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MO5 **Genome-wide association mapping for traits related to drought tolerance and biomass in sugarcane (*Saccharum* spp.) using EST-SSR markers**
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MO6 **Isolation of specific genomic DNA segments from *E. arundinaceus* and chromosome identification**
Yongji Huang, Fan Yu, Ling Luo, Zuhu Deng, Jiayun Wu, Muqing Zhang*

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MO8 **The developmental stages of sugarcane are equivalent between plants of different chronological ages**
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MO9 **Transcriptomic characterization and potential marker development of contrasting sugarcane genotypes in response to leaf abscission, resistance to Pokkah boeng and water stress**
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MO10 **Guidelines for commercial release of transgenic sugarcane in Argentina**

Aldo Noguera, Ramón Enrique, María Francisca Perera, Santiago Ostengo, Josefina Racedo, Diego Costilla, Silvia Zossi, María Inés Cuenya, María Paula Filippone, Björn Welin, Atilio Pedro Castagnaro*

MO11 **Development of transgenic sugarcane associate with increasing biomass, sugar and stress tolerance in Colombia**
Jershon López, Hugo Jaimes, Marcela Franco, Isabel Ocampo, Rocio Barrios, Fredy Salazar, Fredy Garcés*

Poster presentation abstracts (MP)

MP1 **Development of microsatellite markers from sugarcane (*Saccharum officinarum* L.) Phil 97-3933**
John Moises G. Relles, Rimmon T. Armones, and Antonio C. Laurena*

MP2 **Assessment of genetic diversity of first priority parentals of the sugar regulatory administration**
John Moises G. Relles, and Antonio C. Laurena*

MP3 **Transcriptomic analysis of sugarcane callus in response to an *Agrobacterium*-mediated transformation process**
Elaine Cristina Alexandre, Leonardo Cardoso Alves, Renato Vicentini, Monalisa Sampaio Carneiro**

MP4 **Length and nucleotide sequence polymorphism at the *trnL* and *trnF* non-coding regions of chloroplast genomes among *Saccharum* and *Erianthus* species**
Yong-Bao Pan, James R. Todd, Brian E Scheffler, Lionel Lomax, Sheron Simpson, Fanny Liu, Michael P. Grisham*

MP5 **Cancelled**

MP6 **Improvement of sugarcane for stress environments in South Africa**
*Watt DA**

MP7 **Cancelled**

MP8 **A molecular identity database of sugarcane (*Saccharum* spp.) clones constructed with microsatellite (SSR) DNA markers**
Yong-Bao Pan, James Todd, Brian E. Scheffler, Lionel Lomax, Sheron Simpson, Edwis Dufrene, Anna Hale, Michael Grisham, Herman Waguespack Sr., Atticus Finger*

MO1

WORLDWIDE GENETIC DIVERSITY OF *SACCHARUM SPONTANEUM* AND LEVEL OF DIVERSITY CAPTURED IN A SUGARCANE BREEDING PROGRAM

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The hybridization of *Saccharum spontaneum* with *S. officinarum* in the early 20th Century generated hybrids that were embraced by all breeding programs as they were higher yielding had good ratooning and increased disease resistance. These early hybrids were utilized by many of the sugarcane breeding programs around the world but only a small number of *S. spontaneum* clones were used in the generation of these initial hybrids. Around the 1960's there was a renewed interest in introgression breeding due to the realization that there was a limited genetic base in core breeding programs. There was an increased awareness of desirable traits that were present in the wild germplasm collections such as tolerance to drought and waterlogging. Combined with this was the slowing of the rate of genetic gain in breeding programs seen after the spectacular gains achieved after the first interspecific hybridizations. There are two major World collections of *S. spontaneum* and characterization of the available genetic diversity and relationship among the collections would facilitate a more focused choice of clones for further breeding efforts in the future. This study analysed the diversity across 430 *S. spontaneum* accessions collected from 21 different countries using molecular markers. In total 724 markers were used for principle coordinate and cluster analysis, which revealed two main clusters that corresponded to accessions collected north and south of the Tropic of Cancer. The majority of the accessions clustered according to country of origin. Genetic similarity ranged from 0.25 to 0.54 with highest diversity in accessions collected in Indonesia, followed by China, India and Thailand, and the lowest in the Philippines. Despite the low number of *S. spontaneum* clones used for breeding varieties, 51 to 80% of markers were captured in the breeding populations, although many at a low frequency. This is due to the high heterozygosity of sugarcane related to high ploidy and the introgression programs that have been established in a number of sugarcane breeding programs. This study revealed that diversity analysis could be used to guide the selection of wild *S. spontaneum* accessions to increase diversity in the breeding program.

Keywords: *Saccharum spontaneum*, Molecular markers, Genetic diversity, Principal coordinate analysis

MO2

A MONOPLOID REFERENCE SEQUENCE FOR THE HIGHLY COMPLEX GENOME OF SUGARCANE

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Sugarcane (*Saccharum* spp.) is a major crop for sugar and bioenergy production. Its genome poses challenges that have not been addressed in any prior sequencing project due to its highly polyploid, aneuploid, heterozygous and interspecific genome structure (2n ~ 12x ~ 120, ~10Gb). We exploited the colinearity with sorghum to produce a monoploid genome sequence of sugarcane. We applied whole genome profiling (WGP™) to 20,736 sugarcane BAC clones and anchored 11,732 of them onto the sorghum genome. A minimum tiling path of 4,660 sugarcane BAC clones that best cover the gene-rich part of the sorghum genome was selected, sequenced and assembled in a 382 Mb single tiling path (STP) of high quality sequence. A total of 25,316 protein-coding gene models were predicted on the STP, 17% of which displayed no colinearity with their sorghum orthologs. We showed that the two species, *S. officinarum* and *S. spontaneum,* involved in modern cultivars differed by their content in transposable elements (TE) explaining their distinct genome size. We also showed that they differ by a few large chromosomal rearrangements, explaining their distinct basic chromosome numbers while also suggesting that polyploidisation arose in both lineages after their divergence. This BAC-based monoploid sugarcane reference sequence represents an essential resource for genetic and genomic studies and for future whole genome sequence assembly programmes.

BAC and STP sequences as well as gene annotations are available on the sugarcane genome hub (http://sugarcane-genome.cirad.fr).

We acknowledge members of the International Consortium for Sugarcane Biotechnology (ICSB) and Joint BioEnergy Institute (JBEI) for financial support.

Keywords: Reference Sequence, gene annotation, chromosome rearrangements, TE content

MO3

IDENTIFICATION AND CHARACTERIZATION OF GENES RESPONSIBLE FOR THE BROWN RUST RESISTANCE (Bru1) EFFECT

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Brown rust resistance is a major sugarcane innate defence against the fungal pathogen *Puccinia melanocephala*. Bru1 loci are reported to be responsible for brown rust resistance and are being used as a platform for marker assisted selection (MAS) in sugarcane. Sequences for Bru1 alleles have been reported in R570, LA Purple and RB867515. We have assembled the cognate genes in LCP85-834 and SP80-3280. There are 14 genes in the core region, which are present in different copies on different alleles. Every gene was characterised molecularly by domain analysis, structural analysis, homology mapping, phylogenetics, active site analysis and pathway mapping. All but five genes could be excluded, based on their functional annotation. Two of these genes were further excluded, as they were only involved in DNA repair. Genes S6PDH, S6PDHa, CDKA;1 and UCCP were further analysed. CDKA;1 shows unusual convergent evolution between the grass and Brassica cognates. In Arabidopsis CDKA;1 is responsible for pathogen response including epigenetic protection. S6PDH has recently been identified as a key cell death response gene. Uniquely in grasses, sugarcane S6PDHa possesses a strong chloroplast transition peptide, indicating that it may function in sugarcane chloroplasts to downregulate inositol and induce cell death. The functional pathway has been modelled. Transcript mapping indicates that UCCP, CDKA;1 and S6PDH are constitutively expressed but UCCP and S6PDHa are significantly upregulated, while CDKA;1 is down-regulated in response to fungal pathogens. These two major genes (UCCP and S6PDHa), in concert, are responsible for the brown rust resistance phenomenon in sugarcane and could be used directly as gene/transcript markers for MAS. We report, for the first time, the functional mechanism for the Bru1 response in sugarcane.

Keywords: Brown rust, Bru 1, Marker assisted selection, Sugarcane

MO4

ANALYSIS OF QTL RELATED TO RESISTANCE TO SMUT DISEASE USING JAPANESE WILD SUGARCANE (*SACCHARUM SPONTANEUM*)

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Sugarcane smut disease is caused by the fungus *Sporisorium scitamineum*. Once the crop has infected with the fungus, it significantly reduces the economic value of the crop. This research aims to develop an efficient method to select for smut resistant sugarcane clones, and in this report, we discuss about our fundamental research on developing an efficient DNA marker assisted selection system. We performed QTL analysis using 103 interspecific clones derived from three different combinations, using two methods 1) Genotyping Random Amplicon Sequence-Direct (GRAS-Di®) Genotyping system, developed by Toyota, and 2) Composite Interval Mapping (CIM) using 4503 DNA markers. One QTL was detected. Since the LOD value and the phenotypic variance (R^2), values that indicate the effectiveness of the QTL, are greater than 20 and 30%, respectively, at the maximum, it was suggested that the main genes controlling the sugarcane disease resistance are located in this area. By using the DNA markers linked to this QTL, we expect that the efficiency of smut resistant sugarcane clones selection will be greatly improved.

Keywords: Sugarcane smut, QTL, GRAS-Di®, CIM method

MO5

GENOME-WIDE ASSOCIATION MAPPING FOR TRAITS RELATED TO DROUGHT TOLERANCE AND BIOMASS IN SUGARCANE (*SACCHARUM* SPP.) USING EST-SSR MARKERS.

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Every year, the sugarcane production of Thailand is suffering losses caused by drought periods, especially in the North-east province. Several attempts to develop drought-tolerant varieties by conventional breeding have been reported. However, these varieties were never accepted by farmers or industrials because of low yield compared to the most cultivated variety: KK3. As a result, sugarcane breeders are now considering marker-assisted breeding for developing varieties with acceptable cane yield, sugar content and drought tolerance. To do so, the identification of molecular markers strongly associated with these target traits is a major footstep. In this study, genome-wide association mapping for drought tolerance and plant biomass (sugar production and cell-wall biosynthesis) was performed using simple sequence repeat (SSR) markers. Target genes were selected for morpho-agronomical traits such as the leaf rolling, stomatal conductance, plant height, number of tillers or root thickness. Most of the markers used in this work were EST-SSRs found in the literature or developed *in silico* using transcriptomic data available for sugarcane and other closely related species such as *Sorghum* spp. Phenotypic data related to drought tolerance and biomass were recorded on accessions originating from different countries such as Thailand, China, Australia or India. In total, 160 accessions were planted in a lattice square design replicated in two sites. All accessions were genotyped with 40 SSRs related to drought tolerance genes and 40 SSRs related to the plant biomass. The genetic structure of the germplasm was revealed by using STRUCTURE software. The pairwise relatedness coefficients were calculated with the method of Hardy using SPAGeDi (Spatial Pattern Analysis of Genetic Diversity) software. Marker-trait associations were estimated using mixed linear models (MLM) incorporating the population structure Qmatrix as the fixed effect and the relative kinship K-matrix as the random effect covariate. The analysis was conducted using the TASSEL pipeline. The significant marker-wise threshold for associations between loci and traits was set at $P < 0.05$. The experiment-wise threshold was based on Bonferroni correction with $\alpha=0.10$. Minor allele frequency markers ($MAF < 0.05$) were removed from the association analysis, leading the final number of markers to 70. The contribution of each QTL to the phenotypic variance was estimated through the percentage of explained variance (R^2 coefficient). QTLs with relatively high effects on the phenotypic variance (>10 %) were identified. These markers present a good potential for marker-assisted selection on segregating populations.

Keywords: Sugarcane, Association mapping, Simple sequence repeat markers, Drought tolerance, Biomass

MO6

ISOLATION OF SPECIFIC GENOMIC DNA SEGMENTS FROM *E. ARUNDINACEUS* AND CHROMOSOME IDENTIFICATION

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Sugarcane breeders have succeeded in introgressing the *E. arundinaceus* chromatid into sugarcane. A specific chromosome inheritance pattern was found in the BC_1 progeny between sugarcane and *E. arundinaceus*, but the mechanism was not clear. In this study, a specific repetitive DNA library of *E. arundinaceus* was built by suppression subtractive hybridization (SSH). These repetitive DNA clones were identified and selected for chromosome identification by FISH. Based on this, we constructed the FISH chromosome karyotype. In the light of the chromosome identification, we discussed the mechanism in the F_1 and BC_1 progeny between sugarcane and *E. arundinaceus*. The main results and conclusions were as follows: The subtracted library of specific genomic DNA segments from *E. arundinaceus* was constructed by SSH method to remove the homologous sequences and enrich for differences of sequences between sugarcane and *E. arundinaceus*. We obtained 253 specific repetitive DNA sequences of *E. arundinaceus*. The repetitive sequences from the subtracted library were mixed as probe combinations and screened by FISH, good results were obtained from a probe combination. In the probe combination, four repetitive sequences generated a similar banding pattern among the homologous chromosomes, but a distinctive similar banding pattern among nonhomologous chromosome. These four repetitive sequences of the probe combination were successfully developed for simultaneously accurate identification of each of 10 chromosomes. Based on the system of chromosome identification from this probe combination, the specific mechanism in the F_1 and BC_1 progeny between sugarcane and *E. arundinaceus* was investigated. We found that the BC_1 progeny between sugarcane and *E. arundinaceus* inherited distinct chromosomes from their F_1 female. In the light of the relationship of chromosome inheritance between these two generations, the mechanism was analyzed. We determined that hypothesis II（First division restitution + Nondisjunction of sister chromatids）was the possible mechanism, and ruled out hypothesis I（Nondisjunction of homologous chromosomes + Second division restitution）.

Keywords: *Erianthus arundinaceus*, Chromosome inheritance, Suppression subtractive hybridization, Repetitive sequence, Genome-specific molecular marker

MO8

THE DEVELOPMENTAL STAGES OF SUGARCANE ARE EQUIVALENT BETWEEN PLANTS OF DIFFERENT CHRONOLOGICAL AGES.

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Sugarcane stalks sequentially produce internodes with the most immature and mature internodes at the top and base of the stalk, respectively. This results in a range of internodes at different developmental stages in each stalk. When sampling similar developmental internodes, replicate plants may differ in chronological age due to various reasons; these may include different germination rates, regeneration from tissue culture, and treatments affecting growth. To date there has been no research to test if internodes of the same developmental age are equivalent between plants of different chronological age. For example, is internode 4 from a 5 month old plant similar to internode 4 from a 9 month old plant? To address this question, internodes 4, 6 and 11 were assessed in two sugarcane varieties, Q117 and Q208[A]. Plants were germinated monthly to provide a range of chronological ages from 5 – 9 months. This study utilised gene expression (RT-PCR), abundance of metabolites and combined gene/metabolite results to discern chronological/developmental variation. Genes were selected from published research where differential expression was observed between immature and mature internodes. Similarly, specific metabolites were selected from metabolomic profiles, which would assist with providing a unique signature for each internode. These internode signatures were then used to illustrate if there was any variation between developmentally similar but chronologically different age samples. Results included 11 genes and 6 metabolites that were present in both varieties and some that were unique to each variety. ANOVA comparisons were performed on variety and individual internodes for each gene/metabolite and chronological age. There was no significant difference between equivalent internode samples of different chronological age. To capture these results as an overall image, canonical analysis was used to illustrate the lack of spread due to chronological age. The expression of 12 -13 genes or abundance of 10 metabolites did result in similar internode samples clustering together, more so for Q208 than Q117. When the gene and metabolite results were combined the cluster sizes were smaller than gene or metabolite results, further demonstrating that equivalent internodes are similar between plants of different chronological age. These results illustrate that samples labelled as being the same developmental stage are similar between stalks of different chronological age. With this knowledge, researchers can now reliably compare internodes from different plants, with confidence that results are not confounded by plant age.

Keywords: Development age, Chronological age, Internodes, RT-PCR, Metabolite profile

MO9

TRANSCRIPTOMIC CHARACTERIZATION AND POTENTIAL MARKER DEVELOPMENT OF CONTRASTING SUGARCANE GENOTYPES IN RESPONSE TO LEAF ABSCISSION, RESISTANCE TO POKKAH BOENG AND WATER STRESS

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Sugarcane (*Saccharum officinarum* L.) is an important crop for sugar production and bioenergy worldwide. In this study, we performed transcriptome sequencing for six sugarcane genotypes with contrasting leaf abscission patterns, tolerance to pokkah boeng disease and drought stress tolerance. More than 465 million high-quality reads were generated, which were *de novo* assembled into 93,115 unigenes. Based on a similarity search, 43,526 (46.74%) unigenes were annotated against at least one of the public databases. Functional classification analyses showed that these unigenes are involved in a wide range of metabolic pathways. Comparative transcriptome analysis revealed that many unigenes involved in response to abscisic acid and ethylene were up-regulated in the self-defoliation genotype, and unigenes associated with response to jasmonic acid and salicylic acid were up-regulated in response to the pokkah boeng disease in the tolerance genotype. Moreover, unigenes related to peroxidase, antioxidant activity and signal transduction were up-regulated in response to drought stress in the tolerant genotype. Finally, we identified a number of putative markers, including 8,630 simple sequence repeats (SSRs) and 442,152 single-nucleotide polymorphisms (SNPs). Our data will be important resources for future gene discovery, molecular marker development, and genome studies in sugarcane.

Keywords: Sugarcane, Unigenes, Transcriptome, Leaf abscission, Drought stress, Pokkah boeng

MO10

GUIDELINES FOR COMMERCIAL RELEASE OF TRANSGENIC SUGARCANE IN ARGENTINA

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In October 2015, the official approval of commercial release of the first transgenic sugarcane variety, RA87-3RG resistant to glyphosate, was issued by the relevant regulatory bodies in Argentina. However, mainly due to economic reasons, this release was put on hold by the industrial sugar sector of our country and was not resumed until this year. The process of deregulation was initiated in 2011, and was conducted almost entirely by an interdisciplinary research team of the Estación Experimental Agroindustrial Obispo Colombres (EEAOC). During these five years of work, several obstacles related to the release of a transgenic variety of a crop without a precedent neither nationally nor internationally had to be overcome. Our first task was to define which parameters/traits to evaluate, where and for how long a period. This initial elaboration of a work plan was conducted in close collaboration with the National Advisory Commission on Agricultural Biotechnology (Comisión Nacional Asesora de Biotecnología Agropecuaria, CONABIA) in a procedure called Prior Consultation Instance. CONABIA is the official Argentinean advisory committee that evaluates all activities related to genetically modified organisms (GMOs) for agricultural use, including commercial crop varieties. Some of the major concerns that we had to deal with included the complex genetics of sugarcane which prevents the release of a "transgenic event", which is standard procedure for most other crops, such as soybean and maize for instance. Another important issue was that with sugarcane there is no possibility to store "seeds" and that we are dealing with a perennial crop with high area demands for multiplication. Our work prompted a complete revision and reevaluation of deregulation proceedings in CONABIA. These changes were necessary in order to be able to accurately evaluate and release a transgenic sugarcane variety. One of the most important changes that was implemented was the capacity the process of deregulation to include another variety of the same crop, containing the same or essentially the same genetic construction as a previously deregulated cultivar, to enter a streamlined deregulation process. This resolution is essential for the use of transgenic technology in vegetatively propagated crops, because it effectively reduces both cost and time needed for a release as the entire testing procedure is not required for a second variety of the same or essentially the same genetic composition.

Keywords: Commercial deregulation, Glyphosate, Sustainable

MO11

DEVELOPMENT OF TRANSGENIC SUGARCANE ASSOCIATE WITH INCREASING BIOMASS, SUGAR AND STRESS TOLERANCE IN COLOMBIA

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Sugarcane is a major crop in tropical and sub-tropical areas around the world. It is used as an important source of sugar for ethanol production, and for electric power cogeneration. Although sugarcane plant breeding programs have accomplished many important goals, the highly ploidy level, complex genome structure and long-term introgression process have delayed the sugarcane breeding progress. Genetic transformation has become a valuable technology to assist sugarcane breeding programs, in order to transfer genes producing desirable phenotypes such as high sugar yield, high biomass production and water stress tolerance. As a result of the strategic agreement with an experienced partner in plant genetic transformation, CENICAÑA has been evaluating the agronomical performance in relation to sugar yield, biomass production, water stress tolerance (50% reduced water irrigation vs full water supply) and plant pathology evaluations of 500 independent transgenic events belonging to two commercial varieties (CC3 and CC6). There are four different transgenes known to enhance biomass, shoot architecture, sugar accumulation, and stress tolerance. Currently, six independent transgenic events of variety CC3 containing the transgenes are being cultivated in 6 ha. They are maturing successfully in a third cycle of agronomic selection. Some are showing an outstanding field performance, especially in sugar content and increased biomass when compared with checks. Furthermore, a second batch of 220 independent events of seven commercial varieties (including CC3 and CC6) are being subjected to a second cycle of agronomic selection in four additional hectares. The events displaying the better agronomical performance after the current selection cycles will be evaluated by PCR and Southern blots to detect the specific insertion sites of the genes and establishing copy numbers. An additional cycle of agronomic evaluation will be carried out to select promising lines and to determine transgenes stability before planting in a bigger area to commercially measure the impact of the traits.

Keywords: Genetic transformation, *Saccharum* spp, Biomass, Sugar, Water stress

MP1

DEVELOPMENT OF MICROSATELLITE MARKERS FROM SUGARCANE (*SACCHARUM OFFICINARUM* L.) PHIL 97-3933

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Efficiency of commercial farms can be improved through the application of developed technologies such as the use of microsatellite repeats or simple sequence repeats (SSRs) as genetic markers in plant species. This study developed sets of simple sequence repeat markers (SSRs) from Phil 97-3933 variety, a cultivar known to be highly resistant to sugarcane smut and downy mildew. For the library construction, genomic DNA of Phil 97-3933 was extracted and was digested using methyl-sensitive restriction enzymes PstI and AatII, with six base pair recognition sites. Two hundred sequences were obtained from which 27 contained SSR. A total of 27 SSR primers were developed from sugarcane CV Phil 97-3933 using BatchPrimer3 (You *et al*., 2008). SGS P20 had similar sequence identity to Saccharum hybrid cultivar R570 clone BAC 227O17, while SGS P141 had similar sequence identity to *S. officinarum* clone LA154P24. Other SSR primers that returned BLASTn similar sequence identities are SGS P131 (Sorghum hypothetical protein), SGS P76 (*S. officinarum* clone LA34B02), SGS P112 (*Saccharum* hybrid BAC 235G19), SGS P125 (*Sorghum* hypothetical protein), and SGS P139 (*Sorghum* voucher BTx623 locus pSB1123). The rest of the primers identified did not return any BLASTn result. Phil 97-3933 is a cultivar known to be highly resistant to sugarcane smut. Sugarcane smut caused by *Sporisorium scitamineum* is one of the most serious diseases of sugarcane (Comstock, 2000) and has been a long-standing problem in the Philippines. Constructing a genomic library from Phil 97-3933, and developing microsatellite markers from it is a start. Screening and evaluating germplasm collections with SSR markers developed from this local variety could both optimize and facilitate the breeding process in the country.

Keywords: Sugarcane, Microsatellite markers, Genomic library, SSR

MP2

ASSESSMENT OF GENETIC DIVERSITY OF FIRST PRIORITY PARENTALS OF THE SUGAR REGULATORY ADMINISTRATION

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Efficiency of commercial farms can be improved by evaluating the genetic diversity of the Sugar Regulatory Administration First Priority Parentals. In the Philippines, the current basis for the evaluation of genetic diversity in sugarcane germplasm is only based on pedigree records and phenotypic traits. The screening and evaluation of available first priority parentals from SRA with simple sequence repeat markers (SSRs) could both optimize and facilitate the development of varieties resistant to sugarcane smut. A total of 160 SRA First Priority Parentals were extracted and analyzed. DNA profiling was performed using two primers developed from Phil 97-3933 (SGS P20 and SGS P141) and 12 SSRs compiled by UPLB Institute of Plant Breeding (IPB) specific for sugarcane. All the 14 SSR primers utilized were able to distinguish most of the varieties. Three different clusters (I, II, III) were generated at coefficient 0.68. Cluster III had the highest number of varieties (134), followed by Cluster II (16), then Cluster I (10). Principal Component Analysis (PCA) also showed that alleles from the 160 SRA first priority parentals were widely distributed, and grouped accordingly based on their clustering in the dendrogram.

Keywords: Sugarcane, Microsatellite markers, Genomic library, SSR

MP3

TRANSCRIPTOMIC ANALYSIS OF SUGARCANE CALLUS IN RESPONSE TO AN AGROBACTERIUM-MEDIATED TRANSFORMATION PROCESS

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Sugarcane is a major raw material used by the sugar industry and a feedstock for bioethanol production. Transgenic technologies have been applied to sugarcane molecular breeding through Agrobacterium-mediated transformation via somatic embryogenesis (SE). However, different varieties of sugarcane present variations in the efficiency rates of Agrobacterium-mediated transformation, which may be due to genotype-dependent responses. The present work aimed at assembling a transcriptome profile of embryogenic callus tissues of two sugarcane varieties during *Agrobacterium tumefaciens* infection. The Brazilian commercial varieties of sugarcane were selected for this study according of their differences of response efficiency to the process of infection from *A. tumefaciens* (low and high-responsive varieties). Callus induction and maintenance culture medium consisted of MS salts and vitamins, sucrose (30 g/L), citric acid (100 mg/L), 2,4D (3 mg/L) and Phytagel (2.5 g/L). Cultures of globular and friable embryogenic callus, free of microorganisms, were selected at approximately 40 days of in vitro culture. The callus tissues of each varieties were used for infection with *A. tumefaciens*. Treatments consisted of Control (t0), 6 hours (t6) and 12 hours (t12) *after infection*. The libraries, sequenced using the Illumina platform, produced a total of 292,945,992 Paired-Ends (PE) reads from embryogenic callus tissues of the varieties studied. The annotations of the transcripts mapped identified about 14,000 transcripts, where approximately 4,400 were unique elements. More than 80% of transcripts were common elements in both varieties, even when evaluated at different times after infection. However, when raw data were analyzed comparing t6 against t0 and t12 against t6, transcripts with differential expression pattern were observed across times and varieties. High-responsive variety (HRV) exhibit higher expression activity during first 6 hours, while in the low-responsive variety (LRV) was different number of differently expressed transcripts were observed when t12 versus t6 were compared. These data suggest a cellular response process of the HRV occurred mostly in the first six hours, whereas for the LRV this process was longer. The enrichment analysis classified the elements into biological processes, and these functional analyses reveal: (a) comparing the t6 versus t0, there are a higher number of genes up regulated from LRV into GO:0048519 , representing a potential negative regulation of biological processes, e.g. the control of gene expression, protein modification or protein interaction; and (b) comparing the t12 versus t6, almost all of the genes up regulated from HRV were related to response to stimulus and developmental process, while the elements from LRV reflected regulated genes – related to several processes, including developmental, metabolic regulation, immune system, death, and signaling. This study provides insights into the identity genes differentially expressed under the stress caused by Agrobacterium-mediated transformation of sugarcane.

Keywords: *Saccharum*, Transcriptome, *Agrobacterium*, RNA-Seq analysis

MP4

LENGTH AND NUCLEOTIDE SEQUENCE POLYMORPHISM AT THE *trnL* and *trnF* NON-CODING REGIONS OF CHLOROPLAST GENOMES AMONG *SACCHARUM* AND *ERIANTHUS* SPECIES

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The cytoplasmic genomes of all sugarcane (*Saccharum* hybrids spp.) cultivars grown in the world today are derived from *S. officinarum* and show little genetic variability. Since 1997, a few *S. spontaneum* clones have been used as maternal parents at the USDA-ARS, SRU to explore the cytoplasmic genome of *S. spontaneum* in sugarcane improvement. One clone, Ho 02-113, survived a spring freeze in 2003 and was later released as a high-fiber and high-biomass cultivar in 2010. Since 2011, studies were also initiated at USDA-ARS, SRU to investigate the genetic variability of the chloroplast genome among different *Saccharum* species. In this study, four universal PCR primers were used to amplify the *trnL* and *trnF* non-coding regions of chloroplast genomes from 161 clones belonging to six *Saccharum* species, three *Erianthus* species, and nine sugarcane cultivars. These primers were *trnL*-c (5' NED-CGAAATCGGTAGACGCTACG), *trnL*-d (5' GGGGATAGAGGGACTTGAAC), *trnL*-e (GGTTCAAGTCCCTCTATCCC), and *trnF*-f (5' ATTTGAACTG GTGACACGAG), respectively. The fluorescence labeled *trnL*-c/*trnF*-f amplicons were subject to capillary electrophoresis on ABI3730XL (www.appliedbiosystems.com) along with GeneScan™ Size Standards GS1200 (LIZ) to generate <.fsa> files, which were processed by the GeneMarker® software for size calibration. Only five sizes, i.e., 850-, 934-, 950-, 955-, or 996-bp, were observed. The 950-bp amplicon was the most common found in 162 clones (92.60%). The 934-bp amplicon was less common found in eight *S. spontaneum* (4.62%) and one *Erianthus procerus* clone (0.58%). The 850-bp amplicon was lesser common found in one *S. officinarum* clone IM 76-245 and *one spontaneum* clone IND 81-161 (1.16%). The 955-bp and 996-bp amplicons were the least common, found in *S. spontaneum* clone IN84-010 (0.58%) and *Erianthus bengalense* clone IMP9751 (0.58%), respectively. To explore the nucleotide sequence variability, two sets of PCR amplicons were obtained using primer pairs *trnL-c*/*trnL-d* and *trnL-e*/*TrnF-f*. All amplicons were purified with Zymo ZR-96 DNA Sequencing Clean-up Kit (Z4053) (www.zymoresearch.com) and subject to direct sequencing using the BigDye Terminator V3.1 Cycle Sequencing Kit (www.appliedbiosystems.com). The sequencing files were analyzed using DNASTAR's SeqMan® (www.dnastar.com) and DNAMAN® (www.lynnon.com) to determine the exact sizes of the amplicons and sequence variations. While sequencing of *trnL-c*/*trnL-d* amplicons was completed, sequencing of *trnL-e*/*trnF-f* amplicons was not. Among all the *trnL-c*/*trnL-d* amplicons sequenced, eight different sizes, 11 indels, 32 SNPs, and two SSRs were observed. Using these results, we can assess the phylogenetic relationship among *Saccharum* species and track our new breeding lines carrying *S. spontaneum* cytoplasm.

Keywords: Chloroplast genome, *Saccharum*, *Erianthus*, *trnL*, *trnF*

MP6

IMPROVEMENT OF SUGARCANE FOR STRESS ENVIRONMENTS IN SOUTH AFRICA

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In 2016/2017, for the third consecutive season, the South African sugar industry experienced below average rainfall, with an industry average of 20% below the long-term annual mean. Given predictions that this potential impediment to industry sustainability is likely to persist into the future, the research programme of the South African Sugarcane Research Institute (SASRI) focuses research efforts on improving crop resilience to water deficit stress. Of particular relevance are investigations that seek to determine the potential impacts of mid-century climate change scenarios on production and to formulate appropriate resources, technologies, recommendations and best management practices to enable mitigation and adaptation. As such, the SASRI research portfolio is multi-disciplinary and integrated, spanning climate change, variety improvement, crop protection, crop management, water management and bio-energy investigations that aim to deliver outcomes for application to strategic and tactical decision-making at industry, regional, farm and field levels. This poster paper presents an overview of SASRI research that supports the long-term goal of providing sugarcane farmers with sugarcane varieties that have enhanced stress-tolerance. To expedite the improvement of variety stress-tolerance, SASRI undertakes research to develop technologies that are ultimately intended to complement conventional breeding practices, primarily by enabling more targeted selection and providing access to potentially novel genetic sources of stress tolerance. The investigation of selection methods aims to ultimately provide: (a) a high-throughput phenotyping technology for the rapid, semi-automated acquisition and interpretation of stress-response physiology data for use in the early stages of genotype selection; and (b) a marker-assisted breeding technology that uses expression-based SNP markers for the screening and categorisation of genotypes according to stress responses. Prospecting for potentially novel genetic sources of stress tolerance is being conducted through the *in vitro* generation of epigenetic variants by altering DNA methylation patterns with subsequent *in vitro* selection for desirable genotypes. Furthermore, proof-of-concept studies are underway to assess the stress-tolerance phenotype of transgenic lines produced using a gene obtained under the terms of a material transfer agreement with the Japan International Research Center for Agricultural Sciences. The research is intended to provide sugarcane varieties with enhanced water-deficit stress tolerance, which, if cultivated as part of a crop production system focused on moisture conservation and stress reduction, may ultimately provide the production resilience required to sustain the South African sugar industry in the face of a changing climate.

Keywords: Integrated stress management, High-throughput phenotyping, Marker-assisted breeding, Directed mutation breeding, Genetic engineering

MP8

A MOLECULAR IDENTITY DATABASE OF SUGARCANE (*SACCHARUM* SPP.) CLONES CONSTRUCTED WITH MICROSATELLITE (SSR) DNA MARKERS

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The genetic compositions of the nucleus and cytoplasm of sugarcane (*Saccharum* hybrids spp.) clones remain unchanged by using bud-bearing vegetative cuttings of mature stalks during germplasm exchange and field production. However, commercial release of a new cultivar for sugar production requires over a decade of selection. Sugarcane genetic research has been hindered by an extreme level of genotype by environment interaction, the aneupolyploid genome, the lack of a classical genetic map and a reference genome sequence. Although anatomical and morphological descriptors have been used to identify varieties, these descriptors are limited by the stage of growth and influenced by the environment. Proper variety identification is critical, making a molecular identification protocol essential during the breeding process. This paper reports the development and utilization of the first simple sequence repeat (SSR) marker-based sugarcane molecular identity database in the world. Since 2005, we have fingerprinted more than 3,500 clones, including 175 from the Miami World Collection, 2,281 from Louisiana, 45 from Florida, 39 from Texas, 185 from foreign countries, 30 from consultants and companies, and 745 from two genetic mapping populations. High throughput fingerprinting is done on BioRad's C1000 Touch Thermal Cycler (PCR) and Applied Biosystems's ABI3730XL (fragment analysis) using 21 pairs of fluorescence-labeled, highly polymorphic SSR primers that amplify 144 distinctive SSR DNA fragments. Fingerprinting data are visualized using ABI's GeneMapper® or SoftGenetics's GeneMarker® software and manually interpreted due to unacceptable error rates from automatic data output for sugarcane polyploids. The presence (A) or absence (C) of the 144 fragments is recorded in an affixed sequence order as a DNAMAN® sequence file to represent the molecular identity of the clone, which is archived into a local molecular identity database. The molecular identity database is updated annually by fingerprinting of newly assigned varieties and exchanged germplasm. The local database provides molecular descriptors for cultivar registration and clone identification, cytoplasm characterization, paternity determination of polycross progeny, determination of genetic relatedness of parental clones, assessment of cross quality and genetic transmission of SSR markers.

Keywords: *Saccharum* spp., Clone, Database, Fingerprinting, SSR