BREAKING BREEDING AND BIOTECHNOLOGY PARADIGMS - TOWARDS A COMPLEMENTARY APPROACH IN SUGAR CANE RESEARCH: A SUMMARY OF THE 10th ISSCT BREEDING AND GERMPLASM AND 7th MOLECULAR BIOLOGY WORKSHOPS

By

Raul O. Castillo, Asha Dookun-Saumtally, Angelique. D'Hont, Deng Haihua, Anna Hale, Hermann P. Hoffman, Phil Jackson, Erik Mirkov, Candido C. Mota, Guilherme Rossi-Machado, Monalisa Sampaio and Glaucia M. Souza

Introduction

The 10th Sugarcane Breeding and Germplasm and 7th Molecular Biology Workshops have been the biggest attended workshops so far. It was held from 15 to 20 May 2011 in the city of Maceio, state of Alagoas, Brazil and hosted by the Brazilian Society of Sugarcane Technologists, STAB – – East Section. The local organising Committee consisted of Drs Guilherme Rossi Machado Jr, Monalisa Sampaio Carneiro, Cândido Carnaúba Mota and Hermann Paulo Hoffman. The workshops were attended by 148 delegates from 17 different countries (Table 1). The biggest delegation was represented by the Brazilian scientists from all over the country and institutions. The theme of the workshop was "Breaking breeding and biotechnology paradigms - towards a complementary approach in sugar cane research." Perhaps the theme and the joint organization of breeding and biotechnology attracted breeders and molecular biologists to participate in a friendly environment with a high scientific level, merging applications of biotechnology tools, such as molecular markers, to support traditional breeding, or identify genes of interest and perform genetic transformation for specific traits. On Wednesday a visit to the research station of CanaVialis-Monsanto was organised where practical demonstrations complemented the high scientific component of the Workshop.

Table 1 Country of origin and number of attendees of the 10th ISSCT Breeding and Germplasm and 7th Molecular Biology Workshops.

Country	Number	Country	Number
Argentina	12	Germany	1
Australia	8	Guatemala	6
Barbados	2	India	2
Brazil	82	Japan	2
China	5	Mauritius	2
Colombia	2	Reunion	1
Ecuador	2	South Africa	3
Fiji Islands	1	USA	12
France	5	-	-
TOTAL			148

Due to the high number of abstracts received and that could not be accommodated for oral presentation, 35 summaries (17 for Germplasm and Breeding and 18 for Molecular Biology) were presented as posters. For the first time, a flash poster presentation was introduced at this workshop where participants were given the opportunity to show 4-5 slides on their posters. This was well appreciated and will be repeated in future workshops. There were four keynote presentations, two panel discussions, two poster sessions and the business meeting for both sections. The two panel discussions were held on the ISSCT World Germplasm Collections and on Transgenic Legislations.

Opening

On Sunday 15 May, 2011 Dr Guilherme Rossi Machado Jr, member of the organising Committee, welcomed all participants. Dr Asha Dookun-Saumtally, Molecular Biology Committee chairperson made a brief presentation on ISSCT on behalf of the ISSCT Secretariat and emphasised that this was the first joint Workshop of the Germplasm and Breeding and Molecular Biology sections, and encouraged participants to take advantage of this unique opportunity to share ideas amongst the two groups of scientists.

Keynote speakers:

All keynote presentations were well organised and introduced ideas and challenges for breeding and biotechnology. Although the speakers represented private companies, there was a good discussion on how breeders and biotechnologists should orient their research in a network collaboration system. The four topics were:

- Challenges and opportunities for sugarcane Biotechnology. Presented by Dr Ian Jepson, Syngenta
- Breaking paradigms: what do we need, and what do we need to know? Presented by Dr Mike Butterfield, Monsanto.
- Sweet sorghum: A complementary crop for sugarcane. Presented by Dr Walter Nelson, Ceres Inc.
- Biotech R&D for a sustainable Sugarcane Production. Presented by Dr John Lohrenz, Bayer

The program for presentations was arranged in related groups of topics: 1) Breeding for stress tolerance, 2) DNA markers/genetic mapping/QTL detection in sugarcane 3) Crop Improvement, 4) Genetic Transformation, 5) Biometrics, 6) Introgression and Prediction, 7) Diseases and molecular tools and Genomics.

