LI, L. Y. ZHU, H. Y. SU, X. S. GAO, H. LIU, X. YU, L. YANG, X. H. CHENG, and H. X. ZHANG, 2016 Overexpression of the NDR1/HIN1-Like gene NHL6 modifies seed germination in response to abscisic acid and abiotic stresses in arabidopsis. *PLoS ONE* **11**: 1–16.
- BARI, R. and J. D. G. JONES, 2009 Role of plant hormones in plant defence responses. *Plant molecular biology* **69**: 473–88.
- BARRETO, F. Z., J. R. BACHEGA FEIJÓ ROSA, T. W. ALMEIDA BALSALOBRE, M. M. PASTINA, R. R. SILVA, H. P. HOFFMANN, A. P. DE SOUZA, A. A. FRANCO GARCIA, and M. S. CARNEIRO, 2019 A genome-wide association study identified loci for yield component traits in sugarcane (*Saccharum* spp.). *PLoS ONE* **14**: e0219843.
- BENJAMINI, Y. and Y. HOCHBERG, 1995 Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)* **57**: 289–300.
- BERARDINI, T. Z., K. BOLLMAN, H. SUN, and R. SCOTT POETHIG, 2001 Regulation of vegetative phase change in arabidopsis thaliana by cyclophilin 40. *Science* **291**: 2405–2407.
- BINDON, K. A. and F. C. BOTHA, 2002 Carbon allocation to the insoluble fraction, respiration and triose-phosphate cycling in the sugarcane culm. *Physiologia Plantarum* **116**: 12–19.
- BOLGER, A. M., M. LOHSE, and B. USADEL, 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114–2120.
- BOTHA, F. C. and K. G. BLACK, 2000 Sucrose phosphate synthase and sucrose synthase activity during maturation of internodal tissue in sugarcane. *Australian Journal of Plant Physiology* **27**: 81–85.
- CAMPOS, H. and P. D. CALIGARI, 2017 *Genetic improvement of tropical crops*.
- CARSON, D. and F. BOTHA, 2002 Genes expressed in sugarcane maturing internodal tissue. *Plant Cell Reports* **20**: 1075–1081.
- CASU, R. E., C. P. L. GROF, A. L. RAE, C. L. MCINTYRE, C. M. DIMMOCK, and J. M. MANNERS, 2003 Identification of a novel sugar transporter homologue strongly expressed in maturing stem vascular tissues of sugarcane by expressed sequence tag and microarray analysis. *Plant molecular biology* **52**: 371–86.

- CASU, R. E., J. M. JARMEY, G. D. BONNETT, and J. M. MANNERS, 2007 Identification of transcripts associated with cell wall metabolism and development in the stem of sugarcane by Affymetrix GeneChip Sugarcane Genome Array expression profiling. *Functional and Integrative Genomics* **7**: 153–167.
- CASU, R. E., A. L. RAE, J. M. NIELSEN, J. M. PERROUX, G. D. BONNETT, and J. M. MANNERS, 2015 Tissue-specific transcriptome analysis within the maturing sugarcane stalk reveals spatial regulation in the expression of cellulose synthase and sucrose transporter gene families. *Plant Molecular Biology* **89**: 607–628.
- CHABANNES, M., K. RUEL, A. YOSHINAGA, B. CHABBERT, A. JAUNEAU, J. P. JOSELEAU, and A. M. BOUDET, 2001 In situ analysis of lignins in transgenic tobacco reveals a differential impact of individual transformations on the spatial patterns of lignin deposition at the cellular and subcellular levels. *Plant Journal* **28**: 271–282.
- CHIN, C. H., S. H. CHEN, H. H. WU, C. W. HO, M. T. KO, and C. Y. LIN, 2014 cytoHubba: Identifying hub objects and sub-networks from complex interactome. *BMC Systems Biology* **8**.
- CHOI, H., J. HONG, J. HA, J. KANG, and S. Y. KIM, 2000 ABFs, a family of ABA-responsive element binding factors. *The Journal of biological chemistry* **275**: 1723–30.
