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Speaker Abstracts

S-020

Sugarcane Genomics and Genetic Engineering Towards Efficient Conversion of Ligno-cellulosic Sugarcane Residues to Fuel Ethanol

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Sugarcane (*Saccharum* sp. hybrids) is a highly productive C_4 grass used as the main source of sugar and more recently to produce ethanol, a renewable transportation fuel. Down-regulation of lignin biosynthesis pathway enzymes and in planta expression of cell wall-degrading enzymes are promising strategies to increase the efficiency of bio-ethanol production from the abundant ligno-cellulosic sugarcane residues, leaf litter, and bagasse. 4-Coumarate-CoA ligase (4CL) and caffeic acid 3-*O*-methyltransferase (COMT) are key enzymes in the lignin pathway. COMT and 4CL family genes were isolated from sugarcane by a combination of cDNA library screening and PCR-based approaches. Following characterization of the isolated candidates, vectors were generated for RNAi suppression and stably introduced into sugarcane by biolistic gene transfer. Transgenic events were confirmed by PCR, Southern blot analysis, and ELISA. We are currently analyzing the expression of the targeted lignin biosynthetic genes by Northern blot and the lignin content of the corresponding biomass by Klason lignin determination. Xylan is after cellulose, the most abundant polysaccharide in sugarcane residues, and must be hydrolyzed to its component sugars (xylose or xylobiose) before fermentation to ethanol. Endoxylanases are the main enzymes involved in xylan hydrolysis. Constitutive, apoplast- or chloroplast-targeted expression cassettes of the codon optimized, hypothermostable GH10 xylanase from *Thermotoga maritima* (xynB) were generated for in planta expression. Following biolistic gene transfer, co-integration of both *nptII* and *xynB* was confirmed in 83% of the transgenic sugarcane lines. Seventeen transgenic sugarcane lines showed a clearly detectable xylanase activity. TLC analysis confirmed that directly fermentable xylobiose and xylose were the main degradation products from the in planta-produced enzyme, consistent with the activity of the native *T. maritima* enzyme. The comparison of pretreatment requirements and efficiency of ethanol production from transgenic sugarcane biomass expressing xynB and non-transgenic sugarcane will be presented.

S-022

First Transgenic Geminivirus-resistant Plant in the Field: The Development of a RNAi-based Agriculture Technology

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Bean golden mosaic virus (BGMV) belongs to the genus *Begomovirus* whose genome is composed of two single-stranded DNA molecules, designated DNA-A and DNA-B, both of which are essential for infectivity. BGMV is transmitted by the whitefly *Bemisia tabaci* (Gennadius) in a persistent, circulative manner, causing golden mosaic in common bean. This disease is the heaviest constraint on bean production in Latin America, causing significant yield losses ranging from 40% to 100%. Due to the social and economic importance of common bean as a source of protein in the diet of over a billion people worldwide, we have tried to obtain BGMV-resistant engineered lines since the early 1990s. Transgenic lines were produced to express BGMV coat protein gene, the *rep-TrAP-REn* and *BC1* viral genes in antisense orientation and a mutated *rep* (*AC1*) gene, achieving tolerance and delayed and attenuated golden mosaic symptoms. More recently, we have explored the concept of using RNAi construct to silence the *AC1* viral gene, which codes for the only protein strictly essential for viral genome replication. Two transgenic lines exhibited immunity upon inoculation at high pressure and at a very early stage of plant development. Studies on the behavior of transgenic common bean lines under field conditions were conducted and results confirmed the earlier greenhouse observations. Biosafety evaluations are being carried out, taking into account the demands of the Brazilian Biosafety Committee and other regulatory authorities in order to obtain authorization to commercially release the first transgenic bean varieties.