Breeding for stress tolerance

The Breeding program of Louisiana, USDA-ARS breeding program at the Sugarcane Research Laboratory in Houma, Louisiana, U.S.A has to develop varieties in the world's most temperate environments where sugarcane is commercially grown. Researchers have focused on breeding varieties adapted to this unique environment. Selected cold tolerant *Saccharum spontaneum* clones from the basic breeding program have been sent to numerous locations in the U.S.A. to assess their cold hardiness and use as parental materials. The increasing interest in sugarcane as a biofuel feedstock has increased the number of studies aimed to increase levels of cold-tolerance in parental clones.

Breeding sugarcane for water-limited environments is another challenge because water stress is the major environmental limitation to higher sugarcane yields world-wide, and is also a major factor constraining expansion of sugar industries beyond existing boundaries in many countries. In many irrigated regions, higher water use efficiency is also being increasingly targeted as a high priority because of limited water supply and or potential increases in water prices. In 'Cerrado' (savanna) of pasture land characterised by a dry winter with a prolonged water deficit period in Central Brazil, strategies for breeding sugarcane for drought tolerance are being used to meet the ethanol demand in Brazil. Evaluations are carried out using dependable protocols to screen for sugarcane genotypes tolerant to water deficit stress through field trials; greenhouse irrigation withdrawn; *in vitro* and *in vivo* exposure to polyethylene glycol solutions and paraquat exposure. In Australia, water stress normally occurs during the spring and early summer periods in most sugarcane growing regions, and in most years causes serious reductions in yield. Different levels of timing and severity of water stress, and the physiological response mechanisms involved, can cause the ranking of genotypes to vary greatly between different water limited environments. Therefore, selection of a large numbers of genotypes for yield in semi-randomly chosen environments can be ineffective. Measurements of yield components and also monitoring stomatal conductance and relative water content of leaf tissue of all clones, and water use showed significant genetic variation in response to water stress. In Barbados, juice quality evaluation of sugar cane genotypes towards extending the harvest season has shown that juice brix, field brix, pol in juice and purity increased in a linear manner in extended harvesting periods, but also decreased in a linear manner if varieties have increased fibre.

DNA markers/genetic mapping/QTL detection in sugarcane

French researchers at CIRAD, France reported the results of an association mapping study for *Sugarcane yellow leaf virus* based on 334 sugarcane cultivars in a replicated trial carried out in Réunion and Guadeloupe. Four markers were associated with resistance and one with susceptibility to the disease.

In Australia, scientists have screened thousands of DArT markers on 3 sugarcane populations and a set of 384 DArT probes were selected for application in marker-assisted selection.

In Mauritius, mapping with the objective to identify marker(s) linked to yellow spot disease resistance is in progress. Sorghum EST-SSR markers have been placed on a genetic linkage map of cultivar M 134/75, where a major QTL for yellow spot disease resistance has been identified.

Although a number of research laboratories are making progress in genetic mapping of sugarcane, there is still much apprehension on the application of molecular markers for marker-assisted breeding in sugarcane. It is forecast that >15 000 markers would be required to have a saturated sugarcane map and, in order to achieve this goal, high throughput technologies are needed. Other concerns related to sugarcane mapping are that relatively low frequency markers common between mapping populations are available, while a standard nomenclature for designating homologous groups (HGs) has yet to be devised.

The above concern was somewhat dissipated with a presentation from the Toyota Motor Corporation, Japan. A high throughput microarray technology for the construction of a sugarcane linkage map and identification of QTLs was presented. Within six months, a linkage map of sugarcane with some 5000 single dose markers with an average marker distance of 1.2 cM can be obtained. However, the cost of this technology was not disclosed.

A number of posters presented at the Workshop described the routine application of microsatellite (SSR) and AFLP markers to assess the genetic diversity of sugarcane cultivars in a number of breeding programs. Such studies will provide information for more efficient use of the genetic resources, the selection of parents and improving the planning of crosses.