- CONESA, A., S. GÖTZ, J. M. GARCÍA-GÓMEZ, J. TEROL, M. TALÓN, and M. ROBLES, 2005 Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **21**: 3674–3676.
- CORRER, F. H., G. K. HOSAKA, S. G. P. GÓMEZ, M. C. CIA, C. B. M. VITORELLO, L. E. A. CAMARGO, N. S. MASSOLA, M. S. CARNEIRO, and G. R. A. MARGARIDO, 2020 Time-series expression profiling of sugarcane leaves infected with *Puccinia kuehnii* reveals an ineffective defense system leading to susceptibility. *Plant cell reports* **39**: 873–889.
- COUÉE, I., C. SULMON, G. GOUESBET, and A. EL AMRANI, 2006 Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *Journal of Experimental Botany* **57**: 449–459.
- CUNHA, C. P., G. G. ROBERTO, R. VICENTINI, C. G. LEMBKE, G. M. SOUZA, R. V. RIBEIRO, E. C. MACHADO, A. M. LAGÔA, and M. MENOSSI, 2017 Ethylene-induced transcriptional and hormonal responses at the onset of sugarcane ripening. *Scientific Reports* **7**: 1–18.
- DELATTE, T. L., P. SEDIJANI, Y. KONDOU, M. MATSUI, G. J. DE JONG, G. W. SOMSEN, A. WIESEKLINKENBERG, L. F. PRIMAVESI, M. J. PAUL, and H. SCHLUEPMANN, 2011 Growth arrest by trehalose-6-phosphate: An astonishing case of primary metabolite control over growth by way of the SnRK1 signaling pathway. *Plant Physiology* **157**: 160–174.
- DEVAIAH, B. N. and K. G. RAGHOTHAMA, 2007 Transcriptional regulation of Pi starvation responses by WRKY75. *Plant Signaling and Behavior* **2**: 424–425.
- D'HONT, A., 2005 Unraveling the genome structure of polyploids using FISH and GISH; examples of sugarcane and banana. *Cytogenetic and Genome Research* **109**: 27–33.
- DROGE-LASER, W., A. KAISER, W. P. LINDSAY, B. A. HALKIER, G. J. LOAKE, P. DOERNER, R. A. DIXON, and C. LAMB, 1997 Rapid stimulation of a soybean protein-serine kinase that phosphorylates a novel bZIP DNA-binding protein, G/HBF-1, during the induction of early transcription-dependent defenses. *The EMBO Journal* **16**: 726–738.

- EASTMOND, P. J., A. J. VAN DIJKEN, M. SPIELMAN, A. KERR, A. F. TISSIER, H. G. DICKINSON, J. D. JONES, S. C. SMEEKENS, and I. A. GRAHAM, 2002 Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for *Arabidopsis* embryo maturation. *Plant Journal* **29**: 225–235.
- FLUGGE, M., J. LEWANDROWSKI, J. ROSENFELD, C. BOLAND, T. HENDRICKSON, M. . FLUGGE, J. . LEWANDROWSKI, and J. . ROSENFELD, 2017 A Life-Cycle Analysis of the Greenhouse Gas Emissions of Corn-Based Ethanol. *Industrial Biotechnology* **13**: 19–22.
- FOOD AND AGRICULTURE ORGANIZATION, 2021 FAOSTAT.
- GANG WANG, J., T. TING ZHAO, W. ZHI WANG, C. LIAN FENG, X. YAN FENG, G. RU XIONG, L. BO SHEN, S. ZHEN ZHANG, W. QUAN WANG, and Z. XING ZHANG, 2019 Culm transcriptome sequencing of *Badila* (*Saccharum officinarum* L.) and analysis of major genes involved in sucrose accumulation. *Plant Physiology and Biochemistry* **144**: 455–465.
- GARG, A. K., J. K. KIM, T. G. OWENS, A. P. RANWALA, Y. DO CHOI, L. V. KOCHIAN, and R. J. WU, 2002 Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proceedings of the National Academy of Sciences of the United States of America* **99**: 15898–15903.
- GARSMEUR, O., G. DROC, R. ANTONISE, J. GRIMWOOD, B. POTIER, K. AITKEN, J. JENKINS, G. MARTIN, C. CHARRON, C. HERVOUET, L. COSTET, N. YAHIAOUI, A. HEALEY, D. SIMS, Y. CHERUKURI, A. SREEDASYAM, A. KILIAN, A. CHAN, M.-A. A. VAN SLUYS, K. SWAMINATHAN, C. TOWN, H. BERGÈS, B. SIMMONS, J. C. GLASZMANN, E. VAN DER VOSSEN, R. HENRY, J. SCHMUTZ, A. D’HONT, and A. D’HONT, 2018 A mosaic monoploid reference sequence for the highly complex genome of sugarcane. *Nature Communications* **9**: 2638.
- GOLDNER, W., M. THOM, and A. MARETZKI, 1991 Sucrose metabolism in sugarcane cell suspension cultures. *Plant Science* **73**: 143–147.
- GONG, X., Q. SU, D. LIN, Q. JIANG, J. XU, J. ZHANG, S. TENG, and Y. DONG, 2014 The rice OsV4 encoding a novel pentatricopeptide repeat protein is required for chloroplast development during the early leaf stage under cold stress. *Journal of Integrative Plant Biology* **56**: 400–410.
- GOODSTEIN, D. M., S. SHU, R. HOWSON, R. NEUPANE, R. D. HAYES, J. FAZO, T. MITROS, W. DIRKS, U. HELLSTEN, N. PUTNAM, and D. S. ROKHSAR, 2012 Phytozome: A comparative platform for green plant genomics. *Nucleic Acids Research* **40**.
- GRABHERR, M. G., B. J. HAAS, M. YASSOUR, J. Z. LEVIN, D. A. THOMPSON, I. AMIT, X. ADICONIS, L. FAN, R. RAYCHOWDHURY, Q. ZENG, Z. CHEN, E. MAUCELI, N. HACOEN, A. GNIRKE, N. RHIND, F. DI PALMA, B. W. BIRREN, C. NUSBAUM, K. LINDBLAD-TOH, N. FRIEDMAN, and A. REGEV, 2011 Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature biotechnology* **29**: 644–52.
- GRANDBASTIEN, M. A., 1998 Activation of plant retrotransposons under stress conditions.
- GU, Y. and C. SOMERVILLE, 2010 Cellulose synthase interacting protein: A new factor in cellulose synthesis.
- GUILFOYLE, T. J. and G. HAGEN, 2001 Auxin response factors.
- GUILFOYLE, T. J. and G. HAGEN, 2007 Auxin response factors.

- HAGEN, G. and T. GUILFOYLE, 2002 Auxin-responsive gene expression: Genes, promoters and regulatory factors. *Plant Molecular Biology* **49**: 373–385.
- HICHRI, I., F. BARRIEU, J. BOGS, C. KAPPEL, S. DELROT, and V. LAUVERGEAT, 2011 Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. *Journal of Experimental Botany* **62**: 2465–2483.
- HOBO, T., Y. KOWYAMA, and T. HATTORI, 1999 A bZIP factor, TRAB1, interacts with VP1 and mediates abscisic acid- induced transcription. *Proceedings of the National Academy of Sciences of the United States of America* **96**: 15348–15353.
- HU, W., X. HUA, Q. ZHANG, J. WANG, Q. SHEN, X. ZHANG, K. WANG, Q. YU, Y.-R. R. LIN, R. MING, and J. ZHANG, 2018 New insights into the evolution and functional divergence of the SWEET family in *Saccharum* based on comparative genomics. *BMC Plant Biology* **18**: 270.
- IRISH, E. E., 1997 Experimental analysis of Tassel development in the maize mutant Tassel seed 6. *Plant Physiology* **114**: 817–825.
- JADOSKI, C. J. and E. O. ONO, 2011 Physiology aspects of sugarcane production. *Applied Research & Agrotechnology* **3**.
- JIN, J., F. TIAN, D.-C. YANG, Y.-Q. MENG, L. KONG, J. LUO, and G. GAO, 2017 PlantTFDB 4.0 : toward a central hub for transcription factors and regulatory interactions in plants. *Nucleic Acids Research* **45**: 1040–1045.
