# Analysis of enzymatic activity and proteomic profile of class III peroxidases during sugarcane stem development

**Cesarino, I.[1], Araújo, P.[1], Vicentini, R.[2], Leme, A.F.P.[3], dos Anjos, I.A.[4], Souza, S.A.C.D.[4], Landell, M.G.A.[4], Mazzafera, P.[1]**

1 – Departamento de Biologia Vegetal, Instituto de Biologia, Unicamp, Brazil; 2 – Centro de Biologia Molecular e Engenharia Genética, Unicamp, Brazil; 3 - Laboratório de Espectrometria de Massas, Laboratório Nacional de Luz Síncrotron, Brazil; 4 – Centro de Cana, Instituto Agronômico de Campinas, Brazil;

Class III peroxidases are present as large multigene families in all land plants. This large number of genes and the diversity of processes catalysed by peroxidases suggest functional specialization of each isoform. Monolignol oxidation is one of the functions proposed for peroxidases, which allows lignin polymerization by radical coupling reactions. Here we report on the determination of isoperoxidases proteomic profile during sugarcane stem development. *In vitro* enzyme assays were performed using total proteins extracted from six different stem tissues (young, developing and mature internodes, each internode separated in inner and outer tissues) and three distinct peroxidase substrates: guaiacol, syringaldazine and coniferyl alcohol. Such assays clearly demonstrated higher peroxidase activity in outer tissues of developing and mature internodes, for all three substrates. The presence and abundance of class III peroxidases isoforms were analysed by mono-dimensional and bi-dimensional electrophoresis using active staining with guaiacol. High molecular weight peroxidases were found as dominant isoforms in the protein preparation of outer tissues of developing and mature stems, while in all other tissues low molecular weight isoperoxidases were predominantly observed. Moreover, the former tissues also presented a higher number and intensity of individual activity spots. Altogether, our results suggest that the outer tissues of developing and mature stems could be an ideal tissue to study peroxidases correlated to lignification in sugarcane. Many spots were excised from 2DE gels and digested with trypsin, prior to peptide sequencing by LC-MS/MS. The fragment ion spectra were searched against a peroxidase-specific databank (PeroxiBase - http://peroxibase.toulouse.inra.fr/) and against a sugarcane-specifc EST databank (SUCEST – www.sucest-fun.org), which led to the identification of most of the peptides.

*Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP*