11th GERMLASM & BREEDING
8th MOLECULAR BIOLOGY
ISSCT WORKSHOP

Saint-Gilles Réunion Island / 1–5 June 2015

« Pushing the frontiers of sugarcane improvement »

ABSTRACT
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ORAL ABSTRACTS BREEDING (BO)
THE SUGARCANE AND RELATED GRASSES COLLECTION AT USDA/ARS GENE_BANK REPOSITORY IN MIAMI, FLORIDA, USA

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Keywords: Miscanthus, Saccharum, germplasm, barberi, spontaneum, robustum

A world sugarcane collection and related grasses is maintained at the The National Clonal Germplasm Repository (NCGR) in Miami, Florida, USA. This germplasm bank is maintained at least 180 km from the commercial sugarcane growing areas and was established to maintain the collection free of pests and disease. A globally diverse collection of Saccharum and related species (e.g. Erianthus, edule, ravenae, procerum, Miscanthus) germplasm has been assembled and expanded. Distinctive sugarcane genotypes are maintained as growing plants in a 4 hectare area, evaluated and tested for virus and fungus contamination, and partly documented in the Genetic Resources Information Network (GRIN) database. The material is freely distributed to national and international research institutions; however, the requestor is responsible for providing the import permits and shipping and phytosanitary certificate costs. The collection is replanted every 2-3 years around April-May. At present, the collection consists of about 1300 clonal saccharum accessions and over 200 seed lots (maintained at the National Center for Genetic Resources Preservation (NCGRP) representing the major Saccharum species. The collection comprises of S. officinarum (350), S. barberi (37), S. sinense (29), S. robustum (150), S. spontaneum (385), S. hybrids (150) and allied genera (240). The allied genera includes Erianthus arundinaceus, brevibarbe, narenga, edule, ravenae, kanashiroi, rufipilu, procerum, plumosum and miscanthus.

In the early 1990s, the USDA-ARS financed and coordinated the preservation of true seed of S. officinarum and S. spontaneum. Open-pollinated true seed of 78 S. officinarum and 148 S. spontaneum clones were produced at the Breeding Station of the Hawaiian Sugar Planters' Association as well as from over 200 S. spontaneum clones at Canal Point, Florida. More than 100 seeds from each clone were maintained in cold storage at the NCGRP, Fort Collins, Colorado. Work is being done to back up the clones via tissue culture and cryopreserved meristems. Currently 75 clones are stored or undergoing testing in cryopreservation and another 125 are maintained in tissue culture. Recently, the collection has tested negative for most common viruses and rusts but several accessions have tested positive for Sugarcane Yellow Leaf Syndrome. In addition, in the last two years the collection was phenotypically and genotypically evaluated. During the last 5 years, on average, 800 accessions were distributed annually to researchers in the USA and internationally.

Maintenance of the collection (daily maintenance, replanting and repotting of more than 1300 clones) requires a large quantity of resources. Although there have been difficulties in maintaining some species (e.g. S. officinarum) due to disease, labor or weather events, there has been no major loss of clones. At the moment, another 150 accessions have been requested through federal quarantine, and as more material is discovered and becomes available it will be added to the collection.
PRELIMINARY EVALUATION OF THAI ERIANTHUS GERMPLASM

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Keywords: Erianthus Germplasm, Thailand, Evaluation, Basic agronomic traits

Erianthus, one of the genus in \textit{Saccharum} complex, is important germplasm with great potential for improving ratooning, biomass production, adaptability to adverse environments and disease resistance in sugarcane improvement.

KKFCRC and JIRCAS jointly collected 150 accessions of \textit{Erianthus} germplasm from all over Thailand. Tagane \textit{et al.} (2012) reported that the collection consisted of \textit{E. procerus} and three types (Types I, II, III) of \textit{E. arundinaceus}, and showed wide range of variation but clearly divergent in their morphology, ploidy, flowering period and geographic distribution. To further facilitate utilization of this \textit{Erianthus} germplasm collection, we carried out thorough characterization and evaluation of basic agronomic traits at Khon Kaen, Thailand during the period from 2012 to 2014 for plant cane and 2 times of ratooning.

The \textit{Erianthus} accessions showed wide range of variation in their basic agronomic traits e.g. number of stalk (16 - 207/stool), stalk length (230 - 505cm), stalk diameter (8 - 18mm), one stalk weight (100 - 825g), ratio of stem part (21-77\%), Brix (4 - 14\%), ratio of pith (35 - 92\%), dry matter content (44 - 77\%), dry matter yield (1 - 33kg/stool). \textit{E. arundinaceus} Type I accessions are characterized by more stalks, better ratooning and higher yield but low Brix. Type II accessions tend to have weighted stalk with bigger diameter, larger number of leaf and higher Brix. Type III accessions carry weighted stalk with less pith, larger number of leaf but lower dry matter content. \textit{E. procerus} accessions have features of smaller stalk diameter and weight, fewer number of leaf but higher dry matter content. Type II and III tend to have higher ratio of stem part than Type I and \textit{E. procerus}, but even varied within each species and types.

Accessions with distinctive characteristics in stalk number, stalk diameter, ratooning ability, stem ratio, dry matter content, yield, and Brix were identified for further investigation as materials for sugarcane improvement and biomass production.
UTILIZATION OF SACCHARUM AND WILD RELATIVES TO PROMOTE THE SUGARCANE BREEDING IN JAPAN

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Keywords: sugarcane, Saccharum, wild relatives, interspecific hybrids, intergeneric hybrids

Sugarcane products in Japan are produced in small islands of South West Islands. However, the sugarcane yield in this area is highly variable and low, due to adverse conditions including typhoons, drought, and poor soil. In addition, sugarcane has been used only for sugar production though the production costs are very high.

Sugarcane has potential as a biomass resource for multi-purposes such as sugar, ethanol, fiber and fodder. However, biomass productivity under the adverse conditions in the south west islands is challenging. To improve the productivity of sugarcane under these conditions, and maintain the local society and environment, multiple products from improved sugarcane cultivars are needed.

A joint research group of Japan has attempted wide-crossing to overcome unstable yield influenced by adverse conditions, and to improve production of different products. In addition, technological developments to in breeding have been advanced. In this program, flowering inducement techniques were developed to use wide genetic resources. As a result, it has become possible to use to hybridize various genotypes such as commercial varieties, Saccharum and wild relatives. Currently, characteristics of interspecific/intergeneric hybrids and those posterities are being evaluated, and further hybridization is being conducted as a high priority.

In utilization of general materials, the decipherment of the genome information of some commercials, the development of DNA markers for smut etc. are carrying on. We will use DNA markers for our breeding program in near future. In utilization of S. spontaneum, we try to develop the high biomass varieties and utilization process that produces electric powers with more sugar production simultaneously. In the intergeneric hybrids between sugarcane and Erianthus, the adaptability to the adverse condition like a deep root is expected, and the reactiveness to the environment has been evaluated. On the other hand, the decipherment of the genome information of Erianthus, inherited analysis using by GISH etc. are carrying on. We will accelerate the development of DNA marker of important traits.

Japan is a severe region located in the northern limit of the sugarcane production area; we would like to produce sustainable sugarcane production and local society by breaking the breeding paradigms in this area.
**ERIANTHUS HYBRIDS INTROGRESSION TO DEVELOP COMMERCIAL SUGARCANE CULTIVARS**

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**Keywords:** *Erianthus* hybrids, SSR markers, hybridization programme, selection

In E.I.D. Parry (I) Ltd. India, genetic base broadening programs were undertaken for more than a decade and research aimed to produce intergeneric hybrids for sugarcane varietal improvement. Many attempts were made in developing *Erianthus* hybrids using *E. arundinaceus* as pollen donors through conventional breeding. In this study, we developed 26 putative hybrids which were selected based on morphological characters and also subsequently screened by SSR markers at Sugarcane Breeding Institute, Coimbatore. Out of 26 hybrids, 19 were confirmed as *Erianthus* hybrids showing the presence of species specific markers of *Erianthus* and *Saccharum officinarum* in the silver stained PAGE gels following PCR amplification with SSR primers. These confirmed hybrids were planted in the hybridization block at Bangalore for crossing to harness the diverse genetic variability in breeding elite hybrids.

Based on synchronization of flowering ten confirmed hybrids were utilized in hybridization programme as male parents and *S.officinarum* and commercial varieties were used as female parents. A total of 667 seedlings were produced and were evaluated for HR Brix and morphological characters. Though we obtained more number of stage I seedlings from PI 09-0018, PI 09-0020, PI 09-0051 followed by PI 09-0012 and PI 09-0001, we selected the commercial canes which showed high brix, thick canes with good intermodal length and more no. of tillers. The field traits including tillering, stalk population, stalk diameter, suckering, spines, trashingle, and lodging were observed and recorded in stage II selection. From thirty stage II clones, nine promising clones which showed more number of tillers and thick canes were promoted to stage III. Field Brix recorded at the age of 10th month ranged from 16 to 21%.

We were successful in creating the hybrids with commercial traits with the new wild germplasm. The data indicated potential of commercial nature as indicated by high Brix combined with high tillering and high fibre traits transmitted from *Erianthus arundinaceus*. Further the selected clones will be evaluated for preliminary yield trial, a replicated trial at different locations and will be subjected to disease screening. The study would also be expanded to select multipurpose varieties with high biomass suited for bioenergy production.
SUGARCANE BREEDING THROUGH INTERSPECIFIC CROSSING IN JAPAN

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Keywords: wild sugarcane, interspecific crossing, Japan, smut disease

Sugarcane (Saccharum spp. hybrid) is commonly grown for sugar production in Nansei Islands, where is located at the south end of Japan. However, sugarcane has a potential for the alternative use such as ethanol and bagasse-based power generation. The modern sugarcane cultivars were developed from the cross between commercial varieties/lines. In Japan, we have conducted the interspecific crossing by using wild sugarcanes (Saccharum spontaneum) to improve the biomass productivity for the alternative use. As the fruits of our trials, we developed two cultivars as forage use, ‘KRFo93-1’ and ‘Shimanoushie’, and one model cultivar as sugar and ethanol production, ‘KY01-2044’. All of the interspecific cultivars are derived from one oversea wild sugarcane, Glagah Kloet.

For the improvement of our interspecific breeding, we have to utilize wide genetic resources which are suitable to the environment of Japan. Fortunately over 200 accessions of wild sugarcane had been collected in Japan and those are available as the breeding materials. For its efficient utilization, we investigated the characteristics of indigenous wild sugarcanes. Two important characteristics, smut resistance and stem brix, were evaluated. The smut resistance was checked by the pin-prick method. As the results, over 20 accessions were categorized as highly resistant to smut. In addition, the smut infection percentage of F1 progenies between NiF8 and ‘Iriomote 15’, a highly resistant wild sugarcane, was much lower than that of another F1 progenies from NiF8 and ‘Iriomote 37’, a susceptible resistant wild sugarcane.

In the evaluation of stem brix, we could find interesting wild sugarcanes whose stem brix were more than 18%. We are studying now whether these wild sugarcanes could improve the progenies’ stem brix.

These results indicate that wild sugarcanes collected in Japan have the possibility to improve our breeding program. We have to keep evaluating and utilizing the indigenous genetic resources.
INTROGRESSION: EXPLOITING NEW SOURCES OF GENETIC VARIABILITY FOR THE AUSTRALIAN SUGAR INDUSTRY

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Keywords: introgression, breeding, selection

Sugar Research Australia (SRA, formerly BSES) has been breeding sugarcane cultivars for the Australian sugar industry since the 1920’s. SRA’s breeding program has evolved to consist of three stages of selection in each of four regional selection programs (Northern, Burdekin, Central, Southern). Approximately 1500 crosses are made each year, with a total of 100,000 seedlings planted to the first stage, followed by two clonal stages.

As with other breeding programs, the genetic base of the parental and selection populations in Australia is narrow. Introgression of valuable genes from wild germplasm into the breeding population is difficult but has proved successful. In the early 1900’s introgression of *S. spontaneum* resulted in improved productivity, adaptation, vigour, ratoonability, and increased resistance to some major diseases. Further introgression in the 1960’s in Australia with Mandalay (*S. spontaneum*) resulted in many varieties (>20) and a new source of resistance to pachymetra root rot.

Over the last decade, SRA and CSIRO with Chinese collaboration, have made a large number of *S. spontaneum* (BC1, BC2, BC3) and *Erianthus* crosses (BC1, BC2, BC3, BC4) and many progenies from these crosses have been screened for biomass, cane yield, sugar content, diseases and nematode resistance, and are currently being screened for frost tolerance, yield and ratooning ability under harsh conditions. Basic material from the discontinued breeding program of Wilmar (previously CSR) are also being assessed in current SRA projects.

Although Australia is currently exchanging germplasm with more than 15 countries, this is mostly commercial material. New variety exchange agreements, particularly with countries that have basic and early-generation germplasm collections need to be further explored. The importation of true seed also needs to be pursued due to variable flowering of clones and poor seed-set of crosses in Australia.

A long-term introgression strategy is crucial to achieving our aims of efficiently and effectively broadening the genetic base of the SRA breeding population and delivering new parents with beneficial genes. This strategy still needs to be developed and may include a “pre-breeding” program. Advances in molecular and cyto-genetics will assist in which material to import and how best to utilize this material.
CONTRIBUTION OF SUGAR CANE WILD RELATIVES IN THE MAURITIAN SUGAR CANE BREEDING PROGRAMME

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Keywords: wild canes, introgression breeding, germplasm collection, pedigree

An important breakthrough in sugar cane breeding was achieved early in the last century with the use of sugar cane related species as parental lines that led to the creation of a few ‘wonder canes’, such as POJ 2364, POJ 2878, Co 206 and Co 213, with spectacular improvements in cane and sugar yields, pest and disease resistance and adaptation. These varieties became the foundation of modern sugarcane varieties. Basic interspecific hybridization has continued to-date in many sugar cane breeding stations, mostly to broaden the genetic base of sugar cane, to produce new parents with desirable traits, and to identify better cultivars. There is a renewed interest in using wild canes for the creation of new types of sugar cane varieties with higher fibre for bioenergy production. However, information is lacking worldwide on the recent contributions of sugar cane wild relatives in sugar cane breeding. This study focussed on a retrospective analysis of forty years of available data at the Mauritius Sugarcane Industry Research Institute with the objectives of determining the contribution of specific wild clones in the Mauritian sugar cane breeding programme and evaluating their share in the local genetic base broadening programme.