- JU, C. and C. CHANG, 2015 Mechanistic insights in ethylene perception and signal transduction. *Plant Physiology* **169**: 85–95.
- JUN, S. H., M. J. HAN, S. LEE, Y. S. SEO, W. T. KIM, and G. AN, 2004 OsEIN2 is a positive component in ethylene signaling in rice. *Plant and Cell Physiology* **45**: 281–289.
- JUNG, C., S. S. JUN, W. H. SANG, J. K. YEON, H. K. CHUNG, I. S. SANG, H. N. BAEK, D. C. YANG, and J. J. CHEONG, 2008 Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic *Arabidopsis*. *Plant Physiology* **146**: 623–635.
- KAFFKA, S. R. and D. A. GRANTZ, 2014 Sugar Crops. *Encyclopedia of Agriculture and Food Systems* **5**: 240–260.
- KASIRAJAN, L., N. V. HOANG, A. FURTADO, F. C. BOTHA, and R. J. HENRY, 2018 Transcriptome analysis highlights key differentially expressed genes involved in cellulose and lignin biosynthesis of sugarcane genotypes varying in fiber content. *Scientific Reports* **8**: 11612.
- KIDWELL, M. G., 2002 Transposable elements and the evolution of genome size in eukaryotes. Technical report.
- KIM, D., B. LANGMEAD, and S. L. SALZBERG, 2015 HISAT: A fast spliced aligner with low memory requirements. *Nature Methods* **12**: 357–360.
- LANGFELDER, P. and S. HORVATH, 2008 WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics* **9**: 559.
- LANGFELDER, P., P. S. MISCHER, and S. HORVATH, 2013 When Is Hub Gene Selection Better than Standard Meta-Analysis? *PLoS ONE* **8**: e61505.

- LEE, S.-I. and N.-S. KIM, 2014 Transposable Elements and Genome Size Variations in Plants. *Genomics & Informatics* **12**: 87.
- LEMOINE, R., S. L. CAMERA, R. ATANASSOVA, F. DÉDALDÉCHAMP, T. ALLARIO, N. POURTAU, J.-L. L. BONNEMAIN, M. LALOI, P. COUTOS-THÉVENOT, L. MAUROUSSET, M. FAUCHER, C. GIROUSSE, P. LEMONNIER, J. PARRILLA, M. DURAND, S. LA CAMERA, R. ATANASSOVA, F. DÉDALDÉCHAMP, T. ALLARIO, N. POURTAU, J.-L. L. BONNEMAIN, M. LALOI, P. COUTOS-THÉVENOT, L. MAUROUSSET, M. FAUCHER, C. GIROUSSE, P. LEMONNIER, J. PARRILLA, and M. DURAND, 2013 Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in Plant Science* **4**: 272.
- LI, G., H. SIDDIQUI, Y. TENG, R. LIN, X. Y. WAN, J. LI, O. S. LAU, X. OUYANG, M. DAI, J. WAN, P. F. DEVLIN, X. W. DENG, and H. WANG, 2011 Coordinated transcriptional regulation underlying the circadian clock in Arabidopsis. *Nature Cell Biology* **13**: 616–622.
- LIAO, Y., G. K. SMYTH, and W. SHI, 2014 FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**: 923–930.
- LINGLE, S. E. and R. C. SMITH, 1991 Sucrose Metabolism Related to Growth and Ripening in Sugarcane Internodes. *Crop Science* **31**: 172.
- MA, L., T. TIAN, R. LIN, X. W. DENG, H. WANG, and G. LI, 2016 Arabidopsis FHY3 and FAR1 Regulate Light-Induced myo-Inositol Biosynthesis and Oxidative Stress Responses by Transcriptional Activation of MIPS1. *Molecular Plant* **9**: 541–557.
- MAO, G., T. SEEBECK, D. SCHRENKER, and O. YU, 2013 CYP709B3, a cytochrome P450 monooxygenase gene involved in salt tolerance in Arabidopsis thaliana. *BMC Plant Biology* **13**.
