Glaucia Mendes Souza; Marie-Anne Van Sluys. "SUGARCANE GENOMICS AND BIOTECHNOLOGY: STATE OF THE ART, CHALLENGES AND ACTIONS", p.325-332. In Luis Augusto Barbosa Cortez (Coord.). **Sugarcane bioethanol — R&D for Productivity and Sustainability**, São Paulo: Editora Edgard Blücher, 2014.
http://dx.doi.org/10.5151/BlucherOA-Sugarcane-SUGARCANEBIOETHANOL_32

2

SUGARCANE GENOMICS AND BIOTECHNOLOGY:

STATE OF THE ART, CHALLENGES AND ACTIONS

Glaucia Mendes Souza and *Marie-Anne Van Sluys*

Sugarcane belongs to the botanic group known as *Saccharum officianale*, domesticated since 7 000 BC. The process of domestication started in New Guinea from where seedlings were taken to Continental Asia. In China and India, prevailing favorable conditions allowed the development of two different species, *S. sinense* and *S. barberi* respectively. From approximately a century ago, selective breeding produced *Saccharum officinarum,* also known as Nobel Clones. These varieties have been progressively replaced by modern varieties from crossings between *S. officinarum* and *S. robustum,species* which currently predominate worldwide. As sugarcane is a hybrid plant, the identification of genes of greater economic interest is a laborious and lengthy process e.g. it takes about 15 years to release a new commercial variety. This gives an indication of the difficulties involved in developing new commercial varieties.

In Brazil, increasing commercial pressure to improve sugarcane productivity faces the challenge to develop new varieties adapted to the different conditions in the country. For example, it is estimated that to supply internal and external demand, Brazil will have to duplicate sugarcane production within the next 5 to 7 years, requiring investment in new mills, expansion of cultivated area, increase productivity and better cane varieties etc. The expansion of sugarcane to new areas must also include the preservation of unique biomes and biodiversity. In São Paulo State, Brazil's largest producer of sugarcane, increasing productivity with new varieties of higher sucrose content is particularly attractive as this will limit land area use. Land available for sugarcane expansion in the short term is located in the Central Plateau which suffers from water shortages. Therefore the development of new sugarcane varieties resistant to drought will be necessary if sugarcane is to be cultivated in pastures land, to avoid the "cerrado" and tropical forest areas. Producers in the Northeast can also benefit from new cultivars resistant to drought as they could increase productivity quite significantly in this region.

Genetic improvement is, however, limited by two biological barriers: sexual reproduction, in the case blooming plants, and cellular equilibrium that guarantees the correct signaling between the cells, tissues, and organs of the whole plant and, ultimately, its interaction with the environment. In the first case transgenic techniques can be a possible alternative in the short run, but it is necessary to know which gene needs to be modified or introduced. Of the various breeding strategies aimed at the development of varieties with new characteristics, it seems that better understanding of the biological network may effectively lead to cultural management, thus increasing the potential of existing cultures, as well as the development of new ones, through "assisted genomic selection".

The use of biological systems with tools from genomics opens the possibility for integrating know-how on different areas, and together with the use of statistics, can help to improve the management of new sugarcane varieties.

Informatics and the identification of relevant biological characteristics to address specific problems are the pillars of what is currently called "biological systems", or a systematic approach that offers considerable potential to improve crops of economic value. Its application to sugarcane could made a significant contribution particularly to our understanding of the sucrose content, resistance to biotic stress (i.e. pathogens), abiotics (i.e. drought tolerance). The genetic diversity available to breeding programs can be explored using genomic and biotechnological approaches to accelerate the exploitation of the most promising varieties.

Brazil uses sugarcane bagasse in co-generation in sugarcane distilleries or as animal feed that increases significantly the overall efficiency of the system. One challenge will to develop enzymatic and acid hydrolysis processes that can use cellulose and hemicellulose as feedstock, increasing ethanol production even further. From an energy point of view, 2/3 of sugarcane is not used in the production of bio-ethanol.

