10TH GERMPLASM & BREEDING

7TH MOLECULAR BIOLOGY WORKSHOP

MACEIÓ - BRAZIL / 15 - 20 MAY 2011




"Breaking breeding and biotechnology paradigms - towards a complementary approach in sugar cane research"

ABSTRACT

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7Th MOLECULAR BIOLOGY WORKSHOP
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WELCOME MESSAGE

SUMMARY

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ORAL ABSTRACTS
BREEDING (BO)

B01

SWEET SORGHUM – A COMPLEMENTARY CROP TO SUGARCANE

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Keywords: Sweet Sorghum, biofuels industry, production costs.

As a complementary feedstock to sugarcane, sweet sorghum offers the existing sugar-based biofuels industry the opportunity to expand production; more fully utilize existing sugarcane assets and lower production costs. In recent field tests of new sweet sorghum hybrids, yields of both extractable fermentable sugars and biomass have been comparable to sugarcane, with attractive production costs on a per-unit basis. Moreover, sweet sorghum may be grown on land less suited to sugarcane production, and in many regions, harvested prior to the start or following the end of the traditional sugarcane production season. This presentation examines the development of and potential project applications for sweet sorghum as a bioenergy crop.

B02

BREEDING SUGARCANE FOR TEMPERATE AND COLD ENVIRONMENTS

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Keywords: Breeding, Sugarcane, temperate environments

Louisiana represents one of the world's more temperate environments where sugarcane is commercially grown. Since its inception in the 1920s, The USDA-ARS breeding program at the Sugarcane Research Laboratory in Houma, Louisiana, U.S.A. has focused on breeding varieties adapted to this unique environment. Unique selection strategies geared toward early sucrose production have enabled the industry to grow varieties able to produce over 100kg sugar per Mg of cane in seven months. With the growing and harvest period dictated by early- and late-season freezes, cold tolerance is a major trait of interest in the breeding program. The increasing interest in sugarcane as a biofuel feedstock has increased the number of studies aimed at selecting for increased levels of cold-tolerance in parental clones. Clones selected from the basic breeding program have been sent to numerous locations in the U.S.A. to assess their cold hardiness. These locations range as far north as Prosser, Washington (46°12′25″N 119°45′56″W. Selected material has been included in a cold tolerance evaluation in St. Joseph, Louisiana (31°55′7″N 91°14′18″W) to determine the rate of juice degradation following a freeze. Following one year of evaluation, clones from the basic breeding program showed significantly less juice degradation than commercial clones. In 2008, 20 seedling families were planted in Booneville, Arkansas (35°8′23″N 93°55′17″W) and allowed to overwinter in 2008, 2009, and 2010. Results showed differences in survival rates between families, and this information is being used in the breeding program to select parents able to transmit cold tolerance to their progeny. Houma's basic breeding program currently maintains a selection of 50 Saccharum spontaneum accessions. These 50 accessions were screened for cold tolerance using a growth chamber assay. Differences were observed between the clones for shoot emergence after being subjected to freezing temperatures (six days at -7°C). The S. spontaneum accessions identified as having superior cold tolerance are being included as parents in the 2011 crossing season. Ongoing studies at the USDA-ARS-Sugarcane Research Unit aim to further adapt sugarcane to temperate growing regions.

B03

INTEGRATED ESTRATEGIES FOR BREEDING SUGARCANE FOR DROUGHT TOLERANCE

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Keywords: Drought tolerance, gene expression.

To attend the increasing ethanol demand, the sugarcane industry must expand to drought prone regions from 'Cerrado' (savanna), incorporating pastures from Central Brazil, characterized by a dry winter with a prolonged water deficit period. Many sugarcane cultivars have been released in Brazil recently, but few with yield potential under drought-prone conditions. Therefore, elucidation of sugarcane mechanisms (perception, signaling, regulation and response) involved in tolerance to water deficit is valuable to develop productive cultivars, adapted to these marginal lands, assisting in the sustainability of the sugarcane industry. But, this requires integrating knowledge from various areas of plant sciences, such as physiology, genetics, molecular biology and crop breeding. Most of the studies on drought tolerance have focused either on phenotyping (morphology and physiology) or genotyping (genomics and transcriptomics), mostly under controlled conditions, without the corroboration from field assays. Our program intended to develop dependable protocols to screen for sugarcane genotypes tolerant to water deficit stress through various methodologies (field trials; greenhouse irrigation withdrawn; *in vitro* and *in vivo* exposure to polyethylene glycol solutions; paraquat exposure). Field trials were conducted at the 'Jalles Machado' sugarmill (Goianésia, GO) with a prolonged drought period. Our aim was to evaluate a population of 100 clones, screening for standards (contrasting) genotypes for tolerance and sensitivity to drought, and after selecting two contrasting genotypes, confirm their performance by various methodologies of induction/simulation to drought. The cultivar 'IACSP 94-2094 showed enhanced features of drought tolerance (early stomatal closure, maintenance of leaf water potential, osmolytes accumulation), whereas 'IACSP 97-7065' displayed poor performance under drought stress. Ten genotypes previously assessed in field trials were also tested under greenhouse conditions for periods of irrigation withholding,, including the two contrasting genotypes. The two standards were also submitted to *in vitro* and *in vivo* application of PEG, exhibiting similar behavior with the field trials. Structural gene expression levels were also evaluated and used to compare the methodologies. The tolerant genotype showed improved oxidative response when exposed to Paraquat. Generally, most controlled conditions results corroborated with the field trials. These protocols should be adopted for large-scale screening of tolerant elite clones in our sugarcane breeding program.

CAPES, CNPq, FAPESP, FUNDAG, JALLES MACHADO MILL

B04

BREEDING SUGARCANE FOR WATER-LIMITED ENVIRONMENTS

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Keywords: water stress, physiology, water use efficiency

Water stress is the major environmental limitation to higher sugarcane yields world-wide, and is also a major factor constraining expansion of sugar industries beyond existing boundaries in many countries. In many irrigated regions, higher water use efficiency is also being increasingly targeted as a high priority because of limited water supply and or potential increases in water prices. In Australia, water stress normally occurs during the spring and early summer periods in most sugarcane growing regions, and in most years causes serious reductions in yield.

Breeding crops for higher yields under water stress is notoriously difficult, mainly because of large genotype x environment interactions. Different levels of timing and severity of water stress, and the physiological response mechanisms involved, can cause the ranking of genotypes to vary greatly between different water limited environments. Hence, “traditional” selection involving simply screening large numbers of genotypes for yield in semi-randomly chosen environments can be ineffective.

In our research we have hypothesised that a better understanding of the main physiological processes causing genetic variation in response to water stress may lead to more targeted and effective gains from selection, compared with selecting for yield in random water limited environments. To test this, we have screened 133 clones of diverse genetic backgrounds across a range of environments with varying degrees of water stress, including at sites with irrigated and rain-fed treatments. In addition to measurements of yield components we monitored stomatal conductance and relative water content of leaf tissue of all clones, and water use of a subset of clones. Significant genetic variation in response to water stress was observed. As expected, the responses differed across sites, and genetic correlations between yield responses and the traits studied (stomatal conductance, etc) were only small, suggesting that several different physiological processes determining yield are involved.

Based on the data obtained to date, several hypotheses about the major physiological processes explaining observed genotype x environment interactions have been developed, and these will be discussed in the presentation. Genetic variation in these processes is being incorporated into the crop growth simulation model, APSIM-Sugarcane, in order to quantitatively predict responses of particular genotypes across a wider range of environments. These predictions will be tested in further field experiments in coming years. Ways in which potential application of the results to undertake effective breeding for water stress will be discussed.

B05

QUALITY EVALUATION OF SUGAR CANE GENOTYPES GROWN IN BARBADOS TOWARDS EXTENDING THE HARVEST SEASON

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Keywords: *Saccharum* sp., sugar accumulation, maturity, fibre

Quality characteristics (juice BRIX, field BRIX, pol in juice, fibre, moisture and purity) of twelve sugar cane genotypes (five high quality (HQ), four high fibre (HF), and three commercial (C)) were evaluated from six months (April) after planting (possessed at least five internodes) to harvesting (March) in plant cane and first ratoon. The experiment was a randomized complete block design with three blocks and three replicates (44 stools per replicate) per block. Differences between genotypes and genotypic groups with respect to all quality characteristics were significant ($P < 0.001$) during each month. All three genotypic groups had the highest mean juice BRIX and field BRIX in March: Cs-23.1, 24.9; HFs- 20.0, 20.6; and HQs- 24.1, 25.6. From October to March, mean HQ juice BRIX was consistently larger than 21.0. Regression analysis showed that the rate of increase of juice BRIX of the Cs was 1.4 times larger than the HFs and 1.6 times larger than the HQs, but that of the HQs was largest initially. During March, all three groups also recorded their highest pol in juice: Cs (18.6), HFs (13.9) and HQs (19.9). The linear rate of increase of pol in juice of the Cs was 1.7 times that of the HFs and HQs. Mean HQ pol in juice initially (April) was 13.1 (0.8 less than the HFs mean highest value). As early as January, the pol in juice of the HFs was 13.5 and the juice BRIX was 18.0 while that of the Cs and HQs were 16.7, 20.7; and 18.8, 22.2, respectively. The HFs had their highest mean purity in January (74.9), while the Cs and HQs had their highest in February (81.1) and November to January (84.6), respectively. Mean HQ purity was already 76.6 in May, whereas by January, mean C purity was 80.8. The linear rate of increase of purity of the Cs was 2.3 and 3.0 times that of the HFs and HQs, respectively. All three groups recorded their lowest mean moisture in March: Cs (63.7), HFs (58.7) and HQs (64.3). The linear rate of decrease in moisture of the HFs was 1.2 and 2.1 times that of the Cs and HQs, respectively. The HFs and HQs recorded their highest fibre in March (HFs: 27.1, HQs: 16.0) and the Cs in February (18.0). Mean HF fibre was already greater than 20.0 by July and 25.0 by December. The highest mean HQ fibre was only 59.0% that of the HF and 88.9% of the Cs for the same month. The linear rate of increase for fibre of the HFs was 2.7 times larger than the Cs and 5.4 times larger than the HQs, although the HQs and Cs initially had similar values. Correlation analysis showed that juice BRIX, field BRIX, pol in juice and purity increased in a linear manner, but all decreased in a linear manner with increasing fibre. Moisture showed significant negative correlations with fibre only.

B06

BREEDING VARIETIES SUITABLE FOR MECHANICAL GREEN CANE HARVESTING

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Key words: varieties, erect stalks, mechanization, self-trashing, nonflowering

Sugarcane varietal improvement started in India in 1912 with the establishment of Sugarcane Breeding Institute (SBI) at Coimbatore. Sugarcane fields have now been replaced by Co 419, Co 740, Co 7219, Co7527, Co 8014, CoC 671, Co 86032 and CoM 0265. Of these varieties Co 419 and Co 740 have served the Maharshtra State Sugar Industry for more than three decades in such a manner that State lion share in cane and sugar production in the country. Since the last two decades, because of higher sugar content in Co 86032 and CoC671 Maharshtra could achieve average sugar recovery of 11.67%. These two wonder canes have brought socio-economic change in the life of cultivators in the State. But, due to the hike in cane price the area under sugarcane cultivation has drastically increased from 0.7 million hectare to 1.35 million hectares during last three years. This has resulted in increasing pressure on laborers available for harvesting of sugarcane, as a result many a times some of the cane has remained standing in the field even after crushing season. Due to a lodging nature of all the above varieties only manual harvesting was possible and industry was assured to depend on availability of manpower for this purpose which is already in short supply. Therefore, sugarcane breeders tried to breed sugarcane varieties suitable for mechanical green cane harvesting with self detrashing or free trashing nature, high tonnage with high sugar, thick and non lodging canes, less residual ratio, less brittleness, sparse or non flowering, free from common diseases and good ratoon ability etc.

During 1982, SBI developed and released for Peninsular India, an erect growing sugarcane variety Co87025 (Co 7704 X Co 62198) but unfortunately due to higher wax on cane and less sucrose % than Co 86032, it could not influence the cane growers as well as sugar mills. In 1998, from a poly cross with Co8371 as a female, Vasantdada Sugar Institute could select a clone having all these traits as mentioned above in CoVSI 9805 variety which was released recently for general cultivation in Maharashtra State. CoVSI 9805 has given higher cane yield by 22% and sugar yield by 25.18% than the standard check variety Co86032 (0.69 to 0.93 units higher sugar recovery in big mill trials), good cane diameter with higher single stalk weight, good ratoon ability, easy detrashing, erect and nonflowering or late and sparse flowering nature. CoVSI 9805 is spreading faster under drip irrigation and wider planting suitable for mechanical green cane harvesting which has influenced the cane growers to promote drip irrigation and mechanization in sugarcane in Maharashtra. Among the promising clones i.e. CoVSI 03102 has proved its superiority in sucrose% over CoVSI 9805 along with other desirable traits for mechanization and it will be released shortly.

B07

ADDITIONAL SELECTION GAINS FROM PARENT SELECTION ADOPTING A NEW GENETIC EVALUATION SYSTEM

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Keywords: parental selection, statistical analysis, factor analytic, breeding value, economic selection index

BSES-CSIRO sugarcane breeders use data from family trials across four regional programs to estimate breeding values of parents for cross-pollination as well as to determine the potential of a sugarcane family to produce elite progeny. Hence, one way genetic gain to the Australian sugarcane industry can be maximized is through efficient parent selection and choosing specific family combinations to be produced by cross-pollination. Previously, breeding values were estimated using a simple mixed model developed over 10 years ago which relied on an index of cane yield and sugar content called Net Merit Grade. A new statistical approach has been developed to obtain improved estimates of breeding value by addressing many of the limitations of the previous approach, including: modeling site-specific spatial variation; combining family trials across regions using a Factor-Analytic mixed model to exploit the genetic correlations between trials established in different regions; and estimation of breeding values for individual traits, cane yield and sucrose content, separately rather than for Net Merit Grade. Further, as Net Merit Grade no longer reflects current production systems, the improved breeding values were incorporated into a new economic selection index. Differences between the economic index and Net Merit Grade are discussed. Results indicated that the new analytical approaches explained over 75% of the phenotypic variation in both cane yield and sucrose content, while previous methods explained less than 40% of the variation. The breeding values were also predicted with greater accuracy using a genetic evaluation system. Simulated selection using the new evaluation system suggest that additional genetic gains of up to 29 % for cane yield and 10% for sucrose content could be achieved when parents are selected on breeding values estimated using the new approach over the old approach. Additional gains for the industry of $2.34 per tonne of sugar produced, are achieved if parents are selected using the new economic selection index combined with breeding values estimated using the new approach. Sugarcane breeders can therefore select parents with greater confidence when using the new evaluation system outlined for analysis of data from family trials.

B08

GENETIC PARAMETERS FOR HIGH SUGAR CONTENT AND POTENTIAL FOR SUGARCANE IMPROVEMENT IN ECUADOR

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Keywords: *Saccharum* spp., parental clones, crosses, high sucrose content, selection stages.

There is a need to improve varieties with high sugar content under the Ecuadorian lowland of the Guayas River Valley. Therefore, the breeding program of the Sugar Cane Research Center of Ecuador (CINCAE) is developing a population with high sugar content. Two groups of varieties (G1 and G2) from the sugarcane collection were evaluated in plant cane and first ratoon crops. In G1, 11 varieties with high and 2 with low sucrose content were selected. Two groups of crosses obtained among these 13 varieties were evaluated: 27 (CR27) planted with seedlings and a subset of 12 (CR12) using small setts derived from seedlings. These crosses were evaluated in plant cane and first ratoon crop in Stage I, and at the first clonal selection, or Stage II, in plant cane crop. All evaluations for pol % cane and brix % cane were carried out with the parental clones (PROG) and two control varieties (Ragnar and ECU-01).

Parental clones and crosses in Stage I showed similar performance in plant cane and ratoon crop. Estimates of broad sense heritability were higher than 0.7 and similar to those reported in other studies, suggesting that there is genetic variation among parental clones that can be used to establish breeding schemes. Genetic variances for varieties in all characters for parental clones were important in G1, G2 and PROG and variance between plants within families () were the most important in the crosses CR27 and CR12. In plant cane and first ratoon, narrow sense heritability estimates were lower in CR27 (< to 0.3), while in CR12 they were higher (> to 0.6). This suggests that a recurrent selection program may be effective and utilize the interaction effects of dominance due to clonal reproduction of sugarcane.

Varieties CC85-63, BJ65-152 and progenies of crosses with any of them consistently showed high sucrose content through plant cane and first ratoon in both selection stages. Crosses with high sugar content showed a higher frequency of clones that surpassed the parental clones and controls varieties. Crosses between clones that have the variety POJ28-78 as a common ancestor showed a low frequency of outstanding progenies. This suggests that the common genetic basis of these parental varieties have reduced the probability of accumulating favorable genes. Therefore, the establishment of a population of high sucrose content should use parental clones of specific genealogy with good agronomic potential and ability to produce elite progenies.

B09

NEW SELECTION STRATEGY FOR THE SUGARCANE BREEDING PROGRAM AT RIDESA/UFSCar

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Keywords: S*accharum* spp; genetic improvement; genetic gain.

Traits inheritance in sugarcane is controlled by many genes and has strong environment influence. Therefore, genotype x environment interaction studies is essential to maximize the genetic available gains. However, breeders exploit this interaction only in the final selection stages of the breeding program due to the availability of vegetative propagated material to establish experiments under different environmental conditions. Here we summarize the results of a new strategy to capitalize the genotype x environment interaction in stage two of selection, where genetic variability of the population is high. The study was conducted at the experimental stations of the Sugarcane Breeding Program, RIDESA/UFSCar, in Araras (CCA) and Valparaíso (EVA), both in the State of São Paulo. Araras (22º 22' S/47º 23' W) has a predominant Dark Red Latossol Clayey Texture soil, while Valparaíso (21º 13' 20" S/50º 52' 00" W) is a Red Podzolic Sandy texture soil. Nine hundred forty-two mid-late maturity genotypes of the 2004 series (RB04) were evaluated in stage 2 (T2 phase). This group of clones allowed to created an intermediate phase between the early selection phases, T1 and T2, named FM of T1 (multiplication phase of T1). The plot of this new phase was formed by a 4 m single row, without replication, spaced 1.40 m between rows. Data in T2 phase was collected for number of stalks, brix, and 10 stalks weight and kilogram of brix per plot (KBP) and visually evaluated for performance. The new multiplication phase allowed having more stalks available for plant the T2 phase in different locations from the original T1. The FM of T1 also allowed to analyze genotypes in larger plots for visual aspects. The 2004 series was the first of two identical T2 fields with 942 genotypes in the two locations (CCA and EVA), a total of 445 clones were selected in both environments. Only 150 clones were coincidentally selected in both environments whereas 142 clones (31.97 %) were selected at EVA and 153 clones (34.38 %) at CCA. Comparing series 2003 (the traditional selection system) and 2004 (the new strategy), both with same number of initial seedlings, the number of clones selected from T2 was similar in numbers of genotypes and also in visual evaluation. This new strategy resulted in a more secure way to explore the genotype x environment interaction of the T2 phase. In addition, this strategy made possible to reduce the numbers of individuals in T2 fields without compromise their agronomic qualities of the selected population.

B10

REDUCTION IN INTERGENERATION TIME INTERVAL IN SELECTION OF SUGARCANE VARIETIES THROUGH POPULATION TESTING

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KEY WORDS: Selection, sample testing, population testing, intergeneration time interval.

Traditionally, a variety testing scheme spans over a period 13 to 15 years. In most countries all releases are based on small plot (stage 6) trials and small mill analysis. Rarely field planting and large mill tests are carried out with a crush of an hour which is the minimum time required. The current selection programme suffers from old concepts with sample testing and extrapolation of the data, which does not withstand the rigours of field conditions. The gap between the trial plots and field is large on both counts namely Pol% cane and yield. This paper discusses the population-testing concept to facilitate large mill testing and cutting down intergeneration time interval for releasing varieties. In Parry's variety testing scheme, the intergeneration time interval for releasing varieties was reduced from 13 years to 6 to 8 years. This was possible with a change in the variety testing concept. It was a population testing concept which includes early selection based on heritable characters like brix, fibre, pest and disease reactions and later 20 ha and more under field conditions. The best clones were taken for multiplication at three locations using single eye buds. The principle was that instead of using small plots, larger populations at three locations were used. The three varieties viz., PI 96-0151, PI 97-0843 and PI 97-1946 were multiplied along with Co 86-032. These varieties were tested in the large mill for their performance and behaviour under field conditions and compared with the standard variety Co 86-032. All the three varieties recorded higher yield and POCS% when compared with Co 86-032. The early and advance yield trial results are discussed and confirmed. We can release the varieties through large population test, much earlier as the system provides conclusive information on varietal performance under field conditions thus reducing the intergeneration time interval in selection of varieties for commercialization.

B11

BREAKING PARADIGMS: WHAT DO WE NEED, AND WHAT DO WE NEED TO KNOW?

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Keywords: Sequencing, Polymorphism analysis, Bioinformatics, Statistical methodology

The last 15 years has seen an increase in knowledge about genetics and genomics of sugarcane. In the same time period, increases in technology (sequencing, polymorphism analysis, bioinformatics and statistical methodology) have been exponential. Breeding programs for some crop and livestock species have used these technologies to fundamentally change their breeding strategies. Sugarcane breeding, however, has not been able to leverage these technologies in the same way, and has not seen much change in broad strategy and methodology in recent years. Part of this is due to the complex hybrid-polyploid nature of the sugarcane genome, and to the fact that sugarcane has to some extent relied on piggy-backing on research in other crops to leverage advances. In order to make significant progress to break current paradigms, investment will need to be made to solve sugarcane-specific issues. The presentation will examine the key issues that affect the efficiency of sugarcane breeding and molecular breeding, and what research is required in order to take advantage of DNA-based technologies currently available.

B12

INTEGRATION OF SUGARCANE BREEDING AND BIOTECHNOLOGY: AN AUSTRALIAN PERSPECTIVE

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Keywords: Breeding, GM, molecular markers, selection index, tissue culture

Historically, 1921 and 1991 represent landmarks for sugarcane breeding and biotechnology, respectively. The former saw the release of POJ2878, the most notable outcome of the nobilisation work of the Dutch breeders in Indonesia, following Soltwedel's demonstration of interspecific hybridisation. The latter was the production of first genetically modified (GM) sugarcane by Robert Birch in Australia. Both sciences have developed dramatically since, but have been somewhat restricted by the complexity of the sugarcane genome.

Commercialisation of GM sugarcane remains a challenge. Nearly two dozen transgenes with commercial potential have been introduced and expressed in sugarcane. Yet none of them led to any commercial product, highlighting the political, technical and regulatory challenges in commercialising GM sugarcane. Integration of GM technology into breeding programs will involve identification of priority traits, introduction of genes controlling them into best cultivars and parents and developing crossing and selection strategies. The race is on for the first commercial GM sugarcane.

Despite a substantial body of research involving molecular marker development, the use of markers in breeding programs remains elusive. In Australia, we believe a critical step for the implementation of molecular markers is development of a selection index for important traits based on phenotypic and marker data. We have developed EGV (Economic Genetic Value) that combines economic weights and genetic values (BLUPs) of the major traits. Marker data can be incorporated using genetic correlations with the traits of interest, as with any indirect selection method. Simulation modelling is important in determining if or where markers can be used in a breeding program. Our work to date shows that gain per unit cost of using markers at any stage of selection is not viable, at current marker costs. However, the technology has potential in parent development and breeding and possibly in introgression to identify positive and negative effects of large genetic blocks (chromosomes). A trial is currently underway to test the efficacy to DNA markers for selecting parents resistant to smut. DNA fingerprinting has been integrated into the breeding program in two ways: (i) establishing the initial identity of accelerated clones (variety audit) and additional information for Plant Breeder's Rights (PBR); (ii) checking/rectifying possible identity mistakes. Markers are also routinely used in disease diagnostics in domestic and international quarantine.Tissue culture is being used to a small extent for the commercial propagation and release of of new cultivars in Australia, providing disease-free material both directly and indirectly to growers. It is also being used by breeders to reduce the time to obtain disease resistance information and productivity data on foreign clones, after release from quarantine. This paper highlights the areas where integration of different technologies are contributing to the ultimate objective – the release of improved sugarcane cultivars.

B13

SUGARCANE IMPROVEMENT FOR BIO-ENERGY PRODUCTION IN CHINA

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Keywords: bioenergy, cellulosic, bagasse, bioethanol, trash

There has been rapid economic development in China in recent decades and demand for energy has consequently been increasing rapidly. Currently, most energy comes from coal, oil and natural gas, which are non-renewable, possibly increasingly expensive, and give rise to greenhouse gases. Development of clean and renewable energy has been assigned an urgent and high priority in China to help sustain long term and stable development.

Sugarcane is being increasingly used in several countries as feedstock for renewable energy products, and is a major and expanding crop in southern China. The purpose of this paper is to discuss the potential of sugarcane as a feedstock for bio-energy production in China. It includes a review of i) the existing sugarcane industry in China and key bio-physical factors affecting the extent to which sugarcane based industries could supply feedstock for renewable energy production in China, ii) what is being done and what has been done in the breeding programs of GSIRI, iii) the economic and policy factors which affect production of bio-energy from sugarcane in China, and iv) recommendations on actions and policies that may assist with appropriate development of bioenergy production from sugarcane in China.

B14

SIMPLIFIED SELECTION SYSTEM – SSS, A NEW TOOL FOR CLASSIC SUGARCANE BREEDING

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Keywords: early selection; sugarcane; breeding

Several sugarcane selection methods for the initial breeding phases have been developed. Some of them are recognized as the Australian Sequential Selection, Modified Sequential Selection and Combined Selection through Index. However, in terms of expected progress and the time needed to release new cultivars, these methodologies were considered inferior to mass selection (visual), because of the low selection rates and increase of field work and costs (Bressiani, 2001). A Simplified Selection System (SSS) used by some sugarcane breeding programs from Federal Universities of Brazil which are part of Interuniversity Network to Develop the Sugar and Alcohol Sector (RIDESA) has been an efficient tool. The system allows selecting materials through an "Early Negative Selection" before the selection stages are established in the field. Thus, avoiding the evaluation of undesirable first phase -T1 materials, reducing costs, materials and time to release new varieties. The SSS avoids high genotype x environmental interaction due to low environmental variance by managing three controlled distinct phases: sowing, early negative selection and ratoon selection in plastic bottles. Seed planting can be done in a substrate composed of 3 parts of filter mud and one of ash distributed in beds or polyethylene boxes. Germination tests are carried out in order to achieve 2 seedlings per cm^2. Negative selection will be performed with an index of 97% discard after 90 days. The best performing clones are selected considering a fast growth and development, tillering, stalks diameter and disease tolerance and planted in plastic bottles. After 60 days, plants are cut to induce ratoon growth. After 180 days, an early selection of the best ten genotypes per crossing is performed using the mixed model methodology (REML/BLUP). Data analysis is performed for the following yield components: stalk height (m), stalk diameter (cm), number of stalk per stool and genotype mass/stool (kg). The Genetic variances and genetic values of genotypes/family are estimated to perform selection based on the mixed model. Selected stools of superior genotypes are planted in small areas of commercial plots avoiding the use of seedlings. This will allow obtaining significant selection gains and reduction of costs and time to release a new cultivar.

B15

SUGARCANE WIDE HYBRIDS: NEW FEEDSTOCKS FOR ENERGY

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Keywords: Wide Hybrid; Miscanthus; Cold Tolerance

High yielding C4 grasses are naturally well adapted to a range of climates, allowing profitable biomass production and flexibility to growers. The Texas Agilife Reseach Center in Weslaco, Texas has an integrated research focused on the unique germplasm resource of the "Saccharum Complex", aiming at the production of high yielding dedicated bio-energy crops. Besides *Saccharum*, such a complex includes species from *Miscanthus*, which is a perennial grass among the very few plants in temperate climates that use the more efficient C4 photosynthesis process, and is also exceptionally cold tolerant. Because each of these crops has its relative strengths and weaknesses, their inter-specific hybridization is a method to create a unique new species, specifically developed for bio-energy production. Ninety genotypes from the World Colleciton in Miami have been introduced in South Texas and are being characterized for breeding purposes. We have been able to produce *Saccharum x Miscanthus* hybrids, with extreme cold resistance and *Saccharum x Sorghum* hybrids. These hybrids are currently being fast propagated for biomass production assessments. Molecular markers are being developed from genes related to cell wall components and are being applied to these germplasm for marker-assisted selection.

B16

BENCHMARKING BREEDING PROGRAMS – DIFFERENT APPROACHES TO MEASURE THE VALUE CREATED BY NEW VARIETIES

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Keywords: Productivity, benchmarking, genetic gain, new varieties

Benchmarking breeding programs is important in quantifying success and is increasingly demanded by both industry and administrators to justify investment levels in this critical activity. In the BSES-CSIRO Joint Venture Variety Improvement Program, we routinely use a number of benchmark statistics. These include:

• Number of varieties released (Target: 3 varieties/5-year period for each region).

• % production attributed to "new" varieties (Target: 20%). New varieties are defined as those released within six years of the year prior to the production year – ie a new variety in 2010 is one released in 2004 or later. Those released prior to 2004 are defined as "old" varieties.

• % production of varieties with Plant Breeder's Rights (PBR) (Target: >90% of Australian industry planted to PBR-protected varieties by 2011).

• Rate of genetic gain. This is estimated by analysis of available mill data for cane yield, CCS and sugar yield. As previously described, linear regression is used to estimate the average genetic gain as well as the rate of genetic gain over successive 30-year periods for each productivity trait on a state-wide and regional basis. This method has also been used to benchmark breeding programs in Australia, Brazil and South Africa.

These do not provide any estimate of the value of new varieties in terms of prevention of losses through pests and diseases. This is even more important but is often not recognised by industry, mainly because breeders have been so successful in this area.

Better productivity data are now being collected in some regions. For example, for the 2009 season, in the Mackay region, new varieties (those released between 2003 and 2008) yielded 9.1 and 10.6 t/ha more than old varieties in first and second ratoon crop classes, respectively. Other analyses have been performed on new versus old varieties using standard productivity data. Allowance has been made for the higher proportion of plant and early ratoon of new varieties compared with old varieties, which have a higher proportion of older ratoons and less plant and early ratoon. A weighting factor, assuming a 4% reduction per crop class category, was estimated based on the distribution of crop classes for new and old varieties. This was remarkably consistent across the 5 Queensland regions (0.89 to 0.91). The cane yield (TCH) of new varieties was thus adjusted downwards. The value of new varieties was the estimated by the additional sugar produced (hectares new varieties x sugar yield (TSH) difference x sugar price). The total "value" of new varieties for the period 2007-09 was $191.4M. This equates to additional income that the sugar industry would have foregone without a continued investment in Variety Improvement. This is an extremely high return on investment.

B17

UTILIZATION OF BADILA AS A NOBILIZATION PARENT IN THE INTROGRESSION OF WILD GERMPLASM IN MAINLAND CHINA

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Keyworks: Sugarcane; Badila Variety; YC-series Parents.

Important efforts have been made for broadening the genetic base of sugarcane since the establishment in 1953 of The Hainan Sugarcane Breeding Station (HSBS), Guangzhou Sugarcane Industry Research Institute (formerly known as Sugarcane Industry Research Institute, Ministry of Light Industry of China), mainly by using wild germplasm, such as *S. spontaneum* L., *S. robustum* Brandes and Jeswiet ex Grassl and *Erianthus arundinaceus* (Retz.) Jeswiet, and other species. Noble canes, mostly *S. officinarum* clones, were used to nobilize the wild germplasm in the initial crosses. Badila was one of the most successfully used nobilization parents in Mainland China to introduce desirable attributes such as strong ratooning, drought tolerance and disease resistance.

Over the past fifty years, HSBS has produced a number of parental material of YC-series derived from newly used clones of *S. spontaneum*., *S. robustum* and *Erianthus arundinaceus* by using Badila as nobilization parent. Some of them have been commonly used in the national sugarcane breeding program. The successful attempts to cross Badila with *S. spontaneum* were made at HSBS in the crossing seasons of 1957, 1978 and 1981. YC58-43 and YC58-47 were selected from the progeny from Badila×YC *spontaneum* In 1958. In addition, LS-spont (another *spontaneum* clone native to Lingshui，Hainan province) and YC75-2-11(a *spntaneum* clone from Yunnan province) were used in 1978 and 1981 and produced YC79-290 and YC82-108, respectively. From the 1960s to the 1990s, the above-mentioned clones were backcrossed to commercial clones and produced many widely used parents, such as YT 64-395, YC71-374, YC84-125, and YC90-55, and other. To date, 32 commercial varieties, about 1/6 of the total self-bred varieties in Mainland China, can be traced back to these parents derived from Badila×*S. spontaneum* clones.

Although no commercial varieties were produced from crossing Badila with *S. robustum* clones and *E. arundinaceus* clones so far, a number of elite hybrids and back crossing progenies have been selected for further use. Successful progenies obtained from BC1 and BC2 of *E. arundinaceus* symbolized a breakthrough in the utilization of this wild relative in recent years.

B18

A BIPLOT-BASED ANALYSIS FOR EXPLORING INTERACTIONS IN SUGARCANE MULTIENVIRONMENT TRIALS WITH MULTIPLE HARVESTS

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Keywords: mixed linear models, temporal correlations, spatial correlations, triple interaction.

Sugarcane (*Saccharum* spp.) breeding programs involve multienvironment trials (MET), where genotype yields are compared at different crop ages within and across locations. At least two types of correlations are expected in each trial: temporal correlation among yield data from the same plot (consecutive harvests per genotype) and spatial correlations among data from neighboring plots. It is also expected that the residual variances are not equal in different environments, which are identified not only as location (e.i. test sites) but also in relation to crop age. Mixed Linear Models (MLM), which adjust genotype means and standard errors on the basis of lack of independence and homogeneity of variance, turn out to be more appropriate than classical ANOVA models to compare genotype performance and to analyze genotype-location, genotype-age and genotype-location-age interactions. MLM allow obtaining predictors of random terms (BLUPs) of interactions, which are useful to understand genotype-by-environment association (GE). This work proposes a graphical MLM-based approach to study interactions between genotypes and locations considering different crop ages. We worked on data from MET of the Sugarcane Breeding Program of Estación Experimental Agroindustrial Obispo Colombres (Tucumán, Argentina). Twenty clones were compared with respect to cane tons per hectare at six locations through plant cane and first and second ratoon crop ages. A MLM was fitted with heterogeneous residual variances among locations, temporal correlations among data from the same plot for different ages, and spatial correlations among data from plots according to their two-dimensional coordinate position (rows and columns within the spatial arrangement of the field trial at each location). To model serial correlation across crop age beside rows and column correlations derived from the spatial plot arrangement, we use a first order autoregressive model in three dimensions, deriving from the direct product of temporal and spatial correlation matrixes. The adjustments were attained with PROC MIXED SAS v. 9.1. Location effect was considered to be random, so genotype-location-age (GxLxA) interaction was treated as random as well. Triple interaction BLUPs were used to form a GxLxA interaction matrix, which was subjected to principal component and biplot analysis. Results were compared with the ones obtained with an AMMI biplot of GE interaction, where E is held as the combination of location and age factors. According to Akaike Information Criterion and likelihood-based test, the MLM was better than the classical ANOVA model assuming independent data and homogeneous variances. The biplot obtained from the triple interaction BLUPs facilitated the interpretation of genotype-age interactions for each location and genotype-location interactions for different ages.

B19

BREEDING FOR MULTI-PURPOSE SUGAR CANE VARIETIES: APPLICATION OF MULTIVARIATE TECHNIQUES

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Keywords: Biomass, high fibre varieties, interspecific crosses, selection index

The recent revision of EU sugar price entailing a cut of 36% has compelled ACP member states to restructure their respective industries to ensure viability and sustainability. Additionally, with an anticipated increase in the price of fossil fuel, sugarcane with its high biomass potential is increasingly recognised as a stock material for a wide range of products but mainly for sugar, ethanol and electricity through cogeneration.

The Mauritius Sugar Industry Research Institute has thus widened its scope to include a component for the production of different types of canes including the multi-purpose types. About 8-10% of the seedlings is produced each year for the interspecific programme and includes the 1st generation crosses, F1, the 1st generation backcross, BC1, and the 2nd generation backcross, BC2. These are routinely screened to identify different types of high biomass and fibre varieties. Additionally a collection of 60 genotypes that were present in the germplasm collection were pre-selected based on morphological attributes and evaluated in five series of trials - 2 x 5m rows x 3 replicates – over a plant cane and first ratoon crops in a sub-humid, partially irrigated environment. The test genotypes included F1 (21), BC1 (31) and BC2 (6), Erianthus clones (2) and most of the crosses were made with commercial parents as the recurrent parent. A wide range of morphological attributes, cane biomass and quality characters were measured and the data were subjected to multivariate data analyses (MVDA).

Spatial analysis techniques were used to obtain precise and comparable means of all the genotypes planted in the five series of trials. Principal component analysis (PCA) compressed the different characters into five major Principal Components (PCs). The first two explained 79% of total variation. PC1 emphasised on the cane quality traits while PC2 stressed on biomass characters. Cluster analysis defined six major groups in the population. Candidates from three of them were found suitable for either sugar, fibre, or both as the main end-products and these were promoted to the final phase evaluation trials. The results also showed that, while high fibre varieties could be produced from wild and F1 clones, high biomass varieties were obtainable from any of the different generations.

Based on variations in cane quality and biomass traits, four types of canes with different levels of Pol and fibre have been defined for multiple uses: Type 1 (commercial), Type 2 (commercial with enhanced fibre), Type 3 (multi-purpose high fibre), and Type 4 energy canes (fibre > 22% for cogeneration). A selection algorithm has been developed that identified 11 potential high biomass genotypes. The selection index has been extended to the whole selection programme for classifying new sugarcane varieties as from the third clonal stage, where 420 genotypes are routinely evaluated in the first replicated trials.

B20

SELECTION ON SUGARCANE FAMILIES USING REML/BLUP AIMING TO INCREASE BIOMASS

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Keywords: *Saccharum* spp., mixed models, selection, plant breeding.

The use of biomass as feedstock for power generation is of outstanding significance in the search for energy alternatives, given that it is a renewable source of energy. Successful exploration of sugar sugarcane is mainly conditioned breeding programs, in which the aim is to select varieties adapted to the climate conditions of the productive areas for economic needs. The aim of this work was select superior families in sugar cane, originated from biparental crosses, for biomass production, using mixed model methodology (Reml/Blup). 40 families were used originated from crosses made in Serra do Ouro, Murici city, in Alagoas state, from RB01 series. The experiment was installed in Paranavaí Experiment Station, in the north of Paraná state. The Reml/Blup methodology was adopted, whit the Reml used for estimation of the genetic variance, and the BLUP for the estimation of genetic values of families and parental used. The experimental statics design used was incomplete blocks, whit three replications per family. Each replication was composed by fifteens plants. Were used the 38 statistic model of Selegen Reml/Blup computer program, for the indication of promising families. For selections of superior families seeking to increase the biomass were considerate weight of one stalk in kg (W1S), numbers of stalks per plant (NSP) and a weight of stalk per plant in kg (W1P). According to the obtained results, the analyzed characters showed individual heritability () were high (0,42; 0,60 e 0,67). The average genotypic value of the families for W1P was 3,46 kg. Selecting the 20 best families, the estimated average amounts to 13,62%. The selection of families whit genotypic values greater than the average trial it is estimated significant profits of W1P. The best five families for W1P originated from biparental crosses are SP803280 x RB855559, RB72454 x SP70-1143, SP803280 x RB835486, RB72454 x RB835486, SP80-1816 x RB855156, with average gains of 38,77%; 32,49%; 23,58%; 23,53% and 20,15% respectively. The families selection through the mixed model Reml/Blup can be an important strategy to identify families with high genotypic values, where there is a higher probability of selection of potential genotypes for biomass production.

B21

VISACANE, AN INNOVATIVE QUARANTINE TOOL FOR THE EXCHANGE OF PEST AND DISEASE-FREE SUGARCANE GERMPLASM

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Keywords: quarantine, pathogen detection, sugarcane, new viruses, metagenomics.

Sugarcane varietal improvement cannot exclusively rely upon exchange and introduction of genetic resources *via* true seeds (fuzz). It also requires the introduction of vegetative propagation material (cuttings, tissue-cultured plantlets). The continued increase in international and intercontinental trade in plants has led to the enforcement of quarantine measures before introduction into a country because many plant pathogens can be carried and transmitted by vegetatively propagated material.

Visacane is the new name of Cirad's sugarcane quarantine (http://visacane.cirad.fr/en/). It covers three main quarantine procedures: detection of pests and pathogens, elimination of pests and pathogens, and transfer of plant material free of pests and pathogens. It has been devoted to sugarcane quarantining for several decades. Besides phytosanitary constraints, Visacane takes also into account legal constraints and ensures, through appropriate contracts, that plant breeders' intellectual property rights over the transferred material are respected. Unlike most sugarcane quarantines that are essentially used to import sugarcane germplasm into a country, Visacane can import and export varieties from and to most sugarcane growing countries in the world, ensuring that the material is free from any important pest and disease causing pathogen.

Until recently, the sugarcane quarantine process was aimed at detecting known pathogens harbored by the plant material and eliminating these pathogens whenever possible. It is an *a priori* process, because it only takes into account the pathogens that have been previously described and for which efficient detection tools exist. During the last three decades, several new viruses infecting sugarcane have been discovered, including *Sugarcane bacilliform virus*, *Sugarcane yellow leaf virus*, *Sugarcane streak mosaic virus* and the virus associated with Ramu stunt. In addition, the etiology of chlorotic streak, a disease known since 1929, has not been elucidated so far, although there is evidence for its infectious nature. Therefore, it can be assumed that unknown pathogens are still to be discovered in sugarcane, especially if these pathogens do not cause symptoms that can be easily observed. For these reasons, the research team associated with Visacane is setting up a new strategy of diagnostics, the so-called sequence-independent approach which aims at deciphering the virome (= the genomes of all the viruses that inhabit a particular organism). We believe that our forthcoming combined process, that will include our traditional approaches in addition to the metagenomics approach, will drastically improve our routine quarantine diagnostics.

B22

NIR PREDICTION OF SUGARCANE BAGASSE CHEMICAL COMPOSITION

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Keywords: NIRS, sugarcane bagasse, varieties

Presently there is considerable interest in cellulosic biomass as a feedstock for ethanol production. Determining the chemical composition of sugarcane bagasse is becoming increasingly important to give direction to variety improvement, especially in the light of advances in ethanol production technology. The use of wet chemical procedures to measure the chemical constituents is effective but is time consuming and expensive and therefore not applicable in a commercial/routine setting. Near Infra Red Spectroscopy (NIRS) has been used very successfully over the last number of years to analyze sugarcane samples at SASRI for brix, pol, dry matter content and fibre percent. The main advantages of NIRS are the speed, accuracy and cleanliness of analysis. A project was initiated to assess the suitability of NIRS as an alternative to wet chemistry procedures for the estimation of the proportions of lignin, cellulose and hemicellulose in sugarcane bagasse. Stalks of 100 varieties (imported, SASRI pre-released and SASRI released varieties) were shredded and then washed 3 times to remove ~90% of the soluble matter before drying at 35°C. At SASRI, aliquots of the bagasse samples were subjected to NIRS analysis using various sample preparation methods. The remainder (~1kg) of each sample was sent to Stellenbosch University for wet chemistry analysis to determine the chemical composition. Wet chemistry analysis followed the Laboratory Analytical Procedures developed by the National Renewable Energy Laboratory. The main variables measured were lignin, glucan, xylose and arabinose.

The bagasse chemical composition data together with the NIRS spectral data were subjected to multivariate analysis using partial least squares to develop calibration equations. A test set validation was performed, where half the samples were used to develop the calibration equation and the other half the validation. One out of the four sample preparation methods resulted in acceptable coefficient of determination (R^2) values for lignin, xylose, arabinose and glucan, ranging from 80 to 96%, with a root mean square error of estimation (RMSEE) ranging from 0.115 to 1.0. These results indicated that NIRS could provide an accurate predicted measure of the above chemical constituents. The validation R^2 values for the same sample preparation method however were much lower, ranging from 51 to 64%, with a root mean square error of prediction (RMSEP) in the range 0.2 to 1.2. These data indicate that further calibration and/or validation is required to develop a more robust equation. Once this has been developed, bagasse chemical composition data can then be used to create a new selection index for selecting varieties suitable for biofuel production in South Africa.

B23

MULTIVARIATE REPEATED MEASURES: A STATISTICAL APPROACH FOR ANALYSING DATA DERIVED FROM SUGARCANE BREEDING TRIALS

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Keywords: sugarcane, varieties, multivariate repeated measures, univariate, mixed model, model fitness

Data from plots in sugarcane breeding variety trials are collected for several variables over several sequential crop cycles, creating a multivariate repeated measures (MRM) data structure. The MRM analysis accounts for correlations between variables (multivariate) and correlations between crop-years (repeated measures) when computing experimental errors. Currently, univariate analysis (split-plot in time) is used to analyse the data. This approach ignores the correlation between variables and the correlation between crop-years, assuming independence between variables and between crop-years. The assumption of independence could produce incorrect estimates of experimental errors and that could lead to incorrect interpretations. The objectives of this study were to demonstrate the use of MRM analysis on sugarcane breeding variety trial data by determining multivariate effects, covariance structure for crop-years, and comparing univariate to MRM analysis. Data for yield (cane and stalk dry matter (SDM)), quality (ERC % cane and fibre % cane) and agronomic (stalk height and diameter) traits were collected from 16 genotypes planted in four blocks at Mkwasine location and five blocks at Triangle location, and harvested over eight crop-years. The data were analysed using the mixed procedure of SAS. The UN@CS covariance structure was chosen because it used fewer (7) parameters than UN@UN (42) and produced lower Akaike information Criterion (AIC=10406 for yield; 5054 for quality; -309 for agronomy traits) than UN@AR(1) with AIC of 10441 for yield, 5057 for quality and -270 for agronomy traits. At Triangle, cane yield (AIC for MRM=4225 vs Univariate=4417), SDM (MRM=3053 vs univariate=3219) and height (MRM=-233 vs univariate=-234) indicating that MRM produced better model fit than univariate. At Mkwasine, cane yield (AIC for MRM=3026 vs Univariate=3069), SDM (MRM=2118 vs univariate=2154) and height (MRM=-7 vs univariate=14) also indicating that MRM produced better model fit than univariate. The better model fitness for MRM led to greater statistical efficiency during data analysis as most of the variability in the data would be accounted for by the statistical model. There was significant correlation among crop-years for cane yield ($r=0.42$, $P<0.001$), SDM ($r=0.35$, $P<0.001$) and height ($r=0.28$, $P<0.001$) indicating that the source of the efficiency for the MRM was by including the covariance in calculating experimental errors. Quality traits produced non-significant correlation among crop-years. The MRM analysis produced greater discrimination of the differences between experimental genotypes and the control cultivar than univariate analysis for yield traits. At least five varieties categorized as significantly ($P<0.001$) different from the control by the univariate method were found either similar to the control or the difference was at lower levels of probability. For quality traits, the univariate and MRM methods were similar indicating that the univariate method (which is less complex) was adequate.

B24

SUGARCANE BREEDING PROGRESS AND EXPANSION OF CG VARIETIES CHALLENGE

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Keywords: Selection, adoption, released varieties, varietal composition.

The Guatemalan Sugarcane Breeding Program started to work in 1993 to formally develop new varieties for a national industry of 220,000 ha. The program includes four components 1) Develop genetic variability (crosses and introductions), 2) Selection of clones through different stages, 3) release of cultivars, and 4) seed cane production and distribution of the new varieties. In the later stages of selection the program carries out evaluation at eight regional trials and three semicomercial trials. The methodology has allowed to release three CG (CENGICAÑA – Guatemala) varieties and recommended to use two introduced ones. Sugarcane in Guatemala grows in different environments and genotype x environment interaction is an important issue to take into account before any variety release. All the varieties are recommended according to their adaptability to one or more of the four altitude zones and to one or more of the three harvesting periods. The aim of this paper is to show the main characters of the released varieties and analyze the challenge of new sugarcane varieties adoption. Possibilities for rapid adoption of new cultivars have been taking place in the Guatemalan sugarcane industry to meet the challenge of improving the weak varietal composition.

10TH GERMPLASM & BREEDING

POSTER (BP)

POSTER ABSTRACTS BREEDING (BP)

BP2

ESTABLISHMENT OF NEW SUGARCANE BREEDING CENTER AT AMBOLI, SINDHUDURGA DISTRICT OF MAHARASHTRA STATE IN INDIA

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Keywords: VSI's Breeding centre, India, *Saccharum*, ISH, flowering

High sugared varieties with higher fibre content, high water and nutrient use efficiency, resistance in biotic and abiotic stresses and multiratooning ability will have to be produced for meeting the projected sugar and energy requirements. Keeping these objectives in view the Institute has expanded its breeding activities from the year 2006. A sugarcane hybridization facility was established at Amboli (located between 16^0N and 74^0E and 590ft height from mean sea level with average annual rainfall of 14000 mm), in Sindhudurga district of Maharashtra State where good flowering and seed set were observed. A total 1029 clones belonging of the *Saccharum* species (72), related genera to Saccharum (11), Inter Specific Hybrids (166), Indian Hybrids (745) and Foreign Hybrids (35) were planted at SBC, Amboli during January, 2009 to be used as parents in hybridization work.

Sixty three percent of the clones flowered from November, 2009 to January 2010. By using 57 genotypes as parents total 118 biparental crosses and 72 polycrosses were effected. Besides the crosses, 107 General Collections were also made. Data on various aspects of flowering is described. Mauritian cut cane technique developed by Mauritius Sugar Industries Research Institute (MSIRI) will also be implemented soon to get the desired cross combinations as the VSI Scientists got such training in Mauritius during 2009. About more than 0.5 million seedlings were obtained and transplanted in polybags as Ground Nursery-I (2010). In order to select the varieties for different agroclimatic zones of the State, VSI selected in all five locations to conduct the selection trials start from the Ground Nursery to their final selection. These were passed through various stages of selection is mentioned and explained in this manuscript

Seventy nine clones from 2007 batch were under evaluation at two different locations (Manjari Farm) and Vasantdada R & D Farm) and based on yield and quality total twenty one clones viz., 78 188-12 (co 0215 x ISH 29), 190-14 (Co 0215 x Co 0202), 206-35 (CoM 9910 x Co 2000-08), 243-1 (Co 91010 GC) 54-43 (ISH 23 x 97-218), 110-51 (ISH 43x Co 8370), 165-15 (Co 87010 x ISH 29), 65-1 (28 NG 210 x ISH 176) 139-3, 139-6 (Co 1158 x ISH 29) were selected and advanced to Pre-final Varietal Trial (PFVT). Information on best selections will be presented. All the above selected clones are likely to be emerged as a new variety soon after their proper evaluation in the National Streamline of AICRP(S) and State varietal trials in different agroclimatic regions for the selection of the location specific and need based clones as a variety. Finally, looking to the outcome of the sugarcane breeding center (SBC) at Amboli which will be an International level breeding center in future next to Sugarcane Breeding Institute in India. The interested sugarcane research stations in India can send their promising genotypes for the evaluation and confirmation of the flowering at SBC, Amboli.

BP3

ESTIMATION OF OUTCROSSING RATE OF SUGARCANE UNDER NATURAL CONDITIONS THROUGH MOLECULAR MARKERS

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Keywords: polycross, outcrossing rate, molecular marker.

Sugarcane flower and pollen viability are dependent on environmental conditions, specially photoperiod, temperature, air and soil humidity. These factors, when appropriate can induce flowering and maintain pollen viability for sugarcane crosses This study aimed to evaluate the pollen viability of sugarcane commercial cultivars (IACSP95-5000, IACSP91-1099, SP89-1115, RB86-7515) at natural conditions of Ribeirão Preto (SP), Brazil, through the estimation of outcrossing rate obtained by molecular markers. Culms flowered in 2009 were harvest at the Sugarcane Centre ("Instituto Agronômico de Campinas") located in Ribeirão Preto. The stain pollen method with iodide solution (1g of I, 1g of KI dissolved in 100 ml of water) was used to classify the tassel as male or female after microscope reading of percentage of stained blue (male) or white (female) pollen according to a score ranging from 1 (male) up to 9 (female). The scores of cultivars IACSP95-5000, IACSP91-1099, SP89-1115, RB86-7515 were 1 (male), 3 (male), 8 (female) and 4 (male) respectively. After sex characterization of the parents, the culms were placed into an acid solution to maintain the longevity of the flowers, staying for 21 days for polycross and seed maturation. The seeds obtained from SP89-1115 (female) were sown in a box with substrate and 24 progenies were individualized and used for outcrossing rate determination. Six microsatellite primer pairs (SSRs) were used to genotype the 24 progenies and also the four cultivars. These SSRs generated 64 markers (alleles), of which, 28 were absent in SP89-1115 (female parent) and present in the possible male parents. The alleles absent in SP89-1115 identified 22 individuals derived from outcrossing (91.6%), and 2 off types (contaminants). These 22 individuals were identified as derived from the cross between SP89-1115 and IAC95-5000 which received the lowest score (1), that is, a large amount of pollen compared to the other male parents. The SSRs were efficient in the identification of the parents for outcrossing rate estimation. In addition, the results show that it is important to use parents with similar scores to guarantee equal pollen contribution of each male parent in polycrosses.

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BP4

GENETIC DISSIMILARITY AMONG PROGENIES FAMILIES OF SUGAR CANE

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Keywords: *Saccharum* spp., grouping, genetic divergence

Information about the diversity of a germplasm collection serve to increase the efficiency of the improvement works of cultivated species. The aim of this study was to evaluate the genetic diversity among ten families, the RB07 series of sugar cane by means of univariate and multivariate techniques, based on twelve characters agroindustrial. The experimental design was randomized blocks with four blocks and ten families of sugar cane. The experiment was conducted in the agricultural area of the Usina São José - Igarassu / PE during the crop year 2009/2010. The traits average number of culms (AMC), average diameter of culms(ADC), mean height of culms (AMC), average weight of culms (AWC), cane of tons by hectare (TCH), pol of tons by hectare (TPH), brix tons by hectare (TBH), pol in cane (PC), sugar cane in brix (BC), cane % fiber (FIB), total recoverable sugar (TRS) and cane pure (PUR). Analysis of variance was performed for all the characters and the means were grouped by Scott and Knott (1974), the 5% probability ($P < 0.05$). Multivariate analysis was used to quantify the genetic divergence. The Mahalanobis generalized distance was used as dissimilarity measure. Were applied the method of hierarchical links averages (UPGMA) and the optimization method of Tocher. The clustering methods of Tocher and UPGMA hierarchical were partially concordant as the formation of groups. Among the most dissimilar pairs, analysis of the Generalized Distances Mahalonobis identified combinations 3 and 4 (146.75), 3 and 5 (130.02), 3 and 10 (126.58), 4:09 (120.73), 5:07 (117.06) and 3:01 (116.54) as the most divergent pairs of families. The characters that contributed most to the genetic dissimilarity were TCH and TBH. There was formation of four groups by Tocher's optimization method, the two major groups each consisting of three families (30% of the total in each group) and two other groups with two families in each group (20% of the total in each group). According to the dendrogram obtained by UPGMA hierarchical clustering method, the families were gathered in four groups considering cutting about 42% of relative genetic distance. For breeding purposes the use of pairs of more divergent families may result in production of new genetic combinations bringing together a greater number of favorable genes in the crosses between families 10, 2, 3 and 5.

BP5

MORPHOLOGICAL TRAITS AS INDICATORS OF DROUGHT TOLERANCE IN SUGARCANE

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Keywords: *Saccharum* spp., water stress, leaf characteristics, stomatal density

Water stress reduces plant growth and crop production causing problems in most cultivated semiarid regions such as northeastern Brazil and worldwide. The use of improved varieties is an alternative to reverse this condition. Because, there is difficulty in identifying unique characteristics that can be used for selection, it will be appropriate identify tools for selection and quantifiable characteristics to facilitate the process of crop breeding for drought tolerance. This study evaluated the ability of some morphological traits to distinguish between tolerant and susceptible in four sugarcane commercial cultivars grown in two low water regimes. The experiment was carried out in pots containing 22 liters of substrate, in a greenhouse located in the Unit of Research and Development of APTA, Jaú, SP, Brazil. The experimental design was randomized in a factorial scheme 4x2x3 (genotypes x water regimes x evaluation times) with three replicates. At 63 days after emergence, cultivars IACSP95-5000, RB835054, RB928064 and SP80-3280 were exposed to humidity treatments such as no stress (+W) and stress (-W) promoted by 50% of the ideal humidity and evaluated for three times, 0, 42 and 77 days after the initiation of the treatments (DAT). The traits evaluated during this period were: plant height, green leaf number, length and width of leaf + 3, leaf area, specific leaf mass, stomata density, stool and root dry weight. After 42 DAT treatment, plant height, green leaf number, width of leaf +3 and leaf area of drought-stressed plants had declined in all varieties compared to values the well-watered treatments. However, the reductions were more severe in plants of RB83-5054 and SP80-3280. It was not observed any drought effect on specific leaf mass, stomatal density at adaxial and abaxial surfaces. Dry matter production in RB928064 reduced only 29% of root dry weight under drought compared to well-watered treatment. On the other hand, the other varieties had reduction of root dry weight around of 75%. For shoot dry weight, the varieties IACSP95-5000 and RB928064 produced ~50% less, while the varieties RB835054 and SP80-3280 produced ~75% less. Dry mass of shoot showed positive correlation with plant height, green leaf number, width of leaf +3 and leaf area. We concluded that the varieties IACSP95-5000 and RB928064 were tolerant to water stress. And during the selection procedure for drought tolerance when one of the traits plant height, green leaf number, width of leaf +3 or leaf area is maintained or less reduced, makes it feasible to select genotypes with high productivity. The evaluation period of 42 days under drought condition was efficient in evaluating and differentiating drought-tolerant genotypes.

BP6

A NEW SUGARCANE BREEDING CENTRE IS BORN IN BANGALORE INDIA

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Adaptability testing is a major function of any breeder and hence large resources are devoted to this cause. Traditionally this was undertaken by the Sugarcane Research Institute, Regional Government Institutes and Agricultural Universities in India. During 1994, E.I.D.Parry ventured into breeding with a good team and by 2004 had developed 13 precommercials and three commercials for their mill areas. They also participated in all India Co-ordinateTrials. VSI by 2001 commenced their own breeding researches. This was necessitated due to the inability of the Govt. Institutes to cater for individual industries.

During 2007 on retirement from Parry the senior author obtained a set of germplasm from E.I.D Parry and commenced breeding researches. By 2010 three companies joined to finance the research activities. An eighteen acre block was taken in Bangalore where the 1700 clones obtained from Parry were established. This was enlarged to 2000 clones by 2010.The collection currently consists of 2070 clones of hybrids and CO varieties. Collections were mounted in Vietnam in 2008 and 2010. Myanmar was covered in 2009. Myanmar is yet to be completed.Serious crossings were undertaken during 2008=300; 2009=400; and 2010=750.Some 12,000 seedlings were planted. One hundred and ten selections from previous populations were planted at the following sites Andhra Sugar, Raj Shree, DSCL, Chamundeshwari Sugar, Bharati Nagar.

The aim of the exercise is to:

1. Production of commercial varieties and suitable to individual mill sites;
2. Develop parental lines with wider genetic base using *Erianthus* and *Sclerostachya;*
3. Breed Energy cane with higher biomass and increased fermetables;
4. Develop varieties suitable for mechanical harvesting;
5. Train scientists in techniques of crop production suitable to India.

During the last two years good progress has been made in crossing, and preliminary screening of varieties at four locations.

BP7

OCCURRENCE OF AN ANDROGENESIS IN *Saccharum* x *ERIANTHUS HYBRID*

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Key words: Androgenesis, *Saccharum*, *Erianthus*, intergeneric hybrid, cytology

A unique case of parental apomixis (paternal origin) has been recently noticed in the *Saccharum* X *Erianthus* intergeneric cross seedlings. The genetic base of sugarcane varieties is considered to be very narrow and gene introgression studies are being made to transfer genes from wild species or related genera. *Erianthus* a related genus to sugarcane has great potential as a contributor of germplasm to current cultivars for better ratoonability, vigor, wide ecological adaptability, extensive root system, drought and water logging resistance, high biomass and disease resistance. Attempts were made to cross between *Saccharum species* hybrid clone Co 419, Co 7201 and Co 7224 (2n = 108 -112) and *Erianthus arundinaceus* IK 76-91 (2n = 60) and IK 76-99 (2n = 60) to introduce these characters into modern sugarcane cultivars. A total fifty-one seedlings were obtained from these three intergeneric crosses and out of these eighteen seedlings were reported as androgenetic seedlings as the female act as a surrogate mother. At the early stages for seed germination two types of seedlings resulted, one type resembling sugarcane and the other resembling *Erianthus* with deep purple coloration. Morphologically these androgenetic seedlings were similar to the male parents IK 76-91 and IK 76-99 with tall large tufted grass like clumps, vegetative stem with thick reproductive buds, root zone narrow and only one row of root eyes, the lamina gradually passes into leaf sheath with no auricle and dewlaps. Cytological studies were made in these seedlings and during metaphase the somatic chromosome number was observed to be 2n = 60 rather than expected 2n = 85-90. This paternal origin may be due to the ability of *Erianthus* male gametes to produce an embryo within the seed tissues of *Saccharum species* hybrids but without genetic contribution of the seed. Thus, the *in planta* androgenesis is achieved through the combination of the embryogenic behavior of male gametes which has entered in the ovule and the ability of ovules to act as a surrogate mother. They could derive either from the fusion of two male gametes or from the early natural diplodization of the haploid embryo. Such homozygous true breeding seedlings of *Saccharum* X *Erianthus* with hybrid vigor are extremely important in crop improvement. This system provides an unparalleled opportunity to shorten the breeding cycle and fix agronomic traits in the homozygous state. These androgenetic plants with *Saccharum* cytoplasm and *Erianthus* genome are male sterile making them highly useful for further hybridization with sugarcane.

BP8

PHENOTYPIC DIVERGENCE IN RB VARIETIES OF SUGARCANE THROUGH MULTICATEGORIC DESCRIPTORS

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Keywords: Dissimilarity, Hybridization, Breeding, *Saccharum* spp.

The cultivation of sugarcane (*Saccharum* spp.) has contributed significantly to the rise of the Brazilian economy. The genetic breeding has been providing excellent results in the obtaining new varieties of sugarcane that are more productive, with high sucrose content and resistant to major diseases and pests, through the hybridization of divergent parents with desirable traits. This study aimed to characterize and evaluate the phenotypic divergence in accessions of sugarcane, based on multicategoric descriptors. Were evaluated 16 accessions from the germplasm bank of Flowering Station and Crossing in Devaneio (Amaragi - PE, Brazil). The following characters were considered in the analysis: diseases (coal, brown rust, leaf scald and mosaic), sucrose content, useful period of industrialization, maturation, flowering, growth rate, fiber, agricultural productivity and growth habit. The analysis based on multicategoric descriptors revealed the dissimilarity between accessions and RB83102 RB705007. However, the accessions RB70194 and Rb962962, RB002504 and RB962962 showed more similarities. The optimization Tocher method allowed the formation of six groups, in group 1 was framed the majority of accessions, a total of 7, corresponding to 43.75%. Group 2 framed four accessions, corresponding to 25.00%. Group 3 framed three accessions, corresponding to 12.50%. Groups 4, 5 and 6 one access only, totaling 6.25% each of these groups. Whereas the optimization Tocher method maintains homogeneity within groups and heterogeneity between groups, verify, in the present work, the possibility of hybridization between the most divergent individuals included in distincts groups, aimed at the development of genetically superior genotypes.

BP9

BUILDING AND AUTOMATION OF THE FIRST PHOTOPERIOD FACILITY "MADE IN BRAZIL" FOR SUGARCANE BREEDING

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Keywords: breeding, photoperiod facility, sugarcane

Sugarcane breeding in Brazil has always been managed under natural conditions of an ideal climate for the Northeast coastal region where floral induction is profuse. However, flowering synchronizes for specific crosses, such as wild individuals crossing between *S. spontaneum* with *officinarum* for gene introgression has shown difficulties. These crosses are needed to increase the genetic base of sugarcane, increasing the possibilities for superior individual selection with high sucrose as both high fibers. The management of photoperiodic and temperature under controlled conditions has been developed in Australia, Argentina, South Africa and Ecuador. It is known that intermittent nocturnal temperatures lower than 18 °C during floral induction can reduce the intensity of flowering and delayed panicle emergence. Moreover, the frequent occurrence of daytime temperatures exceeding 32 ° C can reduce intensity of flowering or delay it. Through the BIOEN-FAPESP (08/56146-5) project we build in Ribeirão Preto/SP a chamber fully automated capable of simulating the photoperiod and temperature optimum, thereby induces the sugarcane flowering in a controlled manner.We tested three photoperiodic treatments 30", 45" and 1'with daily decay in same varieties starting the treatments on 25/09/2010 where the natural day length was still increasing but induced at the moment the CTC 12, SP89-1115, CTC 8, IACSP96-7569 and IACSP00-8206 varieties where viewed the flag leaf on 18/01/2011. The best treatments were 30"and 45" with daily decay. Thus, it is possible to artificially induce flowering in sugarcane varieties in Brazil to have flowers in February, creating new possibilities of gene combinations in the Brazilian breeding programs through flowering synchronism.

BP10

SELECTION OF SUGARCANE TO BETTER PLANT GROW PROMOTING RIZOBACTERIA (PGPR) ASSOCIATION

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Keywords: *Azospirillum brasilense*, plant-bacteria interaction, plant breeding.

Azospirillum is a genus comprising plant grows promoting rizobacteria (PGPR) that associates with important agricultural crops including sugarcane. *Azospirillum* inoculation can increase crop yield by changes on root morphology resulting in improved water, mineral and microelements uptake. However inconsistent results observed in different field and greenhouse experiments indicate that several parameters are involved in this association which includes plant genotype. Therefore, it is expected that selection of sugarcane clones that present better association with plant grow promoting bacteria (PGPR) can increase the sugarcane yield. Nevertheless, selection of genotypes with this objective had little importance in sugarcane breeding programs. The present work has the objective to select sugarcane families that response better to *Azospirillum brasilense* inoculation. The experiment was carried out at Paranavaí Experimental Station (Paranavaí County, PR – South of Brazil). Fifty four sugarcane families from biparental and polycrosses were planted in random blocks design with tree replications. Each replication had five meters and ten plants. The treatments were: T0 (no inoculated plants), T1 (plants inoculated with *A. brasilense* strain IC26 – $1x10^{10}$ bactéria.ml^{-1}) and T2 (plants inoculated with *A. brasilense* strains Abv5, Abv6 e Abv7 – $3x10^{9}$ bactéria.ml^{-1}). The evaluated parameters after fourteen months were stalk number (SN) average of stalk length (SL), stalk diameter (SD) and average Brix (Brix). Data was analyzed with SELEGEN REM/BLUP software using mixed models. Significant difference between treatments for SN, SL and SD characters were observed and inoculated plants presented superior values compared to non inoculated plants. Significant interaction of family-treatment effect to SD and Brix were observed. Families from crosses between RB867515 x RB977619 with T2 treatment (strain IC26) and RB008309 x RB974115 with T3 treatment (strains Abv5, Abv6 and Abv7) showed positive response to the inoculation. It was also found a negative response to inoculation in cross between RB855511 x RB92606 and RB93509 x RB845257. Clones from responsive families were selected and passed to a new evaluation phase to confirm the obtained results. These results indicate the presence of genetic variability to *Azospirillum* inoculation between the sugarcane families tested. The interaction observed between sugarcane genotypes and inoculation suggests that inoculation of sugarcane genotypes with PGPR should be carried out in Stage I of selection.

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BP11

SIMULTANEOUS SELECTION FOR YIELD AND STABILITY IN SUGARCANE VARIETIES IN VENEZUELA

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Keywords: simultaneous selection, G x E interaction, stability analysis, sugarcane.

Genotype x environment (G x A) interaction was performed by a stability analysis of a group of commercial varieties in Venezuela. The sugarcane variety program from the National Institute for Agronomic Research (INIA-Venezuela) has tested so far 13 groups of genotypes in different locations, cycles and years. One group was comprised of randomly selected varieties: V78-2, CP72-2086, PR61-632, Mex 64-1487, V77-11, PR980, CP74-2005, V77-9, V64-10 and V77-12. The materials were evaluated in eight environments during three harvest cycles. Yield-stability (YSi) statistic, that combines both stability and yield on a single criterion for selecting, was used to evaluate the behavior of this specific group of genotypes considering both cane yield (TCH) and sugar content measured in Pol % cane. The results showed that genotypes V77-12, CP72-2086, CP74-2005, PR980, and PR61632 were the best in cane yield meanwhile the best in Pol % cane were CP74-2005, CP72-2086, V77-12 and V77-9

BP12

STUDIES ON STABILITY OF PERFORMANCE OF NEW SUGARCANE GENOTYPES IN SUGARCANE (SACCHARUM OFFICINARUM L. LEEKE)

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Keywords: sugarcane, environments, stability, economic traits

Genotype-environment interactions are of major importance to a breeder in the process of developing new varieties. As such the interaction of genotype with the environment has an important bearing in breeding improved varieties. Adaptability of genotypes to environmental fluctuations is important for the stabilization of crop production both over regions and years. Estimation of phenotypic stability, which involves regression analysis, has proven to be a valuable tool in the assessment of varietal adaptability. Stability analysis is useful in the identification of adaptable genotypes and in predicting the response of various genotypes over changing environments. It is generally agreed that, the more stable genotypes can somehow adjust their phenotypic responses to provide some measures of uniformity in spite of environmental fluctuations. In a sugarcane breeding programme it is, therefore, important to screen and identify the phenotypically stable genotypes which could perform more or less uniformly under different environmental conditions. In view of scanty information with respect to adaptability of sugarcane genotypes, the present investigation was undertaken to determine genotype x environment interaction and stability of parameters for various economic traits and to identify stable genotype(s). Stability analysis was carried out in eighteen sugarcane genotypes including five commercial checks over four environments (one location two plant and two ratoon crops) to identify phenotypically stable genotypes that could perform more or less uniformly under different environments for various economic traits. Pooled analysis of variance for stability in the performance of different genotypes of sugarcane were highly significant for all the characters viz. cane yield, sugar yield, sucrose% in juice, commercial cane sugar % (CCS%) at 12 months, millable stalk height, single stalk weight, stalk diameter, stalk height, fiber% and pol % cane, indicating that the material selected possessed significant variation for all the characters under study confirming that the environments selected were variable and influenced the expression of most of the characters selected for the stability studies. Mean squares arising due to G x E interaction were significant for most of the characters except single stalk weight and fiber % revealing that most of the characters under study were having significant differential response to the changing environments and the characters showing nonsignificant mean squares revealed, by and large, less effects of the changing environments. In the present study no genotype was found stable for cane yield across the environments. However, genotypes CoVSI 9805, CoVSI 03102, CoVSI 0309, CoVSI 0405 and VSI434 could be recommended for cultivation across environments on the basis of stability of performance of the genotypes for various economic characters.

BP14

THE CHROMOSOME INHERITANCE FOR THE HYBRID PROGENY OF *S. OFFICINARUM* L. AND *ERIANTHUS ARUNDINACEUM*

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Keywords: sugarcane; *E. arundinaceus*; GISH;Disequilibrium hybridization

The use of germplasm with drought and disease-resistant and strong rationing is the most efficient system for variety development and innovation in order to produce high productive varieties. Evaluation and utilization of *Erianthus arundinaceum*, a closely related genus of *Saccharum* has been the most important system for the Germplasm innovation of sugarcane in China. However, crossing materials with *Erianthus arundinaceum* make slow progress because of the unclearness of the genetic background of its chromosomes. GISH analysis was carried out with the F_1, BC_1 and BC_2, in order to discover the genetic of Chromosomes. Our results shows that (1) chromosomes of F_1s YC96-66 and YC96-40 that originated from Badila with *E. Arundinaceus* were both consist of 30 chromosomes from *E. Arundinaceus* and 40 from *S. officinarum*. (2) The chromosomes of BC_1 YC01-134 (clone from the cross of YC96-40 x CP84-1198) showed 29 chromosomes from *E. Arundinaceus* and 85 chromosomes from *Saccharum.spp*. YC01-36 (clone from the cross of YC96-40 x CP84-1198) showed 36 chromosomes from *E. Arundinaceus* and 96 chromosomes from *Saccharum.spp*. This somatic chromosome number transmission is reported for the first time. (3) The chromosomes of BC_2 YC05-179 (clone from the cross of ROC20 x YC01-134) consisted of 14 chromosomes from *E. Arundinaceus* and 96 chromosomes from *Saccharum.spp*. YC03-6 (clone from the cross of YC01-116 x Neijian57-416, YC01-116 is a BC_1 of *E. Arundinaceus*) was consist of 13 chromosomes from *E. Arundinaceus* and 88 chromosomes from *Saccharum.spp*. (4) The phenomenon of distribut of chromosome and chromatin was both found in the 6 progenies of *Saccharum.spp* and *E. Arundinaceus*. The chromosomes of *E. Arundinaceus* always gather together, but not so serious. (5) The results of GISH indicated that the hybrids originated from Badila *(S. officinarum)* and *E. arundinaceus* followed the chromosome transmission pattern of n + n. The chromosome transmission of BC_1 were more complex, YC01-134 with the genetic pattern of 2n + n and YC01-36 with another special kind of genetic pattern. The phenomenon of "Disequilibrium hybridization" was found in BC_1. The chromosome transmission of BC_2 followed the chromosome transmission pattern of n + n.

BP15

TRENDS IN BROAD SENSE HERITABILITIES AND IMPLICATIONS FOR THE SOUTH AFRICAN SUGARCANE RESEARCH INSTITUTE REGIONAL BREEDING AND SELECTION PROGRAMS

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Keywords: broad sense heritability, trends, breeding, selection

Broad sense heritabilities (BSH) refer to the extent which the phenotype of an individual is determined by its genotype. In sugarcane and most vegetatively propagated plants, no segregation occurs after crossing and therefore BSH indicate the potential gains that would be achieved through selection. At SASRI, new research stations were established in 1998 to represent the midlands, coastal hinterland, and coastal average and high potential while the irrigated research station remained unchanged. Urban encroachment and site variability necessitated the need to relocate the coastal research stations. Associated with the new research stations was the move to the RV payment system which led to selection primarily for sucrose content at the early selection stages. The objective of this study was to determine and explain the trends in BSH over time across the regional breeding and selection programs. Data for cane and sugar yield and ERC% cane were collected from several series of trials established at the new research stations since 1998 and analyzed using the mixed procedure of SAS to determine the variance components used to calculate the BSH. The irrigated program produced marginal increase in BSH for yield and slight decrease for ERC% cane. The coastal short cycle produced BSH for cane yield that decreased from 88% in the first six series to 74% in the last five series, 80% to 65% for sugar yield and increased marginally from 80% to 85% for ERC% cane. The BSH for the coastal long cycle increased from 53% to 68% for sugar yield from the first six series to the last four series, 75% to 81% for ERC% cane but remained unchanged for cane yield. For the Midlands program, the BSH decreased from 86% to 80% for cane yield, 78% to 71% for sugar yield but increased marginally from 83% to 86% for ERC% cane. The coastal short cycle and Midlands programs produced trends in BSH whose decrease coincided with varieties selected after the RV system and from seedling raised at the new research stations. The coastal long cycle programs produced the lowest BSH relative to other programs. The breeding and selection programs that produced the highest BSH (Irrigated and Midlands) have also generally produced the largest number of released varieties while those with the least BSH (coastal programs) have produced fewer released varieties. The emphasis on selection for sucrose content appeared to have produced high sucrose but low sugar yield among recently released varieties. The intensive selection for sucrose content also resulted in marginal increases in BSH for ERC% cane but large decreases in BSH for cane and sugar yield. The lower BSH for yield traits could have resulted in the low and non-significant gains through selection for these traits as reflected among the released varieties over time. The intensive selection for high ERC% cane could have potentially narrowed the genetic diversity for yield traits in current and future breeding and selection populations as reflected by lower BSH.

BP16

USE OF SPAD INDEX, CHLOROPHYLL FLUORESCENCE AND PHOTOSYNTHETIC PIGMENTS AS INDICATORS OF DROUGHT TOLERANCE IN SUGARCANE

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Keywords: *Saccharum* spp., water stress, varieties, physiological traits

Water stress is an environmental factor that limits photosynthesis in plants, reducing productivity of important crops such as sugarcane. Analysis of the composition of photosynthetic pigments under drought tolerance has provided essential information to diagnose the integrity of the photosynthetic system, and this integrity corresponds to high photosynthesis and high productivity in different cultures. The method of Spad index has also gained prominence in recent years to enable instant readings, providing a fast, practical and non-destructive assessment in plants. Physiological indicators as tools for selecting drought tolerant genotypes become essential in this process. This study aimed to assess the chlorophyll fluorescence *a*, chlorophyll and carotenoid content in sugarcane leaves subjected to water deficit. The experiment was carried out in a greenhouse located in the Unit of Research and Development of APTA, Jaú, SP, Brazil. Four genotypes of sugarcane (RB855156, RB867515, RB92579, IAC91-5155) were grown in pots containing 22 liters of substrate in a completely randomized factorial scheme 4x2 (genotype x water availability) with three replications. Two treatments of water regime were established from 85 days after planting, control (without water deficit,-D) and severe water stress (+ D), where the genotypes were subjected to a moisture content of 0% promoted for 15 days without irrigation. The variables estimated leaf chlorophyll content via SPAD index; maximum chlorophyll *a* fluorescence ratio, *F*v/*F*m; leaf chlorophyll content (Chl *a*, Chl *b*, Chl *a*/Chl *b* and total), and carotenoids were evaluated. Water stress caused reduction in the values of the variables *F*v/*F*m, SPAD index and Chl *a*, Chl *b* and Chl *a*/Chl *b* in all genotypes. However, the chlorophyll content showed higher reduction in genotype RB855156. While RB867515 and RB92579 showed lower values in Fv/Fm and SPAD index. Water stress caused little reduction in the genotype IAC91-5155 in terms of *F*v/*F*m (3.8%), SPAD index (5.25%), Chl *a* (18.26%), Chl *b* (14.27%) and Chl *a*/Chl *b* (17.12%), suggesting that this variety is more tolerant to drought stress than others. The carotenoid content was also reduced during the water stress with the exception in the IAC91-5155 that maintained the highest value. Carotenoids are pigments that provided protection against photo-oxidation and this trait may have protected the chlorophylls of this genotype. The data suggest that these physiological variables may be established as drought tolerance indicators for selection of varieties in sugarcane breeding programs.

BP17

SUGARCANE BREEDING FOR ALTERNATIVE USE

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keywords: sugarcane, backcrossing, ethanol, feed

Tanegashima Experimental Station, located in Tanegashima island, i.e. the north end of southwest islands, has engaged in sugarcane breeding for domestic sugar production since 1947. Southwest islands, the main production area of sugarcane is characterized by severe condition for agriculture, such as poor soils, drought and typhoon attacks. Cultivars derived from overseas produced well in the early phase of introduction, however, the need of producing cultivars adapted to the local environments were soon recognized. 16 sugar cultivars were released from Tanegashima Experimental Station between 1972 – 2009. These were basically derived from crossings within commercial cultivars/lines. It is known that, in breeding of modern sugarcane cultivars, introgression of *Saccharum spontaneum* to *Saccharum officinarum* played a significant role. Our predecessors were very much aware of the need to introduce genes from *Saccharum spontaneum* as well as other genera to commercial sugar cultivars/lines to tackle the problems in sugarcane cultivation, much of which originated from severe soil and weather conditions in Southwest islands (Nagatomi, 1982; Shimabuku *et al.*, 1989). It is recently that their effort has bore fruit as newly released cultivars using F1, BC1 and BC2. The first feed cultivar of sugarcane in the country, KRFo93-1 was released in 2006 as an F1 between a commercial cultivar, NCo310 and a wild clone, Glagah Kloet (*S. spontaneum*) (Sakaigaichi and Terajima, 2008). The first model cultivar for simultaneous sugar & ethanol production, KY01-2044 was released in 2010 as a BC1 between a commercial cultivar, NiF3 and an F1, KRSp93-14 (Ohara and Terajima, 2010). These cultivars have improved yielding and ratooning ability compared with commercial cultivars released so far. Obviously there is yet room for improvement in yielding and ratooning ability needless to say a need for improvement in a number of traits, such as smut resistance, drought tolerance and cold tolerance. Enhanced utilization of other genera such as *Erianthus*, *Sorghum* and *Miscanthus* is probably a direction to go in. It is, however, more important that the crop be evaluated beyond the scheme of conventional sugar production, as sugarcane is already a crop of wide use and technologies of utilization tend to progress in time.

7TH MOLECULAR BIOLOGY WORKSHOP

ORAL ABSTRACTS
MOLECULAR (MO)

MO1

CHALLENGES AND OPPORTUNITIES FOR SUGARCANE BIOTECHNOLOGY

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Keywords: Genetic engineering, transgenics, transformation, gene expression, traits

Despite sugarcane being a very efficient crop with average fresh weight yields in a number of countries exceeding 80 t/ha, there is still significant opportunity to enhance productivity towards the reported experimental maximum which is approaching 300t/ha. A number of biotic, abiotic and physiological factors limit the productivity of sugarcane. In certain key growing regions such as Brazil, some of these challenges are likely to be more pronounced in the future due to the phasing out of burning before harvest and the expansion of sugarcane cultivation to more challenging environments. While integrated solutions including conventional breeding programs, crop protection products and changes in agricultural practices have partially addressed these limitations in sugarcane crop productivity, a number of unmet needs still exist. In other major crops, biotechnology strategies have been widely adopted to address unmet needs and over the last 15 years genetically modified (GM) crops have significantly changed the face of global agriculture. In 2009, 134 million ha of GM crops were cultivated and the rate of increase of GM adoption is increasing markedly in key sugarcane growing countries. GM sugar beets were launched in the United States in 2008 and reached 95% market share by 2010. The potential benefits of transgenic approaches for sugarcane include enhanced pest and disease resistance, increased tolerance to drought conditions, more efficient use of fertilizers, elevated sugar and ethanol productivity and in the longer term, the production of higher value products including novel sugars, bioplastics or pharmaceutical peptides. One of the most exciting commercial opportunities is the potential to elevate sugar and ethanol yields. While conventional breeding strategies have been successful in enhancing sugarcane productivity, (measured in t/ha), only modest increases in sugar content have been achieved in recent years. We will review a number of molecular strategies that are being investigated to increase sugar levels and describe how they may be utilized to develop improved varieties for sugar and ethanol production. Alternative strategies to elevate ethanol levels from sugarcane include the conversion of bagasse to fermentable sugars using cellulolytic enzymes. The development of a cost efficient process for the conversion of cellulosic materials has proved challenging in part due to the high cost of microbially produced enzymes. We will review the opportunities for plant expressed cellulases to reduce the cost of enzymatic conversion. To date the benefits of biotechnology have not been realized in sugar cane in part due to a number of technical challenges. In this paper we will review some of the methodologies that still require further development including plant transformation, gene expression and gene stacking. Progress towards resolving these issues to allow the benefit of biotech products coming to the market will be presented.

MO2

IDENTIFICATION OF PUTATIVE MARKERS AND DEVELOPMENT OF A RATING SYSTEM FOR RESISTANCE TO SUGARCANE THRIPS

(*Fulmekiola serrata)*

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Keywords: Sugarcane thrips, Fulmekiola serrata, molecular markers, rating system

The sugarcane thrips, *Fulmekiola serrata* Kobus, (Thysanoptera: Thripidae) was first detected in the South African sugarcane industry at Umfolozi (32'08'E, 28' 31'S) in 2004. A widespread and geographically even distribution of infestation suggests that *F. serrata* is well established in South Africa. The main objective of this study was to investigate the effects of thrips infestation on the phenotype of a selected panel of genotypes. Phenotypic assessment of damage caused by thrips was required, not only to develop and standardize a thrips rating system for use in the selection programme, but also to identify putative molecular markers for thrips resistance. Replicated field trials at two different locations were conducted for 80 genotypes plus 10 controls. For all genotypes, data were collated for extent of damage caused by thrips and thrips numbers, in the plant crop (2009) and the first ratoon (2010). Data were recorded from January (three months old crop) at monthly intervals until the sugarcane had grown out of the thrips infestation (by May/June). Thrips damage was estimated based on percentage leaf surface affected, number of leaves affected, and number of 'joined' leaves. One spindle per plot was also sampled to count the number of thrips present. For each trial, the variables were analyzed using a mixed model (REML) with genotype as a fixed effect, and replication as a random effect. Combined analysis was also conducted using replication-within-site as the blocking stratum. The molecular data already available for 80 genotypes were used to identify marker associations by calculating the correlation coefficient between marker data and phenotypes. Results show that there is a large difference between years and sites for both traits. Large differences exist between genotypes for thrips damage (F=22). Although the F value for thrips numbers is also significant (F=3.4), it is much lower than for thrips damage, showing much less discrimination between genotypes for this trait. Analysis suggests that the data for the thrips damage trait are highly correlated across all site/season combinations: i.e. relative damage per genotype is consistent across environments. The correlation for the thrips numbers is poor across sites/seasons, indicating that it is not a reliable trait to differentiate resistance/susceptibility between genotypes. Thrips resistance rating for each genotype was added to the association mapping dataset, and putative markers were identified by correlation analysis. This allows the identification of haplotypes associated with either resistance or susceptibility to thrips. After validation, these markers will be used to make planned crosses for thrips resistance.

MO3

DEVELOPMENT AND CHARACTERIZATION OF SINGLE NUCLEOTIDE POLYMORPHISM MARKERS FOR GENETIC MAPPING IN SUGARCANE

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Keywords: Sugarcane, genetic mapping, EST, SNP, molecular marker, polyploid

The economic importance of sugarcane is increasing significantly worldwide, since it's a source of raw material for producing sugar and biofuels. Breeding programs have concentrated efforts to launch new varieties with agronomic traits that meet the demand for ethanol and sugar. However, the genetic complexity of quantitative traits has hindered the improvement of this important crop. The development of molecular markers and the construction of genetic maps can help to understand the genetic architecture introducing new strategies into breeding programs in order to accelerate the development of new varieties. This study aimed to develop and to analyze SNP markers from expressed sequences derived from SUCEST database and genotype using a mass spectrometry Sequenom®. We selected 2908 sequences with differential expression, then we used the software QualitySNP for discovering the SNPs and the MassArray Assaydesign® to design the primers. With these tools it was possible to develop 943 SNPs. The markers were genotyped in a mapping population of 220 individuals derived from a bi-parental cross of commercial varieties, with IACSP 95-3018 as female parent and IACSP 93-3046 as male parent. Of the total, 790 (84%) SNPs were successfully amplified and of these 245 (31%) had polymorphism between the parents of the mapping population. All SNPs developed were tested for segregation and showed single, double, triple and higher doses in the progeny. They will be used to construct an integrated genetic map with other markers previously developed.

MO4

SCREENING THE R 570 SUGARCANE BAC LIBRARY TO IDENTIFY CLONES CONTAINING QUANTITATIVE TRAIT LOCI OF AGRONOMIC IMPORTANCE

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Keywords: Sugarcane, BAC, quantitative trait loci (QTL), genome sequence

Over the last decade a number of genetic maps have been constructed and quantitative trait loci (QTL) studies carried out for agronomically important traits. These have included traits related to sugar content (Brix, Pol), as well as yield and biomass related traits such as stem diameter, height and stalk number. In parallel, a large association study using the parental population of the Australian sugarcane breeding program has also been conducted targeting disease resistance. These studies have identified many markers that contribute to the phenotypic variation of these traits. The next step is to determine the genetic structure of these regions, and which genes or regulatory elements underlie these QTL. In the last five years a consortium of scientists from a number of countries including Australia have grouped together to form the Sugarcane Genome Sequencing Initiative (SUGESI). The goal of this consortium is to generate a combined monoploid genome sequence of sugarcane. The Australian contribution to this consortium is sequenced BAC clones from the R570 BAC library that underlie the QTLs identified in the numerous studies. The first stage of this work was to establish a strategy to identify which QTL regions to target. QTL regions were selected that had been identified at a significance level of less than p=0.001 and contained more than one marker. Ideally, the selected QTL should also have been identified in more than one population. In the next stage, sets of linked markers were selected to test across the whole QTL region. The selected markers were in most cases SNP or DArT markers with a known sequence. The sequenced markers were BLAST aligned to the sorghum genome to determine the abundance of the sequence. Only those markers that aligned to a single location in sorghum were selected to screen the BAC clones and preference was given to those that also showed alignment to the sugarcane EST collection. BAC clones were then selected using two different approaches: macroarray membrane hybridisation and qPCR using 3-D BAC pools. Of the 81 primer pairs selected for screening across the 3-D pools, 28 (35%) gave less than 10 positive BAC clones when verified using qPCR. The other primers were not selective enough and greater than half the 3-D pools were positive. These will be used to screen the BAC clones using the membrane hybridisation method. The selected BAC clones will be sequenced by second generation sequencing using paired end reads (illumina GA IIx, 76bp) with tagged and pooled BAC clones. Once the sequence is obtained a major outcome will be the development of robust markers, closely linked to causal genes, for use in the Australian sugarcane breeding program.

MO5

INTEGRATED LINKAGE MAP FOR SUGARCANE BASED ON AFLP, EST-SSR AND RETROTRANSPOSONS-BASED MARKERS

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Keywords: Sugarcane, retrotransposon, molecular markers, AFLP, linkage, F_1-based map

Vast amounts of genomic data with ever-increasing speed permitted the development of PCR-based markers designed from characterized sequences. A specific genomic initiative came from the SUCEST project (www.sucest-fun.org) allowing the characterization of sugarcane expressed sequences, including retrotransposons (Domingues, D.S., 2009: *Tese*, IB/USP) and resistance gene analogs (Rossi *et al*., *Mol. Gen. Genomics*, 2003). In the present study, AFLPs and retrotransposon-based markers were used for the construction of an integrated linkage map of sugarcane. Two retrotransposons named *SURE* (SUgarcane REtrotransposon) and *Garapa* were studied. The principle of NBS-profiling technique (Van der Linden *et al*., *Theoret. Appl. Genet*., 2004) was used to generate markers based on these retrotransposon sequences. Markers were analyzed in a F_1-population, composed of 188 individuals, derived from a single cross between the divergent parents, 'IAC66-6' and 'TUC71-7'. Due to the high level of sugarcane ploidy and the available biometric methodologies for the construction of F_1-based maps, only markers that showed a 1:1 or 3:1 segregation ratio were considered; both *the chi-square test* (χ^2) and *Bonferroni method* of multiple comparisons were applied. The integrated genetic map was constructed using the software *OneMap* (Margarido *et al*. *Hereditas*, 2007), specially designed for mapping outcrossing species. Linkage groups (LG) were assembled with a LOD score ≥5 and a recombination fraction ≤ 0.35. Excellent gel profiles of AFLP and retrotransposon-derived markers were obtained; however, for the Garapa element, technical adjustments are still needed. A total of 600 single-dose markers were obtained from 22 AFLP restriction enzyme/primer combinations and six combinations optimized to amplify the SURE-based markers. A map with 107 LGs was constructed, spanning 4,316.5 cM, with a marker density of 8.74 cM. Mapping of SURE-based markers revealed that this element is not uniformly distributed across the groups, and confirmed its low copy number in the sugarcane genome, as suggested in the literature. We propose their distribution as clusters into the LGs. In addition, the validation of these markers was performed by band cloning and sequencing. Then, sequences were aligned using BLAST (Basic Local Alignment Search Tool) and compared with data deposited into NCBI (http://www.ncbi.nlm.nih.gov/). Twenty seven sequences match known nucleotide sequences, 83% of them being similar to retrotransposon-like sequences. Our results support that AFLP data can be used to generate a scaffold of the huge sugarcane map. We also demonstrated that markers derived from repetitive sequences can be detected and mapped using NBS-profiling technique, but mapping depends on their copy number into the host genome.

MO6

LINKAGE MAP OF SUGARCANE USING EST-SSR AND TRAP MARKERS ASSOCIATED WITH DISEASE RESISTANCE

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Keywords: Sugarcane, linkage map, molecular markers, TRAP, *Sporisorium scitamineum,* smut disease

Sugarcane crop is routinely exposed to several pathogens including *Sporisorium scitamineum*, which is responsible for smut disease in Brazil, causing losses in terms of yield, productivity and broth quality. The disease also limits the cultivation of high-yielding sugarcane varieties. It is known that molecular-based approaches can be applied to identify and map resistance genes. Therefore, we used simple sequence repeats (SSR) derived from expressed sequence tags (ESTs) and target region amplification polymorphisms (TRAPs) to provide useful genetic markers for constructing a linkage map that could be used for mapping resistance genes. Good quality DNA was extracted from fresh leaves of both parents 'IAC66-6' (susceptible) and 'TUC71-7' (resistant), and from the segregating F_1 population of 188 individuals using the CTAB-based protocol. Seventeen previously characterized EST-SSR loci were amplified from 'IAC66-6', 'TUC71-7' and a sample of 20 F_1 genotypes using fluorescence-labeled or non-labeled primers. Alleles were detected in a MegaBACE 1000® genotyping system and verified with the Fragment Profiler version 1.2®. Non labeled primers generated products (alleles) that were analyzed by 5% denaturing polyacrylamide gel electrophoresis. Additional 35 EST-SSR loci were amplified and analyzed by polyacrylamide gel electrophoresis for evaluating the polymorphism between the parents. TRAP markers were developed according to Hu & Vick (*Plant Mol. Biol. Rep.,* 2003). Three random primers were used in combination with six fixed primers designed from resistance gene analogs (RGA), which were identified in the sugarcane expressed sequence database (Rossi *et al*., *Mol. Gen. Genomics*, 2003). These bands were resolved by 5% polyacrylamide gel electrophoresis. Each marker was analyzed for the presence or absence of the band (allele) in the segregating population. Due to the high level of sugarcane ploidy and the available biometric methodologies for the construction of F_1-based maps, only markers that showed a 1:1 or 3:1 segregation ratio were considered; both the chi-square test (χ^2) and Bonferroni method of multiple comparisons were applied. Seventeen EST-SSR loci generated 118 alleles, ranging from 3 to 12 per locus. Of them, 57 were identified as single-dose markers. We were able to place 41 of those alleles on a previous 'IAC66-6'*x* 'TUC71-7' map constructed in our laboratory (Palhares, A. C., 2010: www.teses.usp.br). Then, it was possible to associate 31 linkage groups (LG) as homologous groups (HG). From the additional 35 EST-SSRs, 15 showed polymorphism between the parents. These alleles are possible good candidates to identify new HGs. High quality profiles were obtained using the TRAP technique, which generated 95 loci as single-dose markers after applying the Bonferroni correction. Of them, 66 were placed on the previous integrated linkage map. TRAP markers were not evenly distributed along the map, some being allocated near to retrotransposon-derived markers (see Palhares, A. C., 2010: www.teses.usp.br). Our results are promising for mapping quantitative trait loci associated to the response of the F_1-population to *Sporisorium scitamineum* and data are still under analysis.

MO7

BIOTECH R&D FOR A SUSTAINABLE SUGARCANE PRODUCTION

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The ethanol production from sugarcane is recognized as the currently most sustainable way to provide renewable transportation fuel globally. The Brazilian sugarcane ethanol is able to deliver significant reductions in CO_2 emissions by up to 90% as compared to fossil fuel, has the highest productivity per land unit (up to 7,500 l/ha) while being the only renewable fuel that is economically viable without subsidies. These advantages and the strongly increasing demand for ethanol in Brazil as well the rest of the world will lead to a growing production. To meet this demand sustainably in light of the also increasing global demand for food and feed the productivity of land needs to be maximized. In the past much research effort has been undertaken to increase the yield through conventional breeding as well as genetic modification of sugarcane. Breeding by renown research institutions have led to a continuous increase of sugarcane biomass harvested per hectare but have failed to deliver significant improvements of the sugar content.

Bayer has more than 30 years of experience with sugarcane providing innovative products and solutions to the entire segment. Its outstanding portfolio of crop protection solutions comprising herbicides, insecticides as well as growth regulators has supported the remarkable yield gains provided by ever improving agronomic practices thus contributing to today's sustainability.

Besides its continued R&D efforts in chemical pest control Bayer has started to investigate the genetic modification of sugarcane already in the early 2000's. A primary target of such research is to create improved varieties with increased productivity. Bayer today presents a new recombinant DNA technology allowing to significantly increase the sugar yield from sugarcane juice. The new biotech varieties are expected to produce significantly higher amounts of soluble carbohydrates without any negative impact on biomass production: Early research results (greenhouse and open-space) in several varieties consistently indicate the potential of this technology to increase the content of total fermentable carbohydrates in the range of 30-40%. Bayer intends to deliver this technology in best-in-class sugarcane varieties and currently is in intense product evaluation phase on multiple sites.

The new technology is ideally suited for the production of bioenergy as the high-sugar varieties may deliver up to 10,000 l ethanol/ha while reducing the amount of sugarcane to be harvested, transported and processed per liter of fuel. This will further improve the sustainability of sugarcane ethanol as it reduces the need to expand sugarcane area to supply the growing ethanol demand and hence helps to reduce pressure on limited land available for food production.

MO8

APPLICATION OF DNA MARKERS TO ACCELERATE SUGARCANE BREEDING PROGRAMS IN AUSTRALIA

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Keywords: Marker assisted selection, breeding program, sugarcane, DArT markers

Several experiments have been conducted in populations of clones derived from commercial sugarcane breeding programs in Australia over the last 6 years, in order to identify markers linked with commercially important traits, and with the ultimate goal of using marker assisted selection to accelerate gains being made in sugarcane breeding programs. Three populations have been screened with several thousand DArT markers as follows: (i) A set of 480 clones representing a cross section of relatively unselected clones from the Australian sugarcane breeding program. These were measured for cane yield, sugar content and fibre at three sites, and for resistance to smut. (ii) A set of 785 parent clones from the same breeding program. These were measured for breeding value based on progeny performance of up to 10 years at varying numbers of locations, and a high proportion screened for resistance to a range of diseases. (iii) A set of 490 clones from three elite crosses with similar genetic background. Analysis of data was done to detect associations between markers and traits, using models considering effects of population structure as separate source of variation. For most population x trait combinations, more markers showed significance at a range of P values than expected by random chance providing evidence for linkage between QTL for traits and markers. There was limited repeatability of marker effects across populations, although the results were generally reasonably consistent with predictions considering expected rates of type 1 and type 2 errors. Based on results across all populations a set of 384 DArT probes have been chosen for application in a marker assisted breeding program to test the effectiveness of marker assisted breeding. We have considered that the best way markers may be applied in practical sugarcane breeding is via a short generation, recurrent selection program aiming to achieve a more rapid rate of parent improvement than is currently being achieved. This will use a selection index incorporating combined marker and phenotypic data, to identify clones with predicted high breeding value. A simulation model of such a breeding program is used to show under what circumstances such a marker assisted breeding program may be more or less effective than a breeding program based on use of phenotypic data alone. The model predictions will be compared with observed results in coming years

MO9

APPLICATION OF SORGHUM EST-SSR TO THE LINKAGE MAPPING OF SUGARCANE VARIETY M 134/175

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Keywords: Comparative mapping, sorghum, EST-SSR, yellow spot disease

QTL mapping in sugarcane (*Saccharum* spp.) has proven challenging owing to its large genome size and genetic complexity. Comparative genome mapping of sugarcane and sorghum (*Sorghum bicolor*-the model organism for C_4 grasses) has become more informative following the sequencing and assembly of the latter genome. A partial linkage map of sugarcane variety M 134/75 was previously constructed with 557 AFLP and SSR markers. A major QTL for sugarcane yellow spot disease resistance (causal agent: *Puccinia melanocephala*) was identified explaining 24% of observed phenotypic variation of yellow spot resistance in the mapping population of 226 progeny derived from the bi-parental cross M 134/75 x R570.

A set of 600 sorghum EST-SSR primer pairs designed to provide even physical distribution across the aligned rice genome sequence, with the objective of providing good coverage across the sorghum nuclear genome, was tested to act as anchor markers between sugarcane and sorghum. Mapping parents were screened with 425 sorghum EST-SSR primers. The high ploidy level of sugarcane was revealed by the complex PCR product profiles for most primers tested and genetic similarity between the sorghum and sugarcane genomes was confirmed by 95% marker transferability across the two species, with 295 sorghum EST-SSR primers detecting polymorphism for the mapping parent M 134/75 and with an average of 2.6 polymorphisms per primer pair. The progeny population was genotyped with 37 sorghum primers from which 102 single-dose coding markers were scored. These were combined with 985 available non-coding markers and analyzed by GMendel to produce an enhanced map of M 134/75 containing 143 linkage groups (LGs). This enabled integration of 58 sorghum EST-SSR markers into 26 Lgs.

Evidence of gene and chromosome duplication events contributing to the expansion of the *Saccharum* genome was observed. Conservation of gene order in sugarcane, sorghum and rice (*in silico*) was also exemplified in a number of LGs. Based on common genomic SSRs, LGs were grouped into ten homology groups (HGs I - X). HGs I-III and VI-VIII were equated to their respective groups, among which four HGs contained sorghum EST-SSRs. LG 68 containing the yellow spot resistance QTL was assigned to HG VIII, which contains a high concentration of resistance gene analogs. This LG was enhanced with three sorghum markers, Xisep1225, Xisep0203 and Xisep0612.

Following assembly of the sorghum genome sequence, the Phytozome and the **Gbrowse** software (http://www.phytozome.net/sorghum.php) were used to physically map (*in silico*) the sorghum EST-SSR markers on the sorghum genome. HG II was found syntenic to sorghum chromosome SBI-10. HG VIII harboring the highest number of mapped sorghum EST markers showed collinearity to several sorghum chromosomes. These may eventually differentiate into separate HGs upon further enhancement. This project will eventually resolve several issues regarding sugarcane genome arrangement, homology grouping and further describe its synteny with sorghum.

MO10

DEVELOPMENT OF ADVANCED DNA MARKER TECHNOLOGY USING MICROARRAY AND CONSTRUCTION OF A HIGH DENSITY MOLECULAR LINKAGE MAP OF SUGARCANE

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Keywords: Marker, linkage map, QTL, CIM, microarray

Microarray technologies to identify genomic regions in whole-genome-covering have been utilized to develop markers for construction of molecular linkage map and QTL mapping for crops. We developed microarray based high-throughput DNA marker technology. The technology consists of the following two steps; genome complexity reduction and microarray with probes, which length is optimized for hybridization with sample DNA. The evaluation of the accuracy using artificial mutation probes indicated that the approximation formula between mutation rate and signal value was $y=0.0804x^{-0.516}$ ($R^2=0.8068$) and when the mutation rate was 2.9 %, the signal value was reduced to 50%. These results indicate that a few-base pair mutation, SNPs and InDel(s), in the probes can be detected by this technology, and was applied to construct molecular linkage maps of Japanese cultivars of sugarcane, NiF8 and Ni9, using 191 F1 progeny and molecular linkage map construction software “AntMap ver.2.5”. The resulting molecular linkage maps consisted over 3,000 markers and 122 linkage groups for NiF8, and of over 4,500 markers and 123 linkage groups for Ni9, which were similar to the chromosome numbers (100-130) of most modern cultivars of sugarcane. The composite interval mapping (CIM) was performed for early stage culm-length using QTL analysis software “QTL Cartographer ver.2.5”, and six QTLs with a LOD score of over 3.0 were detected on NiF8 and Ni9 molecular linkage maps. These results show that the DNA marker technology is a powerful tool for genetic analysis of the high polyploid sugarcane plant.

MO11

ASSOCIATION MAPPING OF SUGARCANE RESISTANCE TO THE SUGARCANE YELLOW LEAF DISEASE

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Keywords: Genome wide association, *Saccharum* spp., *Luteoviridae*, *Melanaphis sacchari*, population structure, linkage disequilibrium

The Sugarcane Yellow Leaf disease is a major viral disease of sugarcane, caused by a Polerovirus - the SCYLV- transmitted by several aphid species. The disease was described only in the 1990s probably because yellowing of the leaves, the main symptom, may be confused with abiotic stress. Yellow leaf disease is widely distributed in sugarcane growing areas where yield losses have been reported. Varietal resistance, both against the virus and its vectors, is the most practicable control method and a recent objective in breeding programs. In order to analyze the genetic basis of the resistance to SCYLV and to its worldwide aphid vector *Melanaphis sacchari*, we undertook an association mapping study based on 344 sugarcane cultivars. These 344 cultivars were split in two populations sharing 29 common cultivars, and evaluated in two contrasted environments, one in Réunion Island in the Indian Ocean (RUN, 184 cultivars) and one in Guadeloupe in the Caribbean (GUA, 189 cultivars). The RUN (one trial) and the GUA (two trials) populations were evaluated in the field in second ratoon under natural infestation conditions for SCYLV incidence by tissue-blot immunoassay. BRA-PER, CUB and RUN genotypes of the SCYLV were recorded by PCR in the GUA population. SCYLV genotypes detected in the RUN population were BRA-PER and RUN the latter representing the majority of the samples. The Réunion population was also evaluated for *M. sacchari* infestation level during two cropping seasons. Both populations were genotyped with AFLP markers (1367 markers for RUN and 2421 markers for GUA) and DArT markers (1877 markers for RUN and 1560 markers for GUA). Genetic structure of both populations was analyzed by a Principal Component Analysis (PCA) approach with the EIGENSOFT software, using 2092 independent markers for RUN and 2576 independent markers for GUA. Significant marker – trait associations were detected with the TASSEL software, using a mixed linear model taking into account structure and family relatedness. Analysis of marker-trait associations for resistance to SCYLV revealed 16 significant markers in Réunion, defining 11 independent loci. In Guadeloupe, 20 significant associations were detected. Among the markers that were significant either in Réunion or in Guadeloupe, 18 (13 independent loci) were coded in both populations. Among these 13 commonly coded loci, five were confirmed, i.e. detected in one population, with control of GW-type 1 error by permutation procedure, and significant at the 0.05 nominal P value in the second population. Analysis of marker-trait associations for resistance to aphids detected 24 significant markers, defining 19 independent loci. One of these markers was located on a haplotype where a confirmed linkage associated with reduction of SCYLV incidence both in Réunion and in Guadeloupe was identified.

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MO12

COMBINED APPROACHES TO COMBATING ORANGE RUST OF SUGARCANE CAUSED BY *PUCCINIA KUEHNII*

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Keywords: Orange rust, *Puccinia kuehnii*, disease resistance, molecular breeding

Orange rust of sugarcane is an economically important disease caused by the obligate biotrophic pathogen *Puccinia kuehnii*. The disease was first confirmed in the Western Hemisphere in Florida in June 2007 and at a similar time in Costa Rica. Some sugarcane cultivars that were widely grown in the region were susceptible to orange rust meaning that since its initial discovery, the disease has proliferated rapidly. Orange rust has been confirmed in all Central American countries except Honduras and several Caribbean islands. The disease is now present in South America having been confirmed in the Brazilian and Colombian industries. In Florida, a multi-disciplinary research response is being used to minimize the short-term and long-term impact of the disease. Molecular approaches are being applied to improve understanding of variation, spread and epidemiology of *P. kuehnii* to support disease control strategies in the field. Sequence variation in rDNA from isolates of *P. kuehnii* suggests introduction to the Western Hemisphere occurred from a single source. Real-time PCR assays for *P. kuehnii* have been used to monitor spatial and temporal differences in atmospheric concentrations of spores in combination with changes in disease symptoms through the growing season. These data are being used to improve understanding of orange rust disease epidemiology to support field scale disease control strategies. The Canal Point cultivar development program provides many of the regional sugarcane industries with new cultivars and germplasm. Breeding efforts to combat orange rust have focused firstly on identifying sources of resistance among germplasm available to the crossing program. Orange rust susceptibilities in this material have been determined using a combination of naturally occurring infection and artificial inoculations and has included accessions of *Saccharum spontaneum* from the world collection of sugarcane and related grasses, historical and exotic sugarcane cultivars. Resistant clones have been used as parents and high throughput screening methods are being developed to allow the mass selection of resistant genotypes as early in the selection program as possible. Populations segregating for orange rust resistance are being developed to improve understanding of the inheritance of resistance and to support efforts to improve the selection of resistant genotypes through marker assisted selection. Molecular approaches towards marker discovery are being accelerated through the application of massively parallel sequencing. This strategy of applying molecular approaches to support disease control in the field and to improve resistance among new sugarcane cultivars will minimize the impact of orange rust in Florida and regional sugarcane industries.

MO13

LARGE SCALE MOSAIC VIRUS SURVEY IN SUGARCANE REVEALS CONSIDERABLE GENETIC VARIABILITY; IMPLICATIONS FOR THE DESIGN OF A RESISTANCE GENE MEDIATED BY RNA SILENCING

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Keywords: mosaic disease, molecular variability, transgenic viral resistance, RNA silencing

Attractive alternatives to traditional resistance breeding against sugarcane and sorghum mosaic virus (SCMV and SrMV, respectively), both causal agents of mosaic disease, have derived from transgenic approaches exploiting gene silencing. Such approaches, however, have been implemented based upon relatively few available virus sequences, therefore it is possible that infrequent variants escape gene silencing occur and thus a breakdown of resistance. Large scale virus surveys, however, seem necessary to identify possible low frequency virus variants and infrequent virus evolutionary events. We estimated the population structure of potyviruses causing sugarcane mosaic disease throughout the sugarcane growing area of Argentina and neighboring regions in Bolivia, Uruguay and Paraguay by analyzing sugarcane leaf samples showing mosaic symptoms from 103 locations, including commercial and experimental fields. A set of 567 samples from 104 sugarcane genotypes were extracted and analyzed by RT-PCR for the presence of a genomic fragment including most of the SCMV and SrMV coat protein coding regions using reported sets of primers. All but 5 samples resulted in amplification products for the coat protein gene. PCR products were directly sequenced using the same amplification primers. Sequence analysis demonstrates that SCMV is the predominant mosaic virus infecting sugarcane in the region, represented in 94% of samples. SrMV was present only in 2.8% of samples, with low (0.5%) coinfection rates. SCSMV, a third causal agent of mosaic, was not detected. We found that 403 out of 450 SCMV nucleic acid sequences obtained were unique, and these were clearly grouped into two cohesive clusters. The first, widely predominant cluster grouped 97.7% of the sequences, which showed identity values higher than 95% with previously reported SCMV-SCE group. Remarkably, a second, previously unreported cluster consisting of only 9 SCMV sequences was also identified. We sequenced and analyzed the full genome of a sample of this new, SCMV-W group, 95% identical to SCMV-A (isolate for Brisbane), the only fully sequence genome of a reference strain for SCMV-SCE. Interestingly, the SCMV-W genome displays a common pattern of silent base substitutions confined within the highly conserved core coat protein region of the genome. Two samples were identified as belonging to the SCMV-MZ group, to our knowledge, not previously reported to infect sugarcane under natural conditions. A resistance gene was designed to trigger silencing mediated resistance against all virus variants found in the large scale survey. Three viral fragments: a P3 gene fragment and two non-overlapping fragments from the coat protein gene, were cloned in opposite directions separated by an intron, and placed under the maize UBI promoter. Transgenic sugarcane plants of 5 varieties were obtained and putative events are being tested in the greenhouse with artificial inoculation and in field trials at Chacra Experimental under natural infection conditions. We propose that large scale sequence-based studies are the adequate tool for identifying infrequent virus variants and to select target regions for RNAi-mediated resistance in transgenic plants.

MO14

CHARACTERIZATION OF THE BROWN RUST RESISTANCE LOCUS BRU1; DISTRIBUTION IN SUGARCANE GERMPLASM

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Keywords: Brown rust resistance gene, molecular diagnostic markers, germplasm

Modern sugarcane cultivars have a particularly complex genome, being highly polyploid (100-130 chromosomes), aneuploid, and of interspecific origin. *Bru1*, a major dominant gene conferring resistance to brown rust, has been identified in the cultivar R570. This gene was shown to confer resistance to all eight isolates of *Puccinia melanocephala* H. & P. Syd. from Brazil, Colombia, Florida (three isolates), Guadeloupe, Réunion, and Zimbabwe that we tested. *Bru1* is the focus of a map-based cloning approach that so far resulted in the development of (i) a high-resolution genetic map and (ii) a partial physical map of the target haplotype that features an insertion specific to this haplotype. This insertion is associated with a local reduction of the rate of meiotic recombination. Several markers surrounding *Bru1* in R570 were surveyed in 380 international sugarcane cultivars that were phenotyped for rust resistance in Réunion Island (Mascarene) or Guadeloupe Island (West Indies). They exhibited strong linkage disequilibrium in the target region. Two PCR markers were found completely associated with *Bru1* in the whole population and thus represent efficient molecular diagnostic makers for *Bru*1 detection. The results suggest that *Bru*1 is the main source of brown rust resistance in modern cultivars. Only 6.6 % of the resistant cultivars tested did not shown the *Bru1* haplotype; they represent alternative sources of resistance to brown rust. These diagnostic markers are used to trace the origin of the chromosome insertion that includes *Bru1*. Preliminary results suggest that the insertion is old and could have been transmitted to modern cultivars via the Indian canes (barberi) which are natural hybrid between *S. officinarum* and *S. spontaneum*.

MO15

OPTIMIZATION OF GENETIC TRANSFORMATION IN SUGARCANE AND DEVELOPMENT OF GENOTYPES TOLERANT TO HERBICIDES IN ESTACIÓN EXPERIMENTAL OBISPO COLOMBRES (TUCUMÁN, ARGENTINA)

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Keywords: Biobalistic, breeding, glyphosate, transgenesis

Conventional breeding has always involved genetic manipulation of crops through crossing and selection cycles. However, several interesting traits are not found in the cultivated germplasm or the one directly related to it, which thus limits their transfer through sexual transmission. Recent development of genetic transformation methods, which allow transferring one or a few traits to improved cultivars, overcomes this limitation. In sugarcane breeding, transgenesis is a valuable tool, especially when considering its high ploidy level and vegetative propagation, which enables the transfer and stable propagation of transgenic material. There are different methods to transform plants and the choice depends on the species, the plant material, its regeneration capacity and transformation efficiency. The biobalistic technique has been successfully used with several plant species, including sugarcane. It involves a process in which DNA-coated microparticles are accelerated by compressed gas and introduced into plant cells. Since 2006, EEAOC Biotechnology Department has been working on sugarcane breeding so as to introduce traits of agronomic importance into local commercial varieties RA 87-3 and TUCCP 77-42. To evaluate the efficiency of the biobalistic technique, expression assays of a reporter gene (uidA) were carried out to visually detect the introduction of the gene into the plant genome. This allowed adjusting involved parameters, not only optimizing the transformation process of calli (undifferentiated tissue) with the gene of interest, but also the regeneration of plants from these calli. To obtain embryogenic calli, discs of immature sugarcane leaves were cultured *in vitro*. Calli were bombarded with tungsten particles, in which a lineal segment of plasmid DNA containing the EPSPS and *NPTII* genes was precipitated. The former codes for an enzyme that confers tolerance to the herbicide glyphosate, and the latter confers resistance to geneticin, which enables *in vitro* selection of transformed cells. Transgene presence was evaluated by PCR using specific primers. Transgenic plants were micropropagated and later planted and kept in the greenhouse, in accordance with the legislation passed by Comisión Nacional Asesora de Biotecnología Agropecuaria in Argentina (CONABIA; Exp Nº S01-0231880/2007). To determine levels of tolerance to glyphosate, several doses of herbicide were tested and the different events were classified according to herbicide tolerance and multiplied for field evaluation tests.

MO16

TRANSGENIC SUGARCANE PLANTS EXPRESSING *SACCHAROMYCES CEREVISIAE* INORGANIC PYROPHOSPHATASE DISPLAY ALTERED CARBON PARTITIONING IN THEIR SINK STEMS AND INCREASED PHOTOSYNTHETIC ACTIVITY IN THEIR SOURCE LEAVES

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Keywords: Inorganic pyrophosphatase, carbon partitioning, photosynthetic activity

Sugarcane (*Saccharum officinarum L.*), is not only the most important sugar crops, but also an important energy crop in the world. The main goal of sugarcane breeding is to increase the sugar content in sugarcane. Enhancing the ability for sucrose synthesis in sugarcane is an efficient method to increase the sugar content in sugarcane. Sucrose synthesis in sugarcane takes place in the mesophyll cells. During sucrose synthesis, a large amount of inorganic pyrophosphate - PPi is produced. The accumulation of PPi in mesophyll cells can largely inhibit sucrose synthesis, and thus ultimately increase the sugar content in sugarcane. The PPi can be cleaved by inorganic pyrophosphatase - PPase. Transferring the PPase gene into sugarcane and its expression in mesophyll cells can decrease the accumulation of PPi and therefore increase sucrose synthesis and thus ultimately increase the sugar content in the sugarcane. We report here the expression of *Saccharomyces cerevisiae* inorganic pyrophosphatase gene in sugarcane which displays altered carbon partitioning in their sink stems and increase photosynthetic activity in their source leaves. The expression of the transgene was under the control of mesophyll cell specificity expression promoter rbcS and transferred into sugarcane by *Agrobacterium tumefaciens* EHA105. Compared with non-transgenic plants, the net photosynthetic rate and sucrose content of the transgenic plants were increased by about 67% and 43% respectively in source leaves; the content of sucrose, fructose and glucose of the transgenic plants were increased by up to 25%, 39% and 39% respectively in the stems. These results indicate that induction of ppa activity in the cytosol affects carbon partitioning between source and sink organs and also that the concomitant increase in inorganic phosphate - Pi caused the accumulation of carbon metabolites and raised photosynthetic activity. Moreover, the relationship between the accumulation of soluble sugar and an increase in photosynthetic activity may be explained by a disturbance in transport pathways; hence, not only via the activity of sucrose transport via phloem but also due to a disruption of the exchange between plastid and cytosol. These results provide direct evidence that modifying the level of PPi can cause metabolic changes and that this is probably related to metabolic transport in sink stem. Future studies of altered carrier activities may help to further elucidate the relationship between carbon metabolite accumulation and increase photosynthetic activity in ppa transgenic sugarcane plants.

MO17

EXTENT OF CANE YIELD PENALTY IN TRANSGENIC SUGARCANE PLANTS

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Keywords: Transgenic, transformation, field performance, tissue culture

Sugarcane transgenic technology is now well-established and practised in all major sugar producing countries. However, production of commercially useful transgenic cultivars in the desired genetic backgrounds remains a significant challenge. Despite extensive research a systematic investigation of the impact of transgenesis on sugarcane crop production has not been reported. The aim of this work was to evaluate the field performance of transgenic sugarcane plants generated by different methods of transformation and understand the extent of variation in crop yield and transgenic trait expression. In contrast to previous studies, here a large number of independent transgenic lines produced either by *Agrobacterium*-mediated or biolistic transformation were screened. The lines selected for field trials include those produced by four different *Agrobacterium* strains (AGL0, AGL1, LBA4404 and EHA105) and those developed by microprojectile bombardment using either minimal DNA transgene cassettes or circular plasmid vectors of the same transgenes. Field trials were conducted according to the selection scheme used in the BSES-CSIRO plant improvement program. The results of a combined analysis of all clones showed a reduction in growth and cane yield in plants produced by both biolistics and *Agrobacterium*-mediated transformation methods compared to wild-type (not gone through transformation or tissue culture). However, when individual lines were analysed, growth and yield of 10-15% of transgenic lines were comparable to wild-type suggesting that both transformation methods could be used to produce agronomically suitable clones. Despite considerable growth variation in most transgenic lines, crop ratoonability was not affected by transgenesis. Transgene expression analyses over time and crop class suggest that it remained stable over different crop cycles and increased with plant age. No correlation between transgene copy number and trait expression was evident; and both biolistics and *Agrobacterium*-mediated transformation produced low copy transgenic events. Molecular genomic analysis using three most commonly used DNA fingerprinting technologies failed to detect any significant genetic changes even in phenotypically different somaclones.

MO18

GM SUGARCANE IN ARGENTINA AND IN SUGARCANE PRODUCER COUNTRIES: LIMITATIONS OF REGULATORY SYSTEM

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Keywords: GMO, sugarcane, regulatory system, genetic engineering

Several agronomical traits of interest can be achieved using genetic engineering (for example herbicide tolerance, virus resistance, pest resistance, drought tolerance, etc.) which is a very useful tool for breeders and many approaches are being conducted in biotechnology in sugarcane producing countries. However, until now no institution has been able to deregulate GM sugarcane because the regulatory process is too long and expensive. It generally takes about ten more times money and years to bring a genetically modified crop to market than a non-genetically modified one. This prejudices particularly small research institutions that have to invest a large amount of resources in the deregulation process (and in last instance benefits multinational companies).

In Argentina regulations regarding GMO crops consist of three main steps:

I - CONABIA: evaluates environmental biosafety of GM crops that will be released for field trials.

II - SENASA: determines alimentary aptitude and lack of toxicity of GM crops.

III - Once SENASA reaches a decision, evaluation goes to the International Market Direction which determines the economical impact of the commercialization of the GM crop.

The criteria used in Argentina to conduct the evaluation process is in agreement with criteria applied by the most important and strict regulatory agencies of the world (European commission, Australia and New Zealand, Canada and Japan). Worldwide GMO regulations were first developed for large scale productive crops such as soybean and maize - and evaluations are conducted on a case-by-case basis. This approach is not a big obstacle for crops like maize, cotton or soybean, because researchers can generate a GMO line or progenitor that can be easily transformed and used as progenitor in a breeding program to obtain a commercial hybrid or variety containing the trait of interest. This is very difficult to achieve in a species like sugarcane because of the complexity of its genome.

At present, regulators focus their decisions based on the way the GMOs were obtained. However, there are traits (like herbicide tolerance) that have been shown to be safe for more than 15 years. We contend that it is more practical and reasonable for new genetically modified crops to be regulated not according to how they are bred, but according to their novelty.

The way regulations are drawn makes deregulation of GM sugarcane very difficult. In the first place sugarcane has a complex genome and the probability of obtaining a progeny with good agronomical performance and the trait of interest is very low. Second, fermentation of sugarcane stalk and syrup occurs very fast, so researchers have to make the evaluations in a short time period or need good storage facilities. Given that biotechnology is widely accepted as a very useful tool, it would be valuable to develop a forum where sugarcane researchers and regulators can find a way to make this tool profitable, without neglecting biosafety.

MO19

CELLULASES EXPRESSED IN SUGAR CANE: TOWARDS ECONOMICALLY VIABLE CONVERSION OF BAGASSE TO FERMENTABLE SUGARS

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Keywords: Biofuel, hydrolysis, ethanol, enzyme, transgenic

Biofuels, including ethanol, are derived from renewable, biological feedstocks. The transition from petroleum to biological feedstocks for production of liquid transport fuels offers the opportunity to improve energy security, reduce greenhouse gas emissions, and enhance rural development. The majority of fuel ethanol is produced by fermentation of sugars naturally present in sugarcane juice or molasses, or derived from the enzymatic hydrolysis of corn starch. In addition to these conventional sugar sources, plant cell walls present a vast but as yet untapped source of fermentable sugars for the production of ethanol and other biofuels as well as other high value products produced via microbial fermentation. However, there are several roadblocks to developing an economically viable cellulosic ethanol process based on biochemical conversion which includes feedstock collection and transport, pre-treatment of the feedstock, enzyme cost and robust fermentation systems to convert the liberated sugars to ethanol. Transporting feedstocks and the cost of enzymes required for hydrolysis to fermentable sugars are currently two of the major cost limitations to developing an economic industrial cellulosic ethanol process. Sugar cane bagasse is an ideal feedstock since it is already collected and concentrated as a result of the sugar milling process. Currently, cellulolytic enzymes are produced in microbes and as a result the production costs of these enzymes are high. The expression of cellulolytic enzymes *in planta* is one way to substantially reduce production costs. Cellulolytic enzyme production in sugar cane will have a substantial impact on the economics of lignocellulosic ethanol production from bagasse. Our research program seeks to produce the main enzymes required for hydrolysis of sugar cane bagasse during plant development. The core enzymes required for efficient and complete degradation of cellulose to glucose are derived from three functional classes; (i) endoglucanase (EG) which creates free chain ends; (ii) cellobiohydrolase (CBH) which cleave cellobiose units from free chain-ends, and (iii) b-glucosidase (BG) which hydrolyse cellobiose to glucose. Enzymes from the EG and CBH classes were chosen for expression in sugar cane as these are required in the largest quantities. To determine if cellulolytic enzymes could be successfully expressed in sugar cane we generated transgenic lines using the maize *PepC* promoter and different sub-cellular targeting signals. The expression results for the plant and ratoon crop will be presented and are the first demonstration of expression and accumulation of recombinant CBH and EG in sugar cane. This work represents a significant first step toward optimisation of cellulolytic enzyme expression in sugar cane for the economic production of lignocellulosic ethanol.

MO20

REGULATION OF SUCROSE ACCUMULATION IN SUGARCANE GENOTYPES

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Keywords: Sucrose content, regulation, microarray, genotypes, sugarcane

Sugarcane is an important model for studies of source-sink relations due to its ability to store high concentrations of sucrose in the culms. The use of recombinant DNA technology tools as a strategy to raise the concentration of sucrose content in sugarcane requires a broad knowledge related to metabolism and regulation associated with sucrose accumulation. The main objective of this study is to understand the behaviour of physiological processes, such as photosynthesis and yield, in high brix genotypes. To this end, two different populations of the RIDESA breeding program grown in the field were analyzed: *(i)* 4 sets of F1 progeny grown since 2005 (stage T1) as a result of crosses between contrasting parents in relation to soluble solids content (Crossing I: SP83-2847 x TUC71-7; Crossing II: SP70-1143 x RB925211; Crossing III:SP80-3280 x RB855156 and Crossing IV: RB855002 x RB855035) and *(ii)* a T3 progeny derived from two cycles of selection, which aimed to increase the concentration of favorable alleles for brix enrichment. The parameters measured were: brix content, diameter of internode 3 to the last base of stem, plant height, culm length, leaf area and photosynthesis rate. All measurements were done from seven months old field grown sugarcane plants. Among the data collected, there seems to be a slight trend in the assimilation of CO_2 in relation to brix value (higher brix correlates to a lower rate of carbon dioxide assimilation). However, there are cases where genotypes considered as high brix exhibited greater rates of assimilation and vice versa. This is an interesting finding that might indicate a path towards adding more sucrose on sugarcane by increasing the photosynthetic rate. The correlation between yield and brix also seems to reveal a slight trend in which higher brix is related to greater productivity for some genotypes. Thus, our data indicate that some genotypes seem to have achieved a desensitization of source-sink relationships, where higher brix genotypes showed a higher rate of CO_2 assimilation than those reported for genotypes with lower brix. Previous studies suggest that stored sucrose prevents increases in the rate of CO_2 assimilation in genotypes of high sucrose, through a negative feedback not fully explained yet. The genotypes were also expression profiled using oligoarrays. The regulatory pathways leading to activation or deactivation of photosynthesis and sucrose accumulation will be used in the generation of transgenic plants and in search for molecular markers useful for the selection processes.

MO21

MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF SUGARCANE DURING LEAF SENESCENCE WITH EMPHASIS ON PLANT CELL WALL

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Keywords: Sugarcane, leaf senescence, cell wall monosaccharides

Sugarcane is cultivated in several countries, with Brazil being the first worldwide producer of this crop. The crop is mainly used for the production of ethanol, sugar and electricity from bagasse. Increases of greenhouse effects and dimishing reservoirs of natural oil have prompted the search for new feedstocks for energy production. Efforts aiming at disrupting sugarcane cell wall polymers for bioethanol production from lignocellulosic material have been made. To develop new technologies for producing bioethanol, it is crucial to understand the biochemical and molecular processes involved in the synthesis and modification of plant cell wall polymers that occur during plant development, particularly during leaf senescence when there are many structural and metabolic changes in plant cells. The aims of this study were to look at the biochemical and molecular characterization of the sugarcane leaf senescence process, with emphasis on the cell wall, and to identify genes involved in this last stage of development. Leaf senescence in sugarcane was characterized by the remobilization of nutrients such as Zn, Cu, N, Fe, K, P and the reduction in chlorophyll content. Analysis of the monosaccharide profile in cell walls indicated that the process of senescence does not alter the cell wall composition as the sugarcane plant ages, however, differences in the proportions of hemicelluloses and pectins were found between the base, middle and tip of leaves whether they were senescent or not, which might be related to the structure of sugarcane leaf. The gene expression profile of α-arabinofuranosidase, α-xylosidase, β-glucosidase, cellulase genes showed variations in expression levels throughout the day and between base, middle and tip, which can be correlated with the differences obtained in the profile of monosaccharides. The gene expression profile of xyloglucan endotransglucosilase and cysteine protease genes indicated a higher expression of these genes in late afternoon in senescent leaves. Using GeneSnare™ technique it was possible to obtain five genes that were differentially expressed between non-senescent and senescent leaves. Four (*GS1*, *GS2*, *GS4* e *GS5*), out of the five genes obtained, proved to be more expressed in not senescent leaves and only one gene (*GS3*) showed low expression in leaf +1, with an increase in senescent leaves +2 to +8. The comparison of sequences with the *Gene Index* database indicated that the *GS1* gene had similarity to a phosphatidic acid phosphatase, the *GS2* gene was similar to the response regulator 7 (*RR7*), the *GS3* gene has no similarity to the sequences in the database, the gene *GS4* was similar to a protein expressed in *Oryza sativa* without known function and the last gene, *GS5*, had similarity to a putative histone protein.

7TH MOLECULAR BIOLOGY WORKSHOP

POSTER ABSTRACTS
MOLECULAR (MP)

MP1

GENETIC DIVERSITY IN SUGARCANE CULTIVARS ASSESED BY DNA MARKERS AND MORPHOLOGICAL TRAITS

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Keywords: AFLP, breeding program, sugarcane germplasm bank, morphological descriptors, similarity coefficient, SSR

Knowledge of sugarcane genetic diversity should provide useful information concerning genotype value for breeding programs and contribute to the improved use and conservation of genetic resources, especially as this crop is a high genetic complex model. For the aforementioned reasons and also for protecting new sugarcane varieties and intellectual property rights, varietal identification is essential. Ideally, the identification method should be rapid, simple and inexpensive. Morphological descriptors are traditional tools to characterize varieties; however, sometimes they do not yield clear data due to ambiguous differences or phenotypic modifications caused by environmental factors. Molecular markers are becoming important for genotype identification and diversity estimation, because they are accurate, abundant and not affected by the environment. The aim of this work is to evaluate integrally the genotypes most widely used as parents by the Breeding Program of Estación Experimental Agroindustrial Obispo Colombres (EEAOC) (Tucumán, Argentina) by using different molecular techniques jointly (AFLP, SSR and ISSR), studying morphological traits and comparing data analysis softwares. All cultivars were grouped in one main cluster in dendrograms with both NTSys and InfoStat programs. At least 150 data were included in the analysis to obtain the same cluster with both programs. Local genotypes grouped together with USA varieties: no clear genetic differentiation could be found comparing these programs, due to constant germplasm exchange. Although morphological traits only reflected external resemblance, the dendogram topology that revealed genetic relationships was not modified when molecular and morphological methods were used jointly. Therefore, both methods should be used together to estimate diversity, and molecular traits should be included in the characters established internationally for sugarcane variety protection.

MP2

EXPLORING THE INHERITANCE MECHANISM OF SSR MARKER IN ANEUPOLYPLOID SUGARCANE

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Keywords: Capillary, electrophoresis, genotyping, sugarcane, SSR Marker

Using a capillary electrophoresis-based genotyping platform and fluorescence-labeled primers, the distribution of five molecular alleles (*6-154*, *6-167*, *6-169*, *6-171*, and *6-175*) of the SSR marker SMC336BS among 93 single pollen grains of the sugarcane variety L 99-233 and its 165 progeny from a bi-parental cross was investigated. Among the 93 pollen samples, the five alleles were segregated at a ratio of presence to absence of 34:59 (*6-154*), 42:51 (*6-167*), 44:49 (*6-169*), 55:38 (*6-171*), and 37:56 (*6-175*), respectively. Except for the allele *6-154* that segregated at 1:2, the other four alleles segregated at 1:1. The latter four alleles also segregated independently by two-point-test. The number of genotypes detected among the 93 pollen grains was 23 at frequencies varying from 1.8% to 11.8% and number of alleles per genotype ranging from one to four. On the other hand, six alleles, namely, *6-154*, *6-166*, *6-167*, *6-169*, *6-171*, and *6-175*, were detected among the 165 progeny and these alleles segregated at an ratio of presence to absence of 72:93 (*6-154*), 73:92 (*6-166*), 74:91 (*6-167*), 163:2 (*6-169*), 117:48 (*6-171*), and 71:94 (*6-175*), respectively. Allele *6-166* originated from the maternal parent HoCP 00-950, alleles *6-154*, *6-167*, *6-171*, and *6*-175 were of paternal origin from L 99-233, while allele *6-169* was found in both parents. Alleles *6-154*, *6-166*, *6-167*, and *6-175* segregated at 1:1, allele *6-171* segregated at 3:1, and allele *6-166* segregated at 83:1. Although at least 500 genotypes would be expected by theory, only 36 genotypes were detected among the 165 seedlings at frequencies that varied from 0.61% to 8.48% and the number of alleles per genotype ranging from one to six. The molecular data indicates that the majority of SSR alleles may inherit in a 1:1 fashion and that not all gametophytes' genotypes were able to pass into the progeny. More research is needed for complete understanding of the inheritance mechanism of SSR markers in sugarcane.

MP3

GENETIC DIVERSITY AND POPULATION STRUCTURE IN SUGARCANE ASSESSED BY SSRs AND AFLP.

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Keywords: sugarcane, SSR, AFLP, genetic similarity, population structure

Association between markers and sugarcane important agronomic traits is a useful approach for marker assisted selection in breeding programs. Association mapping is a method to test this association based on linkage disequilibrium (LD). LD values depend on many factors, such as genetic diversity and recombination rate. However, one obstacle to LD mapping application is the structured populations, which may lead to spurious associations. The objectives of the present study were to investigate the population structure and genetic diversity in a set of 87 sugarcane genotypes, including commercial varieties and also superior clones at advanced stage of selection from the Sugarcane Breeding Program of the "Instituto Agronômico de Campinas".

Genetic variation was studied by screening in this collection with 17 microsatellites (SSRs) primers and one AFLP selective combination (Eco AGC/Mse CGT) primer pair. Population structure was examined by phylogenetic analysis, using Jaccard coefficient to estimate the genetic similarity among the genotypes and the UPGMA method to establish the dendrogram.

The SSRs primers produced a total of 158 polymorphic alleles ranging from 2 to 19 alleles per locus, while the AFLP primer combination detected 30 polymorphic markers. The polymorphism information content (PIC) derived from the SSRs ranged from 0.38 to 0.93, with an average of 0.8, while the PIC value detected by the AFLP combination was 0.31. The coefficient of similarity ranged from 0.25 (between the IACSP97-2055 and SP97-3391) to 0.76 (between IACSP93-3046 and its female parent SP79-1011), with a mean of 0.45. Despite of the genetic diversity, no sub-group was observed in the dendrogram.

The results suggest a lack of genetic structure that will be well investigated with more markers that will be added to this mapping population.

Financial support: FAPESP/Bioen.

MP4

FUNCTIONAL GENETIC DIVERGENCE OF CLONES AND RB VARIETIES OF SUGARCANE BY MICROSATELLITE MARKERS

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Keywords: Biotechnology, hybridization, breeding, *Saccharum* spp.

Studies involving genetic variability have been conducted in sugarcane. These studies aim at exploiting efficiently the genetic resources, selection of plants resistant to certain pests and diseases and for the identification of divergent parents with favorable agronomic features to achieve highest heterotic effect and a higher number of alleles related to yield in subsequent selection cycles. In this study we evaluated the level of genetic variation between and within progenies of sugarcane and quantified the genetic divergence between the functional genetic material considered for the selection of parents to be introduced in our breeding programme. A population of 20 progeny derived from the selfing of 3 commercial varieties RB863129, RB943365 and RB867515 were evaluated. These were obtained from the Flowering Station in Devaneio (Amaragi, PE, Brazil) and belong to the breeding program of Interuniversity Network for Development of Sugar and Alcohol - RIDESA. DNA was extracted following the CTAB protocol with some modifications and amplified with 6 genomic microsatellite primer pairs, and 7 EST-SSR primers. To estimate the genetic variation among the progenies of sugarcane we proceeded an analysis of molecular variance (AMOVA). The quantification of genetic divergence was obtained by using the arithmetic complement of the Jaccard coefficient and a dendrogram was built using the hierarchical method of UPGMA – Unweighted Pair Group Method using Arithmetical Averages. The data were processed by Genes software. AMOVA revealed high genetic variation level within the progenies confirming a wide genetic variability among clones and varieties for improvement. Through the quantification of genetic divergence, it was possible to identify divergent parents for breeding, so, hybridizations between genotypes UFRPE10, UFRPE08, UFRPE09, RB863129, RB867515 with UFRPE14, UFRPE16, URPE17, UFRPE18, UFRPE19 and UFRPE20 can result in obtaining of new clones genetically superior with favorable agronomic traits of interest to sugarcane industry.

MP5

GENETIC DIVERSITY ASSESSMENT OF *SACCHARUM* SPECIES AND ELITE CULTIVARS FROM CHINA USING SSR MARKERS

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Keywords: Simple sequence repeat (SSR), *Saccharum*, fingerprinting, capillary electrophoresis, genetic diversity

Genetic diversity amongst 52 sugarcane clones including *Saccharum* species and cultivars (used for breeding and commercial production since the beginning of 20th century) was assessed using 21 Simple Sequence Repeat (SSR) primer pairs. The PCR products were visualized using Capillary Electrophoresis (CE) technique, instead of traditional agarose gel electrophoresis. Use of the 21 SSR primers resulted in amplification of 327 distinguishable SSR markers with an average of 15.6 bands per primer ranging from 7 to 24. A total of 141 polymorphic SSR markers were scored and used for the construction of a fingerprinting database and the assessment of genetic diversity. The UPGMA algorithm with SSR markers showed four distinguishable clusters, each with genetically similar species and varietal clones. The highest genetic homology was 87%, observed between ROC 16 and TY 1 and a few other closely related cultivars. Further, the use of CE in combination with PCR revealed that, most of the sugarcane clones bred from genetically similar parents during several successive breeding programs led to their grouping into one cluster. The results also indicate that the CE platform is an efficient tool for genetic fingerprinting and diversity studies. It is also important that breeding programs be tailored to exploit a wider range of *Saccharum* species germplasm in order to obtain better varieties especially for disease and pest resistance.

MP6

GENETIC DIVERSITY FROM THE BRAZILIAN PANEL OF SUGARCANE GENOTYPES (BPSG) BY SIMPLE SEQUENCE REPEAT (SSR) MARKERS

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Keywords: Saccharum spp., breeding, genetic similarity, germplasm, molecular markers

Sugarcane breeding programs have focused their efforts to release new superior varieties to those currently in commercial cultivation. However, this is a long process that usually exceeds 10 years and therefore very costly for breeders. Molecular markers may be an important strategy in reducing the time and the costs involved. The objective of this work was to evaluate the genetic diversity of 94 sugarcane genotypes belonging to the Brazilian Panel of Sugarcane Genotypes (BPSG) using microsatellite markers. This panel brings together genotypes from different geographic regions of the world, which represent the genetic basis of Brazilian breeding programs, and also aims at association mapping studies in sugarcane. For constructing this panel, were adopted the following criteria: most planted varieties, most used varieties as progenitors, important ancestral species, genotypes from the genetic mapping programs, and newly released varieties by breeding programs in Brazil. Genetic diversity among all the genotypes, 2 to 2, was estimated by Jaccard coefficient using the software R. Thirty-nine SSR markers (28 derived from EST-SSRs and 11 genomic) generated a total of 667 bands, of which 604 were polymorphic (90%). Owing to the high ploidy level of sugarcane, most of analyzed SSRs produced more than two alleles, ranging from 4 to 36, with an average of 16 alleles per marker. The polymorphism information content (PIC) ranged from 0.50 to 0.96, with an average of 0.88. The power of discrimination (PD) ranged from 1 to 0.62, with an average of 0.97. Molecular data allowed to obtain a dendrogram composed of various correlation groups, contained into two large subdivisions originated from *Saccharum barberi*. Bootstrap analysis (1000 estimates) were performed for each pair of genotypes in random samples of SSRs. Based on the dendrogram and the genetic diversity matrix, it was observed that, in general, genotypes from the same crossing (full-sib genotypes) presented superior genetic similarity when compared to those from only one joint parental (half-sib genotypes). For example, full-sib genotypes from the crosses RB72454 x NA56-79, RB72454 x SP70-1143 and SP71-1088 x H57-5028 presented an average genetic similarity over 60%, whereas this same parameter for the half-sib genotypes from the crosses NA56-79 x ? and IAC48-65 x ? remained, in general, below 55%. These results are preliminary, since other sugarcane genotypes from the Brazilian panel as well as more markers will be considered for obtaining these estimates. The obtained data will also be used to analyze the extent of linkage disequilibrium and to subsequently perform association mapping in genotypes of interest for genetic improvement in Brazil.

Financial support: INCT/Bioetanol (CNPq)

MP7

PRELIMINAR EVALUATION OF SSR AND AFLP MARKERS FOR BROWN RUST RESISTANCE IN SUGARCANE

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Keywords: Sugarcane, *Puccinia melanocephala*, brown rust resistance, EST-SSR

One of the biggest challenges for sugarcane breeding programs is to obtain genotypes resistant to pathogens. Sugarcane brown rust (*Puccinia melanocephala*) is one of the major sugarcane diseases that account to decline in productivity and its control is mainly through the adoption of resistant varieties. Molecular markers such as microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) are powerful tools for mapping and marker trait association analysis. In the present work, SSR and AFLP markers scored in a bi-parental (IACSP95-3018 x IACSP93-3046) mapping population of 200 individuals were analysed for marker trait association for brown rust resistance in plant cane and ratoon cane. The individuals were scored based on a diagrammatic scale of leaf symptoms ranging from 0 (most resistant) to 9 (most susceptible). A total of 488 single dose markers (1:1 or 3:1) were obtained and used for the single marker trait association analysis at 1% ($P<0.01$) and 5% ($P<0.05$) significant levels. Among the 61 marker trait associations detected, 30 were observed in plant cane, 3 in ratoon cane and 10 in both crop cycles. Only two marker trait associations were detected at $P<0.01$ in both crop cycles and assumed as most consistent. In plant cane 37% of the marker trait association detected contributed to decrease the rust rate score while in ratoon cane 41%. The phenotypic variation (R^2%) explained by the markers detected ranged from 1.86 to 7.2%. These preliminary results indicate the existence of QTLs for rust resistance in the population being studies, that will be mapped as more markers will be screened on the mapping population.

MP8

PRELIMINARY EVALUATION OF THE GENETIC VARIABILITY IN A SUGARCANE GERMPLASM COLLECTION ASSESSED THROUGH MICROSATELLITE MARKERS

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Keywords: Microsatellites, germplasm, genetic variability

Sugarcane worldwide germplasm collections are potential valuable source of new genes that can be introduced in breeding programs. However, their uses depend on a well characterization and management of the accessions. Molecular markers can be used to estimate the levels of genetic variability among germplasm accessions and also in the management of collections by checking duplicate or mislabeled accessions. In the present work, 12 microsatellites (SSRs) were used in a preliminary molecular characterization of 42 accessions of sugarcane from the IAC Sugarcane Collection, in order to evaluate the potential of these SSRs to detect the genetic variability among them, as also to identify duplicated accessions in the collection. The 12 SSRs produced a total of 186 alleles with an average of 15.5 alleles per loci. The average genetic similarity (GS) among the accessions estimated based on Jaccard Coefficient was 38% with the lowest GS value (8.3%) obtained between SES205A (*S. spontaneum*) and Badila de Java (*S. officinarum*) and the highest GS (85%) between Caiana Roxa and Flor de Cuba, both *S. officinarum* clones. The dendrogram showed that accessions having a pedigree relationship were grouped together as observed between Fiji162 and Fiji 119, and among NG57213, NG7792, NG7718, as also between Krakatau and SES205A, both *S. spontaneum* clones. Although the 12 SSRs initially investigated were able to assess the genetic polymorphism among the accessions evaluated the coefficient of variation estimated by bootstrapping (1000 resamplings) reached 16.3% indicating that more markers will have to be added to increase the accuracy of genetic similarity estimation. Financial support: INCT/Bioetanol (CNPq)

MP9

VALIDATION OF THE EFFECTIVENESS OF AN EMASCULATION TREATMENT IN SUGARCANE BY USING MOLECULAR MARKERS

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Keywords: Emasculation, SSR, marker

Sugarcane cultivars are developed by means of three procedures: assembling a described population of parental clones; generating variable progenies by cross-pollination and selecting outstanding clones. Parents used in crosses are classified as male or female based on the relative amounts of viable pollen produced. High pollen production determined by both genotype and environmental conditions reduces "female inflorescence" availability and restricts the possibility of cross combinations. However, male parents could be employed as female parents when an efficient emasculation treatment is used. This technique implies pollen sterilization by immersion of the panicle in hot or cold water, chemical products or steam. On the other hand, an ideal approach for hybridity testing is the use of molecular markers, especially SSR. To determine the effectiveness of an emasculation treatment (immersion of the panicle in water at 50ºC for 5 minutes), six cross combinations between two varieties commonly used as males, LCP85-384 and RA87-3, were evaluated using SSRs. After the emasculation treatment, pollen absence in the two varieties was confirmed with a magnifying glass, whereas pollen was detected in the same varieties without treatment. The crosses were performed successfully and no viable seeds were obtained when LCP85-384 and RA87-3 were emasculated and selfed, respectively. Fifteen SSR primers pairs were evaluated to identify the one producing polymorphism in the two varieties. DNA of the progeny of each cross combination was screened with the primer that best differentiated the parents (MSCUR19, unpublished sequence, D'Hont 2005), and the presence or absence of the specific markers was scored. This primer produced seven polymorphic and three monomorphic bands between the two progenitors. Segregation analysis showed that each marker segregated in a Mendelian fashion (as evaluated by $\chi 2$ tests, $P \leq 0.05$) for each cross combination. Results indicated that the treatment was successful to emasculate RA87-3 and LCP85-384 varieties completely and it did not cause a serious reduction in both the stigma and ovary viability. Also, this method is simpler, faster and cheaper than other emasculation techniques and it will allow the expansion of bi-parental crosses. Besides, the SSR technique allowed assessing the fidelity of sugarcane crosses. Thus, both implemented tools will improve crossing efficiency of sugarcane breeding programs.

MP10

ESTIMATION OF OUTCROSSING RATE OF SUGARCANE UNDER NATURAL CONDITIONS THROUGH MOLECULAR MARKERS

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Keywords: Polycross, outcrossing rate, molecular marker

Sugarcane flower and pollen viability are dependent on environmental conditions, specially photoperiod, temperature, air and soil humidity. These factors, when appropriate can induce flowering and maintain pollen viability for sugarcane crosses. This study aimed to evaluate the pollen viability of sugarcane commercial cultivars (IACSP95-5000, IACSP91-1099, SP89-1115, RB86-7515) under natural conditions of Ribeirão Preto (SP), Brazil, through the estimation of outcrossing rate using molecular markers. Culms flowered in 2009 were harvest at the Sugarcane Centre ("Instituto Agronômico de Campinas") located in Ribeirão Preto. The stain pollen method with iodide solution (1g of I, 1g of KI dissolved in 100 ml of water) was used to classify the tassel as male or female after microscope reading of percentage of stained blue (male) or white (female) pollen according to a score ranging from 1 (male) up to 9 (female). The scores of cultivars IACSP95-5000, IACSP91-1099, SP89-1115, RB86-7515 were 1 (male), 3 (male), 8 (female) and 4 (male) respectively. After sex characterization of the parents, the culms were placed into an acid solution to maintain the longevity of the flowers, staying for 21 days for polycross and seed maturation. The seeds obtained from SP89-1115 (female) were sown in a box with substrate and 24 progenies were individualized and used for outcrossing rate determination. Six microsatellite primer pairs (SSRs) were used to genotype the 24 progenies and also the four cultivars. These SSRs generated 64 markers (alleles), of which, 28 were absent in SP89-1115 (female parent) and present in the possible male parents. The alleles absent in SP89-1115 identified 22 individuals derived from outcrossing (91.6%), and 2 off types (contaminants). These 22 individuals were identified as derived from the cross between SP89-1115 and IAC95-5000 which received the lowest score (1), that is, a large amount of pollen compared to the other male parents. The SSRs were efficient in the identification of the parents for outcrossing rate estimation. In addition, the results showed that it is important to use parents with similar scores to guarantee equal pollen contribution of each male parent in polycrosses.

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MP11

PRODUCTION OF HEALTHY SEED CANE AT EEAOC (TUCUMÁN, ARGENTINA)

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Keywords: Genetic purity, healthy plant, micropropagation, meristems

Since 2001, Estación Experimental Agroindustrial Obispo Colombres (EEAOC) has been working on "Vitroplantas Project". On average, 55,000 sugarcane seedlings of commercial sugarcane varieties are produced annually through *in vitro* meristem culture. The micropropagation technique is widely used to eliminate systemic diseases, especially viral ones. Hence, this project guarantees health and genetic purity of seedlings. The project involves the stages presented in this work. Sanitation of plant material is achieved through *in vitro* culture of apical meristems from donor plants, hydro-heat-treated previously and held for 3 years under greenhouse conditions with an anti-aphid screen. Also, systemic diseases are evaluated in both meristem donor plants and micropropagated seedlings using sensitive, rapid and reproducible molecular tools. PCR protocols to detect two bacterial diseases; ratoon stunt and leaf scald, and RT-PCR protocols to detect SCMV and SrMV (causal agents of sugarcane mosaic disease) and ScYLY (causal agent of yellow leaf disease) are routinely applied. On the other hand, *in vitro* culture can produce somaclonal variation, which consists of genetic modifications in cultured cells and tissues. Thus, a molecular methodology based on molecular markers to quantify and detect somaclonal variation in the propagation scheme is routinely applied as a complement of phenotypic evaluation in the field. In cases where this variation occurs, it is possible to detect it before releasing the material propagated. The seedlings produced in the laboratory undergo an acclimatization process in the greenhouse. In order to avoid dehydration, this process takes place in an environment with high relative humidity (RH = 80 - 100%) and low light intensity during the first two weeks. After that, RH is gradually reduced and the intensity of light gradually increased. Under these conditions, this critical stage that defines the commercial viability of the whole process lasts 90 days on average. Then, the material goes through two more stages of conventional propagation in the field (Basic and Registered Nurseries) before being distributed among sugarcane growers. Regarding productivity, efficiency and safety, propagated plants from meristems are quite advantageous. In the short term, old and/or infected materials will be replaced with this healthy material of high yield potential in Tucumán, Argentina. This state-of-the-art technology, which is widely spread in sugarcane growing countries, has been incorporated by EEAOC to obtain healthy seedlings for release among local growers.

MP12

ISOLATION AND CHARACTERIZATION OF FULL-LENGTH cDNAs ASSOCIATED WITH DROUGHT AND WATER EXCESS TOLERANCE IN SUGARCANE

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Keywords: drought stress, flooding stress, full-length cDNA, gene expression

Drought and flooding tolerance are desirable breeding characteristics for Colombian sugarcane cultivars due the high cost (approximately 50% of the cost of production) of irrigation and drainage, which are an integral part of sugarcane agriculture in the country. It is estimated that, of the 218 000 ha of sugarcane grown in the Cauca valley river, approximately 207 000 (95%) are grown under irrigation, while almost 174 000 (80%) of this cultivated land will, at some point, need a drainage system. One of the focus of current sugarcane research in Colombia deals with the understanding of the physiology of tolerance of Colombian varieties to stress such as drought and excess water. Besides we have begun the isolation and characterization of cDNAs of interest so that breeding strategies based on plant transformation could be implemented in the near future. Our main interests are the DREB (Dehydration Responsive Element Binding) and IPT (IsoPentenyl Transferase) gene families since previous data suggest that they could modulate cellular processes leading to tolerance to drought in the case of DREB and to drought and water excess in the case of IPT. As part of our methodology, CC 85-92 plants were maintained in a growth chamber at 28 °C, with a 13-h light (100 $\mu Em^{-2}\ s^{-1}$) and an 11-h dark period, with constant irrigation (50% soil humidity), for two months. After this period, plants were divided in four groups and subjected in an independent manner to either drought (samples were collected when soil humidity was 40%, 30% 20%, 10% and 5%), or excess water (anoxia) or increased photoperiod (16 h light at 100 $\mu Em^{-2}\ s^{-1}$, and 8 h darkness). The fourth group was kept under conditions as described above and was used as control. Leaves and roots were collected from plants of each treatment and total RNA from leaves was extracted using the TRIzol® reagent. Primers to amplify partial or full-length cDNAs of interest were designed based on the information obtained from the SUCEST and GenBank databases. Amplified targets were cloned in the vector pCR 2.1 TOPO® and were sent for sequencing to the company Macrogen USA. Among our results we have isolated fragments of cDNAs with high similarity to the DREB and the IPT gene families. Currently we are working in standardizing methodologies such as RACE and Real-Time PCR in order to obtain full-length sequences of the different isoforms of these gene families and to characterize their levels of gene expression. Also, our strategies has allowed us to amplify full-length sequences of cDNAs highly similar to the gene families dehydrins, cysteine proteases, alcohol dehydrogenases and glutamine synthetases, which have been reported in the literature as relevant in abiotic stress tolerance and might prove important for this investigation.

MP13

SUGARCANE TRANSCRIPTS RESPONSE TO DROUGHT STRESS

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Keywords: RT-qPCR, SAGE, water stress

The cultivation of sugarcane (*Saccharum* spp.) is of great economic importance to Brazil for the production of sugar and ethanol, but water deficit is one of the main factors limiting growth and productivity of this crop. Growing demand for biofuels has required the development of varieties of sugarcane genetically improved and more efficient in water use. Based on data from Serial Analysis of Gene Expression (SAGE) in sugarcane previously annotated, from stem libraries of plants grown in the field, and contrasting to the rainy or dry seasons, identified *in silico* transcripts potentially associated with responses to drought stress. Of these, twenty-one associated genes (including three reference genes) were selected for gene expression analysis and validation by reverse transcription followed by real-time PCR (RT-qPCR) using two varieties of sugarcane contrasting to tolerance to water deficit stress; RB92579 (tolerant) and RB855113 (susceptible). Experiments were performed in greenhouse under four different water treatments. For each gene, three biological replicates were used, and each RT-qPCR reaction was performed in triplicate. The specificity and absence of contamination were assessed by dissociation curve of the PCR reactions and the presence of negative controls, respectively. For normalization and RT-qPCR data processing, we used three reference genes which were analyzed for stability using the software *GeNorm*. From the collection of 46,536 SAGE tags analyzed *in silico*, 45.0% showed inhibition by drought, 6.3% were drought-induced and 48.7% showed no significant variation ($0.5 < \text{ratio} < 2.0$). Out of the 21 transcripts selected for experimental validation as potentially responsive to water stress, only 15 could be monitored by RT-qPCR in leaves and 12 genes in roots. Results pointed that 66.6% (Dhyn_98, ERD4, Sip, DGT, MARK, Pox, Hd-Zip, Hsp70, and DnaK PPlase) of the genes differentially expressed in drought treatment ($p < 0.05$), while 33.3% of them showed similar expression in tolerant and susceptible varieties, respectively, in similar levels as previously detected by SAGE. Moreover, according to the results of RT-qPCR, two genes associated with drought tolerance were selected to determine their complete nucleotide sequence. Therefore, it is expected that these genes associated to drought tolerance may not only be used in breeding programs of main crop plants of economic importance, but also may help researchers to understand the genetic networks involved in stress tolerance.

MP14

MOLECULAR CLONING AND EXPRESSION ANALYSIS OF A *NPR1* GENE FROM SUGARCANE

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Keywords: Sugarcane, *NPR1*, smut, disease resistance, gene expression

The *NPR1* genes play a pivotal role in systemic acquired resistance in plants. In this study, a full-length sugarcane *NPR1* gene, designated as *ScNPR1*, was isolated and identified. The full-length cDNA was 2184 bp in length with a 1758 bp open reading frame which encoded a 586 amino acids protein. Homology analysis suggested that the ScNPR1 protein shares significant similarity to ZmNPR1 of maize and shared common features with *NPR1* obtained from other plants. Real-time quantitative PCR (RT-qPCR) results indicated that the expression of *ScNPR1* gene was obviously up-regulated after treatment with salicylic acid and inoculation with smut disease fungus *Ustilago scitaminea*, while was reduced after methyl jasmonate and ethylene treatments. In addition, higher accumulation of *ScNPR1* transcripts in leaf and stalk tissues of sugarcane cultivars resistant to smut disease were observed. These results clearly demonstrated that the *ScNPR1* gene was likely to be involved in SA-mediated signaling pathway and might play a role in the defense response to sugarcane smut disease.

MP15

SUGARCANE GENETIC TRANSFORMATION FOR TRANSPOSABLE ELEMENTS SILENCING

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Keywords: Transgenic plants, biolistic, tissue culture, leaf disks, embryogenic callus, transposition

Sugarcane is one of the major agro-industrial crops of Brazil and is being widely cultivated for sugar and ethanol production. Its genetic transformation beyond of contributing to study functional genes is an important method to introduce new traits into elite cultivars. The process of obtaining sugarcane transgenic plants generally involves a long period during callus phase, which is known to increase transposable elements (TEs) activity. TEs transposition may cause variability in the plant genome by altering patterns and gene functions due to unlikely insertions, then confronting the genetic fidelity of the transgenic cultivars obtained. Based on the importance of reducing the period of tissue culture and controlling TEs activity during *in vitro* development, this work sought alternatives to control and reduce the time for plant regeneration, especially from leaf disks, as well as to genetically transform the varieties RB835089 and RB835486 with *Ddm1* Arabidopsis gene to silence the TEs in sugarcane. Particle bombardment-mediated co-transformation with the *neo* and *AtDdm1* genes resulted in 34 independent transgenic events. Our results showed that genetic transformation with the *AtDdm1* gene and the fast regeneration of plants from leaf disks provided suitable conditions to minimize TEs expression in sugarcane.

MP16

TWO SUGARCANE GENES INDUCED BY DROUGHT CONFER TOLERANCE TO DROUGHT AND SALT STRESS IN TRANSGENIC TOBACCO PLANTS.

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Keywords: Sugarcane, drought, saltless, transgenic, tolerance

Sugarcane varieties differ in their tolerance to drought, a major abiotic stress affecting crops worldwide. Due to the exploitation of sugarcane as an energy crop there is a need to increase sugarcane production and this includes cultivation on lands with lower water availability, such as the Brazilian Cerrados. We are interested in finding sugarcane genes that could be useful to produce transgenic sugarcane plants with increased tolerance to drought. To this end we used genetical genomics, comparing the transcriptome changes in sugarcane varieties differing in their tolerance to drought, to identify genes associated to drought stress. Among the genes up- or down-regulated by drought, we selected two genes encoding proteins with unknown functions, which may give insights in new mechanisms that plants use to overcome drought. The genes were named *Scdr1* and *Scdr2* (for sugarcane drought-responsive). Transgenic tobacco plants were used as a first step to evaluate the role of these two genes for drought tolerance. *Scdr1* encodes a putative 248 amino acid protein rich in proline and cysteine residues. Phylogenetic analysis indicated that this gene is found only in monocots. *Scdr2* encodes a putative protein containing 77 amino acids and homologs were found in monocots and dicots. We found that the germination of wild type seeds was completely inhibited at 300 mM mannitol, while 20% of the *Scdr1* overexpressing seeds were able to germinate. Under 175 mM salt stress, wild type seeds did not germinated, while 50% of the *Scdr1* seeds germinated. Seeds from *Scdr2* plants showed a similar pattern, although they presented a higher germination rate (60%) under 300 mM mannitol, compared to wild type seeds (20%). *Scdr1* and *Scdr2* transgenic and wild type adult plants were watered with mannitol or NaCl for 10 days and then watered with tap water for three days, while control plants were watered regularly over the entire period with tap water. We found that stomatal conductance, transpiration, photosynthesis and internal CO_2 concentration were affected in both wild type and transgenic plants during drought and salt stress. However, plants overexpressing *Scdr1* and *Scdr2* showed a trend indicating they were less affected. Interestingly, after rewatering, a recovery of these parameters was observed in *Scdr1* and *Scdr2* plants, which appeared normal after the three days of rewatering, while wild type plants did not recover. Our data indicate that both *Scdr1* and *Scdr2* play a role in drought and salt tolerance in sugarcane. These genes have the potential to be used for producing sugarcane varieties with greater tolerance to both drought and salt stress. Work underway is focused in the understanding on the pathways controlled by these genes.

Financial support: CNPq and FAPESP

MP17

Improving low-temperature tolerance in sugarcane by expressing the isopentenyltransferase (*ipt*) gene under control of a cold-induced promoter

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Keywords: *Saccharum* spp., *COR15a* promoter, cold stress, cytokinins, senescence.

Sugarcane is cultivated in tropical and subtropical and it is generally considered as a cold-sensitive plant. Low temperatures result in lower yields and reduced industrial quality of the stalks. In order to enhance cold tolerance in sugarcane, the gene encoding isopentenyltransferase (*ipt*), a key enzyme in cytokinin synthesis, was transferred via biolistic transformation into sugarcane cv. RB855536 under control of the cold-inducible gene promoter AtCOR15a. Semi-quantitative RT-PCR showed increased expression of the *ip*t transgene under low temperatures and even 24 h after the cold stress. Detached leaves of genetically modified plants subjected to low temperatures had visible reduction of leaf senescence in comparison to non-transgenic control plants. Experiments with whole-plant sugarcane transgenic events in growth chamber conditions showed that low temperature-induced *ipt* gene enhanced cold tolerance of non-acclimated plants cultivated. Leaf total chlorophyll contents of plants subjected to freezing temperature were up to 31% higher in transgenic plants compared to non-transformed controls. MDA (malondialdehyde) levels and electrical conductivity analysis also indicated that less damage was inflicted to the cell membrane of transgenic plants. Thus, our findings showed that the expression of *ipt* driven by the stress inducible COR15a promoter did not affect plant growth under normal conditions and provided greater tolerance to cold stress.

MP18

TRANSGENIC SUGARCANE HABOURING NPK1 PRODUCED BY PARTICLE BOMBARDMENT AND *AGROBACTERIUM*-MEDIATED TRANSFORMATION

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Keywords: *Saccharum* spp., abiotic stress, protein kinase, NPK1

Sugarcane (*Saccharum spp*.) yields are severely limited by abiotic stresses, including drought and extreme temperatures. Classical breeding approaches have many challenges in order to provide sugarcane varieties more tolerant to abiotic stresses due to the complex genome of this crop and environment interactions. Complementarily, biotechnogical strategies are relevant to speed up sugarcane breeding for tolerance to abiotic stresses. NPK1 is a mitogen-activated protein kinase kinase kinase (MAPKKK) from tobacco that plays important roles in auxin signal transduction, cytokinesis and responses to multiple stresses. Previous studies have shown that expression of NPK1 activated an oxidative signal cascade that led to the tolerance of multiple abiotic stresses in transgenic tobacco and maize. The objective of this work was to obtain sugarcane transgenic events expressing NPK1 in order to enhance stress tolerance in sugarcane. Somatic embryos and leaf sheath meristematic tissues from *in vitro*-grown plantlets of cv. RB855536 were used as explants for particle bombardment and *Agrobacterium*-mediated transformation protocols, respectively. We used the binary vector pSHX004, containing an expression cassette carrying a modified CaMV 35S promoter, an 800 bp DNA fragment encoding the kinase domain of *Nicotiana* protein kinase (NPK1), and the nopaline synthase terminator. The selectable marker cassette contained a CaMV 35S promoter, a tobacco etch virus 5' untranslated region, the *bar* gene that confers resistance to the herbicide ammonium glufosinate, and a soybean vegetative storage protein terminator. In both transformation methods, selection was carried out on 5 mg/L ammonium glufosinate. Transgenic plants were selected by PCR analysis using *NPK1* and *bar* derived primers. Putative transgenic sugarcane plants were recovered from both *Agrobacterium* and biolistic transformation methods – three and seven events, respectively. RNA blot analysis using *bar* as a probe detected transcriptional variation among the selected events, where *Agrobacterium*-derived events displayed higher transcriptional activity of the *bar* gene when compared to biobalistic-derived events. Further analysis of these transgenic events will be carried out to evaluate their tolerance to different abiotic stresses.

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