- MATTIELLO, L., D. M. RIAÑO-PACHÓN, M. C. M. MARTINS, L. P. DA CRUZ, D. BASSI, P. E. R. MARCHIORI, R. V. RIBEIRO, M. T. V. LABATE, C. A. LABATE, and M. MENOSSE, 2015 Physiological and transcriptional analyses of developmental stages along sugarcane leaf. *BMC plant biology* **15**: 300.
- MEDEIROS, C., T. W. ALMEIDA BALSALOBRE, and M. S. CARNEIRO, 2020 Molecular diversity and genetic structure of Saccharum complex accessions. *PLoS ONE* **15**: 1–17.
- MING, R., P. H. MOORE, K. K. WU, A. D'HONT, J. C. GLASZMANN, T. L. TEW, T. E. MIRKOV, J. DA SILVA, J. JIFON, M. RAI, R. J. SCHNELL, S. M. BRUMBLEY, P. LAKSHMANAN, J. C. COMSTOCK, and A. H. PATERSON, 2010 *Sugarcane Improvement through Breeding and Biotechnology*, volume 27.
- MITCHELL, H. J., J. L. HALL, and M. S. BARBER, 1994 Elicitor-Induced Cinnamyl Alcohol Dehydrogenase Activity in Lignifying Wheat (*Triticum aestivum* L.) Leaves. *Plant physiology* **104**: 551–556.
- MOCKAITIS, K. and M. ESTELLE, 2004 Integrating transcriptional controls for plant cell expansion.
- MOORE, P. and F. BOTHA, 2014 *Sugarcane : Physiology , Biochemistry , and*.
- MOORE, P. H. and D. J. COSGROVE, 1991 Developmental changes in cell and tissue water relations parameters in storage parenchyma of sugarcane. *Plant Physiology* **96**: 794–801.
- NODA, K. I., B. J. GLOVER, P. LINSTED, and C. MARTIN, 1994 Flower colour intensity depends on specialized cell shape controlled by a Myb-related transcription factor. *Nature* **369**: 661–664.

- NOVER, L., K. BHARTI, P. DO, S. K. MISHRA, and A. GANGULI, 2001 Arabidopsis and the heat stress transcription factor world : how many heat stress transcription factors do we need ? Cell Stress Chaperones **6**: 177–189.
- OGAWARA, T., K. HIGASHI, H. KAMADA, and H. EZURA, 2003 Ethylene advances the transition from vegetative growth to flowering in Arabidopsis thaliana. Journal of Plant Physiology **160**: 1335–1340.
- ONODERA, Y., A. SUZUKI, C. Y. WU, H. WASHIDA, and F. TAKAIWA, 2001 A rice functional transcriptional activator, RISBZ1, responsible for endosperm-specific expression of storage protein genes through GCN4 motif. The Journal of biological chemistry **276**: 14139–52.
- PAPINI-TERZI, F. S., F. R. ROCHA, R. Z. N. VÊNICO, J. M. FELIX, D. S. BRANCO, A. J. WACLAWOVSKY, L. E. V. DEL BEM, C. G. LEMBKE, M. D. L. COSTA, M. Y. NISHIYAMA, R. VICENTINI, M. G. A. VINCENTZ, E. C. ULIAN, M. MENOSSE, and G. M. SOUZA, 2009 Sugarcane genes associated with sucrose content. BMC Genomics **10**: 120.
- PARTIDA, V. G. S., H. M. DIAS, D. S. M. CORCINO, and M. A. VAN SLUYS, 2021 Sucrose-phosphate phosphatase from sugarcane reveals an ancestral tandem duplication. BMC Plant Biology **21**: 1–13.
- PATRO, R., G. DUGGAL, M. I. LOVE, R. A. IRIZARRY, and C. KINGSFORD, 2017 Salmon provides fast and bias-aware quantification of transcript expression. Nature Methods **14**: 417–419.
- PIPERIDIS, G., N. PIPERIDIS, and A. D'HONT, 2010 Molecular cytogenetic investigation of chromosome composition and transmission in sugarcane. Molecular Genetics and Genomics **284**: 65–73.