Some varieties of sugarcane have been developed by breeding programs (i.e. the Inter-University Network for the Development of the Sugar/Alcohol Sector – Ridesa, the Sugarcane Center of Technology – CTC, and the Sugarcane Centre of the Agronomy Institute of Campinas – IAC), adapted to different Brazilian conditions such climate and soil, that are highly productive and with high sugars or fiber content. A good example of RIDESA breeding program is the Centre of Agricultural Sciences (CCA) at the Federal University of São Carlos – UFSCAR.

The sugarcane breeding program (PMGCA) has 36 years of research experience. Over 16 years the PMGCA/CCA/UFSCAR has developed 48 new improved sugarcane varieties suitable for differing environmental conditions and mechanical harvesting, all of which have been made available to the sugarcane sector. Currently Ridesa and the CTC are jointly responsible for about 95% of the varieties planted in Brazil (CTC for 30%, and Ridesa for 65%). This shows that the public sector is able to create and spread the technology to the sugar/alcoholic sector. The success is due to the close partnership among universities and the mills/distilleries, and good communication channels between research and industrial sectors. Currently RIDESA has the largest public collection of sugarcane genotypes of Brazil. CTC (ex-Copersucar) has probably one of the most important private germoplasm collections of the world.

The use of molecular techniques based on the systemic analysis of genomics data, can help in the selection and trials of cultivars adaptable to specific environments; and create the conditions for acid and enzymatic hydrolysis that will be one of the main challenges of sugarcane biotechnology.

Another major challenge will be to develop new strategies in biological research to create the basis for the development of biofuels in Brazil. It would be necessary to stimulate the creation of integrating mechanisms between the sugar and alcohol sectors with genetic improvement programs of sugarcane and advance biological research, integrating genomics, molecular, biochemical and physiological data, as a response to new environment requirements. A good example is the project Sucest, supported by Fapesp through the Onsa Genome Program, which is the outcome of more than 200 researchers of 50 research groups who have identified and analyzed 238,000 ESTs of sugarcane from 26 libraries of cDNA from 13 cultivars [1-3]. However, the impact of this study is still rather limited, and therefore it is necessary to implement an efficient program to identify research gaps and develop a basic research to increase productivity, in the medium and long term, in the productive sector.

The efficient use of sugarcane as a source of renewable energy depends on the integration of characteristics that control plant growth, the volume of biomass and its adaptation to the environment. In all cases, the genetic stability associated to the agronomic characteristics of the variety is fundamental. A better understanding of the plant growth and the optimized use of the nutrients is necessary to develop an efficient and environmentally sustainable agriculture. Within this perspective it is important to provide research funding to support diversification of control mechanisms for efficient use of carbon and sugars, in angiosperms through comparative analysis involving sugarcane and other models. The results should contribute to tuned manipulation of homeostasis of energy by crossing or transgenesis.

The process of energy and biomass generation has been analyzed only at cellular or on micro-organism level and has shown a complex net of reactions. Genes related to sucrose content were identified[4] and through a study with transgenic plants was found that they are responsible for the increase of the synthesis of sugar. This work was carried out by researchers at the Institute of Chemistry and the Institute of Biosciences of USP, in collaboration with CTC[5-7]. Moreover, studies of transcriptome in sugarcane identified around 700 genes associated to sucrose content, lignin and drought response[8-9]. Despite the fact that the role of several individual genes has been demonstrated, the biggest structure of the system remains unknown. After 50 years of research with sugarcane, the process and enzymes involved in the accumulation of sucrose have been identified and used to produce transgenic plants, but the results are not encouraging, probably due to the complex systems regulations. Transgenic sugarcane plants have been obtained with several agronomic characteristics of commercial interests[10-26], including water stress[27-28]; varieties, with regeneration capacity; and transgenic plants of Brazilian sugarcane genotypes have already been described[12, 17, 29, 30]. The development of transgenic plants is a necessary step to better understand and control the regulating routes of the plant's carbon metabolism.

