From the genome to the factory

The Bioen Program helps to promote knowledge about sugarcane and biofuels

Fabrício Marques

In 2011, researchers at the University of São Paulo (USP) and the State University of Campinas (Unicamp) discovered approximately 10.8 billion sugarcane DNA base pairs. This is 33 times the result obtained after two years of work on the Sugarcane Genome project, which mapped the explicit genes of this plant and ended in 2001. This result is part of a thematic project to sequence sugarcane genes, coordinated by molecular biologist Glaucia Souza of USP, and scheduled to end in 2013. Given the complexity of the genome, 300 regions are already organized into sections larger than 100 thousand bases, which contain from 5 to 14 contiguous sugarcane genes, in a thematic project by geneticist Marie-Anne Van Sluys, who is also a professor at USP. The researchers want to go beyond the Sugarcane Genome project, both in terms of the quantity of data and in questions on the functioning of the genome for this plant, which has become synonymous with renewable energy. Studies of grasses like sorghum and rice have shown that in order to improve plant productivity, it is necessary to know how gene activity is controlled, which is a function of DNA segments called promoters.

This research is an example of how knowledge about sugarcane and ethanol has advanced in recent years. Through the FAPESP Program for Research on Bioenergy (Bioen), begun in 2008 and coordinated by Glaucia Souza, the Foundation has been sponsoring a major investigative effort to bring together researchers from different fields to raise productivity of Brazilian ethanol and to advance basic science and technology related to the generation of biomass energy.

One of Bioen's objectives is to overcome technological bottlenecks and enhance productivity of first generation ethanol, which uses a third of the sugarcane's biomass and is made by fermenting sucrose. Another goal is to participate in the international race to find second generation ethanol, produced from cellulose. This would make it possible to obtain this biofuel from bagasse and cane straw, as well as other raw materials. The program has five divisions. One of them is research on biomass, focused on improving sug-

Sugarcane plantation in Lins, in the state of São Paulo: increasing productivity

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periment that confirms the function in this calculation," says Ricardo Vêncio.

A software program called OneMap, which focuses on the use of genetic markers in improvement programs, exploring the genetics and physiology of the sugarcane, was developed by Augusto Garcia from the Luiz de Queiroz School of Agriculture (Esalq), at USP. "This is one of the major expectations for obtaining cultivars more rapidly," says Glaucia Souza. Every year the Sugarcane Technology Center (CTC) tests one million seedlings to find more productive plants. It takes twelve years for two or three promising varieties to appear.

Growth of biofuels in Latin America does not compromise food production

Manual harvesting in Cordeirópolis (SP), in 2010: studies aim to learn about and reduce economic and social impacts

"ENERGY PLANT"

Current sugarcane productivity, which averages 80 tons per hectare/year, could reach 381 tons per hectare/year with the development of varieties engineered for bioenergy production. These varieties would offer high levels of productivity, high sugar content, drought tolerance and resistance to pests and diseases, etc. This calculation was published in a scientific article written by a group of researchers from Bioen and the Hawaii Agriculture Research Center. According to the study, the so-called 'energy plant' is fast growing, requires fewer inputs to grow and can be harvested mechanically. To reach this unprecedented theoretical calculation, the study associated technological data on sugarcane production with information on the plant's physiology (photosynthesis, growth, development and maturation of the sugarcane), and used functional genomic studies to recommend possible target genes for improvement involving carbon partitioning, which is how the sugarcane distributes the carbohydrates that it produces from photosynthesis.

Published in the *Plant Biotechnology Journal* in April 2010, the study showed how the cane could be adapted with

arcane. The second is the biofuel manufacturing process. The third is linked to ethanol uses in automobile and aviation engines. The fourth is linked to studies on biorefineries, synthetic biology, sugar chemistry and alcohol chemistry. And the fifth division refers to the social and environmental impacts of the use of biofuels.

GENETICS

In the nearly four years of its existence, the results of the Bioen Program are tangible and varied. Due to their experience in genomics, Marie-Anne Van Sluys, from USP, and Anete Pereira de Souza, from Unicamp, led projects designed to generate a partial sequencing of two sugarcane cultivars (R570 and SP80-3280) and support the development of molecular tools capable of aiding in the understanding of this genome. One of the targets is the study of the so-called transposition elements, which are DNA regions that can be transferred from one region of the genome to another, and which may or may not leave behind a copy in the location where they had previously been. "Improvement programs may also benefit from access to molecular information with the potential to develop markers," says Van Sluys, a professor at the USP Bioscience Institute and one of the Bioen coordinators.