- PIPERIDIS, N. and A. D'HONT, 2020 Sugarcane genome architecture decrypted with chromosome-specific oligo probes. Plant Journal **103**: 2039–2051.
- PREMACHANDRAN, M. N., P. T. PRATHIMA, and M. LEKSHMI, 2011 Sugarcane and polyploidy - A review. Journal of Sugarcane Research **1**: 1–15.
- RAE, A. L., C. P. GROF, R. E. CASU, and G. D. BONNETT, 2005 Sucrose accumulation in the sugarcane stem: Pathways and control points for transport and compartmentation. In *Field Crops Research*, volume 92, pp. 159–168, Elsevier.
- RANOCHA, P., N. DENANCÉ, R. VANHOLME, A. FREYDIER, Y. MARTINEZ, L. HOFFMANN, L. KÖHLER, C. POUZET, J. P. RENOU, B. SUNDBERG, W. BOERJAN, and D. GOFFNER, 2010 Walls are thin 1 (WAT1), an Arabidopsis homolog of Medicago truncatula NODULIN21, is a tonoplast-localized protein required for secondary wall formation in fibers. Plant Journal **63**: 469–483.
- ROBINSON, M. D., D. J. MCCARTHY, and G. K. SMYTH, 2010 edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics (Oxford, England) **26**: 139–140.
- SAUTER, M. and H. KENDE, 1992 Levels of β -glucan and lignin in elongating internodes of deepwater rice. Plant and Cell Physiology **33**: 1089–1097.
- SEGARRA, G., S. VAN DER ENT, I. TRILLAS, and C. M. PIETERSE, 2009 MYB72, a node of convergence in induced systemic resistance triggered by a fungal and a bacterial beneficial microbe. Plant Biology **11**: 90–96.
- SHANNON, P., A. MARKIEL, O. OZIER, N. S. BALIGA, J. T. WANG, D. RAMAGE, N. AMIN, B. SCHWIKOWSKI, and T. IDEKER, 2003 Cytoscape: A software Environment for integrated models of biomolecular interaction networks. Genome Research **13**: 2498–2504.

- SIBOUT, R., A. EUDES, G. MOUILLE, B. POLLET, C. LAPIERRE, L. JOUANIN, and A. SÉGUIN, 2005 Cinnamyl alcohol dehydrogenase-C and -D are the primary genes involved in lignin biosynthesis in the floral stem of arabidopsis. *Plant Cell* **17**: 2059–2076.
- SIMÃO, F. A., R. M. WATERHOUSE, P. IOANNIDIS, E. V. KRIVENTSEVA, and E. M. ZDOBNOV, 2015 BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31**: 3210–3212.
- SLEWINSKI, T. L., 2012 Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance, and biofuel production. *Journal of Experimental Botany* **63**: 4647–4670.
- SUNG, S. J., D. P. XU, and C. C. BLACK, 1989 Identification of actively filling sucrose sinks. *Plant physiology* **89**: 1117–21.
- SUNG, T. Y., C. C. TSENG, and M. H. HSIEH, 2010 The SLO1 PPR protein is required for RNA editing at multiple sites with similar upstream sequences in Arabidopsis mitochondria. *Plant Journal* **63**: 499–511.
- TAYLOR, N. G., R. M. HOWELLS, A. K. HUTTLY, K. VICKERS, and S. R. TURNER, 2003 Interactions among three distinct CesA proteins essential for cellulose synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 1450–1455.
- THIRUGNANASAMBANDAM, P. P., N. V. HOANG, A. FURTADO, F. C. BOTHA, and R. J. HENRY, 2017 Association of variation in the sugarcane transcriptome with sugar content. *BMC Genomics* **18**.
- THIRUGNANASAMBANDAM, P. P., P. J. MASON, N. V. HOANG, A. FURTADO, F. C. BOTHA, and R. J. HENRY, 2019 Analysis of the diversity and tissue specificity of sucrose synthase genes in the long read transcriptome of sugarcane. *BMC Plant Biology* **19**: 160.
- TOBIAS, C. M. and E. K. CHOW, 2005 Structure of the cinnamyl-alcohol dehydrogenase gene family in rice and promoter activity of a member associated with lignification. *Planta* **220**: 678–688.