Pedigree analysis helped to retrace the parentages of elite interspecific derived genotypes that reached the final stages of selection in the last four decades. Results confirmed the high prevalence of a few wonder canes, namely POJ 2878, Co 421, Co 312 and Co 281, among the ancestors of Mauritian varieties. Among the wild relatives, five *S. spontaneum* (MAUR 1937, Kletak, IS 76216, IK 7610 and Mandalay), two *S. robustum* (NG 50208 and Molokai), and one *Erianthus* (IK 7647) clones were involved in generating promising genotypes. The wild cane species contributed mainly by producing one or two specific clones that later became important parents of elite varieties. For example, MAUR 1937 produced an F₁ progeny named “Uba Marot” that was an important parent in the 1950-70s. In the 1980s, Kletak produced two F₁ genotypes, M 376/84 and M 386/84, with good breeding potential. IS 76216 generated M 1000/86 that in turn gave rise to a BC₁ offspring, M 422/91, that was much involved in producing promising varieties in the last decade. The contribution of *S. spontaneum* clones IK 7610 and Mandalay are relatively recent with few BC₁ clones evaluated at the variety trial stages. Among the *S. robustum* clones, NG 50208 was identified through M 5/75 (F₁) and its offspring M 2077/78 (BC₁). Molokai made its way relatively recently through two BC₁ progenies, M 467/84 and M 816/86. Crosses between *Erianthus arundinaceous* clone IK 7647 and existing commercial varieties in the late 1990s produced several progenies with potentially high biomass. However, the F₁ clones proved to be selfings of the wild cane used as parent. Overall, three BC₂ and 13 BC₃ progenies were released in the past for industrial exploitation. Few early generation hybrids are being evaluated at the final stages of selection.
YIELD STABILITY IN GENOTYPES DERIVED THROUGH BASIC BREEDING

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Keywords: Sugarcane, Breeding, Introgression, Yield Stability

The sugarcane variety ‘LCP 85-384’ was derived through basic (introgression) breeding, and after its release in 1995, the variety quickly gained acreage in the state of Louisiana. The primary reason for the popularity of the variety was the plant vigor and increase in the number of ratoon harvests over the other leading varieties at the time. This variety is a frequently-used parent in Louisiana breeding programs because of its wide adaptability to the local environment, and currently a majority of varieties in the state are related to LCP85-384.

Today, there is a need to develop the next generation of parental varieties with a broader genetic base. The current practice in the commercial variety testing program drops test material after a poor performance in a plant-cane or first-ratoon crop; thus many promising varieties are never tested into later ratoons. The lack of data on ratooning ability for many of the parental clones limits a breeder’s ability to consider late-ratoon stability when making cross combinations. The efforts of the basic breeding program are ongoing, and new parental material is advanced to the commercial program on a regular basis. Seven breeding lines that were recently advanced to the commercial program were tested alongside the three leading commercial varieties in the state. The test was planted on a heavy clay soil, which typically is difficult to cultivate and puts added stress on ratoons, and cultural practices which increase plant-stress were also utilized.

The average yield reduction in basic varieties between plant cane and first ratoon was 33 percent while the average reduction of the commercial varieties was 40 percent. Data from the plant-cane and first-ratoon harvest demonstrates the potential ability of some newly-advanced basic varieties to compete with commercial standards. The study is ongoing, but results thus far indicate many of the varieties derived from the basic breeding program have yield stability that surpasses that of the leading commercial varieties.
COMPARISON OF WHOLE CANE ANALYSIS OF 34 FAMILIES GROWN IN BARBADOS, BELIZE AND JAMAICA USE IN DATA FROM A SPECTRACANE HIGH SPEED ANALYSER

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Keywords: family selection, GxE interactions, Spectracane, NIR analysis

Family selection procedures are used in seedling selection in Barbados, Jamaica and Belize. The seedling families derive from crosses made at the West Indies Central Sugar Cane Breeding Station in Barbados. Although most of the crosses are specific to a particular country, each year there are a set of families that are evaluated across all of the countries in the network.

Thirty four families were evaluated at the seedling stage. Each family was represented by a single fifty seedling plot. Each family was sampled by collecting one cane from each seedling to give a bulk sample of 50 canes. This bulk sample was then divided into 10 five cane samples to pass through the Spectracane high speed NIR cane analyser. The mean of these ten results was then used as representing the family mean. The characters recorded were Brix in Juice, Pol in juice and fibre percent fresh weight of cane.

Since families were not replicated within each country a formal GxE analysis was not possible. The data are presented here as pair wise country by country rank correlations across the families. Clear and major differences in rank are apparent, indicating considerable interaction across environments as was expected. Fibre showed the least interaction and pol the greatest. Belize and Jamaica were more similar to each other than to Barbados in all three characters.

These results suggest that it may be worthwhile testing more families across the environments that the breeding programme serves. A more formal trial, replicating families within environments is planned.
A SOFTWARE APPLICATION FOR PARENTAL SELECTION IN CROSSING STAGE AT CHACRA EXPERIMENTAL SUGARCANE BREEDING STATION

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Keywords: parental selection, crossing value, mixed linear model prediction, software application.

Parental selection in a sugarcane breeding program should take into account multiple traits. In order to improve sugar and alcohol production, high-yielding disease-resistant parent varieties are preferred when making new crosses. Particularly in subtropical areas prone to frosts, precocity is also an important trait to evaluate new hybrids. Using data from CHACRA Breeding Program, an easy-to-use software application was developed for selecting parents for crosses with high expected genetic value for a given trait. To set up the algorithm we used CHACRA data for the 1997-2014 period corresponding to the first two clonal stages and late variety trials. The statistical protocol involved calculation of the Best Linear Unbiased Predictor (BLUP) for male and female effects of each parent from historical data of the derived progeny. For this purpose, a mixed linear model with fixed effect of crossing series and random effects of female, male and their interactions was run for each clonal stage and the BLUPs of parental effects were obtained. The BLUP of kg.Brix.ha⁻¹ (TBxH), tons.cane.ha⁻¹ (TCH), early Brix (EBx), and average Brix (Bx) of consecutive samplings taken before the period with frosts in the clonal stages and four times during the crop cycle in the variety trials, were calculated. A variance component analysis was also performed to weigh information from the different breeding stages for parental selection. According to these analyses parents with high EBx (> 95th percentile) in the first clonal stage, high TBxH, TCH and Bx in the second clonal stage, and those that produced varieties with good performance in the variety trials in advanced breeding stage were selected. BLUP values of the selected parents were then used to obtain a Predictor of the Crossing Value (PCV) for all potential crosses between the selected parents. Correlation between PCV and historical crossing data adjusted for series effect were significant and high. The parental selection algorithm and PCV calculations were compiled in an interactive web application using a package with R statistical analysis software [1]. By using this software application, combining historical information was straightforward.

IMPROVING SELECTION ACCURACY IN CLONAL ASSESSMENT TRIALS BY ACCOUNTING FOR SITE VARIABILITY

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Key words: variety selection trials, selection accuracy, site variability, electrical conductivity

One of the essential requirements in the field experiments of clonal selection is that clones within a block or replicate are managed under the same condition so that the differences among clones are mainly the reflections of their genetic worth. When breeders suspect that such an assumption is invalid, they often use blocking techniques to ensure a homogenous environment within a block. However, it is difficult when the environmental factors are unknown or not apparent to researchers. For example many soil properties are often unknown or are quite variable within a particular site unless an extensive survey is done, which is impractical in routine selection trials due to limited research resources.

In sugarcane breeding programs, the problem described is more evident in the early stage of selection where a large area is often required to test many clones. For example, for the clonal assessment trials (CATs), the second stage of selection in the breeding program of Sugar Research Australia, about 2,500 clones are often tested for their cane yield, sugar and fibre contents. Even with a partially replicated design or p-design currently adopted in CATs, only about 20% of test clones are replicated, more than 4.5 ha of land is required assuming a plot size of 15 m² (10 m x 1.5 m). Trials with such a large area will be difficult to meet the requirement of the homogeneity of environmental factors.

Electrical conductivity (EC) has been widely used in precision agriculture where soil variability could be identified and then managed for achieving optimal productivity. This capacity of identifying variation in the field could be useful in variety selection trials, because the differences identified by EC can be used as a method of adjustment in analysing yield data.

In two CATs, one in the Burdekin region and another in the Central region, we demonstrated that EC could be used to improve the selection accuracy. When each site was grouped into four zones based on EC measured for 0-90 cm of soil, the difference of average cane yield between high and low performing zones was 32 tonnes per hectare in the Burdekin site and 16 tonnes in the Central site. However we found no significant difference for sugar content among different zones. If such variation in cane yield was ignored in clonal evaluation, selection would be biased to high performing zones and clones planted in poor soil zones would be less likely to be selected.
DEVELOPMENT OF NIRS METHOD FOR ROUTINE ASSESSMENT OF SUGARCANE QUALITY IN REUNION ISLAND

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Keywords: NIRS, breeding assessment, calibration

In a joint project with Cirad, eRcane laboratory facilities have been equipped with a semi-automatic Near Infra-Red Spectroscopy (NIRS) device (a Bruker Matrix-F and linked CPS conveyor) dedicated to fresh cane analysis. The breeding programme at eRcane requires analysis of 8,000 sugar cane samples per year. Conventional methods to determine cane quality at eRcane consist of shredding stalk cane samples, pressing the resulting pulp in a hydraulic press and collecting plug and juice obtained. The plug is then weighed for determination of fibre content (fibre% cane) while juice is filtered for determination of brix% and pol%. Extracted Sugar (ES% cane) is computed using the data collected. Currently, the characterisation of 8,000 sugarcane varieties requires the mobilisation of five workers simultaneously, two days and a half per week, during five months. An experiment was conducted to evaluate if NIRS could be a feasible alternative for an easier and more rapid assessment of cane varieties quality.

After a period of first handling, the semi-automatic system proved to be able to handle higher work rate than the conventional method, with only 2 operators. A preliminary calibration was developed using a 200 samples dataset. 75% of the dataset was used for calibration and 25% for validation. The prediction of Brix% juice, Pol% juice and of ES% cane exhibited reliable results, since the coefficients of determination ($r^2$) of regressions between predicted and observed values exceeded 0.9 for these three traits and Ratio Performance to Deviation (RPD=Standard deviation/standard deviation of residuals) was higher than 3. The calibration has already reached interesting accuracies statistics for fibre% cane ($r^2>0.7$, RPD >2). Prediction of Juice purity (Pol% juice / Brix% juice) showed very poor accuracy so far ($r^2<0.5$, RPD<1.5). Juice purity prediction will not be sought further. Standard Error of Laboratory (SEL) was calculated and it turns out that it was really low: 0.01 for Brix% juice and Pol% juice; 0.03 for ES% cane and 0.15 for fibre% cane. Hence, Standard Error of Prediction (SEP) although rather acceptable compared to bibliography, was higher than SEL: 0.62 for Brix% juice, 0.61 for Pol% juice; 0.56 for ES% cane and 1.85 for fibre% cane. Improvement of NIRS prediction equations will be sought in order to have a completely automated tool, able to accurately predict simultaneously all quality parameters required for variety comparisons.
RELATIVE CONTRIBUTION OF GENETIC AND ENVIRONMENTAL EFFECTS ON NONSUGAR COMPOUNDS OF JUICE CANE

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Keywords: sarch, ash, phosphate, phenolic compounds, sugarcane juice.

The juice of the sugar cane is composed of sugars, water and insoluble substances known as non-sugar compounds. These last compounds are important in the factory because they are responsible for adverse effects in the quality and recovery of sugar. For example, increases in the ash and starch content may affect sucrose recovery and crystallization while low phosphate concentrations (<300 mg/kg) may cause clarification defects. On the other hand, anthocyanin and phenolic compounds affect the juice color and the settling velocity. In this work, the relative contribution of genotypic, environmental and interaction effects on the total variability of these non-sugars compound and their genetic correlations were assessed.

Special determinations associated with non-sugar components were performed in a set of 10 sugarcane genotypes evaluated in a multi-environment trials conducted in six sites of the target region of the sugarcane breeding program of Tucumán, Argentine. We analyzed starch, ash, phosphates, phenols and color of sugarcane juice. The measurements were taken during the beginning of harvest season in two consecutive years. For each variable, a mixed linear model was adjusted in order to estimate variance components associated with the effects of genotype, environment and their interaction on the total trait variability. Genetically determined correlations were assessed through biplot analysis.

For ash, starch and juice color, the main component of variance was associated with genotype main effects, with contributions of 78\%, 63\% and 48\% of non-error variability, respectively. These three variables showed negative genetic correlations with sucrose content. In the case of phosphate and phenolic compounds, environment effects were the principal source of variation. Identifying the main source of variability for each non-sugar component will facilitate the development of useful strategies for characterization of the sugarcane genotypes regarding industrial quality.
SUGARCANE FOR WATER LIMITED ENVIRONMENTS. GENETIC VARIATION IN YIELD AND PHYSIOLOGICAL CHARACTERS

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Keywords: cane yield, stomatal conductance, genotype-by-environment interaction, heritability, adaptation, genetic correlation

Water limitation is a major production constraint for sugarcane worldwide but to date there has been little investigation of patterns of genetic variation for response to water stress in sugarcane. Field experiments were conducted over three years under fully irrigated and managed water stress conditions at three locations in Northern Queensland in Australia. One hundred and thirty one clones representing commercial and introgression lines (genetically diverse) were evaluated for their yield and physiological performance. Water stress treatments reduced cane yield (tonnes cane hectare⁻¹ - TCH) and total dry matter (TDM) by 17 to 52% and 20 to 56%, respectively, compared with irrigated treatments in the same experiments. Cane yield, CCS and most of the morphological and physiological parameters studied showed substantial variation within the test population under moderate to severe water stress conditions. There was little G×E for cane and sugar yield under mild and moderate water stress conditions; however, under severe water stress, there was a significant GxE effect. This situation was rare under commercial production where nearly 50% yield reduction in cane yield was recorded. Commercial cultivars out-performed unselected clones (introgression lines) under both stressed and non-stressed conditions. Though sugar content in a few clones crashed to very low levels under severe stress conditions, in general, it was less affected than cane yield in stressed plants. Leaf and stalk elongation and leaf senescence were the most affected morphological characteristics in stressed plants. This was true even under moderate stress. Notably, stalk thickness did not change significantly across variable moisture environments. Stomatal conductance (gs) was affected by moderate to severe water stress. The G×E variation for conductance was smaller than the clone (G) variation in many occasions. High genetic correlations (γg = -0.29 - 0.94) for gs were observed across test environments in all 3 different production regions. Canopy conductance (gc) based on gs and leaf area index (LAI) showed a stronger genetic correlation than the gs with cane yield at 12 months (mature crop). The regression analysis of input weather data for the duration of measurements showed the predicted values of γg were more closely correlated with the maximum temperature (r=0.47) during the measurements than the other environmental variables. Stress index (crop water supply-demand ratio) and vapour pressure had significant effects but were lower than maximum temperature. These results confirmed that the canopy conductance has immense potential as a selection criterion for early-stage selection of clones for efficient water use and biomass production in sugarcane.
IMPROVING TRANSPERSION EFFICIENCY IN SUGARCANE

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Keywords: Transpiration efficiency, water stress, photosynthesis, sugarcane breeding, selection

Transpiration efficiency may be defined as the ratio between biomass produced per water loss through transpiration. Achieving high transpiration efficiency is important in both rainfed and irrigated crops. Developing sugarcane varieties with high transpiration efficiency is one approach to help achieve this goal. However, sugarcane breeding programs currently do not explicitly target improved water use efficiency. Some efforts have been made to select for transpiration efficiency in major crop species, but limited progress has been made generally, and it has been debatable on whether specific focus on TE in selection will lead to desired outcomes. The main problems arise because transpiration use efficiency is often negatively genetically correlated with stomatal conductance, which in turn is related with reduced growth rates.

We have conducted pot experimentals in both Australia and China to examine genetic variation in transpiration efficiency. Important genetic variation in whole plant transpiration efficiency have been found and will be presented. Measurements of leaf gas exchange using a LICOR 6400 instrument have been shown to predict whole transpiration efficiency, with a genetic correlation of -0.90±0.30 between internal leaf CO2 levels and whole plant TE observed in a diverse group of 50 genotypes when observations from a narrow range of stomatal conductance levels is considered.