A project led by Ricardo Zorzetto Vêncio, from the USP Medical School at Ribeirão Preto, developed the pilot version of a software program designed to describe the functions of sugarcane genes. This project took an innovative approach, and was not limited to attributing functions already observed in similar sequences in different living beings to the sequence of genes in a given organism. Rather, the idea was to use algorithms that include the uncertainty contained in this association. "Instead of just saying that a gene has a specific function, we want to determine the probability of it having this function, and take into account different evidence such as the evolutionary relationship it has with other genes, or whether there is any ex-

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the use of biotechnology tools to create new varieties. The magazine editor, biologist Keith J. Edwards of the University of Bristol in England, sent the authors a letter informing them that the article had already been downloaded more than 1.6 thousand times, which is an extremely high amount for a specialized journal. Glaucia Souza, the article's main author, and a professor at the USP Chemistry Institute, notes that interest in this article reveals a new level of research on sugarcane. "A few years ago, it was hard to publish articles on sugarcane biotechnology because sugarcane was considered an exotic plant that only grows in sub-tropical regions. Today, since many countries are seeking to develop energy from biomass, sugarcane studies are becoming increasingly important," she says. The popularity of the article also shows how the Bioen Program is advancing in the field of genetic improvement. "We are managing to bring the genome to the field," says Souza.

Brazil occupies a special position in the international debate on biofuels, since it is the only country that has undergone a large-scale substitution of ethanol for gasoline. The Brazilian experience and studies in the bioenergy field conducted by institutions have enabled researchers in the state of São Paulo to participate in the Global Sustainable Bioenergy Project (GSB), an international initiative to discuss the feasibility of large scale global biofuel production and seek a scientific consensus on this issue. The GSB held meetings among scientists in the energy industry in five countries. The intention was to discuss whether it is possible to supply a substantial part of energy demand through the production of biomass without compromising the food supply, the preservation of natural habitats and the quality of the environment. It also sought to propose strategies for the transition to a new energy matrix; a matrix that is more balanced and renewable. The Latin American workshop was organized by FAPESP in March 2010, and discussed the technological challenges involved in obtaining ethanol from cellulose at a competitive price, the possibility of replicating the successful case of Brazilian sugarcane ethanol in other countries, and

3% is the annual productivity gain of Brazilian ethanol over the past four decades

60% of domestic ethanol production comes from the state of São Paulo

Sugarcane field in Guaíra (SP): biofuel production may increase agribusiness earnings the concern that competition from biofuels could compromise other agricultural crops. The resolution approved at this meeting emphatically affirmed the potential for expanding bioenergy production in Latin America without compromising food production, the environment and biodiversity. Lee Lynd, coordinator of the GSB Project, from the Thayer School of Engineering at Dartmouth College in the United States praised the willingness of the Brazilian researchers to look for sustainable ways to produce biofuels saying "Other countries should face the problem the way Brazil is facing it. For example, the United States is more defensive in relation to sustainability mechanisms, even though it leads in ethanol production."

AVIATION FUEL

One of the program's most important goals is the development of biofuels for aviation. Last April, representatives from FAPESP, Boeing and Embraer began a study into the main scientific, technological, social and economic challenges facing the commercial and executive aviation industries in Brazil in developing and adopting biofuels. This study, which is expected to last from nine to twelve months, will be guided by a series of eight workshops scheduled for this year to collect data with researchers and members of the biofuels production chain, as well as representatives from the aviation industry and the government. After concluding the study, FAPESP, Boeing and Embraer will conduct a joint research project on the most important topics indicated in the study and will issue a call for proposals to establish a commercial aviation biofuel research and development center involving the three institutions, based on the model of the Research, Innovation and Dissemination Centers (Cepids) at FAPESP, focused on developing research on the frontiers of knowledge. This research project is part of an agreement between the institutions, signed in October 2011, within the scope of Bioen, coordinated by Luís Augusto Barbosa Cortez, professor at Unicamp and adjunct coordinator of FAPESP Special Programs.

An innovative process for biokerosene production from different types of vegetable oil, which could make the fuel used in planes less polluting and cheaper, has already been developed at Unicamp's School of Chemical Engineering (FEQ). After extraction and refining, the oil is placed in a reactor with a specific quantity of ethanol and a catalyzer, which is responsible for accelerating the chemical reactions. "The greatest contribution to the process of obtaining biokerosene is the high purity content of the final product," says Rubens Maciel Filho, professor at FEQ and study coordinator. This method makes it possible to obtain oxygenated aviation biofuels with a freeze point that allows them to be used as jet fuel. Another process enables the propduction of hydrocarbonate type biokerosene through an international patent application that has already been filed and registered. Another of Maciel Filho's contributions is a project to create compounds with high economic value based on sugarcane substrates. This project has been obtaining good results in the production of acrylic acid and propionic acid from lactic acid. Maciel Filho says "It is possible to develop products with values 190 thousand times higher than that of sugar."

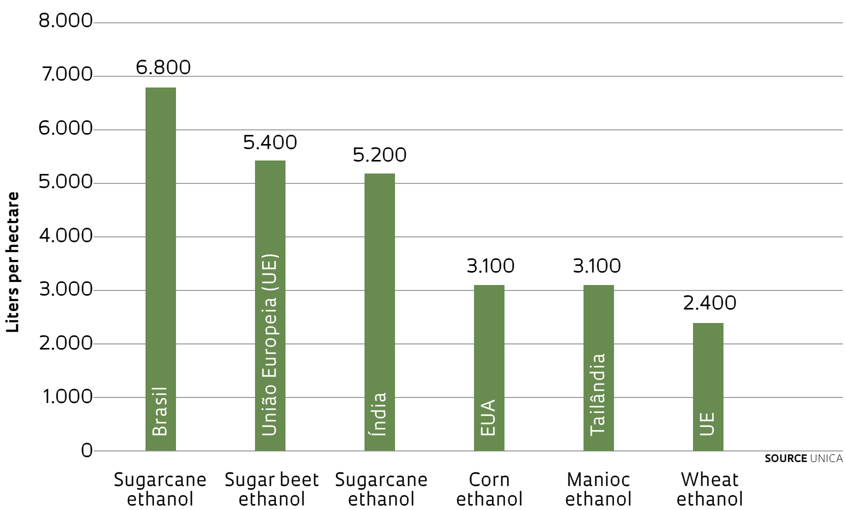
ECONOMIC AND SOCIAL IMPACTS

Bioen seeks to measure the social and economic impacts of a society based on biomass energy. "We have groups of researchers studying economic models capable of evaluating the changes in land use caused by large scale production of biofuels," says Glaucia Souza. "There are also studies on economic bottlenecks in the production of biofuels, agro-ecological mapping and impact on biodiversity, to mention a few examples," she says further. Aside from new knowledge, Souza emphasizes the potential contribution of biofuels to the fight against poverty, saying "Sugarcane contributes to rural development, but this crop brings little profit to the producers. Biofuel production can add value to agribusiness; for example, by allowing the industry to generate its own power and sell any excess, thus contributing to regional development and the fight against poverty."

Other countries question the fact that while Brazil is strong in food production, it uses land that is good for food production to plant sugarcane. However, this is not a real problem, according to a study by the group led by economist André Nassar of the Institute for International Trade Negotiations (Icone), which was financed by FAPESP through Bioen. According to

Ethanol productivity

Brazilian biofuel has the highest yields



Pile of sugarcane bagasse at Costa Pinto Plant in Piracicaba (SP): extraction of ethanol from cellulose may multiply production of this Brazilian fuel

PHOTO HÉLVIO ROMERO/AE

the scenario forecast by the institute, the area planted with sugarcane is expected to cover 10.5 million hectares in 2022, compared to 8.1 million hectares in 2009. This 30% growth in the size of sugarcane plantations will likely take place in Brazil's Southeastern region, principally in areas where cattle graze, and in the Center-West region, where it will replace traditional grain crops and pasture. "Today, cattle farmers produce more meat per hectare. In 1996, 6 million tons of meat were produced on 184 million hectares. Ten years later, production totaled 9 million tons of meat, produced on 183 million hectares. During this period, the herd jumped in size from 158 million to 206 million," says researcher Leila Harfuch from Icone. "The amount of pasture land is expected to decrease by some five million hectares between 2009 and 2022, accommodating part of the expansion in grains and sugarcane."

CLIMATE CHANGES

Some studies focus on how sugarcane will respond to climate change. This knowledge may help to develop varieties that are more resistant to possible increases in precipitation and heat, as well as the expected increase in pests. We already know that a high concentration of carbonic gas produces an increase in photosynthesis and in the volume of biomass, which enables us to predict an increase in productivity. "On the other hand, little is known about the mechanisms of hormonal control, their relation to carbon metabolism and the associated networks of genetic transcription," says Marcos Buckeridge, a professor from the Botany Department at the USP Life Sciences Institute, and scientific director of the Brazilian Bioethanol Science and Technology Laboratory (CTBE). "Knowledge of these processes has the potential to show which aspects of sugarcane metabolism could be modified to produce varieties with the potential to adapt to climate change," Buckeridge states. The search for biofuel production sources that are not harmful to nature, such as obtaining ethanol from polysaccharides from native tree seeds grown among the sugarcane plantations, is also underway. "Agro-forest systems can represent a new model capable of increasing renewable energy production, in a manner compatible with bringing social benefits while minimizing environmental impact," says Buckeridge.