Research on generation of vegetative transformation methodologies must be encouraged. The process of biobalistic transformation is not very efficient with sugarcane, depending on the gene or genotypic and plant generation efficiency. The genetic transformation by *Agrobacterium tumefaciens* needs also to be investigated and applied to sugarcane[11, 31-34]. With proper manipulation and control of conditions of *in vitro* culture, age/type/stage of embryogenic culture and methods of transformation, and by improvement of the virulence of the genealogy *A. tumefaciens,* it has been demonstrated that it is possible to increase the efficiency of the transformation of this specie via *A. tumefaciens.* Despite the intrinsic difficulties of this method in sugarcane, further attention should be paid to the advantages offered by *A. tumefaciens,* as it has the advantage of be able to transfer relatively long segments of DNA with few resetting, integration of a short number of copies of the transgene, simplicity and lower procedural cost.

Whatever the desirable agronomic trait maybe, this will have to be incorporated to breeding programs by means of crossbreeding or transgenesis. So, efficient markers and the know-how of the genetic potential associated to the cultivars are essential. Sugarcane has a complex genome that varies from cultivar to cultivar as the genome is hybrid and poliploide. With about 760 to 926 Mbp, the size of the basic monoploid genome of *Saccharum* is about twice the size of rice (389 Mbp), similar to the *Sorghum bicolor* Moench (760 Mbp), and significantly smaller than the corn (2500 Mbp). The size of the genome of one somatic cell (2C) of the modern culture R570 (2^{nd} = about 115) was estimated as 10,000 Mb[35].

In function of such characteristics, focused access to allelic diversity in germplasm database, through the use of the traditional genetics, becomes extremely difficult. The SUCEST project opened up a new perspective in the development of breeding programs with advanced molecular techniques. After the ESTs progression, genetic maps were obtained from cultivars of interest and molecular markers have been developed, that further support breeding programs[36-39]. The complete genome of a sugarcane cultivar is not yet available and should be promoted by Brazilian research groups to improve biotechnological research on sugarcane, while at the same qualifying Brazilian geneticists and breeders. The progression of the sugarcane genome and the comparison of the genome of several cultivars will create the conditions to establish a platform for in-depth studies on the diversity of the germoplasm of *Saccharum* genus. Contrary to the ESTs sequence, the genome sequencing will permit the identification of regulation areas, comparative studies of the evolution of chromosomal regions and other plants pattern, and identification of potential allelic variation observed among cultivars. The understanding of the genomic structure of sugarcane in terms of physical location of the genes and allelic proportion, as well as genetic ambiance where they were introduced, will improve our understanding of the genetic ambiance where they have been inserted; it will also enable a better understanding of the epigenetic effects over the genetic expression of sugarcane.

Consequently, it will be possible to increase the efficiency in the use of biotechnological tools for a better result for this culture e.g. the transformation of plants. It is possible through sequencing of a complete genome, to identify the regulatory regions of the genes (promoters) and genomic sequences that allow the transgenic expression in a controlled way, and tissue-specific. Several sugarcane promoters have already been identified e.g. tissue-specific expression data[40, 41], induced by insects[42] and of transposons[43]. Studies of this nature are important as they provide greater freedom to operate and to have intellectual property rights in an area dominated by foreign biotechnology companies.

The identification of molecular markers associated to phenotypes of interest is extremely important for the genetic improvement of sugarcane, avoiding the crossing between related parents. In this way it is possible to take maximum advantage of heterosis, based on the information from the identified quantitative characteristics – QTL. The molecular markers will also serve as tools in the evaluation and guidance for the introduction of the genetic variety in *Saccharum.* Genetic variety is fundamental to obtain cultivars resistant to pests and diseases and improving the most important economic characteristics such as sucrose and biomass volume.

The result of such studies could be used by public and private institutions with banks of sugarcane germplasms, to better understand their genetic variability. This will allow the establishment of *core collections* of available germplasms, and identify new ones. The genetic change by transgenics will enable existing species of sugarcane to improve their commercial lifetime, particularly in new sugarcane growing areas.

