Cell wall proteins identified in sugarcane young and mature culm internodes: a focus on lipid transfer proteins (LTPs)

Maria Juliana Calderan-Rodrigues[1], Juliana Fonseca Guimarães[1], Elisabeth Jamet[2,3], Thibaut Douché[2,3], Maria Beatriz Rodrigues Bonassi[1], Thaís Regiani Cataldi[1], Fabricio Edgar de Moraes[1], Hélène San Clemente[2,3], Rafael Pont-Lezica † [2,3], Carlos Alberto Labate[1]

calabate@usp.br

[1] Departamento de Genética, Laboratório Max Feffer de Genética de Plantas, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Av. Pádua Dias 11, CP 83, 13400-970, Piracicaba-SP, Brazil.
[2.] Université de Toulouse; UPS; UMR 5546, Laboratoire de Recherche en Sciences Végétales; BP 42617, F-31326 Castanet-Tolosan, France
[3.] CNRS; UMR 5546; BP 42617, F-31326 Castanet-Tolosan, France

INTRODUCTION

Second generation ethanol has become a great promise for supplying the fuels demand worldwide, making possible the production increase without widening the planted area. However, the cell wall recalcitrance and the high costs of the enzymes to access the sugars make difficult the production of such a biofuel with a reasonable price.

Information dedicated to elucidate the mechanisms of plant cell wall plasticity is still scarce, wherein proteins play an important role in this compartment[1]. Therewith, a better description of the proteins present in cell wall (CWPs) at several developmental stages could help unraveling the processes involved in cell wall growth, providing knowledge that could assist the development of improved cultivars.

Therefore, this work identified common proteins in sugarcane 2- and 4-month-old culm internodes, also addressing major differences between them.

MATERIAL AND METHODS

Cell wall proteins were obtained through vacuum infiltration protocol, based on the method developed by Boudart *et al.* (2005)[2], using the salts $CaCl_2$ and LiCl. After extraction, acquisition of MS data used a Synapt G2 HDMS equipped with an ion mobility cell and a NanoLockSpray source in the positive ion and `V´ mode (Waters®). The bioinformatics analysis was performed as described[1].

The 2- and 4-month-old experiments were conducted independently.

RESULTS and CONCLUSION

Altogether, 258 different CWPs distributed into 8 functional classes were identified in 2- and 4-month-old internodes (young and mature ones). From these, 47 appeared to be identified at both stages. The number of CWPs identified was almost 3 times higher in 4-month-old plant, than in 2-month-old plant, which can be explained by the type of tissue or the performance of individual experiments.

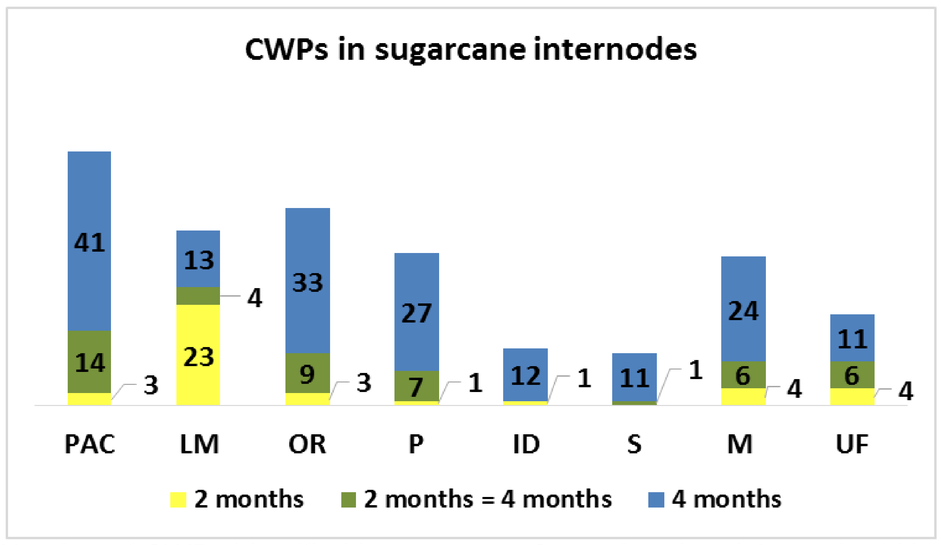

Figure 1. Number of CWPs identified in sugarcane 2 month-old and 4 month-old internodes.