Traditional crop improvement

The presentation of M. Cox from BSES, Australia on Benchmarking Breeding Programs, was an stimulating start for discussions. Breeders should quantify the success in order to justify investment levels to both industry and administrators in this critical activity. The BSES-CSIRO Joint Venture Variety Improvement Program uses the following benchmark statistics: 1) Number of varieties released (Target: 3 varieties/5-year period for each region), 2) Percentage production attributed to "new" varieties (Target: 20%). New varieties are defined as those released within six years of the year prior to the production year, 3) Those released prior to 2004 are defined as "old" varieties, 4) Percentage production of varieties with Plant Breeder's Rights (PBR) (Target: >90% of Australian industry planted to PBR-protected varieties by 2011), and 5) Rate of genetic gain. This is estimated by analysis of available mill data for cane yield, CCS and sugar yield.

A revised statistical approach to identify best performing parental materials was presented by breeders from BSES, Australia. Improved estimates of breeding values by modeling site-specific spatial variation; combining family trials across regions using a Factor-Analytic mixed model to exploit the genetic correlations between trials established in different regions; and individual traits, cane yield and sucrose content, separately rather than for Net Merit Grade were used. Results indicated that the new analytical approaches explained over 75% of the phenotypic variation in both cane yield and sucrose content, while previous methods explained less than 40% of the variation. The breeding values were also predicted with greater accuracy using a genetic evaluation system.

The tropical lowland areas of the Guayas River valley in Ecuador show adverse environmental conditions for sugar accumulation. Therefore, there is a need for developing parental materials to accumulate sugar in new clones. Crosses with high sugar content showed a higher frequency of clones that surpassed the parental clones and control varieties. Crosses between clones that have the variety POJ2878 as a common ancestor showed a low frequency of outstanding progenies. This suggests that the common genetic basis of these parental varieties has reduced the probability of accumulating favourable genes. Scientists from RIDESA/UFSCar, Brazil, have suggested a new selection strategy based on capitalizing the genotype x environment interaction in stage two of selection, where genetic variability of the population is high. Comparing series 2003 (the traditional selection system) and 2004 (the new strategy), both with the same number of initial seedlings, the number of clones selected from stage 2 (T2) was similar in numbers of genotypes and also in visual evaluation. This new strategy resulted in a more secure way to explore the genotype x environment interaction of the T2 phase, reducing the numbers of individuals in T2 fields without compromising the agronomic qualities of the selected population.

A population-testing concept to facilitate large mill testing and cutting down the intergeneration time interval for releasing varieties was proposed by scientists from the Sakthi Sugarcane Research and Consultancies Pvt. Ltd. Research Centre in India. In Parry's variety testing scheme, the intergeneration time interval for releasing varieties was reduced from 13 years to 6 to 8 years. This was possible with the use of population testing which includes early selection based on heritable characters like brix, fibre, pest and disease reactions and later 20 ha and more under field conditions to allow a selection of the best clones for multiplication at three locations using single-eye buds. A simplified selection systems (SSS) method as a new tool for classical breeding was presented by researchers from the Universidade Federal Rural de Pernambuco (UFRPE), Brazil. Materials are selected through an "Early Negative Selection" before the selection stages are established in the field. This avoids the evaluation of the undesirable first phase -T1 materials, reducing costs, materials and time to release new varieties. The SSS avoids high genotype x environmental interaction due to low environmental variance by managing three controlled distinct phases: sowing, early negative selection and ratoon selection in plastic bottles.

Breeding sugarcane for bio-energy production is carried out at Texas A&M, USA at Weslaco by developing wide hybrids of feed stocks for bio-energy. Besides *Saccharum* species, the program includes *Miscanthus*, a perennial grass that in temperate climates uses the more efficient C4 photosynthesis process, and is also exceptionally cold tolerant. Because each of these crops has its relative strengths and weaknesses, their inter-specific hybridisation is a method to create a unique new species, specifically developed for bio-energy production. Molecular markers are being developed from genes related to cell wall components and will be applied to this germplasm for marker-assisted selection. Scientists from China showed that sugarcane is being increasingly used in several countries and will be used as a feedstock for renewable energy products in China, becoming a major and expanding crop in southern China.