The results suggest that improvement in TE may be possible in sugarcane without necessarily sacrificing yields under no or mild stress environments. Possible approaches in breeding programs to undertake practical improvement of TE and performance under water limited environments are suggested. These involve concurrent measurement of yield under relatively non stress conditions and canopy cover and temperature in early stages of selection, and appropriate weighting of these two variables in an optimal selection index. Appropriate weightings may be determined through either or both modelling and empirical estimates (obtained from trial data) of genetic correlations between measurements and yield in a range of targeted environments.
ERCANE REGIONAL SELECTION: SUCCESS IN REUNION ISLAND AND NEW DEVELOPMENTS IN CENTRAL AND WEST AFRICA

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Keywords: sugar cane breeding, selection, agro-climatic diversity, local adaptation

eRcane is a private research institute owned by the sugar industry in Reunion Island. Its core activity is sugar cane breeding. From 1929 to the late 1980’s, most of the selection stages were planted in one location, in La Bretagne station, in the north of the island. In 1984, after an audit of the sugar cane industry, considering the great diversity of cane growing conditions in Reunion Island, 5 selection stations were set up. These stations are representative of contrasting soil and climatic conditions of the island justifying selection program organised in a multilocal approach beginning at seedling stage.

The results of this new selection program based on an early multilocal approach were very promising: from a mean of one elite clone produced each year in the last years of the previous program, we went to over 10 elite clones per year. The breeding program has increased tremendously its capacity to produce high yielding varieties.

Based on these results, eRcane, which has many partnerships in Africa, proposes to accompany sugar cane estates to develop, for their production area, their own selection from fuzz. Instead of importing elite clones selected in a foreign country, eRcane’s partners could obtain varieties specifically adapted to their local conditions, from crosses made specifically for them in Reunion Island.

The first fuzz supplies were conducted in 2005, for Cameroon and Chad. In 2015, 7 selection sites, representing 10 different environments are operational in Chad, Cameroon, Republic of Congo, Senegal and Ivory Coast, three of them with 2 different target environments (irrigated and rainfall). The first results are very promising, and the first varieties from these partnerships will be released in 2015 or 2016.
ENERGY CANE SELECTION IN NORTHEAST REGION OF BRAZIL

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Keywords: Saccharum spp., breeding, biomass, renewable energy.

Brazil has long experience and expertise in producing sugar and ethanol from sugarcane. However, in recent years, breeders have turned attention to selecting genotypes, termed energy cane, with high ability in biomass yield and specially intended to produce ethanol from lignocellulosic and, also, electricity. In this work, we investigated yield potential of some genotypes in northeast region of Brazil. Several crosses between wild species of Saccharum spontaneum and S. robustum and other interspecific hybrids were performed in 'Serra do Ouro' station (Murici, Alagoas, Brazil, 09°13’S; 35°50’W) in 2013. From the first selection stage 741 clones were taken to the next stage. The next experiment was planted in February, 2014 in Caeté sugar mill (São Miguel dos Campos, Alagoas, Brasil, 09°42’S; 36°06’W). Plots comprised a single row of five plants, raised from one eye setts, spaced by 0.8m between plants and 1.9m between rows. In January 2015, selection based on growth and disease resistance was done, and number of stalks, weight of 30 stalk, soluble solids (%Brix) and fiber content were measured. 141 genotypes were selected (19.03%). Among the top ten clones for fiber, the estimates varied from 21.68% to 25.27%, with mean of 23.07%, which represents an increase of 60.88% compared with the commercial check variety used (RB92579). If one considers the top ten selected genotypes for total solids (Brix + Fiber content) represented in t.ha⁻¹, estimates varied from 57.08 to 74.56, with mean of 62.66, representing an increase of 153.03%, compared to RB92579. It can be also stressed that these same top ten genotypes for total solids, showed yields varying from 167.35 to 229.90, with a mean of 192.09, which represents an increase of 159.43% in comparison with the check. These preliminary results suggest that selection for energy cane showed promising results for the Northeast region of Brazil, characterized as tropical savanna climate (Aw), and performing better than RB92579, a well adapted genotype for this condition.

We consider in a medium-term RIDESA will be able to realize new commercial variety of energy cane, helping with the supplying the demand for renewable energy in the Brazilian energy matrix, especially for the production of second generation ethanol and electricity.
OPTIMIZATION OF SUGARCANE CROSSING AND SOWING PROCESSES IN REUNION ISLAND

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Keywords: Sugar cane breeding, flower fertility, hybridization practices, seeds and seedlings production.

In recent years, the eRcane breeding programme has had to face different challenges: (i) a need to improve fertility of crosses, (ii) the need for a higher number of seedlings to supply a new selection station in Reunion Island, and (iii) an increasing need for specific crosses for seven emerging selection schemes in Africa.

All steps of the crossing and sowing processes were analyzed and optimized from the implantation of the flowering fields to the seedlings rearing and management. Flowering fields were planted in three locations, and germination rates were compared. Flowers harvested in one location (Saint Benoît) gave a significantly higher seed production. To maximize the fecundation success, (i) pollen staining was added to refine the estimation of the level of the male fertility of panicles, (ii) the concentration of the Hawaiian solution was increased, (iii) a better attention was paid to males and female panicle development timing (by reinforcing male number in lanterns occasionally). An improvement of seed conservation was obtained by drying hybridized panicles during 48 hours instead of 24 hours. To optimize and accelerate fuzz germination, a germination facility was set up. Among three different temperatures tested, 32°C was the most efficient one. Three different germination substrates were also tested but did not show any difference in germination. The easiest preparation was chosen to be used in routine. Moreover, the glasshouse was equipped with temperature and humidity probes to control environmental conditions during crossing and seedlings rearing after germination. To improve traceability and security of the whole hybridization and sawing processes, a bar code system has been developed along with the eRcane breeding data base.

To conclude, from 2010 to 2014, the cumulative effect of all these technical optimizations and innovations allowed (i) an increase of the number of crosses with the same human resource (from 2000 to 2500 per year), (ii) an 11% decrease of unfertile crosses, and (iii) a twofold increase in the mean germination rate (from 44 to 82 seedlings per gram of fuzz), (iv) a gain of time in the realization of the campaign production of seedlings.
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GERmplasm characterisation for the high sucrose and early-ripening characteristics

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Keywords: sugarcane, high sucrose content, early ripening, sucrose accumulation pattern, evaluation, characterization

There is an increasing need to develop new types of varieties that are better suited to the challenges facing the sugarcane industries in many parts of the world. As a result of the drastic reduction in sugar price and associated needs to become more cost effective and profitable, Mauritius has embarked on the process of centralizing milling activities. Hence, an earlier start to the harvest period is being implemented, and this has prompted efforts to initiate development of genotypes with high sucrose early in the harvesting period. The objective of this study was to characterise and screen for early high-sucrose parent varieties in the available germplasm that can be utilised in crossing and development of high-sucrose commercial varieties that can be harvested early in the season. A methodology for categorizing the germplasm was developed using eight varieties tested in three contrasting environments. The criteria developed, based on pol % cane dry matter, was used to screen 199 parental varieties planted in 2010 in a replicated trial in a humid irrigated environment at Réduit with an annual normal rainfall of 1464 mm in a low humic latosol (L2). Cane quality characters for Brix, Pol % cane and fibre % cane on a fresh weight basis were assessed, with Pol % cane dry matter derived over three harvest dates (mid-May, mid-August and mid-November) during the first-ratoon crop.

The first-ratoon results showed that sucrose accumulation differed among the 199 parent varieties. Pol % cane fresh weight ranged from 8.1 to 14.8 in May and improved at a faster rate for varieties with the lowest sucrose content, such that Pol% cane fresh weight ranged from 11.7 to 20.4 in November. The trend was similar for juice purity which increased from t 69.6 - 89.5 in May to 84.3 - 95.3 in November, and for Pol % cane dry matter which improved from 29.8 - 55.4 in May to 38.6 - 61.8 in November. The wide range in Pol % cane dry matter showed that it was the best criterion for use in categorizing the 199 varieties based on their sucrose accumulation patterns at the three different harvest dates. Twenty four varieties were potentially categorized as “early ripening,” representing 12% of the population. Of these, 18 (9%) were classified as “high sucrose type”. Moreover, 16% of the total parental varieties were categorized as being “mid-season” varieties whereas 62% were identified as “late season type”. Within the mid-season parent varieties, 21 (11%) were classified as being high sucrose type whereas for the late season varieties, 85 varieties (43%) belonged to the high sucrose type. The results showed that opportunities are present to screen for early high sucrose parental varieties which can be adopted for developing high-sucrose, early-ripening commercial varieties.
IMPORTANCE OF FOREIGN SUGARCANE \textit{(Saccharum spp)} CLONES IN THE IMPROVEMENT OF SUGAR CONTENT IN CG CULTIVARS

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Keywords: introducing sugarcane germplasm, high sucrose content, breeding value.

Introducing foreign sugarcane (\textit{Saccharum spp}) clones into the Sugarcane Breeding Program at CENGICAÑA in Guatemala to broaden the genetic base of the crop has been a major effort. Because high sugar content makes sugar production more economically feasible in the Guatemalan sugar industry, it is a major breeding objective in the sugarcane breeding program. Therefore, the primary objective of importing foreign varieties into Guatemala is to increase genes for sugar content in the local breeding populations. In sugarcane, studies have suggested that sugar and juice brix content are under additive genetic control. Therefore the ability of the parents to transmit these traits can be estimated by their breeding values. To incorporate high-sugar genes from foreign varieties through traditional breeding, a wide range of new germplasm must be evaluated to determine which parents are best adapted to Guatemala. Superior parents are crossed, and the progeny are assessed. The objectives of this study were to identify superior parents and verify the breeding value for sugar content (percent Brix) and other agronomically important characteristics.

The work was conducted by CENGICAÑA’s Sugarcane Breeding and Development Program, and started with the evaluation of clones in the Sugarcane Germplasm collection. This collection mainly consists of germplasm introduced from the USDA-ARS Sugarcane Field Station in Canal Point, Florida. Five crossing campaigns were performed, and the progeny were evaluated by family selection each year. Data recorded included percent cane Brix, tonnes of cane per hectare (TCH), tonnes of sugar per hectare (TSH), reaction to major diseases, and flowering rate. The best families were identified by using a selection index. From a total of 2,265 accessions only 54 showed sugar content 10-20\% higher than the check variety CP72-2086. Of these 54 accessions, 53 were foreign clones and 45 (83\%) were clones imported from Canal Point. According to family selection experiments and to the selection index, 68 superior crosses were identified along with 102 parents that produced offspring with superior Brix, TCH, TSH, and good disease resistance. This study confirms that high sugar content and other traits from foreign parents were effective in producing high sugar content progeny; thus demonstrating the successful identification of superior parents and crosses for the Sugarcane Breeding Program at CENGICAÑA.
SELECTION FOR ELDANA SACCHARINA BORER RESISTANCE IN EARLY STAGES OF SUGARCANE BREEDING IN SOUTH AFRICA

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**Keywords:** Eldana saccharina, family selection, logistic regression

_Eldana saccharina_ (Lepidoptera: Pyralidae) is the most wide-spread and damaging stem borer of sugarcane in South Africa, causing losses estimated at R900 million per annum. The current Integrated Pest Management approach includes the cultivation of resistant varieties. Since the 1980s, the breeding strategy for resistance has included the selection of resistant parents at crossing and the screening of progeny during the final stages of genotype testing. To date there has been no early-stage selection where large variability can be expected. Family selection involves positive selection of whole populations of seedlings based on data derived from family plots. Family data have been used to estimate breeding values of parents. Logistic regression models have been proven to enhance selection gains in non-replicated plots for yield and quality.

The objectives of this study were to examine the potential of evaluating sugarcane families and parents using data collected from the seedling stage (Stage I) of sugarcane breeding programmes and to determine the potential of using logistic regression models to further enhance selection for resistance to _E. saccharina_ in non-replicated early stage (Stage II) genotype plots. Data were collected from Stage 1 trials (BML12 and FML13) at Bruyns Hill and Pongola research stations, respectively, and Stage 2 (BSL12 and SSL12) at Bruyns Hill and Glenside research stations. Stage I trials were established as 1 m row plots while Stage II were 8 m single-row plots. In Stage I, 20 stalks were randomly sampled from the first 20 genotypes in a family plot. Samples were examined for _E. saccharina_ entry and exit holes and the number of bored stalks. For Stage II, 12 stalks were randomly sampled per plot. There were significant family effects for BML12 (P=0.0029) and FML13 (P=0.0003), indicating the existence of significant differences for _E. saccharina_ damage among families. Family variance for BML12 (P=0.0144) and FML13 (P=0.0878) was significant indicating presence of variability among families. Broad sense heritability of 0.52 (BML12) and 0.51 (FML13) indicated the effectiveness of selecting elite families. The predicted gains were 19.93% (BSL12) and 68.89% (FML13), indicating the value of family selection. There were significant female effects (BML12, P=0.0017; FML13, P=0.0041) indicating the dominance of maternal effects. Male effects were non-significant (BML12, P=0.088; FML13, 0.1464). The female*male interaction effects were non-significant (BML12, P=0.1532) and significant (FML13, P=0.0442) suggesting non-additive genetic effects. Logistic regression analysis produced significant coefficients (BSL12, P<0.0001; SSL12, P=0.0232) suggesting that selecting for _E. saccharina_ was effective. Sensitivity analysis validated discriminating ability for _E. saccharina_ damage. Family selection would be effective in enhancing breeding for _E. saccharina_ resistance. Further, logistic regression models incorporating _E. saccharina_ damage in un-replicated plots would enhance selection against _E. saccharina_ damage. Adopting family evaluation and logistic regression models in early stages of sugarcane breeding will increase efficiency of breeding for _E. saccharina_ resistance in South Africa.
REVIEW OF THE UPOV TECHNICAL GUIDELINES FOR SUGARCANE

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Keywords: descriptors, PBR, harmonized

Plant Breeder’s Rights (PBR) is the exclusive commercial rights to a registered variety or cultivar. In Australia, the PBR scheme is administered by the Plant Breeders’ Rights Act 1994, which conforms to the 1991 revision of the UPOV convention. The UPOV Convention provides options and guidance (Technical Guidelines) for the methods used to examine Distinctness, Uniformity, and Stability (DUS) in a harmonized way. Australia has adopted the “breeder-based testing system” for determining DUS for sugarcane PBR. Since 1995, BSES and now SRA has been protecting the Australian sugarcane industry’s investment in breeding new sugarcane cultivars by registering new cultivars for PBR. This involves planting of a comparative trial each year to compare the new cultivar with the most similar cultivars of common knowledge. The trial is used to measure the descriptors (quantitative and qualitative) and states of expression outlined in the Technical Guidelines and this information is then submitted via an online Interactive Variety Description System set up by the PBR office. The Technical Guidelines for sugarcane were developed in consultation with breeders from Australia and Brazil and were adopted by UPOV in 2005. The guidelines consist of 45 qualitative and 9 quantitative traits, but not all of these traits are essential for submission in PBR applications. Nine of these traits are denoted with an asterisk which recognises them as important for the international harmonization of variety descriptions and should always be examined for DUS. The advantages and disadvantages of these nine descriptors will be discussed and proposed for revision.
STRATEGY FOR IMPORTING AND ASSESSING FOREIGN VARIETIES IN THE SRA BREEDING AND SELECTION PROGRAM

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Keywords: breeding, selection, variety exchange

The Australian sugar industry has greatly benefited from the importation of varieties from overseas breeding programs. Foreign varieties dominated the Queensland industry up to the late 1940’s, continuing to play a major role until the late 1980’s. Since then, foreign varieties have generally not performed well compared to SRA (formerly BSES) bred varieties and today contribute less than 1% of production.