- TOKUNAGA, N., T. KANETA, S. SATO, and Y. SATO, 2009 Analysis of expression profiles of three peroxidase genes associated with lignification in Arabidopsis thaliana. *Physiologia Plantarum* **136**: 237–249.
- USADEL, B., T. OBAYASHI, M. MUTWIL, F. M. GIORGI, G. W. BASSEL, M. TANIMOTO, A. CHOW, D. STEINHAUSER, S. PERSSON, and N. J. PROVART, 2009 Co-expression tools for plant biology: Opportunities for hypothesis generation and caveats. *Plant, Cell and Environment* **32**: 1633–1651.
- VAN DIJKEN, A. J., H. SCHLUEPMANN, and S. C. SMEEKENS, 2004 Arabidopsis trehalose-6-phosphate synthase 1 is essential for normal vegetative growth and transition to flowering. *Plant Physiology* **135**: 969–977.
- VAN HA, C., D. T. LE, R. NISHIYAMA, Y. WATANABE, S. SULIEMAN, U. T. TRAN, K. MOCHIDA, N. VAN DONG, K. YAMAGUCHI-SHINOZAKI, K. SHINOZAKI, and L. S. P. TRAN, 2013 The auxin response factor transcription factor family in soybean: Genome-wide identification and expression analyses during development and water stress. *DNA Research* **20**: 511–524.
- VATÉN, A., J. DETTMER, S. WU, Y. D. STIERHOF, S. MIYASHIMA, S. R. YADAV, C. J. ROBERTS, A. CAMPILHO, V. BULONE, R. LICHTENBERGER, S. LEHESRANTA, A. P. MÄHÖNEN, J. Y. KIM, E. JOKITALO, N. SAUER, B. SCHERES, K. NAKAJIMA, A. CARLSBECKER, K. L. GALLAGHER, and

- Y. HELARIUTTA, 2011 Callose Biosynthesis Regulates Symplastic Trafficking during Root Development. *Developmental Cell* **21**: 1144–1155.
- VERMA, A. K., S. K. UPADHYAY, P. C. VERMA, S. SOLOMON, and S. B. SINGH, 2011 Functional analysis of sucrose phosphate synthase (SPS) and sucrose synthase (SS) in sugarcane (*Saccharum*) cultivars. *Plant Biology* **13**: 325–332.
- VICENTINI, R., A. BOTTCHER, M. DOS SANTOS BRITO, A. B. DOS SANTOS, S. CRESTE, M. G. DE ANDRADE LANDELL, I. CESARINO, and P. MAZZAFERA, 2015 Large-scale transcriptome analysis of two sugarcane genotypes contrasting for lignin content. *PLoS ONE* **10**: 1–19.
- WACLAWOVSKY, A. J., P. M. SATO, C. G. LEMBKE, P. H. MOORE, and G. M. SOUZA, 2010 Sugarcane for bioenergy production: An assessment of yield and regulation of sucrose content. *Plant Biotechnology Journal* **8**: 263–276.
- WAESE, J., J. FAN, A. PASHA, H. YU, G. FUCILE, R. SHI, M. CUMMING, L. A. KELLEY, M. J. STERNBERG, V. KRISHNAKUMAR, E. FERLANTI, J. MILLER, C. TOWN, W. STUERZLINGER, and N. J. PROVART, 2017 ePlant: Visualizing and exploring multiple levels of data for hypothesis generation in plant biology. *Plant Cell* **29**: 1806–1821.
- WANG, L., Z. FENG, X. WANG, X. WANG, and X. ZHANG, 2010 DEGseq: An R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* **26**: 136–138.
- WANG, L. and Y.-L. RUAN, 2013 Regulation of cell division and expansion by sugar and auxin signaling. *Frontiers in Plant Science* **4**: 163.
- WANG, W., W. TANG, T. MA, D. NIU, J. B. JIN, H. WANG, and R. LIN, 2016 A pair of light signaling factors *FHY3* and *FAR1* regulates plant immunity by modulating chlorophyll biosynthesis. *Journal of Integrative Plant Biology* **58**: 91–103.
