

## CONTROL OF LIGNIN BIOSYNTHESIS IN SUGARCANE: MANY GAPS STILL TO BE FILLED

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FAPESP Process 2008/58035-6 | Term: Sep 2009 to Aug 2014 | Thematic Project

Although significant knowledge on lignin in plants has been obtained, we still do not know to which extent plants can survive without this polymer. Lignin content may vary in response to several biotic and abiotic stresses and understanding how this occurs may help to understand the control of lignin biosynthesis. We know “almost nothing” about lignin in sugarcane. However, taking in account the information accumulated for other plants and the agronomical practices and problems in sugarcane cultivation, we may have enough hints to plan several studies on how sugarcane modulates lignin composition and content. Therefore, the aim of this project is 1) to cultivate contrasting sugarcane genetic material for lignin content in 5 locations well characterized for temperature, water availability and irradiance and analyze lignin, sucrose and cellulose, and then, based on these results to study gene expression and perform a more detailed study of lignin composition; 2) to search the SUCEST database for ESTs coding transcription factors known to be involved in lignin metabolism in model plants and use this information in controlled studies (on water supply, nitrogen fertilization, light intensity and low temperatures under field and greenhouse conditions, and growth chamber) to establish correlations between transcription factors regulation and lignin content; 3) search the SUCEST database for ESTs coding orthologs to peroxidases and laccases and use this information in the controlled studies to evaluate the involvement of these enzymes in lignin biosynthesis; 4) to perform a system biology study of regulatory network involved in lignin biosynthesis. With this information we may get some valuable knowledge on the lignin biosynthesis in the complex sugarcane genome.

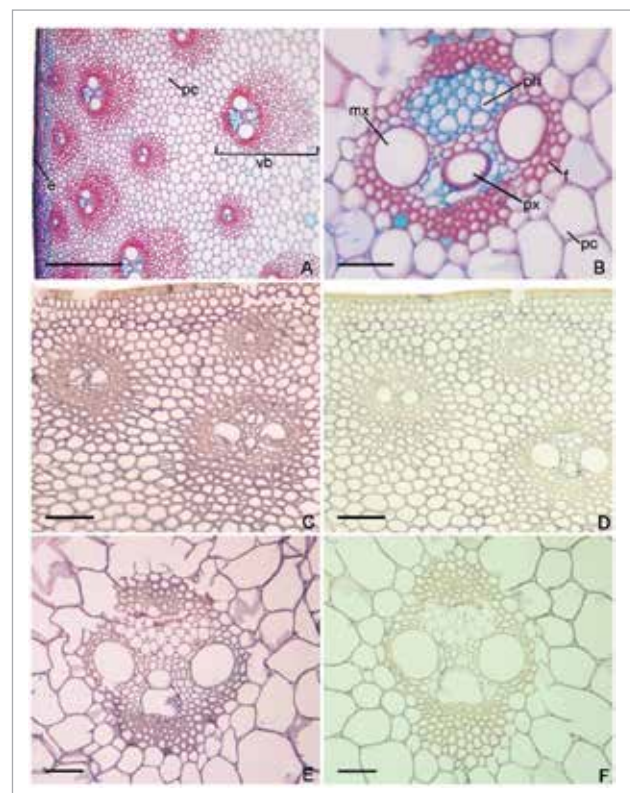


Figure 1. Anatomic cuts of a sugarcane stalk showing stained vessels for lignin (A and B) and in situ expression of dirigent proteins (C-F).

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The main result of this BIOEN project was the identification of 6 genes that can be used to produce genetic modified sugarcane for lower or modified lignin. They were two of the lignin biosynthetic pathway – ferulate 5-hydroxylase (F5H), hydroxycinnamoyl transferase (HCT) – a dirigent protein – DIR1 – and two transcription factors – NHS and MYB58. Other very important result was the realization that there is variation in the ratio S/G in the pith and rind of the sugarcane stem during its development. This was initially observed in two contrasting genotypes for lignin growing in the greenhouse and then in four genotypes grown in the field. This shows that during cane ripening S are mainly synthesized in the cortical cells, which is desirable, since lignin with S units is more easily removed by chemical treatments, increasing the efficiency to obtain cellulose. This result defined the choice of the F5H gene, which is in the unit S biosynthetic branch of lignin pathway, as well as transcription factor MYB58, which showed strong correlation with the expression of F5H. It is worth mentioning that the control F5H by MYB58 bypasses the control or interaction with other transcription factors. In the rind, either on new stems or mature culms, S/G ratio remains low. Thus, as a consequence the following questions become very important: In relative terms, how important is the rind and pith lignin in the total lignin content of the sugarcane? What is the amount found in both tissues and this would affect saccharification?

The control of gene expression in the pith is certainly different of the cortex, since they have different S/G ratio, but would be feasible to manipulate gene expression in a way that S/G was also high in the rind? Transformed sugarcane for F5H and MYB58 gene could provide an answer to this. The project also allowed developing methods for determination of lignin oligomers and S/G ratio.

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