The need for new genes to widen the genetic basis of sugarcane hybrids has led to the use of Badila, one of the most successfully used nobilisation parents in Mainland China to introduce desirable attributes such as strong ratooning, drought tolerance and disease resistance. Also, the Hainan Sugarcane Breeding Station (HSBS), Guangzhou Sugarcane Industry Research Institute, is using wild germplasm, such as *S. spontaneum* L., *S. robustum* Brandes and Jeswiet ex Grassl and *Erianthus arundinaceus* (Retz.) Jeswiet, and other species. Noble canes, mostly *S. officinarum* clones, were used to nobilise the wild germplasm in the initial crosses. Back-crossed progenies have been selected for further use from crosses between Badila with *S. robustum* and *E. arundinaceus*.

Biometrics

Sugarcane improvement requires efficient experimental designs and data analysis. Breeding programs evaluate clones in multi-environment trials (MET), where genotype yields are compared at different crop ages within and across locations. A graphical MLM-based approach to study interactions between genotypes and locations considering different crop ages using data from MET of the Sugarcane Breeding Program of Estación Experimental Agroindustrial Obispo Colombres (Tucumán, Argentina) was shown. The biplot obtained from the triple interaction BLUPs facilitated the interpretation of genotype-age interactions for each location and genotype-location interactions for different ages. Another paper showed the application to select families using REML/BLUP aiming to increase biomass to develop varieties for biomass as feedstock for power generation. This methodology was adopted, with the REML used for estimation of the genetic variance, and the BLUP for the estimation of genetic values of families and parentsl used.

The Mauritius Sugar Industry Research Institute has widened its scope of selection to include multi-purpose clones applying multivariate techniques. Based on variations in cane quality and biomass traits, four types of canes with different levels of Pol and fibre have been defined for multiple uses: Type 1 (commercial), Type 2 (commercial with enhanced fibre), Type 3 (multi-purpose high fibre), and Type 4 energy canes (fibre > 22% for cogeneration). Researchers from South Africa showed that a confounding effect of ‘seed type’ can occur when the relative performance of families or individual seedlings differs between the seedling and clonal stages. Statistical methods that model simultaneously the potential and repeatability of performance between the seedling and clonal stages will help to mitigate possible adverse confounding effects to provide a more robust tool for family selection. Application of a Random Coefficient Model (RCM) analysis can be implemented as a separate program to evaluate new families and that information used to rank elite families as well as decide what proportion of seedlings from these elite families to plant in the core breeding program.

Genetic transformation

Although genetic modified (GM) sugarcane research is progressing in a number of countries, there is a need for multiple and effective gene introduction using a robust high capacity transformation system, preferably *Agrobacterium* in order to produce thousands of events. Gene expression tools to enable selection of stable events are also needed.

The work carried out by Syngenta to produce sugarcane lines expressing cellulases for the conversion of cellulosic material for future economical conversion of bagasse to fermentable sugars is showing promising results. Currently, cellulolytic enzymes are produced in microbes and the cost of production of such enzymes is high. Expressing cellulases *in planta* could greatly reduce production costs for second generation ethanol production.

Bayer Crop Science AG in partnership with CTC in Brazil is exploiting the possibility of converting the sucrose reserve in sugarcane into higher value novel carbohydrates via genetic transformation technology. Inulin type fructans is a particular interest due to its health-promoting effect. Bayer has today a new recombinant technology allowing significant increase in total sugar yield without affecting the biomass yield of the plant.

Field performance of transgenic sugarcane plants issued by different transformation systems were compared with non-transgenic and tissue culture plants in Australia. No differences were observed and therefore transgenic technology could be used to produce economically suitable clones in the future. Moreover, no correlation was found between transgene copy and trait expression and also both *Agrobacterium* and the biolistic system produced low copy transgenic events.

For Sugarcane mosaic virus (SCMV) and Sorghum mosaic virus (SrMV), gene silencing technology was exploited by Chacra Experimental Agricola in Argentina as an alternative approach to obtain resistant clones. Following a large scale survey for mosaic variants, a resistance gene was designed to trigger gene silencing mediated resistance against all virus variants. Transgenic plants of five varieties have been produced and are being evaluated for resistance to mosaic virus.