Studies on environmental interaction of sugarcane and the investigation of molecular mechanisms on physiologic responses and biochemical characteristic of the different cultivars are extremely important for the basic understanding of sugarcane. The identification and characterization of genes related to herbivores and pathogens is also one of the biggest challenges facing functional genomics. Studies have already been carried out on the characterization of the transcriptome of sugarcane to biotic stresses (sugarcane borer and endophytic nitrificant bacteria), abiotic stresses (dry and phosphate deficiency) and hormones responses[8]. Many candidate genes tolerance to insects have already been tested and incorporated to the main genotypes of sugarcane[15, 44-50]. Resistance proteins as a soybean inhibitor protease[17] or the *cry protein of Bacillus thuringiensis*[51] increase resistance to the borer *D. saccharalis,* the most recurrent pest in Brazil. Moreover, the characterization of cysteine proteases inhibitors brought a new perspective in the control of pathogens e.g. growth inhibitors of the fungus *Trichaderma reesei*[52], suggesting that this can also be used in the control of sugarcane fungus infections. The interaction plant-insect is considered as a dynamic system, subject to continuous variations. Plants develop different mechanisms to reduce insect attachs, including specific responses to activate different metabolims, changing considerably their chemical and physical characteristics. On the other hand, insects have also developed several strategies to overcome the plants' defensive barriers, permitting their feeding, development and reproduction. Studies have shown adaptation of insects and proteases inhibitors through general increase of the expression of proteasis genes. Therefore it is important to continue to investigate this matter so that we understand the behavior of the borer, one of the most serious plagues of sugarcane in the Northeast Region; and more recently also in the Southeast region; it is very important therefore to develop new diseases and pest control strategies.

Several aspects point to changes in sugarcane production and productivity in the near future and this requires a serious study of the potential impacts of climatic change on sugarcane, to put it in the right track. It is already known that there is an increase in photosynthesis (50%) and biomass in the stem (60%), in response to higher CO_2, which suggests productivity increases over the next few years[53]. At the same time very little is known about the mechanisms of temperature increases, for example. A further aspect relates to climatic changes associated to high consumption of fossil fuels and hence worldwide pressure to produce fuels of biological origin. Thus, it is important that Brazil starts to use sustainable renewable energy in harmony with the environment that accrues also social and environmental benefits.

A further possibility is to produce biofuels from polymeric compounds of cellular walls. Cellular walls are constituted of cellulose, hemicelluloses and pectins, intertwined in such a way that it is extremely difficult to extract energy in their chemical connections efficiently. It will be necessary to understand not only how the wall is built (biosynthetic processes), but also how the degradation processes works, if we are to dismount the cellular wall and learn how to use the energy that it contains. Within this context we can also apply our knowledge from studies of hydrolases, fungi and ligno-cellulolytic microorganisms that degrade the vegetable walls from the diverse Brazilian biodiversity. This can be done by identifying and purifying new fungi, enzymes, microorganisms and insects that can degrade cellulose and other constitutions of the wall. This should be in conjunction with industrial and fermentation process so that the new technologies have immediate commercial applications in the sugarcane sector.

Based on research experience in genomics in the State of São Paulo, we believe it is necessary to integrate the efforts of biologists, physiologists, biochemists, mathematicians and computer scientists to develop an integrated Research Program in Bioenergy. With biological sugarcane mapping, using biological system tools we believe it is possible to shape responses adapted to existing cultivars; and as last instance, contributing to increasing productivity necessary in a context where keeping environmental equilibrium is fundamental for future generations. It is important and urgent that Brazil acquires expertise in these fundamental aspects, to increase our capacity of “Freedom to Operate” in Biotechnology, training personnel to work in universities, and innovation agencies of the private and public sectors.

REFERENCES

1. VETTORE, A. L.; SILVA, F. R. da; KEMPER, E. L.; SOUZA, G. M.; SILVA, A. M. da; FERRO, M. I.; HENRIQUE-SILVA, F.; GIGLIOTI, E. A.; LEMOS, M. V.; COUTINHO, L. L. *et al*. **Analysis and functional annotation of an expressed sequence tag collection for tropical crop sugarcane**. *Genome research*, 2003, 13(12):2725-2735.
2. VINCENTZ, M.; CARA, F. A.; OKURA, V. K.; SILVA, F. R.; PEDROSA, G. L.; HEMERLY, A. S.; CAPELLA, A. N.; MARINS, M.; FERREIRA, P. C.; FRANCA, S. C. *et al*. **Evaluation of monocot and eudicot divergence using the sugarcane transcriptome**. *Plant physiol.*, 2004, 134(3):951-959.
3. ARRUDA, P. **Sugarcane transcriptome. A landmark in plant genomics in the tropics.** *Genetic and molecular biology*, 2001, 24:1-4.
4. PAPINI-TERZI, F. S.; FELIX, J. M.; ROCHA, F. R.; WACLAWOVSKY, A. J.; ULIAN, E. C.; CHABREGAS, S.; FALCO, M. C.; NISHIYAMA-JR, M. Y.; VÊNCIO, R. Z. N.; VICENTINI, R.; MENOSSI, M.; SOUZA, G. M. **The Sucest-FU/Project: identifying genes that regulate sucrose content in sugarcane plants**. *Proc. Int. soc. sugarcane technol*, 2007, 26.
5. SOUZA, G. M.; PAPINI-TERZI, F. S.; ROCHA, F. R.; WACLAWOVSKY, A. J.; VÊNCIO, R. Z. N.; MARQUES, J. O.; FELIX, J. M.; TEIXEIRA, M. M.; ULIAN, E. C. **Sugarcane with increased sucrose levels**. In: United States: Universidade de São Paulo, Universidade de Campinas, Fundação de Amparo à Pesquisa do Estado de São Paulo, Centro de Tecnologia Canavieira e Central de Álcool Lucélia, 2006.
6. SOUZA, G. M.; PAPINI-TERZI, F. S.; ROCHA, F. R.; WACLAWOVSKY, A. J.; VÊNCIO, R. Z. N.; MARQUES, J. O.; FELIX, J. M.; TEIXEIRA, M. M.; BUCKERIDGE, M.; SOUZA, A. P.; ULIAN, E. C. **Genes associated to sucrose content**. In: USPTO. Universidade de São Paulo, Universidade de Campinas, Fundação de Amparo à Pesquisa do Estado de São Paulo, Centro de Tecnologia Canavieira e Central de Álcool Lucélia, 2006.
7. SOUZA, G. M.; PAPINI-TERZI, F. S.; ROCHA, F. R.; WACLAWOVSKY, A. J.; VÊNCIO, R. Z. N.; MARQUES, J. O.; FELIX, J. M.; TEIXEIRA, M. M.; BUCKERIDGE, M.; SOUZA, A. P.; ULIAN, E. C. **Genes associated to sucrose content**. In: USPTO. Universidade de São Paulo, Universidade de Campinas, Fundação de Amparo à

Pesquisa do Estado de São Paulo, Centro de Tecnologia Canavieira e Central de Álcool Lucélia, 2007.

8. ROCHA, F. R.; PAPINI-TERZI, F. S.; NISHIYAMA, M. Y., JR., VENCIO R. Z.; VICENTINI, R.; DUARTE, R. D.; ROSA, V. E. de, JR., VINAGRE F., BARSALOBRES, C.; MEDEIROS, A. H. *et al*. Signal transduction-related responses to phytohormones and environmental challenges in sugarcane. *BMC genomics*, 2007, 8:71.
9. SOUZA, G. M. Transcriptoma da cana-de-açúcar. In: Universidade de São Paulo, Universidade Estadual de Campinas: Fapesp, CTC e Centralcool, 2004.
10. GALLO-MEAGHER, M. I. J. Herbicide resistant transgenic sugarcane plants containing the bar gene. *Crop Sci*, 1996, 36:1367-1374.
11. ENRIQUEZ-OBREGON, G. A. V. P.; PRIETO, S. D. L.; RIVA-GUSTAVO, A. D. L.; SELMAN, H. G. Herbicide resistant sugarcane (Saccharum officinarum) plants by Agrobacterium-mediated transformation. *Planta*, 1998, 206: 20-27.
12. FALCO, M. C. T-NA; ULIAN, E. C. Transformation and expression of a gene for herbicide resistance in a Brazilian sugarcane. *Plant cell reports*, 2000, 19:1188-1194.
13. LEIBBRANDT, N. B. S. S. Stability of gene expression and agronomic performance of a transgenic herbicide-resistant sugarcane line in South Africa. *Crop sci*, 2003, 43:671-678.
14. SNYMAN, S. J. M. G.; RICHARDS, J. M.; HARICHARAN, N.; RAMGAREEB, S.; HUCKETT, B. I. Refining the application of direct embryogenesis in sugarcane: effect of the developmental phase of leaf disk explants and the timing of D/A transfer on transformation efficiency. *Plant cell rep.*, 2006, 25:1016-1023.
15. ARENCIBIA, A. V. R.; PIETRO, D.; TELLEZ, P.; CARMONA, E. R.; COEGO, A.; HERNANDEZ, L.; DE LA RIVA GA. Transgenic sugarcane plants resistant to stem borer attack. *Mol breed*, 1997, 3:247-255.
16. ARENCIBIA, A. C. E.; CORNIDE, M. T.; CASTIGLIONE, S.; O'RELLY, J.; CINEA, A.; ORAMAS, P.; SALA, F. Somaclonal variation in insect-resistant transgenic sugarcane (Saccharum hybrid) plants producd by cell electroporation. *Transgenic Res.*, 1999, 8:349-360.
17. FALCO, M. C. S-FM. Expression of soybean proteinase inhibitors in transgenic sugarcane plants: efects on natural defense against Diatraea saccharalis. *Plant physiol. and biochem.*, 2003, 41:761-766.
18. INGELBRECHT, I. L. I. J.; MIRKOV, T. E. Postranscriptional gene silencing in transgenic sugarcane. Dissection of homology dependent virus resitance in a monocot that has a complex polyploid genome. *Plant physiol. and biochem.*, 1999, 119:1187-1197.
19. BUTTERFIELD, M. K. I. J.; VALDEZ, GARCIA M.; MIRKOV, T. E. Inheritance and segregation of virus and herbicide resistance transgenes in sugarcane. *Theor Appl. Genet.*, 2002, 104:797-803.
20. MCQUALTER, R. B. D. J.; HARDING, R. H.; MCMAHON, J. A.; SMITH, G. R. Production and evaluation of transgenic sugarcane containing a Fiji disease virus (FDV) genome segment S9-derived synthetic resistance gene. *Aust J Agric. Res.*, 2004, 55:139-145.
21. ZHANG, L.; XU, J.; BIRCH, R. G. Engineered detoxification confers resistance against a pathogenic bacterium. *Nat. biotechnol.*, 1999, 17(10):1021-1024.
22. MCQUALTER, R. B.; CHONG, B. F.; MEYER, K.; VAN DYK DE, O'SHEA, M. G.; WALTON, N. J.; VIITANEN, P. V.; BRUMBLEY, S. M. Initial evaluation of sugarcane as a production platform for phydroxybenzoic acid. *Plant biotechnology journal*, 2005, 3(1):29-41.
23. VICKERS, J. E. G. C.; BONNETT, G. D.; JACKSON, P. A.; KNIGHT, D. P.; ROBERTS, S. E.; ROBINSON, S. P. Overexpression of polyphenol oxidase in transgenic sugarcane results in darker juice and raw sugar. *Crop sci.*, 2005, 45:354-362.
24. VICKERS, J.; GROF, C.; BONNETT, G.; JACKSON, P.; MORGAN, T. Effects of tissue culture, biolistic transformation, and introduction of PPO and SPS gene constructs on performance of sugarcane clones in the field. *Australian Journal of agricultural research*, 2004, 56(1):57-68.
25. VICKERS, J.E. G. C.; BONNETT, G. D.; JACKSON, P. A.; MORGAN, T. E. Effects of tissue culture, biolistic transformation, and introduction of PPO and SPS gene constructs a perfomance of sugarcane clones in the field. *Aust. J. agric. res.*, 2005, 56:57-68.
26. WANG, M. L.; GOLDSTEIN, C.; SU, W.; MOORE, P. H.; ALBERT, H. H. Production of biologically active MCSF in sugarcane: a secure biofactory. *Transgenic res.*, 2005, 14(2):167-178.
27. ZHANG, S-Z YB-P.; FENG, C-L.; CHEN, R-K.; LUO, J-P.; CAI, W-W.; LIU, F-H. Expression of the Grifola frondosa trehalose synthase gene and improvement of drought-tolerance in sugarcane (*Saccharum officinarum L.*). *Journal of integrative plant biology*, 2006, 48:453-459.
28. MOLINARI HBM, C. J.; DAROS, E.; CAMPOS, M. K. F.; CARVALHO, J. F. R. P.; BESPALHOK FILHO, J. C.; PEREIRA, L. F. P.; VIEIRA, L. G. E. Evaluation of the tressinducible production of proline in transgenic sugarcane (*Saccharum* spp.): osmotic adjustment, chlorophyll fluorescence and oxidative stress. *Physiologia plantarum*, 2007, 130:218-229.

29. LIMA, MAC G. R.; MARTINS, G. S.; MANSUR, E. Morfogênese *in vitro* e susceptibilidade de calos de variedades nacionais de cana-de-açúcar (Saccharum officinarum L.) a agentes seletivos utilizados em sistemas de transformação genética. *Revista Brasil Bot.*, 2001, 24:73-77.
30. CIDADE, DAP G. R.; DUARTE, A. C.; SACHETTO-MARTINS, G.; MANSUR, E. Morfogênese *in vitro* de variedades brasileiras de cana-de-açúcar. *Pesq. agropec. bras.*, 2006, 41:385-391.
31. ARENCIBIA, A. D. An efficient protocol for sugarcane (*Saccharum* spp. L.) transformation mediated by Agrobacterium tumefaciens. *Transgenic res.*, 1998, 7:213-222.
32. ELLIOTT, A. R. C. J.; DUGDALE, B.; BRETELL, R. I. S.; GROF, C. P. L. Green-fluorescent protein facilitates rapid in vivo detction of genetically transformed plant cells. *Plant cell rep.*, 1999, 18:707-714.
33. ELLIOTT, A. R. C. J.; BRETELL, R. I. S.; GROF, C. P. L. Agrobacterium-mediatd transformation of sugarcane using GFP as a screenable marker. *Aust J. plant physiol.*, 1998, 25:739-743.
34. MANICKAVASAGAM, M.; GANAPATHI, A.; ANBAZHAGAN, V. R.; SUDHAKAR, B.; SELVARAJ, N.; VASUDEVAN, A.; KASTHURIRENGAN, S. Agrobacterium-mediated genetic transformation and development of herbicide-resistant sugarcane (Saccharum species hybrids) using auxiliary buds. *Plant cell rep.*, 2004, 23(3):134-143.
35. D'HONT, A.; GLASZMANN, J. C. Unravelling the genome structure of polyploids using FISH and GISH; examples of sugarcane and banana. *Cytogenet genome res.*, 2005, 109(1-3):27-33.
36. PINTO, L. R.; OLIVEIRA, K. M.; MARCONI, T.; GARCIA, A. A. F.; ULIAN, E. C.; SOUZA, A. P. de. Characterization of novel sugarcane expressed sequence tag microsatellites and their comparison with genomic SSRs. *Plant breeding*, 2006, 125:378-384.
37. PINTO, L. R.; OLIVEIRA, K. M.; ULIAN, E. C.; GARCIA, A. A.; SOUZA, A. P. de. Survey in the sugarcane expressed sequence tag database (Sucest) for simple sequence repeats. *Genome*, 2004, 47(5):795-804.
38. GARCIA, A. A.; KIDO, E. A.; MEZA, A. N.; SOUZA, H. M.; PINTO, L. R.; PASTINA, M. M.; LEITE, C. S.; SILVA, J. A.; ULIAN, E. C.; FIGUEIRA, A. *et al*. Development of an integrated genetic map of a sugarcane (*Saccharum* spp.) commercial cross, based on a maximum-likelihood approach for estimation of linkage and linkage phases. *Theor appl genet*, 2006, 112(2):298-314.
39. OLIVEIRA KMP, L. R.; MARCONI, T. G.; MARGARIDO, G. R. A.; PASTINA, M. M.; TEIXEIRA, L. H. M.; FIGUEIRA, A. V.; ULIAN, E. C.; GARCIA, A. A. F.; SOUZA, A. P. Functional integrated genetic linkage map based on EST-markers for a sugarcane (*Saccharum* spp.) commercial cross. *Molecular breeding*, 2007, 20(3):189.
40. PAPINI-TERZI, F. S.; ROCHA, F. R.; VENCIO, R. Z.; OLIVEIRA, K. C.; FELIX, J. M.; VICENTINI, R.; ROCHA, C. S.; SIMOES, A. C.; ULIAN, E. C.; di MAURO, S. M. *et al*. Transcription profiling of signal transduction-related genes in sugarcane tissues. *DNA res*, 2005, 12(1):27-38.
41. SOUZA, G. M.; ROCHA, F. R.; MARQUES, J.; WACLAWOVSKI, A. J. Sequências de nucleotídeos de cana que promovem a expressão de genes. In: INPI. Universidade de São Paulo, Central de Álcool Lucélia, 2007.
42. SILVA-FILHO MCM, D. S.; BARATA, R. M.; MEDEIROS, A. H.; POMPERMAYER, P. Promotor do gene de defesa de cana-de-açúcar induzido por herbivoria e sua utilização. In: INPI, 2005.
43. VAN-SLUYS MAR, M.; ARAÚJO, P. G. Retroprom: método de obtenção e uso. In: INPI, 2004.
44. BRAGA, D. P. V.; ARRIGONI, E. D. B.; BURNQUIST, W. L.; SILVA-FILHO, M. C.; ULIAN, E. C. A new approach for control of Diatraea saccharalis (Lepidoptera: Crambidae) through the expression of an insecticidal CryIa(b) protein in transgenic sugarcane. *Proc. int. soc. sugarcane technol.*, 2001, 24(2):331-336.
45. NUTT, K. A.; ALLSOPP, P. G.; MCGHIE, T. K.; SHEPHERD, K. M.; JOYCE, P. A. Transgenic sugarcane with increased resistance to canegrubs. *Proc. austr. soc. sugarcane technol.*, 1999(21):171-176.
46. WENG, L. X.; DENG, H.; XU, J. L.; LI, Q.; WANG, L. H.; JIANG, Z.; ZHANG, H. B.; LI, Q.; ZHANG, L. H. Regeneration of sugarcane elite breeding lines and engineering of stem borer resistance. *Pest management science*, 2006, 62(2):178-187.
47. CHEN, P. H.; LIN, M. J.; XUE, Z. P.; CHEN, R. K. A. Study on genetic transformation of G/A gene in sugarcane. *Acta agriculturae universitatis jiangxiensis*, 2004, 26(5):740-743.
48. JOYCE, P. A.; MCQUALTER, R. B.; BERNAD, M. J.; SMITH, G. R. Engineering for resistance to SCMV in sugarcane. *Acta hort*, 1998, 461:385-391.
49. INGELBRECHT, I. L.; IRVINE, J. E.; MIRKOV, T. E. Posttranscriptional gene silencing in transgenic sugarcane. Dissection of homology-dependent virus resistance in a monocot that has a complex polyploid genome. *Plant physiol.*, 1999, 119(4):1187-1198.
50. MCQUALTER, R. B. H. R.; DALE, J. L.; SMITH, G. R. Virus derived transgenes confer resistance to Fiji disease in transgenic sugarcane plants. *Proc. int. soc. sugarcane technol.*, 2001, 24(2):584-585.

51. BRAGA, D. P. V.; ARRIGONI, E. D. B.; SILVA-FILHO, M. C.; ULIAN, E. C. Expression of the cry1Ab protein in genetically modified sugarcane for the control of Diatraea saccharalis (Lepidoptera: Crambidae). *J. new seeds*, 2003, 5:209-221.
52. SOARES-COSTA, A.; BELTRAMINI, L. M.; THIEMANN, O. H.; HENRIQUE-SILVA, F. A sugarcane cystatin: recombinant expression, purification, and antifungal activity. *Biochem biophys res commu*, 2002, 296:1194-1199.
53. SOUZA, A. P.; GASPAR, M.; SILVA, E. A.; ULIAN, E.; WACLAWOVSKY, A. J.; NISHIYAMA-JR, M. Y.; VICENTINI, R.; MENOSSI, M.; SOUZA, G. M.; BUCKERIDGE, M. S. Elevated CO_2 induces increases in photosynthesis, biomass, productivity and modifies gene expression in sugarcane. *Plant, cell and environment*.