- WHITTAKER, A. and F. C. BOTHA, 1997 Carbon Partitioning during Sucrose Accumulation in Sugarcane Internodal Tissue. *Plant Physiology* **115**: 1651–1659.
- XIANG, C., Z. MIAO, and E. LAM, 1997 DNA-binding properties, genomic organization and expression pattern of *TGA6*, a new member of the TGA family of bZIP transcription factors in *Arabidopsis thaliana*. *Plant Molecular Biology* **34**: 403–415.
- YILMAZ, A., M. Y. NISHIYAMA, B. G. FUENTES, G. M. SOUZA, D. JANIES, J. GRAY, and E. GROTEWOLD, 2009 GRASSIUS: A platform for comparative regulatory genomics across the grasses. *Plant Physiology* **149**: 171–180.
- YOUNG, M. D., M. J. WAKEFIELD, G. K. SMYTH, and A. OSHLACK, 2010 Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biology* **11**: R14.
- YUAN, Y., X. XU, Z. GONG, Y. TANG, M. WU, F. YAN, X. ZHANG, Q. ZHANG, F. YANG, X. HU, Q. YANG, Y. LUO, L. MEI, W. ZHANG, C. Z. JIANG, W. LU, Z. LI, and W. DENG, 2019 Auxin response factor 6A regulates photosynthesis, sugar accumulation, and fruit development in tomato. *Horticulture Research* **6**: 1–16.
- ZHANG, J., X. ZHANG, H. TANG, Q. ZHANG, X. HUA, X. MA, F. ZHU, T. JONES, X. ZHU, J. BOWERS, C. M. WAI, C. ZHENG, Y. SHI, S. CHEN, X. XU, J. YUE, D. R. NELSON, L. HUANG, Z. LI, H. XU, D. ZHOU, Y. WANG, W. HU, J. LIN, Y. DENG, N. PANDEY, M. MANCINI, D. ZERPA,

J. K. NGUYEN, L. WANG, L. YU, Y. XIN, L. GE, J. ARRO, J. O. HAN, S. CHAKRABARTY, M. PUSHKO, W. ZHANG, Y. MA, P. MA, M. LV, F. CHEN, G. ZHENG, J. XU, Z. YANG, F. DENG, X. CHEN, Z. LIAO, X. ZHANG, Z. LIN, H. LIN, H. YAN, Z. KUANG, W. ZHONG, P. LIANG, G. WANG, Y. YUAN, J. SHI, J. HOU, J. LIN, J. JIN, P. CAO, Q. SHEN, Q. JIANG, P. ZHOU, Y. MA, X. ZHANG, R. XU, J. LIU, Y. ZHOU, H. JIA, Q. MA, R. QI, Z. ZHANG, J. FANG, H. FANG, J. SONG, M. WANG, G. DONG, G. WANG, Z. CHEN, T. MA, H. LIU, S. R. DHUNGANA, S. E. HUSS, X. YANG, A. SHARMA, J. H. TRUJILLO, M. C. MARTINEZ, M. HUDSON, J. J. RIASCOS, M. SCHULER, L. Q. CHEN, D. M. BRAUN, L. LI, Q. YU, J. WANG, K. WANG, M. C. SCHATZ, D. HECKERMAN, M. A. VAN SLUYS, G. M. SOUZA, P. H. MOORE, D. SANKOFF, R. VANBUREN, A. H. PATERSON, C. NAGAI, and R. MING, 2018 Allele-defined genome of the autopolyploid sugarcane *Saccharum spontaneum* L. *Nature Genetics* **50**: 1565–1573.

ZHU, Y. J., E. KOMOR, and P. H. MOORE, 1997 Sucrose accumulation in the sugarcane stem is regulated by the difference between the activities of soluble acid invertase and sucrose phosphate synthase. *Plant Physiology* **115**: 609–616.

ZOU, M., Y. GUAN, H. REN, F. ZHANG, and F. CHEN, 2008 A bZIP transcription factor, OsABI5, is involved in rice fertility and stress tolerance. *Plant Molecular Biology* **66**: 675–683.