Diseases and Molecular tools

One paper was presented on sugarcane orange rust by the group of USDA-ARS, Canal Point in USA. This fungal disease caused by *Puccinia keuhnii* has recently made its appearance in the Western hemisphere. Initially observed in Florida in 2007, it has now been confirmed in almost all countries in Central America and in countries in South America, namely Brazil and Colombia as well as in the African Continent; Cameroon, Ivory Coast. In Florida, in addition to classical breeding to find resistant cultivars, molecular approaches towards marker discovery are being accelerated.

For brown rust disease, caused by *Puccinia melanocephala*, a major dominant resistant gene-*Bru1* has been identified by researchers from the CIRAD, France. Following a thorough survey in a number of countries, *Bru1* was found as the main source of resistance in modern cultivars. Only 6.6% of the resistant clones surveyed were not associated with the *Bru1* haplotype, indicating alternative source(s) of resistance to the brown rust.

Genomics

Sugarcane sequencing is also taking much importance in a number of countries namely Brazil, Australia, France, South Africa and USA. With future sequencing data from *S spontaneum*, *S. officinarum* and hybrid cultivars, much information will be disclosed on the complex sugarcane genome that would assist the sugarcane community to improve this complex plant species.

Integrating breeding and biotechnology

Australia is applying genetic transformation in sugarcane with emphasis on an integration of genetic modified (GM) technology into breeding programs which involves the identification of priority traits, introduction of genes controlling them into the best cultivars and parents and developing crossing and selection strategies. A critical step for the implementation of molecular markers is development of a selection index for important traits based on phenotypic and marker data. Marker data can be incorporated using genetic correlations with the traits of interest, as with any indirect selection method. DNA fingerprinting has been integrated into the breeding program in two ways: 1) establishing the initial identity of accelerated clones (variety audit) and additional information for Plant Breeder's Rights (PBR); 2) checking/rectifying possible identity mistakes. Markers are also routinely used in disease diagnostics in domestic and international quarantine. Tissue culture is being used to a small extent for the commercial propagation and release of new cultivars, providing disease-free material both directly and indirectly to growers. Commercialisation of GM sugarcane remains a challenge because of political, technical and regulatory challenges in commercializing. Nearly two dozen transgenes with commercial potential have been introduced and expressed in sugarcane but none of them led to any commercial product.

Quarantine, prediction and variety adoption

Three important papers were presented in this topic, including the Visacane program of CIRAD for sugarcane quarantine (http://visacane.cirad.fr/en/) a system that has been in place for several decades. It covers three main quarantine procedures: detection of pests and pathogens, elimination of pests and pathogens, and transfer of plant material free of pests and pathogens. In addition to phytosanitary constraints, Visacane also takes into account legal constraints and ensures, through appropriate contracts, that plant breeders' intellectual property rights over the transferred material are respected.

In South Africa there is considerable interest in cellulosic biomass as a feedstock for ethanol production. Therefore, determining the chemical composition of sugarcane bagasse is becoming increasingly important in the variety improvement plan, especially in the light of advances in ethanol production technology. Near Infra Red Spectroscopy (NIRS) has been used very successfully over the last number of years to analyse sugarcane samples at SASRI for brix, pol, dry matter content and fibre percent. The main advantages of NIRS are the speed, accuracy and cleanliness of analysis. Correlations between data from the Laboratory Analytical Procedures at the National Renewable Energy Laboratory of lignin, glucan, xylose and arabinose and those from NIRS showed that NIRS could provide an accurate predicted measure of the above chemical constituents. However, some sample preparation methodology and further calibration and/or validation are required to develop a more robust equation to create a new selection index.

The Guatemalan Sugarcane Breeding Program started in 1993 selecting local varieties. In the later stages of selection, the program carries out evaluation at eight regional trials and three semi-commercial trials. The methodology has allowed the release of three CG (CENGICAÑA – Guatemala) varieties and recommended the use of two introduced ones. Sugarcane in Guatemala grows in different environments and genotype x environment interaction is an important issue to take into account before any variety release. The released varieties are all rapidly adopted in the recommended environments.

ISSCT World Germplasm Collections

There has been concern regarding the conservation and distribution of the World collection held in Coimbatore, India and Miami, USA. A panel discussion on this topic was arranged with the two curators: Dr. Vijayan Nair, Director of Sugarcane Breeding Institute, Kannur, India and Dr Tomas Ayala, curator at ARS, USDA. Both germplasm collections for the ISSCT are available in Coimbatore, India and in Miami, USA. The report from India shows that the collection consisting of 1819 clones is well maintained and is clonally propagated every year. Clones are characterised and catalogues were available from Dr. Nair on request. Up to now, germplasm has been sent to more than 256 locations. Varieties can be obtained following an official request before June (one shipment of not more than 25 clones in any year per country). If any breeder is interested, start requesting information to Dr V. Nair at vijay52in@yahoo.com and director@sugarcane.res.in or tel. +91-422-2472986, 2472621. For the Miami collection, Dr T Ayala, curator at ARS, USDA informed the meeting that a database on the 887 sugarcane clones is available and can be consulted at the following website address: www.ars-grin.gov. Clones can also be obtained from this collection, but freight charges need to be paid by the importing country. Information on the variety notes from Dr. Rossi Machado should also be the basis to start including information all around the world, accessed by members only.

Transgenic Legislations

Genetic transformation is gaining importance in a number of sugarcane research programs and 16 countries are currently involved in sugarcane genetic modification (GM) technology. Moreover, recently, several of the large multinational companies that commercially exploit GM technology in other crops have shown much interest in GM sugarcane development. So far no commercial GM sugarcane is grown in the field as the regulatory process is too long and expensive, but it is expected that GM cane will be planted in the coming years. For this to materialise, a regulatory framework for the purpose of GM sugarcane varieties will have to be developed. Presently, regulations available are based for large scale production crops such as maize and soybean, and evaluations are based on a case by case basis. However, this approach does not suit sugarcane due to the complexity of the crop.

Argentina has already 15 years experience in the deregulation of other GM crops and GM regulators are currently preoccupied in the preparation of legislation for GM sugarcane. Brazil is also addressing this issue. The main traits of interest introduced so far are drought resistance, disease and pest resistance and herbicide tolerance.

The sugarcane community should give more attention to the building up of a database on the chemical composition of sugarcane, as this will be an important component for the food safety procedures for sugar produced from GM varieties.

Field visit

During the field day, participants visited CanaVialis-Monsanto Breeding Station in Maceió. This new station in the northeast of Brazil is well situated to meet sugarcane flowering requirements and seed setting. The station produces around 3 million seedlings annually from some 2000 crosses. Crosses are carried out in a controlled manner under plastic lanterns, where parentage can be guaranteed. The program is supported by software that controls all the processes for crossing; in fact all stalks of parents are bar-coded. A germplasm collection made up presently of some 2000 varieties is also available.

Closing Ceremony

The closing ceremony was held during the Workshop dinner on Thursday 19 May. Dr Raul O. Castillo, chairman of the Germplasm and Breeding Committee of ISSCT thanked all the local organisers for the highly successful and well organised Workshop. He then made a brief summary on the outstanding career in breeding sugarcane of Dr Nils Berding, former breeder at the BSES Ltd, Australia and invited him to give a talk on his "45 Years of Sugarcane Breeding". This was followed by a gorgeous show with local native dances and a copious dinner. Dr Guilherme Rossi Machado Jr. once again thanked all participants and those who were involved in the organisation of the Workshop.

Conclusion

The sugarcane genome is among the most complex of all cultivated crops and this complexity has somewhat hindered our understanding of sugarcane genetics and its full potential for exploitation. Today with the interaction of classical breeding and biotechnology, more knowledge on the crop is being acquired. This joint Germplasm & Breeding and Molecular Biology Workshop was a unique opportunity for the two groups of scientists to move towards a complementary approach in sugarcane research. This will no doubt allow further contribution to the knowledge on the sugarcane genome in the future.

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