Abbreviations: CWPs: Cell Wall Proteins, PAC: Proteins acting on polysaccharides, LM: Proteins related to Lipid Metabolism, OR: Oxido-reductases, P: Proteases, ID: Proteins with interaction domains (with proteins or polysaccharides), S: Signaling, M: Miscellaneous proteins, UF: Proteins of Unknown Function and LTPs: Lipid Transfer Proteins.

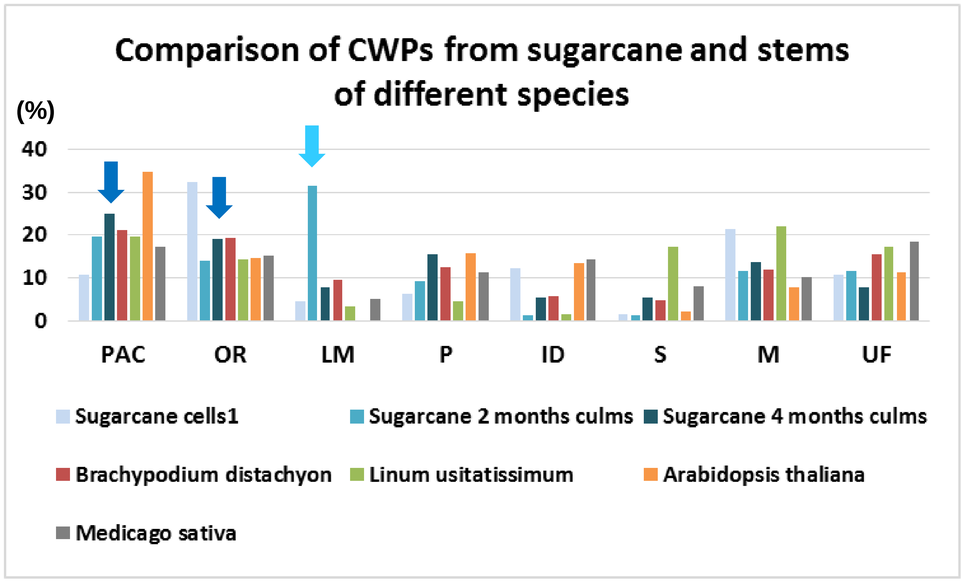

Figure 2. Percentage of CWPs identified in stems of different plant species distributed into functional classes. The results obtained with sugarcane cell suspension cultures are shown for comparison.

About 54,6% of the 2 month-old CWPs and 21,5% of the 4 month-old CWPs could be identified at both ages. Attention should be driven to the LM functional class, the only more represented in 2 month-old internodes (Figure 1), and also the class presenting the highest difference between all species (Figure 2) , mostly comprising LTPs.

The functions of LTPs are very diverse. They can transfer lipids in cell walls[4], play roles in defense against pathogens, cutin deposition and pollen tube adhesion. They have also been assumed to bind hydrophobic molecules yet to be identified in cell walls which could enhance cell wall loosening, thus facilitating wall extension[5]. Therefore, the high number of LTPs identified in young internodes (2 month-old) could be linked to the fact that these organs have a higher rate of extension than mature ones (4 month-old). These data are in agreement with previous studies, since LTPs are frequently detected in young tissues[5].

In addition, the ORs are more represented in 4 month-old internodes, as expected since they could contribute to the cross-linking of cell wall polymers at the end of the growth period. Conversely, a higher number of PACs in mature plants was not expected since the cell walls of young stems are supposed to undergo more rearrangement processes (Figure 2).

This work suggests focusing genetic manipulation studies not only on PACs[1], but also on LTPs, especially the ones found in young tissues.

REFERENCES

[1] CALDERAN-RODRIGUES *et al.* Proteomics. **2014**, 14: 738-49.
[2] BOUDART *et al*. Proteomics. **2005**, 5: 212-21.
[3] CALDERAN-RODRIGUES *et al.* BMC Plant Biol (submitted).
[4] SAMUELS *et al.* Ann Rev Plant Biol. **2008**, 59: 683-707.
[5] NIEUWLAND *et al.* Plant Cell. **2009**, 17: 2009-19.
[6] DOUCHÉ *et al.* Proteomics. **2013**, 13: 2438-54.
[7] VERDONK *et al.* Front Plant Sci. **2012**, 3: 279.
[8] DAY *et al.* Proteomics. **2013**, 13: 812-25.
[9] MINIC *et al.* J Exp Bot. **2007**, 58: 2503-12.

ACKNOWLEDGEMENTS

Processes:
USP-COFECUB: 10.1.1947.11.9
CNPQ: 142784/2007-9
FAPESP: 2007/59327-8 and 2012/1212521-2
BIOEN/PRONEX FAPESP Proc. 2008/56100-5