Foreign varieties are also included in the SRA parental breeding population being an important source of desirable gene combinations. Over the last ten years, 16 of 48 varieties released had one foreign variety as a parent.

This shift in importance from commercial to breeding application of foreign varieties, together with changes in the quarantine procedure, advances in propagation techniques, and stricter exchange agreements has necessitated a re-think of the SRA strategy on using foreign varieties.

A new foreign strategy has been developed placing more emphasis on assessing foreign varieties for disease resistance and determining their breeding value from progeny performance. The advantages and practical implications of this new strategy will be discussed.
VISACANE, AN UP TO DATE CIRAD QUARANTINE TOOL FOR EXCHANGING DISEASE-FREE SUGARCANE VARIETIES IN COMPLIANCE WITH PROPERTY RIGHTS


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Keywords: sugarcane, pathogen, detection, quarantine, transfer of varieties, property rights

Sugarcane varietal improvement requires the introduction of vegetatively propagated material. The continued increase of international and intercontinental trade of plants has led to the enforcement of quarantine measures in many countries before the import of vegetatively propagated material because many plant pathogens can be carried and transmitted by them.

Visacane, the CIRAD’s sugarcane quarantine service, has been devoted to sugarcane quarantining for several decades. It covers detection and elimination of pests and pathogens and the transfer of pest and pathogen-free plant material. At the present time, Visacane detects 12 major sugarcane diseases caused by viruses and bacteria. Visacane imports and exports varieties from and to about 30 sugarcane growing countries in the world, and ensures that the material is free from any well-known important pest and disease causing agent.

Besides phytosanitary constraints, Visacane also takes into account legal constraints and ensures that plant breeders’ intellectual property rights over the transferred material are respected. All the shipments of varieties are covered by a Material Transfer Agreement which specifies the conditions for use of the varieties. Breeding centers can exchange sugarcane varieties with other breeding centers or disseminate varieties through the Visacane network in compliance with their property rights.

Because it is integrated into a pathology research unit studying various aspects of plant-pathogen interactions, and thanks to its collaborations within a network of sugarcane technologists, Visacane regularly updates its expertise and provides plant material exhibiting the best possible phytosanitary quality. The last update follows the discovery of a novel sugarcane mastrevirus using a metagenomics approach (new strategy for virus identification).
EFFECTS OF SUSTAINED GIBBERELLIC ACID 3 AND 5 APPLICATIONS ON SUGARCANE VEGETATIVE GROWTH AND FLOWERING

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Keywords: GA3, GA5, flowering

The affect of gibberellins on plant growth was first noted in rice that was infected with the fungus Gibberella fujikuroi resulting in rice plants with elongated stems, slender leaves and stunted roots (Yabuta and Sumiki 1938). There are many forms of gibberellic acid, identified in plants, fungi and bacteria, which are integral to many plant developmental processes including stem elongation and induction of flowering (Achard and Genschik 2009, Davière and Achard 2013). The main form of gibberellic acid (GA) used in research to manipulate plant growth is GA3 (MacMillan 2004), though GA1, GA4, GA5, GA6 and GA7 are also major bioactive components within the GA signalling pathway (Botha, Lakshmanan et al. 2013, Davière and Achard 2013).

Various gibberellins have been detected in sugarcane shoots, leaves and meristems (vegetative and reproductive). Gibberellic acid has been applied to sugarcane as a ripener, to improve sucrose content that may be low at the time of harvest due to unfavourable growth conditions, though without success either due to inconsistent sucrose yields or lack of meeting other criteria. While the GA signalling pathway has been linked with flowering in other plant species no direct link has been observed in sugarcane.

While investigating sugarcane flowering it was apparent that sugarcane can revert to a vegetative phase at various stages along the floral developmental pathway (Julien 1973, Glassop, Rae et al. 2014). This reversion suggested that sugarcane requires a persistent ‘activator’ or a succession of various ‘activators’ in order to produce a floral structure. We hypothesised that sustained application may also be required to achieve success with GA application in sugarcane; with previous experiments reporting significant differences in stalk height, internode (length/weight), leaves and sugar concentrations between controls and GA sugarcane but not long lasting results.

We applied GA3 and 5 to mature Q208^A sugarcane plants (3-4 visible internodes) once or twice per week for a period of 9 weeks. This report will present results on stalk length and weight, internode length and diameter, leaf sheath length and leaf length, area and weight; as well as sugar concentrations in the internodes.
BREEDING FOR MULTIPURPOSE CANES

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Keywords: fibre, multipurpose, sugarcane, breeding, diversity

Sugarcane is a source of tremendous genetic diversity. This diversity has implications for what would be the most suitable type of variety to efficiently produce a given end product.

The germplasm at the West Indies Central Sugarcane Breeding Station contains a good range of this diversity as we have noble cane, *Saccharum spontaneum*, commercial varieties and high-quality accessions, as well as clones derived from various combinations of these. As we exercise greater use of the whole cane plant for products other than sugar, attention is being paid to characteristics such as fibre that have increasing value for the production of energy from biomass. Recently, clones with genetic combinations suited to fibre production have gained importance. There is renewed interest in the outcomes when various types of high fibre clones are used to produce new populations.

This study involved testing populations that were generated by crossing clones from various sections of the diversity spectrum, to ascertain how fibre content and the fibre vs sugar content are affected. The objective was to confirm which combinations most efficiently produce a high frequency of multipurpose clones.

All genetic combinations have the potential to produce various types of clones but, as predicted, the choice of parents is highly correlated to the frequency of genuine multipurpose progeny.
PARENTAL SELECTION BASED ON HISTORICAL SERIES OF EXPERIMENTS

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Keywords: mixed models, GE interaction, RCBD, unbalanced data

Parental selection is critical to the success of the sugarcane breeding program. Several criteria can be considered, such as, genealogy, breeding values estimated by progeny test, and also per se performance evaluated along multi-environmental trials (MET). This kind of information can be easily found in databases of well-established breeding programs. The objective of this work was to infer the genetic potential of the different genotypes through MET, evaluated in advanced stages in the Sugarcane Breeding Program of Federal University of São Carlos, integrant of the Interuniversity Network for the Development of Sugarcane Industry (RIDESA). It is also possible to study the genotype by environment interaction, to predict best parents for several environmental conditions. In this work, we considered 23 experiments located in different sites in Brazil. They were installed from 1995 to 2009, in which all were installed under randomized complete block design. The number of repetitions varied from three to four and the harvest from two to five, along these experiments. Overall, 262 genotypes was considered and evaluated for tons of cane per hectare (TCH) and sugar content (SC). Statistical analysis was performed using the mixed model approach, in which we select the best matrix for genetic (G) and residual effects (R). Model selection was considered by Akaike Information Criterion (AIC). Analyses for both traits provided similar results for model selection in mixed model. The final model contained fixed and random effects. The best covariance matrix for R considered heteroscedasticity between trials and for G matrix, a kronecker product of two matrices, one considering heterogeneous compound symmetry for trails and a second one modeling harvest under autoregressive of order one.

The predicted means for genotypes considering SC varied from 13.37\% to 16.45\%, with an overall mean of 15.12\%. Also, for TCH varied from 82.7 ton.ha\textsuperscript{-1} to 123.7 ton.ha\textsuperscript{-1}, with mean of 101.1 ton.ha\textsuperscript{-1}. The correlation between these traits was -0.17, however it was possible to find 56 genotypes with showed values over the mean for both traits, simultaneously. If one considers a threshold, as the third quantile, we still able to find ten genotypes. This result is very promising because it suggests a transgressive segregation may be found in order to increase both traits. We highlight that RB867515 the most cultivated genotype in Brazil is in this group. Another result that can guide the parental selection is the interaction genotypes by harvest, specially for TCH, where some genotypes have lower yield reduction over harvest then others, which can suggest breeding can be done to extent the number of harvest.
GERMLASM REVIEW – EVIDENCE AND APPROACHES THROUGH ACP-SRP FUNDED PROJECTS TO IMPROVING GENETIC DIVERSITY IN FIJI CROSSES

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Keywords: genetic resources, varieties, nobilization, introgression.

Genetic resources are vital for efficient running of a conventional plant breeding program. In Fiji, numerous efforts had been undertaken to exploit and maintain genetic resources (Daniels et al, 1965; Brown et al, 1969; Singh et al, 2012). In 1990’s, there was an interval to this work after departure of experienced cane breeders. As a result, lack of genetic diversity was realized through results attained from a prototype trial in 2011 that was carried towards gaining experience and awaiting funds for an ACP-SRP funded project being a comparative study of family and individual mass selection methods as early selection criteria. There were 50 local crosses planted in this trial in 4 replicates, 20 seedlings per cross. It was found that there was no significant differences in %pocs, sugar and cane yield in the random samples taken from the crosses used with p values of 0.2, 0.6 and 0.3 respectively. This had prompted recommendations which resulted in importation of 52 varieties from Australia, Mauritius and Vietnam as well as 304 packets of fuzz from West Indies from 2011-2013. 42 of these varieties are already in the breeding plots and have been used in the sugarcane crosses in the last 2 years whereas 10 varieties have been planted for flowering this year. All of the West Indies fuzz were sown in 2012 and 2013 and the germinated seedlings have been utilized in the above mentioned ACP-SRP project which are ongoing whereas excess seedlings will undergo routine selection for inclusion in the breeding plots. Rigorous nobilization work has also been initiated from 2009 using Erianthus arundinaceus under another ACP-SRP funded project being Nobilization of Erianthus spp. and a total of 320 crosses have been made to date. From these crosses, 270 crosses have been sown however no authentic hybrid have been produced and the program is still being pursued. From the above findings, it is realized that it is essential to customarily introduce foreign varieties in the gene pool and also to run an introgression program in parallel with current program whereby authentic hybrids could be introduced consistently into the breeding program.
IMPLEMENTATION OF A SUGARCANE (Saccharum spp.) VARIETY TRANSFER MANAGEMENT MODEL AND DETERMINATION OF ADOPTION RATES OF VARIETIES FOR THE 2014-15 HARVEST PERIOD IN THE PANTELÓN-CONCEPCIÓN CORPORATION, GUATAMALA.

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Keywords: sugarcane variety transfer management model, adoption, sugarcane Guatemala

The Sugarcane Breeding and Development Program of CENGICAÑA is the entity responsible for the creation and development of new sugarcane varieties adapted to the local Guatamalan environments. The objective of the program is to increase sugar, economic gains and crop sustainability. Genetic variability is obtained through crossing and the importation of clones. Clones are selected in five stages, and new varieties are promoted through specific CENGICAÑA publications. To date the program has released several varieties for commercial use. However, a widespread problem of slow adoption is a critical issue that adversely affects economic benefits and may reduce the useful life of a released variety. The sugarcane variety transfer management model-SCVTMM is proposed as the strategy to accelerate the transfer of information for each of the varieties to the decision makers at mills associated with CENGICAÑA. The objectives of this study are a) describe the sugarcane variety transfer management model-SCVTMM and b) show the results of SCVTMM implementation expressed as adoption rates of recommended varieties using the 2014-15 harvest of the Pantaleón-Concepción corporation as a model. Adoption rates will be determined analyzing production data containing the variety recommended in the SCVTMM and the planted variety on farms. Adoption rates will be expressed in performance indicators (relationship between the recommended and the planted variety in a lot) and the percentage of planted area with the recommended variety in the model.
COMPARISON OF MATURITY CURVES OF SUGARCANE GENOTYPES

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Keywords: pol% cane, maturity curve parameters, mixed non-linear models.

The maturity curve of a sugarcane variety is given by the temporal evolution of the sucrose content during the harvest season. The initial and final Pol%cane levels and the rate of sucrose accumulation at different times during the maturity process are important features to compare genotypes. However, these parameters are affected by environmental variations. Mixed Nonlinear Models (MNLM) provides an adequate framework to model growth curves including random effects to take into account for special sources of variability. In this work we propose first to fit piecewise regressions to model sucrose accumulation of sugarcane varieties with an environmental random effect to account for the effect of different environments of a breeding program’s target region. Second, to obtain the BLUPs of the curve parameters for each genotype as an indicator of the variety performance. We worked with Pol%cane values from nine sugarcane varieties recorded at 3 to 11 times of the harvest period (May to October) in different years and sites of Tucumán, Argentina. Therefore, data analysis was performed in two stages. First, a two-pieces non linear regression model was adjusted for each variety with parameters associated to the Pol%cane initial level, the rate of sucrose accumulation in the initial and final phase of the harvest period, and the threshold time where the curve slope changes. A random effect of environment (year and site combination) was considered to take into account for environmental variability. A predicted genotypic population curve was obtained for each genotype. In a second stage, the same piecewise regression model was adjusted on the genotypic predicted curves of Pol%cane and a random effect of genotype was included on each model parameters to account for genetic variability. Predictors of random genotype effects (BLUPs) for each maturity curve parameter were used to compare maturity profiles among varieties. According to these BLUPs, two varieties showed relatively high maturity values during the beginning of harvest, and others two were those with positive effects for the slope at the initial phase of the harvest period (high speed of sucrose accumulation), while another variety was the best regarding sucrose at the final of the maturity period. The proposed analytic approach was useful to compare maturity profile characteristics among sugarcane varieties.
DROUGHT TOLERANCE OF SUGARCANE CROSSES AND PARENTS

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Keywords sugarcane, drought tolerance; cross, parent

In order to understand the impact of drought on sugarcane, and screen crosses and parents with good drought tolerance, three groups’ family trails were carried out under natural drought condition. Correlation, heritability and BLUP analysis was used to investigate the relationship in different traits. It was found that there were significant differences in green leaves number after drought and rewatering among crosses, and this trait was significantly correlated with most of the agronomic traits. It is easy and simple to evaluate drought tolerance of sugarcane by selecting the total number of green leaves after drought and rewatering treatment. Twenty crosses and 14 parents with good drought tolerance were selected by MCMCglm analysis based on the drought tolerance related data of crosses and parents.
THE SRA CROSSING PROGRAM: THE IMPORTANCE OF PHOTOPERIOD CROSSING DURING YEARS OF POOR FIELD FLOWERING

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Keywords: arrowing, cross pollination, photoperiod

Effective selection of parents and specific cross-combinations is fundamental to achieving genetic gain in any sugarcane breeding program. SRA has been producing new sugarcane varieties through cross pollination at Meringa, near Gordonvale, since 1926. Between 1,200 and 1,500 cross-combinations are produced on average each year through field crossing (arrows collected from parent blocks planted in-field). However, field arrowing at Meringa can be very sparse and unreliable when field conditions during floral initiation in February-March are not ideal because of moisture and/or temperature stress during this time inhibiting the floral initiation process. This often results in poor field flowering with only an average of 30-35% of parents flowering naturally each year. This has been the case in most recent years where Meringa has experienced one or both types of environmental stresses: in 2011 Meringa had a major cyclone (Cyclone Yasi) plus record rains during the growing season leading up to the cyclone (2010); in other recent years Meringa has experienced below average rainfalls in addition to very high daytime temperatures (up to 38-40 degrees Celsius) for several consecutive days during the initiation period. This has resulted in fewer flowers, and hence fewer crosses made in recent years: 2010 = 740 crosses; 2011 = 345 crosses (second smallest crossing year on record); 2012 = 705 crosses; 2013 = 455 crosses; 2014 = 879 crosses.

Poor field flowering at Meringa means that SRA Sugarcane Breeders can only take an opportunistic approach to cross-pollination, and only make the best specific cross-combinations given the few parents available each day, regardless of what your breeding strategy might be. For this reason, SRA and the Australian sugar industry has invested in three large photoperiod facilities in Meringa, constructed in 1986, 1998 and 2008 respectively. The benefits of photoperiod crossing include our ability to control the initiation process and environmental in which the parents are grown (such as day length, nutrients, moisture and temperature), resulting in as many as 90% of our parents consistently flowering in the photoperiod facilities (compared with an average of 30-35% flowering in the field). In addition to improving the level of successful flowering in parents, we have the ability to impose different photoperiod treatments to parents, depending on their natural time of flowering, to synchronise early and late-flowering parents. This means we can undertake a more directed or strategic approach to crossing (rather than opportunistic in the field) and increase the number of possible cross-combinations we can make, many of which are not possible in the field, to explore different genetic possibilities.

Crossing strategies conducted specifically in the SRA photoperiod facilities, and how they complement field crossing strategies, will be discussed.
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CAN CYTOGENETIC AND PCR MARKERS ASSIST SELECTION OF HIGH VALUE ERIANTHUS-DERIVED SUGARCANE CLONES?

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Keywords: Introgression, cytogenetic molecular marker Erianthus hybrids

Intergeneric hybrids between Saccharum and Erianthus are the newest exotic addition to the Australian sugarcane breeding program. The wild species Erianthus arundinaceus, a close relative of sugarcane could be an important candidate to enlarge the gene pool of the Australian parent populations. E. arundinaceus is reported to contain numerous traits of agronomic importance including pest and disease resistance and tolerance to drought and water-logging conditions. Based on genomic in situ hybridisation (GISH) results on the Erianthus introgression clones, we have developed a molecular method to allow selection of hybrids at an early stage that have incorporated sought-after traits. So far, five generations of fertile hybrids from intergeneric crosses between S. officinarum, E. arundinaceus and modern sugarcane cultivars have been produced. The chromosome composition of F1, BC1, BC2, BC3 and BC4 hybrids was studied by GISH and revealed a reduction of Erianthus chromosomes as well as a loss of chromosomes from the BC1 generation. We also revealed the formation of recombinant chromosomes between both genera. Individuals with low number of Erianthus chromosomes or recombined chromosomes could be an important addition to the breeding program if these chromosomes are inherited stably during crossing. The stability of recombined chromosomes is currently under investigation by studying the transmission of these recombined chromosomes in further crosses. Nevertheless, as the number of Erianthus chromosomes in the BC3 (and BC4) are lower or equivalent to the basic number of E. arundinaceus (x=10), we are aiming to develop a simple method based on PCR molecular markers which will allow the identification of individual Erianthus chromosomes in the backcross hybrids. If important traits, such as nematode and/or pachymetra resistance can be linked to Erianthus specific chromosomes, then this method could become a valuable tool for sugarcane breeders as an effective selection screening method.
OPTIMISING FLOW CYTOMETRY FOR THE CHARACTERISATION OF SUGARCANE INTROGRESSION CROSSES

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Commercial sugarcane varieties are a complex interspecific cross between S. officinarum and S. spontaneum. However, the use of very few clones in the initial hybridisation of these parental genotypes, resulted in the narrowing of the sugarcane genetic base. Introgression breeding is being used worldwide to increase the genetic diversity in plant breeding programmes and utilises cytogenetics as a tool. Introgression breeding is a novel approach at SASRI which has two main objectives: (1) to widen the genetic base of germplasm and (2) to introduce novel traits. Flow cytometry is a technique for studying cytogenetics in plants and animals that has become popular for hybrid verification and ploidy screening, but little work has been done on plants, especially sugarcane. There is therefore a need to optimise this technique for studying sugarcane cytogenetics. The study objectives were to optimise flow cytometry for estimating chromosome numbers and nuclear DNA content in sugarcane, to infer ploidy levels from chromosome numbers and predict progeny chromosome numbers from parental data. Six sugarcane varieties were chosen for optimisation, two wild species, and four commercial varieties. Three areas of optimisation were selected: firstly sample source (leaves and roots), secondly nuclear counts, and lastly flow rates. Saccharum interspecific hybrids (F1) and back-crossed progeny (BC1, BC2) were selected for characterisation. Plant tissue was mechanically homogenized in buffer solution and stained with a fluorescent dye (DAPI). DNA content was calculated from peak fluorescent ratios using maize as the internal standard. Chromosome numbers were determined from the Partec PA flow cytometer. The data were analysed using ANOVA to determine differences in 2C-values among genotypes, and regression analysis to predict chromosome numbers. There were highly significant differences in the DNA content among genotypes, indicating accuracy of measurements after calibration. Tissue source, flow rates and nuclear counts did not significantly affect DNA content. Regression analysis indicated a significant association between chromosome numbers and DNA content ($r^2 = 96.3$) indicating that DNA content can be used to predict chromosome numbers. This study showed that chromosome number of progeny can be predicted from their parents. Future studies will enable breeders to understand the relationship between genomic size and phenotypic traits.
CHARACTERIZATION OF CHROMOSOME INHERITANCE OF THE INTERGENERIC PROGENY BETWEEN *Saccharum* spp. and *Erianthus arundinaceus*

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**Keywords:** *Saccharum* spp.; *Erianthus arundinaceus*; chromosome translocation; inheritance

*Erianthus arundinaceus* is one of the most closely related genera to *Saccharum* and has many desirable agronomic traits for sugarcane improvement. To investigate the introgression of the *E. arundinaceus* genome into sugarcane and to uncover the chromosome inheritance of the intergeneric progeny between *Saccharum* spp. and *E. arundinaceus*, intergeneric progeny between *Saccharum* spp. and *E. arundinaceus* were studied using the genomic in situ hybridization (GISH) technique. The main results were as follows:

1. Five clones of *S. officinarum × E. arundinaceus* showed a total of 68 to 69 chromosomes, of which 40 were derived from *Saccharum officinarum* (2n = 80) and 28 to 29 were derived from *E. arundinaceus* (2n = 60). The result revealed that all F₁ clones were product of a n + n transmission.

2. In the 13 BC₁ progeny, the total numbers of chromosomes were 120 to 130, of which 22 to 35 were derived from *E. arundinaceus*. Due to their female parent contained *E. arundinaceus* chromosome numbers were 28 to 29. A complex chromosome inheritance existed in the intergeneric progeny between *Saccharum* spp. and *E. arundinaceus*. The results revealed that nine BC₁ clones were product of 2n + n transmission and the other four resulted from more than 2n + n transmission.

3. The BC₂, BC₃ and BC₄ resulted from n + n chromosome transmission, the number of *E. arundinaceus* chromosomes was halved along with backcross generations. However, a special phenomenon of chromosome inheritance existed in BC₃ progeny YCE05-164. The *E. arundinaceus* chromosome number in YCE05-164 was more than YCE03-16 as the male parent.

4. Chromosome translocation occurred in 8 of 37 progeny of *Saccharum* spp. and *E. arundinaceus*. Among them, there were 2 translocation lines in BC₁, 2 translocation lines in BC₂, and 4 translocation lines in BC₃. According to the pedigree, the translocated chromosomes could be transmitted to their progeny. For instance, YCE01-92 was the male parent of YCE03-378 and YCE03-168 was the female parent of YCE06-111.
TOWARD A REFERENCE SEQUENCE OF THE SUGARCANE GENOME

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Keywords: Sugarcane, genome, sequencing, reference, genes

The sugarcane genome poses challenges that have not been addressed in any prior genome sequencing project. The main difficulties reside in the high polyploidy (2n ~ 12x ~ 120), and the high level of heterozygosity of cultivars which make an assembly of the genome very challenging through classical whole genome shotgun sequencing approaches.

We develop an approach based on previous studies that demonstrated that sugarcane hom(e)ologous chromosomes share a very high level of micro-colinearity among themselves and show good micro-colinearity with sorghum. We used the Whole Genome Profiling (WGP™) technology of Keygene to analyze a set of 20,736 BACs from cultivar R570 and map them on the sorghum genome. An average of 37.2 sequence tags per BAC was generated that allowed anchoring 11,732 of the analyzed R570 BACs on the sorghum genome. A core set of 5000 BAC representing the minimum number of BAC to best cover the gene rich part of the sorghum genome was selected. This set of 5000 BAC is currently being sequenced through international collaborations. The aim is to obtain a high quality sequence for each BAC, which mean an assembly in one or very few contigs. So far, half of the 5000 BAC have been sequenced. A sugarcane web portal is currently being developed together with friendly tools to make BAC sequences and gene annotations available through an exploitable form to the sugarcane community.

These 5000 BAC sequences will correspond to the gene rich part of the sugarcane genome and will represent a very important resource for genetic, structural and functional genomic studies in sugarcane. This high quality frame will be essential to build a whole genome sugarcane sequence when improved sequencing and assembling methods are available.

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THE AUSTRALIAN CONTRIBUTION TO THE R570 SUGARCANE GENOME SEQUENCE

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Key words: Next generation sequencing, mosaic monoploid genome

An international consortium of researchers has commenced work on the generation of the sugarcane genome sequence. Known as SUGESI (Sugarcane Genome Sequencing Initiative), the consortium includes researchers from Australia, Brazil, France, South Africa who are working together to produce a reference sequence of the sugarcane cultivar R570. We present here the Australian contribution to the R570 genome sequence. A BAC by BAC sequencing approach is being used to generate a mosaic monoploid genome. We present data on sequence generated from over 1000 R570 BAC clones, these were selected using a number of approaches and were in part targeted to important QTL identified in Australian germplasm. In a parallel approach, we have also generated large amounts of whole genome shotgun (WGS) sequence from variety R570 and used advanced computational methods to assemble this sequence into a number of scaffolds. This WGS data was generated from a range of DNA fragment sizes between 180 bp and 32,000 bp, which should enable even complex regions of the genome to be ordered. In addition to this PacBio long read technology has been used to generate 31.7 Gbp of data with an average read length of 7282 bp. This has been used to help resolve repeats and increase scaffold lengths. In total the data covers the complete genome sequence to a level of 73-fold, representing roughly 1000-fold monoploid genome coverage, which highlights sequence variation at each locus. We present here the analysis of the multiple alleles of genes that are present in the polyploid genome of sugarcane. Analysis of this data has identified large numbers of single nucleotide polymorphisms (SNPs), which are currently being tested for association with desirable traits amongst a population of plant lines. A defined genome sequence will be used by many researchers to identify the basis of traits and to capitalise on knowledge of traits from related crops such as sorghum. Previous work has identified quantitative trait loci (QTL) for traits such as biomass in sugarcane. Bioinformatic tools can now identify the underlying gene sequences from the sugarcane genome sequence. In a similar way, the sugarcane homologues of genes that are known to enhance productivity in other species can now be identified. In addition to revealing underlying biological mechanisms, these genes will be valuable as targets for selection or genetic modification to enhance variety development.
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FLUORESCENCE IN SITU HYBRIDIZATION IN SUGARCANE OR FISH-ING IN THE GENOMIC WILDERNESS

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Keywords: Cytogenetics, FISH BAC sugarcane

Cytogenetics applied to sugarcane has brought our fundamental understanding of the sugarcane genome to a new level. In the mid-nineties, Genomic in situ Hybridisation (GISH) was first applied to sugarcane to determine the specific composition of the modern cultivar R570. GISH revealed the chromosomal composition of R570 was 80% Saccharum officinarum, 10% S. spontaneum and 10% of recombined chromosomes. The Australian counterpart Q165, revealed a slightly different species composition as 75%, 15% and 10%, respectively. Both R570 and Q165 genetic maps have portrayed a partial coverage of linkage groups (LG) despite the large number of molecular markers invested in the maps. It also shows that S. spontaneum chromosomes seem to have a better vertical coverage than S. officinarum chromosomes as the S. spontaneum genome is more polymorphic. To gain a better understanding of the genome composition in terms of LG number per homology group (HG) and species attribution of the LG, we applied BAC-FISH to sugarcane. Bacterial Artificial Chromosomes (BAC) consist of large chromosome segments (around 100kb). BAC from the Sorghum or Saccharum genomes were used as anchorage points on the sugarcane cultivars to identify homologous/homeologous chromosomes for each HG. We will present some examples of results of BAC-FISH applied to several cultivars for at least 4 different HG. The determination and comparison of the number of chromosomes per HG to the number of LG from the genetic maps will determine the saturation level of the genetic maps. This will help us to obtain critical knowledge of the horizontal chromosome distribution for a particular cultivar and compare its structure to another cultivar. Eventually we will have a better understanding of the distribution of the chromosomes during crossing and this will help breeders to make more informed and targeted choices in their selection programs.
GENERATION OF A 345K SUGARCANE SNP CHIP

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**Key words:** Sugarcane SNP chip, Axiom

DNA markers can enhance rates of genetic gain in breeding programs and are currently being applied in many animal and crop species. However, application of DNA markers in sugarcane remains very challenging and still lags behind other species. In particular, the very large sugarcane genome means that many current “best bet” markers associated with agronomic traits of interest are probably not in very close linkage with underlying causal genes. This will limit gains from future applications of markers in sugarcane breeding. Single nucleotide polymorphism (SNPs) are now the molecular marker of choice in animal breeding programs and important crops because massive numbers per genotype can be accurately screened for, and a proportion of these markers are usually directly within the genes causing genetic variation in traits of interest. The development of SNPs markers in sugarcane can overcome the current limitations as large numbers throughout the genome can be easily screened across many genotypes. To generate a SNP chip for sugarcane with large enough numbers of polymorphic single dose markers that would be useful in the Australian and Brazilian breeding programs 16 lines were selected based on their contribution to these breeding programs. A reduced representation method (RRS) was used to generate sequencing libraries and two samples per lane were run on an Illumina Hi-seq to generate an expected coverage of at least 50x of a given genomic region. Sequences were aligned to a de novo reference RRS contig assembly generated previously. SNPs were selected using categories designed to maximise low dose (single/double) and polymorphic SNPs across all the sixteen lines. To determine genome-wide distribution, the selected SNPs were aligned to the sorghum genome and distribution was as expected across all chromosomes and with a bias away from centromeric regions. Analysis was also carried out to determine that no bias had been introduced in the selection of low dosage SNPs based on origin of clones. Further, Affymetrix Axiom technology was identified as the most appropriate technology to use for sugarcane SNP screening. SNPs were finally selected from the low dose categories according to the Affymetrix design protocol. Due to the difficulty in identifying low dose SNP markers, a twostep approach was taken with the first chip containing 345K SNPs. Then a subset of 70K high quality informative markers will be selected for the production of a smaller SNP chip that will be more cost effective. We will present preliminary data for the screening of an association mapping population of 480 individuals across the 345K SNP chip.
MICROSATELLITE MARKERS – CAN WE AGREE ON AN INTERNATIONAL SET FOR SUGARCANE VARIETY IDENTIFICATION?

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Keywords: Quality assurance, genotyping, microsatellite, SSR

For more than a decade, SRA has undertaken a variety audit trail to provide a quality-assurance system for delivery of new varieties to the industry. An important component of this is to DNA fingerprint new varieties at critical stages of the selection program on the path to variety release. In mid-2005, SRA (formerly BSES), in collaboration with the Australian Genome Research Facility Ltd (AGRF), initiated a system to incorporate microsatellite fingerprinting techniques into the variety audit trail and quality assurance pipeline. A set of six highly-selected microsatellite primers was provided to the AGRF and a web-based searchable index of allele sizes of sugarcane cultivars was developed. The database is routinely accessed with approximately 1,000 assays per year. The success of this work in resolving and preventing potentially costly field identification errors has led to the incorporation of the test as a routine step in the SRA Variety Audit system.

Microsatellites (also named SSR) appear to be the marker of choice internationally for sugarcane variety identification. However most sugarcane institutes or research teams have selected different sets of SSRs for this purpose. An attempt was previously made to identify an agreed set of SSR for sugarcane identification at an international level. However, this proved difficult for several reasons including difficulties and differences in the systems used to reveal the markers (silver staining, radioisotopes, autoradiographs), challenges associated with allele calling, and mislabelling of varieties. With the advent of new platforms for revealing SSR markers, and advanced software for identifying alleles these issues may now be easier to resolve. Such a marker system would have great benefits for the international sugarcane community and would overcome many of the problems we face with regard to the unequivocal identification of sugarcane cultivars. It would also be valuable, given the amount of sugarcane germplasm that is exchanged internationally each year.
TRANSCRIPTOMIC STUDIES TO IDENTIFY GENES RELATED TO DROUGHT AND FLOODING TOLERANCE IN SUGARCANE

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**Keywords:** drought stress, flooding stress, gene expression, complex networks, support vector machine analysis.

Irrigation and drainage are an integral part of sugarcane agriculture in Colombia. This is due, in part, to the unfavorable rainfall pattern in the Cauca river valley region, where most of sugarcane in Colombia is grown, and the local harvesting system, which is designed for an all-year round milling season. It is estimated that, of the 230,000 ha of sugarcane grown in the region, approximately 223,000 (97.2\%) are grown under irrigation, while almost 116,000 (50.5\%) have drainage infrastructure.

While irrigation and drainage favor yield it also increases the cost of production, and thus the development of flooding and drought tolerant sugarcane hybrids have become an objective for the Colombian industry. Previous works on the characterization of Cenicaña’s germplasm collection led to the identification of genotypes that displayed little or no yield decrease (TCH and TSH) under the stress conditions of interest. With this information, a comparative transcriptomics study began as an attempt to identify genes contributing to the traits of interest.

As part of the gene expression studies, RNA-Seq libraries (a total of 72) from leaf and root tissues, from tolerant and susceptible genotypes, were constructed and sequenced. The data was initially analyzed to identify differentially expressed genes (DEGs) finding 2,170 exclusively in the plants subjected to drought, 1,376 exclusively in the plants subjected to flooding and 2,374 shared between both experiments. Because of the high number of DEGs identified, and given that environmental effects non-associated with the condition of interest (drought of flooding stress in this case) often introduce noise when selecting candidate genes, in this investigation the DEGs were also analyzed to establish relationships where expression levels were not prevalent. As a result co-expression networks where created and machine learning algorithms, such as support vectormachines (SVMs), where implemented. Also, because it was important to identify the genes activated in metabolic pathways modulating process of carbon assimilation, photosynthesis and sucrose synthesis, all the genes were annotated and mapped to the Kyoto Encyclopedia of Genes (KEGG). Finally, in order to consolidate the most important results of each analysis, a selection system, currently involving 19 categories, was generated and is currently being used to select the genes for functional characterization. The development of this project is a valuable opportunity to test the use of complex networks and machine learning techniques for gene selection in agricultural studies, which have not been explored enough within this context.
INTEGRATING TRANSCRIPTOMICS AND EXPRESSION ANALYSIS: THE SASRI APPROACH

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Keywords: Transcriptome, pipeline, assembly, annotation

After having fully committed to be part of the sugarcane genome sequencing project, SASRI is now embarking on using Next Generation Sequencing technologies to progress its understanding of the genetics of sugarcane. Our current drive is to use transcriptomics to help understand some of the molecular mechanisms associated with specific biological challenges to sugarcane. We are therefore setting up a modular transcriptomics analysis pipeline that will initially integrate all publically available data from relevant plant species (NCBI). The two main components of the pipeline, viz. assembly and annotation, will draw mainly on Saccharum, but will also utilize Sorghum and Miscanthus data (genomes and transcriptomes) to act as references to aid assembly. In addition, Arabidopsis, rice and wheat genomic data from a local EnsEMBL mirror will be utilized for transcriptome annotation. Data that will be generated from our own Saccharum hybrids will be mapped to our reference transcriptome (Unigene clusters). Mitochondrial and chloroplastic sequences will be binned by using locally built reference plastid genomes and gene sets. Ultimately, this pipeline will allow for differential analysis of any type of transcriptomics data sets for the discovery of novel sugarcane transcripts, as well as for generating SNP markers that, in conjunction with genomic data, could eventually be linked to eQTLs. This pipeline will yield the largest integrated sugarcane transcript dataset generated to date.
MO11

GENOME-WIDE ASSOCIATION STUDIES (GWAS) IN SUGARCANE CONTEXT: EXPERIENCE FROM A CASE STUDY AND QUESTIONS ABOUT WAYS OF OPTIMIZATION OF GWAS

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Keywords: association mapping, SCYLV, population structure, kinship, candidate genes

A Genome-Wide Association Study (GWAS) was undertaken to prospect for sources of resistance to Sugarcane yellow leaf virus (SCYLV) transmitted by aphid vectors. To this end, a panel of 189 sugarcane cultivars representative of the breeding germplasm was fingerprinted with 3,949 DArT and AFLP markers and was phenotyped for SCYLV infection in leaves and stalks under natural disease pressure prevalent in Guadeloupe in two repeated trials in two crop cycles. Mixed linear models including co-factors representing population structure fixed effects and pairwise family random relatedness effects provided an efficient control of the risk of inflated type-I error at a genome-wide level. Six independent markers were significantly detected in association with SCYLV resistance phenotype. Among them, two DArT markers were detected repeatedly across the GWAS exercises based on the different disease resistance parameters. These two markers could be blasted on Sorghum bicolor genome and candidate genes potentially involved in plant-aphid or plant-virus interactions were localized in the vicinity of sorghum homologs of sugarcane markers. The low frequency of all markers in the panel (8-20%) combined with a high virus incidence mean reflects (1) the absence of selection in breeding programs due to the recent spread of the disease and (2) a probable scarcity of sources of resistance available in modern sugarcane germplasm. All markers explained individually between 9 and 14% of the disease variation of the cultivar panel. The cumulative effects on disease resistance variation of the six detected markers were estimated with stepwise multiple regression models. Depending on trials and resistance parameters considered, between three and five markers were captured in multiple regressions. They explained a maximum of one third of disease resistance variation in the panel. Development of efficient marker-assisted breeding applications will depend on the ability to detect robust associations more or less numerous in GWAS experiments. Our work represents a case study illustrating questions we should paid attention for when designing GWAS experiments to optimize statistical power of association tests. Our presentation will review several considerations relative to statistical power including size and structure of mapping population, efforts invested in phenotyping, methodology of association tests, presumable architecture of target traits and marker technology so far available in sugarcane context.
MO12

IMPROVING SUGARCANE WITH GENOMIC SELECTION

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Key words: sugarcane improvement, selection accuracy, genomic selection, DArT

Molecular markers have become an obvious tool in both animal and plant genetic improvement programs, but the implementation of markers in commercial breeding programs has been slow and even rarer in sugarcane. However, rapid development of new methods in all areas of molecular marker research is now seeing the commencement of application of molecular techniques. For example, in animal breeding programs, 25% genetic bulls in New Zealand to 70% in Netherlands were from genomic selection. In sugarcane, simulation studies in Australia suggested a potentially valuable role for markers in parental improvement, and testing of this has commenced in Australia. Rates of parental improvement using traditional breeding approaches have been apparently slow for several decades because of low narrow sense heritabilities and long generation intervals. Marker data may both improve prediction of breeding value and reduce generation intervals.

Since 2012, we have started to investigate the effectiveness of genomic selection. So far, it has produced some promising results. Selection accuracy is defined as the correlation between predicted genetic values and observed genetic values. Selection accuracy of around 0.5 for cane yield and 0.4 for sugar content has been observed with current data sets. This was based on the same population for the single marker association mapping studies we reported previously; there were only about 480 clones in the population and each clone was genotyped with 15,360 continuous DArT markers and 1,531 discrete DArT markers. These accuracies were encouraging in that even higher prediction accuracies should be attainable with larger training populations and marker numbers. Based on published results with other species and our concurrent studies on the different proportion of training versus test populations, we may expect accuracies >0.6 from larger training populations and a larger number of markers. A chip with 50K SNPs is currently under development that should improve the accuracy further. This level of accuracy would be expected to greatly increase generation-wise gain in sugarcane breeding compared with that achieved in recent decades. All of these developments could provide sugarcane breeders exciting and more importantly realistic opportunities to improve genetic gains.
QTL MAPPING OF SUGAR CANE ENHANCED BY HIGH THROUGHPUT RADSEQ GENOTYPING

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Keywords: RADseq, QTL mapping, yellow spot disease, early ripening

Abstract
Restriction site associated DNA sequencing (RADseq) was applied to two sugar cane mapping populations derived from the crosses M 134/75 x R 570 (144 individuals) and CP 67412 x M 245/76 (105 individuals) in order to identify markers linked to yellow spot disease resistance and to early ripening/high sucrose QTL(s) respectively. Each genotype was represented by an average of 3 million read of 100nt. Sequence data were processed using the software STACKS, aligned to *Sorghum bicolor* reference genome using Novoalign and SNP calling was carried out using Samtools.

The SNP marker distribution varied between 5354 to 12157 across the four mapping parents. Markers with more than 15% missing data were excluded and only markers segregating as single dose were used to construct linkage maps of the four mapping parents using Joinmap 4.1. A further 800 available gel-based markers (AFLP and SSR) were combined to 2500 RADSeq derived SNP markers of variety M 134/75 to construct its linkage map. The four linkage maps constructed for CP 67412 (3293 markers), M 245/76 (3066 markers), M 134/75 (2426 markers) and R 570 (1768 markers) were of size 8103 cM, 6606 cM, 12580 cM and 1768 cM respectively, with an average marker distance of 2.15 to 5.1 cM. In most cases, the sugar cane linkage groups (LGs) were found to consist of markers of similar sorghum chromosomal origin, thus confirming the collinearity between the two species. Based on sorghum chromosome 1-10 marker origin, LGs were assembled into homology groups HG1-HG10. Manual alignment of sugar cane LGs to their corresponding sorghum chromosome across each HG showed that the *Saccharum* hybrid genomes consist of up to 10 copies of homeologous chromosomes.

QTL analysis for yellow spot disease resistance identified a major QTL on LG28 of variety M 134/75 which was flanked by EST-SSR markers SAT2033 and SAT2036 and which explained 30% of the phenotypic variation. On the same LG, two additional QTLs were located near a RADseq marker CHR_5-13658719-G which explained 14.9 of the phenotypic variation and an SSR marker CIR12284 which explained 13% of the phenotypic variation. Several minor QTLs were also detected explaining less than 5% of the phenotypic variation. Through the application of RADseq, the challenge faced in the construction of dense sugar cane linkage maps has been considerably reduced. In addition to the lowered cost and high throughput, the sequence associated information of the markers has proved extremely useful for comparative mapping studies.
MO14

GENOME WIDE ASSOCIATION MAPPING OF AGRO-MORPHOLOGICAL AND DISEASE RESISTANCE TRAITS IN SUGARCANE

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Key words: GWAS; Saccharum spp.; population structure; family relatedness.

The objectives of the study were to assess genome wide association study (GWAS) for sugarcane on a panel of 183 accessions and to evaluate the impact of population structure and family relatedness on QTL detection. The panel was genotyped with 3327 AFLP, DArT and SSR markers and phenotyped for 13 traits related to agro-morphology, sugar yield, bagasse content and disease resistances. Marker-trait associations were detected using i) general linear models (GLM) that took population structure into account with either a Q matrix from STRUCTURE software or principal components (PC) from a principal component analysis added as covariates, and ii) mixed linear models (MLM) that took into account both population structure and family relatedness estimated using a similarity matrix K* computed using Jaccard’s coefficient. With GLM analysis, test statistics were inflated in most cases, while MLM analysis allowed the inflation of test statistics to be controlled in most cases. When only detections in which both population structure and family relatedness were correctly controlled were considered, only 11 markers were significantly associated with three out of the 13. Among these 11 markers, six were linked to the major resistance gene Bru1, which has already been identified. Our results confirm that the use of GWAS is feasible for sugarcane in spite of its complex polyploid genome but also underline the need to take into account family relatedness and not only population structure. The small number of significant associations detected suggests that a larger population and/or denser genotyping are required to increase the statistical power of association detection.

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MO15

GENOME-WIDE ASSOCIATION MAPPING OF QUANTITATIVE TRAITS IN A BREEDING POPULATION OF SUGARCANE

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Keywords: Biomass; Linkage Disequilibrium; Population Structure; Quantitative Trait Loci; *Saccharum sp*.; Sugar.

The aim of this study was to establish an appropriate genome-wide association (GWA) analysis to detect molecular markers associated with high biomass and sugar yield in order to be able to improve productivity in sugarcane. Clones of a local breeding population were evaluated for cane yield (CY) and sugar content (SC) at two contrasting locations during three successive crop cycles and genotyped with molecular markers (DArT and TRAP). GWA mapping was applied within a mixed-model framework considering the possible population structure by using Principal Component Analysis to minimize spurious associations. Sequences from sugarcane DArT markers significantly associated with the traits were used to determine their similarity and position on sorghum genome. Markers significantly associated with CY and SC in plant-cane, ratoon-1 and ratoon-2, respectively, were detected. The high gene microlinearity between sorghum and sugarcane was confirmed since sequences of some sugarcane DArT markers showed high similitude and e-value with coding sequences of *Sorghum bicolor*. Interestingly, sequences of DArT marker associated with trait of interest were aligned in sorghum chromosomal regions where QTLs for plant height, tiller number, stem diameter and stem biomass yield, have been previously reported. The methodology presented in this work that combines existing phenotypic trial data and genotypic marker characterizations within a Linkage Disequilibrium (LD) approach including population structure as covariates, has proven to be highly efficient to find molecular markers significantly associated with the measured traits. Therefore, GWA mapping could be a valuable tool in order to assist sugarcane improvement to help supply future demand that has been projected for the upcoming decades.
GENETIC DIVERSITY AND ASSOCIATION MAPPING FOR BRIX IN BRAZILIAN PANEL OF SUGARCANE GENOTYPES

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Keywords: breeding, microsatellites, germplasm, population structure.

Sugarcane breeding programs have focused their efforts to release new superior varieties and this process has been very costly for breeders in terms of its long period, which usually exceeds 12 years. Molecular markers may be an important strategy in reducing that time and its costs involved and identification of marker–trait associations is the first step towards marker-assisted selection in plant breeding. This study evaluated a collection of 258 sugarcane genotypes (mainly 160 cultivated and 98 ancestral genotypes) bred in worldwide countries (Brazil, USA, Argentina and Southwestern Asia). This collection was named the Brazilian Panel of Sugarcane Genotypes (BPSG). These clones of BPSG do not form any predesigned mapping population. These genotypes have played an important role in the sugarcane genetic background, and the panel represents the genetic history of sugarcane improvement programs in Brazil. These clones of BPSG were planted and maintained at Centre for Agricultural Sciences from Araras – Sao Paulo in the Federal University of São Carlos (UFSCAR/RIDESA). The field experiment was carried out in a RCB design, with four replicates. The clones were evaluated in terms of stalk diameter (SD in mm), stalk height (SH in m), stalk number (SN), stalk weight (SW in kg), soluble solid content (BRIX), sucrose content of cane (POL%), sucrose content of juice (POL%), purity (PUR), fiber (FIB), cane yield (tons of cane per hectare, TCH), sucrose yield (tons of POL per hectare, TPH) and resistance to brown rust. The subset of 134 genotypes PBGCA was evaluated for 100 microsatellites. The program R was used to calculate PIC (polymorphic information content) and PD (discrimination power). Population Structure was investigated using the program STRUCTURE. The number of subgroups (K) was set from 1 to 10 based on models characterized by no admixture, ploidy level 1 and independent allele frequencies. Association between markers and brix was performed using a mixed linear model (MLM), including population structure and kinship information (model Q+K), implemented in the software TASSEL v.3.0. The significant marker-trait associations were declared by P≤0.01 and the magnitude of the QTL effects were evaluated by r²-marker. One hundred SSR markers generated a total of 1483 markers with PIC value ranged from 0.5 to 0.96 and PD value ranged from 0.62 to 1. The model-based analysis using SSR markers, identified an underlying population structure comprising four (DK=4) sub-populations. A total of 36 markers was significantly associated with brix of these 34 markers had a positive effect on the trait. Two markers had negative effect on brix and showed homology with an enzyme responsible for a greater accumulation in the vegetal cell wall. Others two markers showed high positive effect on brix, and these markers have been classified with high homology for proteins involved in the sugar accumulation of the Zea mays and Sorghum. However, this study may be the first step for implementation of marker-assisted selection in sugarcane breeding.
IDENTIFYING MARKERS FOR RESISTANCE TO ORANGE RUST (*Puccinia kuehnii*) VIA SELECTIVE GENOTYPING AND CAPTURE SEQUENCING

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Key words: association mapping, capture sequencing, orange rust, selective genotyping

Sugarcane orange rust, caused by the fungus *Puccinia kuehnii*, is a serious disease of sugarcane in many parts of the world. The most effective strategy to combat the disease is to develop resistant sugarcane cultivars. Phenotypic screening for resistance to orange rust is a laborious and time-consuming process, and breeders would greatly benefit from molecular markers linked to resistance. The objective of this research was to identify, via association mapping, markers linked to resistance to orange rust. From the germplasm available in the breeding nursery at Canal Point, 726 genotypes were screened for orange rust resistance via artificial inoculation of field-grown plants. A frequency distribution was generated from the disease reaction scores, and the most susceptible and resistant genotypes, comprising the lower and upper 5 percent of scores, respectively, were chosen for marker analysis. This technique, known as selective genotyping, can be an effective method to identify markers of interest, and is less expensive than genotyping the entire population for a large number of markers. These individuals were sequenced via capture sequencing using approximately 32,500 sugarcane probes anchored to the sorghum genome. A total of 148,603 SNPs were initially identified in the dataset. Filtering to eliminate SNPs with excessive missing data and/or low minor allele frequencies reduced this number to 46,946. In addition, five individuals were eliminated due to excessive identity by state (IBS). Initial exploration of the filtered dataset, including correction for population structure, suggests the presence of SNP markers associated with orange rust on chromosome 5 in particular, but also chromosomes 1, 3, and 6. Those SNPs most likely to be associated with orange rust will be evaluated in the full mapping population. This evaluation will test whether the SNPs identified via selective genotyping are indeed associated with orange rust, and provide estimates on the effect of each SNP on the trait.
EXPERIMENTAL ASSESSMENT OF THE ACCURACY OF GENOMIC SELECTION IN SUGARCANE

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Key words: Saccharum spp., genomic selection, morphological traits, technological traits, disease resistance, statistical models.

Sugarcane cultivars are interspecific hybrids with an aneuploid, highly heterozygous polyploid genome. The complexity of the sugarcane genome is the main obstacle to the use of marker assisted selection in sugarcane breeding. Given the promising results of recent studies of plant genomic selection, we explored the feasibility of genomic selection in this complex polyploid crop. Genetic values were predicted in two independent panels, each composed of 167 accessions representing sugarcane genetic diversity worldwide. Accessions were genotyped with 1499 DArT markers. One panel was phenotyped in Reunion Island and the other in Guadeloupe. Ten traits concerning sugar and bagasse contents, digestibility and composition of the bagasse, plant morphology and disease resistance were used. We used four statistical predictive models: bayesian LASSO, ridge regression, reproducing kernel Hilbert space and partial least square regression. The accuracy of the predictions was assessed through the correlation between observed and predicted genetic values by cross-validation within each panel and between the two panels. We observed equivalent accuracy among the four predictive models for a given trait, and marked differences were observed among traits. Depending on the trait concerned, within-panel cross validation yielded median correlations ranging from 0.29 to 0.62 in the Reunion Island panel and from 0.11 to 0.5 in the Guadeloupe panel. Cross validation between panels yielded correlations ranging from 0.13 for smut resistance to 0.55 for brix. This level of correlations is promising for future implementations. Our results provide the first validation of genomic selection in sugarcane.

**MATE: A PROMISING GENE FOR ALUMINUM TOLERANCE IN SUGARCANE**

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**Keywords**: Model plant, Acid soil; Aluminum tolerance; *Setaria viridis*

Over 50% of world’s potentially arable soils are acidic. Aluminum toxicity (Al³⁺) is the main consequence of soil acidity. Al toxicity is an important limit to agricultural productivity worldwide. In low pH, aluminum is converted to soluble and toxic form of Al³⁺ that stops root growth and affects the plant development. One region in Brazil that presents such problems is the Cerrado biome which is considered the main region for sugarcane expansion area. The Cerrado is a savannah-like biome with climate classified as Aw type (Koppén-Geiger), with long period of drought. The soil is predominantly latosol, acid with low fertility. Plants utilize physiological mechanisms for Al³⁺ tolerance, and the main mechanisms is the exudation of organic acids through transporters located in the cell membrane of root tips. A Multidrug and Toxic Compound Extrusion Family (MATE) gene isolated from *Sorghum bicolor* codifies a membrane transporter responsible for citrate efflux, activated in Al³⁺ presence. Due to the complexity of sugarcane genome, the time to obtain transgenic events and low transformation efficiency, *Setaria viridis* is being used as a model plant for concept-proof. *S. viridis* is phylogenetically related to sugarcane, it is small, has a short life cycle and established genetic transformation protocol. In this study, *S. viridis* plants overexpressing *MATE* isolated from *Brachypodium distachyon* (BdMATE) were generated. Thirty events were confirmed by PCR and the gene expression was analyzed by RT-qPCR using BdMATE specific primers. The results showed that relative abundance of BdMATE transcripts ranged from 150 to 2043-fold in comparison with non-transgenic plants. Physiological analysis of relative root growth was conducted in two homozygous transgenic lines, submitted to 20 µM Al³⁺ (free activity). The similarity matrix between the root growth rate and gene expression is presented by the Pearson product-moment correlation coefficient (r) value was $r = 0.98$ in the second day. The result suggests a positive correlation between the two variants one molecular and other phenotypic. The results showed that the BdMATE increased aluminum tolerance in *S. viridis*. Altogether, the results indicate that the use of *MATE* gene could offer an alternative solution for sugarcane cultivation in the Cerrado.

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KNOWLEDGE TO SUPPORT RISK ASSESSMENT FOR DEPLOYMENT OF GM SUGARCANE VARIETIES

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Keywords: Biosafety, environmental risk assessment, fuzz, Saccharum, seed germination

GM sugarcane is currently being developed by a number of Australian and international research organisations and companies. Before GM varieties can be grown in Australia, they will need approval from several regulatory authorities, depending on the nature of the modification. Scientific evidence will be used by The Office of the Gene Technology Regulator (OGTR) and Food Standards Australia and New Zealand (FSANZ) to assess risks to the environment and the safety of foods derived from GM varieties respectively.

As part of the assessment, GM varieties will be compared to the baseline knowledge of conventional sugarcane varieties to determine whether they are more likely to spread and establish outside cultivation. However there is limited baseline data describing the sexual reproduction of sugarcane. Sugarcane is a vegetatively propagated crop and consequently the production of seed and their fate in the environment has not been studied until recently. We have been studying each stage of sugarcane reproductive biology from flowering to seedling establishment to facilitate assessment of biosafety for future GM sugarcane.

This presentation will summarise the new findings on physiology of sugarcane seed and seedlings and their ability to overcome the constraints to survival in and around sugarcane fields. We conducted a series of field-based experiments where we quantified the proportion of sugarcane seed in a natural soil seed bank and studied seed persistence in an artificial soil seed bank. Sugarcane represented a very small proportion of the soil seed bank in the area where sugarcane fertility is the highest. The seeds were short-lived (<1 year), with an exponential decline of viability, resulting in 50% loss of viability within one month. No seed dormancy was found and environmental conditions at the time of seed production were not always a limiting factor for their germination (Pierre et al. 2014). Whilst some fertile seeds are produced in regions where they could germinate, their relatively short longevity and lack of competitive advantage means they have a low weediness potential.

In assessment of food safety, a key approach is comparison of the nutritional composition with the range of compositions found amongst conventional varieties. The aim of our study was to produce a comprehensive set of composition data for conventional sugarcane varieties, using varieties that are currently grown in Australia. The samples included five commercial varieties grown in three different locations and harvested at different crop ages. Ranges of concentrations for the proximates (moisture, crude fibre, protein, fat, ash and N-free extractives), and forage values were obtained. The results indicated that the variation in nutritional composition due to environmental conditions or developmental stage was greater than the variation between cultivars. These results will provide a baseline for comparison of GM varieties during food safety assessment.
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AN ANALYSIS OF ShSUT1 IN TRANSGENIC SUGARCANE PLANTS: CHANGES TO ALLELE EXPRESSION PROFILES AS A RESULT OF RNAi.

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Keywords: allele, polyploidy, RNAi, sucrose transporter, sucrose accumulation

One of the key aims of sugarcane researchers is to increase the amount of sucrose stored within the plant. Consequently the role of sucrose transporters has been investigated to determine their role in sucrose accumulation and to test whether they can be manipulated to increase sucrose concentrations.

Firstly identified in microarray analysis of three sugarcane cultivars, ShSUT1 demonstrated a greater than twofold increase in expression in mature over immature internodes (Casu, Grof et al. 2003). ShSUT1 was cloned and yeast complementation/Xenopus oocytes experiments showed specificity to sucrose (Rae, Grof et al. 2005, Reinders, Sivitz et al. 2006). Detailed expression analysis confirmed high levels of expression in mature leaves, less in young leaves and no expression in roots (Rae, Perroux et al. 2005). Expression of ShSUT1 in the stalk showed no expression in immature internodes 1-3, a peak of expression in internodes 5-6 which then decreased with increasing internode maturity (10-11 and 22-23) (Rae, Perroux et al. 2005). With strong expression in the mature leaves and internodes 5-6 it was purported that ShSUT1 may be involved in sucrose transition from tissue that exports sucrose, mature leaves, to storage tissue within the internodes. In-situ hybridisation results localised ShSUT1 expression in mature leaves to the vascular parenchyma and inner bundle sheath; and within internode 5 to sclerenchymatous cells that surround vascular bundles; these regions were also identified with ShSUT1 antibody immunolabelling (Rae, Perroux et al. 2005). These results further suggested roles for ShSUT1 in efflux of sucrose from the symplasm to the apoplasma and the supply of sugars to cells that are undergoing rapid wall thickening.

To confirm the role of ShSUT1 in sucrose mobilisation/storage in sugarcane, RNAi technology was employed to produce transgenic sugarcane with reduced expression of ShSUT1. Five transgenic plants were produced with reduced expression ranging from 2-10 fold less than control plants. These plants were assessed for various phenotypes and displayed a range of altered sugar measurements, fibre, moisture content and ShSUT1 expression. No single phenotype was consistent between all five transgenic lines. ShSUT1 allele expression profiles in the transgenic plants were investigated to determine whether differential suppression of alleles could explain the observed phenotypes. Six expressed ShSUT1 alleles have been detected over a region of 400bp containing 7 SNP’s. MiSeq analysis of the ShSUT1 alleles within the transgenic lines and controls show a variation in allele expression; which may explain the range of phenotypes. Detailed results on the transgenic phenotypes and the ShSUT1 alleles will be presented.
CROP IMPROVEMENT BY METHYLOME REMODELLING OF SACCHARUM SPP

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Keywords: methylation, breeding, crop improvement

This study aims to explore the utility of demethylation as a mechanism for increasing the variability present in germplasm collections. An essential component of any agricultural industry is the development of new and improved varieties. The rate at which new varieties can be developed is directly proportional to the amount of variation which is present in the parental material available to the plant breeder. The Plant Breeder's Equation is R = h²S where R is the response to selection, h is the heritability of the trait and S is the selection differential. The heritability of a trait is biologically fixed, which means that to improve the rate of crop improvement, i.e. the response to selection R, then S is the variable that has to be increased. S is itself directly related to the amount of variation present in a population and the size of the population. Increasing the size of the population is often not possible, leaving altering the variation available to the breeder as the best mechanism to improve the efficiency of the breeding programme.

Methylation is the addition of a methyl group, primarily to the cytosine of DNA. The addition of methyl groups can repress gene expression and thus alter phenotype. Changes in methylation and gene silencing have been shown to result from polyploidization; and treatment with demethylating agents can restore gene expression for silenced genes. Furthermore these change in methylation status are heritable.

It is our hypothesis that allelic variants of agronomically important traits may have been silenced, as a result of high copy number, in modern sugarcane cultivars; and that a demethylation treatment may allow for reactivation of these silenced allelic variants and alter gene expression and thereby alter the phenotype. This question is being approached from two angles: the first is by treating true seeds with demethylating agents and seeing if it can alter the population statistics for sugar and fibre of the resulting populations. The other is to demethylate individual varieties by treatment in tissue culture. This will allow us to see if changes in methylation can directly alter the value of agronomic traits, particularly sugar or fibre.

To date we have treated seed from 17 crosses with two demethylation agents 5-azacytidine and 5-aza-2'-deoxycytidine which we will analyse using Spectracane NIR spectrophotometer to access variability in sugar and fibre between treated and untreated populations of plants from the crosses. We have also begun to develop a protocol to produce demethylated varieties through tissue culture, these will be used to assess the effect of demethylation in reference to known varieties.
MO23

EXPRESSİON OF GLUTAMİNE SYNTHETASE İN ASSİMLİATİON OF AMMONİUM AND NİTRATE İN SUGARCANE

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Nitrogen is an essential part of many important molecules for plants. In the production area of sugarcane in Colombia there are regions where waterlogged conditions prevailing during part of the year that can create anaerobic conditions that decrease the concentration of nitrogen in nitrate form and increase it in its ammonium form. These conditions are conducive to toxicity by high concentration of ammonium (NH₄⁺) resulting in reductions of biomass and symptoms such as chlorosis of leaves, growth suppression among others. Nitrogen assimilation involves several biochemical reactions; one of the key enzymes in this process is the glutamine synthetase (GS), which assimilates ammonium (NH₄⁺) into amino acids. There are two isozymes of GS, cytosolic or type 1 and plastid or type 2. Specific primers were designed to evaluate the expression of both GS gene by real-time PCR technique. Three varieties of sugarcane were placed in a hydroponic culture, with two different nutrient solutions: solution 1 = 85%NO₃⁻/15%NH₄⁺ and solution 2 = 15%NO₃⁻/85%NH₄⁺. Plants were grown for 35 days in solution 1 and then, half of the plants were placed in solution 2, the other half remained in solution 1. Samples of leaf and root were taken at different times after the change of solutions (0, 4, 24 and 72 hours). To analyze relative expression GAPDH was used as reference gene and we evaluated two sugarcane genes scGS1.a and scGS2 as target genes. We found that both genes were differentially expressed depending on the variety, time and tissue, reflecting that the gene expression depends greatly on the tissue; therefore, it was decided to perform statistical analysis for each tissue separately. The interactions were established, the confidence intervals for the mean relative expression were created and finally the significant differences were determined. It was found that the GS1 at 4 hours had higher expression in roots, while GS2 was activated at 24 hours. In the leaf, GS1 gene showed significant differences between the tolerant variety (CC 93-4181) and susceptible (CC 01-678) at 72 hours (p = 0.05) and 4 hours (p = 0.0231), observed that in the susceptible variety GS1 was activated at 4 hours but decreased over time, whereas in the tolerant variety, GS1 was expressed gradually increasing with time (72 hours). In the root, the GS1 showed a peak at 4 hours, with a tendency to decrease over time. The GS2 was differentially expressed at 72 hours in leaf of CC 01-678, which was expressed 0.76 times more than at 0 hours. In contrast, in the leaf of CC 93-4181, GS2 was activated at 24 hours. GS2 was activated at 24 hours in the root of CC 84-10 and CC 93-4181, showed significant differences with CC 01-678, which had a slight increase at 72 hours. In general, expression patterns of GS1 and GS2 confirm that sugarcane plants capture nitrogen in the roots, assimilating both nitrate and ammonium efficiently.
MO24

MOLECULAR CHARACTERIZATION AND FIELD EVALUATION OF GLYPHOSATE TOLERANT SUGARCANE: A STEP TOWARDS COMMERCIAL RELEASE

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Keywords: Saccharum hybrid, glyphosate, CP4 epsps gene, herbicide tolerance.

Sugarcane commercial variety RA 87-3 was transformed with a genetic construct harbouring the epsps gene from Agrobacterium strain CP4 conferring tolerance to glyphosate and nptII gene for kanamycin-selection. Herbicide-tolerance of transformed lines was evaluated at different concentrations of glyphosate in the greenhouse. All herbicide-tolerant (HT) lines were field tested to confirm glyphosate-tolerance and perform preliminary evaluations of phenotypic resemblance to parental cultivar. All field-tested transformed lines maintained herbicide-tolerance but many showed phenotypic changes and/or growth aberrations. Ten HT-lines, showing close growth resemblance to RA 87-3, were analyzed using nine compulsory morphologic markers proposed by the International Union for the Protection of New Varieties of Plants (UPOV) and 339 molecular markers. Out of the ten HT-lines tested, six showed minor morphologic and genetic variations and were selected for field-testing over two vegetative crop cycles (plant-cane and first ratoon) at two production areas in Argentina. The six field-tested HT-lines were found to be almost indistinguishable when comparing agronomic and industrial characteristics, antinutrients content (dhurrin) and chemical composition. Stable heritance of the CP4 epsps gene and glyphosate-tolerance throughout different clonal generations were confirmed by RT-qPCR and Southern blot. Flanking sequences to inserts in the genome of a candidate line were determined by using Targeted Sequence Enrichment and Next Generation sequencing (NGS) technologies. Taking into account all results, one out of the six lines tested was selected for a possible commercial release. Our study confirms the utility of genetic transformation as a complementary tool to classical breeding procedures and highlights the usefulness of UPOV traits together with molecular markers for early selections of transgenic events that closely resemble their parental genotype.
8th MOLECULAR BIOLOGY

POSTER ABSTRACTS MOLECULAR (MP)
DNA PROFILING OF PARRY CULTIVARS USING SUGARCANE SPECIFIC STMS PRIMERS

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Keywords: fingerprinting, STMS primers, identification, introgression

Sugarcane is an important commercial crop cultivated across the world for food and energy. Marker assisted selection is one technique which can accelerate and improve the efficiency of sugarcane breeding. The construction of molecular fingerprints will support the protection of plant breeder’s rights and identification of genotypes to be used as parents in molecular breeding program. We have made an initial attempt in moving towards molecular breeding to hasten our ongoing program apart from using for identification of our varieties.

Our ongoing breeding program has resulted in development of commercial cultivars which are high yielding, high sugar and resistant to prevailing diseases. We do have a good germplasm (around 2300) is diverse with different species and general and we do have an ongoing introgression program also. In the present study (supported by Sugarcane Breeding Institute) we studied nine cultivars viz., PI 08-0391, PI 08-0021, PI 14-001, PI 14-002, PI 14-003, PI 14-004, PI 14-005, PI 14-007 and PI 00-1110 were analysed with fifteen sugarcane specific STMS primers for clone identification in sugarcane. DNA was PCR amplified and products were resolved on 8% non-denaturing polyacrylamide gels and silver stained to derive their specific fingerprints. The cultivar PI 08-0391 has amplified a unique band with the STMS primer NKS-2 and also another primer NKS-48 amplified a different banding pattern for the clone PI 08-0021. The parry entries PI 04-007 and PI 00-1110 shares a similar molecular profile with the primer NKS-57.

In this study most of the STMS primer pairs produce complex banding patterns with all the nine cultivars. It is estimated that using more primers will bring out better characterization of sugarcane clones for establishing their genetic relationship and clone identification. Though we have a set of morphological descriptors for all the nine sugarcane genotypes, we are also looking at combination of morphological, biochemical and molecular methods for selection of the parents in our breeding programme where we plan to diversify and introgress by using hybrids developed through our inter specific and inter generic crosses. These molecular profiles will support in identifying these cultivars without any ambiguity. We would be interested to find out QTL markers for sugar and fiber so that we can select for multipurpose varieties for energy purpose.
COMPARISON OF SSR AND SNP GENETIC DIVERSITY ANALYSES IN A POPULATION OF 220 SUGARCANE GENOTYPES


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Keywords: SNP, RADSeq, SSR, genetic diversity, GBS.

A detailed knowledge about the variability available in germplasm collections is essential for breeding programs. In sugarcane, most of genetic diversity studies have been developed using classical DNA markers such as microsatellites (SSR), AFLP or RFLP. Single nucleotide polymorphism (SNP) markers occur at a much higher rate in the genome compared to DNA markers and thus offer the possibility to have a closer look into the diversity present in sugarcane germplasm collections.

In this work a group of 220 genotypes, 130 representing the phenotypic diversity of the Cenicaña’s germplasm collection and 90 of high value for the breeding program, were selected and analyzed by SSR and SNP markers. In the case of microsatellites, three primer pairs were used (CV 29, CV 37 and CV 38), yielding a total of 67 bands. In the case of the SNP markers, 32,567 variants derived from NGS-RADSeq data were used. Genetic distances for both sets of data were calculated using the Nei coefficient and phylogenetic trees were constructed using the Neighbor-Joining method. Results obtained from SSR data revealed 19 clusters with an average of 12 genotypes per cluster, while those derived from SNP markers showed 9 clusters, with an average of 24 genotypes per cluster. Both markers were able to differentiate genotypes from *S. officinarum*, *S. spontaneum* and *Erianthus*, separating them into different clusters. Similarity indices generated from SNP showed less variation (SD: 0.07, range: 0.41-0.97) than those calculated from SSR data (SD: 0.15, range: 0.01-1.00). Importantly, in one specific case the SSR markers showed two different genotypes (EPC 47493 and EPC 47650) with a genetic similarity index of one (1.0), erroneously indicating that these two genotypes were identical. These same genotypes showed a similarity coefficient of 0.87 when calculated from SNP data.

Overall, our results confirm the utility and robustness of SNP markers, which were more accurate in representing the genetic diversity of the genotypes under study. Finally, as part of this study SNP markers derived from the GBS methodology have been produced for the same 220 genotypes. These data are still under analysis with the main objective of comparing the utility of both sequencing platforms, RAD and GBS, for genetic studies in a complex species such as sugarcane.
WATER DEFICIT INDUCED EXPRESSION OF AREB GENE IN SUGARCANE

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Keywords: Transcription factor, Stress-inducible promoter, Drought tolerance

Drought is the most important environmental factor that affects the sugarcane production. In some cases, yield losses caused by drought bring down nearly 50% of total crop production. In recent years, the knowledge in the molecular and biochemical mode of action of drought-responsive transcription factors reveals that the overexpression of these proteins may be an alternative strategy to improve transgenic plant drought tolerance. In this context, some of the most promising transcription factors for drought tolerance are the abscisic acid-responsive element binding proteins (AREBs). Transgenic plants of rice and soybean overexpressing one AREB gene (here code-named as TFA1) also showed increased drought tolerance. In the present study, embryogenic calli of sugarcane were transformed by biolistic with a vector containing the TFA1 gene under control of the maize stress-inducible promoter (MSIP) and the herbicide resistance gene (bar) as selective marker. Three transgenic plants were obtained, showing a transformation frequency of 0.43% (number of PCR positive plants/total number of inoculated callus x 100). Water deficit stress trials are being conducted under greenhouse conditions. Phenotypic, physiological and detailed molecular analyses will be presented.

Financial support: Embrapa; Capes.
METABOLITE CHANGES IN TRANSGENIC SUGARCANE EXPRESSING DROUGHT TOLERANCE RELATED GENE


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Keywords: HPLC, Gas chromatography, Mass spectrometry, GABA, Tricarboxylic acid

Transgenic plants of sugarcane with drought tolerance related gene (here code-named as TF2A) driven by a maize stress-inducible promoter (MSIP) showed water deficit tolerance under greenhouse conditions. RT-qPCR analysis revealed a basal activity of the MSIP promoter in the transgenic plants during the whole plant cycle under well-watered conditions. In order to evaluate the metabolic changes in different tissues, 8-month-old transgenic and non-transgenic plants grown under well-watered regime in the greenhouse were harvested. Samples of leaf (+1, +2 and +3) and internodes I1-I6, I7-I12 and I13-I18 (top, middle and bottom portion, respectively) were collected and cut into approximately 1 cm pieces. All samples were frozen in liquid nitrogen as quickly as possible. Primary metabolite profiling by GC-MS could not show a clear pattern of changes in sugars or sugar alcohols between the transgenic and non-transgenic tissues. However, sucrose level increased in immature internodes (I1-I6). Considering organic and amino acids, a decreasing trend in some key metabolites, such as those of the TCA cycle and related pathways was observed. Such a trend was more evident on leaves and immature internodes, where metabolism is more active. Together, these results indicate a slight decrease on the metabolism of immature internodes of the transgenic, resulting in accumulation of carbon as sucrose in those tissues. Another point to be stressed is the changes in the content of GABA, which is closely related to drought tolerance. Interestingly, GABA levels decreased in immature internodes in the transgenic and increased in the mature ones, which suggests that its role on drought tolerance is more pronounced on the older parts of the plant. More detailed studies on metabolic changes in transgenic plants under well-watered and water deficit conditions are in progress.

Financial support: Embrapa; Capes
IDENTIFICATION AND ANALYSIS OF FLANKING SEQUENCES FROM TRANSGENIC SUGARCANE WITH MULTIPLE INSERTS BY USE OF TARGETED SEQUENCE CAPTURE AND NGS TECHNOLOGIES

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Keywords: Sugarcane, targeted sequence capture, NGS, flanking sequences

A prerequisite for commercial release of a transgenic crop in Argentina and other countries in the world is the identification and sequence analysis of the transgene(s) insertion site in the plant genome. When analyzing a glyphosate-tolerant transgenic sugarcane variety RA 87-3 pre-selected for commercial release, by Southern blot assays a high number of transgenes inserts (nine) was found in the transgenic line. Using an approach of targeted sequence enrichment coupled with next generation sequencing (NGS) technology we were able to identify and subsequently analyze all flanking sequences to these multiple transgene insertions in the sugarcane genome. First, a library of genomic regions containing foreign DNA was isolated from the complex sugarcane genome using the Targeted Sequence Enrichment system by NimbleGen (Roche). The aforementioned library was thereafter sequenced using the Roche 454 GS FLX Titanium XL+ platform. Finally bioinformatics analyses were carried out to assess the putative flanking sequences to the corresponding insert. Out of 29 candidate contigs, 18 were confirmed as junction sequences by means of PCR reactions and Sanger sequencing. The outlined methodology using targeted sequence enrichment coupled with NGS and bioinformatic analyses, represents a novel and highly efficient strategy for the characterization of flanking sequences corresponding to inserts of a transgene in a large and complex genome such as sugarcane.
OVEREXPRESSION OF *AtDREB2A CA* GENE IN SUGARCANE

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Keywords: Drought tolerance, Transcription factor, constitutive promoter

The production and use of ethanol from sugarcane in Brazil is a global model for bioenergy production, distribution, and use, and is recognized as one of the most efficient in the world. Drought is among the greatest limits to productivity and geographic distribution of crops. Due to drought stress, yield could be reduced by 50% or more. The DREB genes are well studied transcription factors that regulate the expression of stress-related genes. The DREB proteins interact with *cis*-acting dehydration-responsive element/C-repeat (DRE/CRT) present in the promoter region of many functional genes related to drought. In the present study, immature leaf segments of 6–8-month-old plants of sugarcane variety RB855156. Transverse segments 2–3 mm wide were excised above the apical meristem and placed on solid MS medium supplemented with 3 mg L⁻¹ of 2,4-D. Embryogenic calli were selected and cultured at 3-week intervals on the same medium prior to bombardment. We gene generated four independent events expressing *AtDREB2A CA* under the control of a constitutive promoter (*ZmUbi*) via biolistic method showing a transformation frequency of 0.5% (number of PCR positive plants/total number of inoculated callus x 100). The overexpression of *AtDREB2A CA* in sugarcane led to the up-regulation of known stress-related genes. Water deficit stress trials are being conducted under greenhouse conditions. Phenotypic, physiological and detailed molecular analyses will be presented. Currently, three out four generated transgenic sugarcane events are being multiplied in order to establish field trials.

Financial support: Embrapa; Capes.
SUGARCANE DIVERSITY AND EXOME SEQUENCE VARIATION

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The World Collection of Sugarcane and Related Grasses (WCSRG) presumably contains genes controlling important agronomic traits. However, the WCSRG has not been fully exploited by breeders mainly due to its lack of characterization. To optimize the utilization of the sugarcane genetic resource, a thorough phenotypical and genotypical evaluation of the WCSRG was carried out. A wide range of phenotypic variance and a total gene diversity of 0.304 in a range of 0 to 0.5 were revealed. The WCSRG was grouped into three clusters with all S. spontaneum in one cluster, S. officinarum and S. hybrids in the second cluster, and mostly non-Saccharum spp. in the third cluster. A core collection of 300 accessions was identified which captures most of the genetic diversity in the WCSRG. To catalogue the sequence variation, we implemented hybridization-based target enrichment approach to capture the exome regions of 12 phenotypically diverse accessions in the WCSRG. The chromosome number of the 12 accessions representing nine different species ranged from 48 to 115. In total 55,946 120-mer probes targeting 35% sorghum transcripts and spanning 6.7 Mb target sequence regions were designed from gene sequences of sugarcane and sorghum to capture the 12 samples’ exome regions. The captured regions were deep sequenced generating approximately 411 million reads with 83% of the cleaned reads mapped to the sorghum genome which represent nearly 53 times coverage of the sugarcane exome sequences. Approximately 115 thousands of SNPs (1/5,723 bases) were called and majority (96.7%) of them were synonymous and existed in the non-coding regions of the exome. These SNPs were dispersed across the entire genome in consistence with the distribution of probes. Fifty-seven SNPs were randomly selected for validation by Sanger sequencing and 89% of SNPs were confirmed. The high-density SNPs provide a valuable resource for defining genotype variation across the genome, and serve as a great tool for traits mapping and marker assisted breeding.