The bagasse is burned to generate energy at the plants and used in animal feed production

CELLULOSE ETHANOL

Only sucrose, which represents a third of the sugarcane biomass, is used to produce sugar and fuel alcohol. Brazil in fact uses the bagasse to generate energy at plants and produce animal feed, which also resulted in a noteworthy gain in efficiency. The greatest challenge faced is to also convert the cellulose present in the bagasse and sugarcane straw into ethanol. This requires enzymatic hydrolysis or physical-chemical processes that would enable the hemicellulose and cellulose carbon to also ferment. Domain over cellulose usage technologies is at the center of the global race to produce energy from renewable sources. Today this process is not yet economically feasible. If researchers find ways to reduce costs, the use of cane cellulose could dramatically increase ethanol production.

One highlight in the search to obtain ethanol from cellulose is a project that evaluates how to break down the cell wall resistance of lignified plants like sugarcane by using enzymatic hydrolysis. Lignin is a macromolecule associated with cellulose in the cell wall of plants whose purpose is to provide strength and resistance. Finding a way to break it down in order to obtain ethanol from cellulose is challenging. "To understand how the removal of lignin can reduce cell wall recalcitrance, both commercial and hybrid varieties of sugarcane have been evaluated with contrasting lignin contents," says Adriane Milagres, a professor at the Lorena Engineering School of USP, one of the project's coordinators. "When materials are treated with selective methods, removal of 50% of the original lignin raises the conversion level of the cellulose to 85-90%." According to Adriane, the project also focuses on assessing which enzymatic cocktails are best suited to obtaining a high level of enzymatic hydrolysis using sugarcane bagasse that has been pre-treated with the lowest possible severity. "This is because the initial lignin content of the selected plants is already low," she explains.

FIRST GENERATION

Brazil enjoys significant advantages in first generation ethanol production, made from sucrose fermentation. Sugarcane productivity has increased greatly over the past 30 years, at an average rate of about 4% a year. This productivity could grow even more if some technological challenges are overcome. A project conducted by researchers from the Polytechnic School of the University of São Paulo (USP) in collaboration with research groups from the Federal University of Santa Catarina (UFSC) and Delft University of Technology in Holland demonstrated that there is room for advancement through genetic improvement of the microorganisms used in the conventional process of industrial production of biofuel by fermentation, using yeast from the species *Saccharomyces cerevisiae* to convert the sucrose (sugar) into ethanol.

By using metabolic engineering strategies, the group was able to increase ethanol yield on a bench scale by 11% over sucrose using genetically modified yeast. "This experiment has not yet been tested in an industrial environment. But taking into account the large volume of current production, an increase of just 3% in the alcohol fermentation yield would mean an increase of one billion liters of ethanol per year today, just in Brazil, using the same amount of sugarcane. This alone would be an extraordinary gain," says Andreas Karoly Gombert, professor at USP's Polytechnical School and project coordinator. The project began through the initiative of Professor Boris Ugarte Stambuk of UFSC, who developed in his laboratory and patented a metabolic engineering strategy that alters the topology and energy of sucrose metabolism in the yeast *Saccharomyces cerevisiae*.

INTERACTION WITH THE PRIVATE SECTOR

A leap forward in the level of interest in sugarcane and ethanol research occurred in April 1999, with the advent of the Sugarcane Genome project, which mapped 250 thousand functional sugarcane gene fragments (see report on page 54) and was characterized by private sector interaction. After the program ended, the interest in this research on the part of companies did not drop off. In 2006, in partnership with the BNDES, FAPESP signed an agreement with Oxiteno, from the Ultra group to develop cooperative projects that investigated topics ranging from the enzymatic hydrolysis process of sugarcane to obtain sugars to the production of cellulose ethanol. The following year, Dedini Indústrias de Base signed an agreement with FAPESP to finance projects on techniques to convert sugarcane bagasse into ethanol. In early 2008, FAPESP and Braskem also signed an agreement to develop biopolymers.

The growing economic importance of sugarcane helped increase interest among the researchers. In 2009, Brazil

Boilers at the Santa Elisa Plant in Sertãozinho (SP): electricity production from sugarcane bagasse harvested 569 million tons of sugarcane, almost double the 1999 harvest, according to data from the Sugarcane Industry Union (Unica). Half of this production, or about 27 billion liters, was transformed into ethanol, making Brazil the second largest global producer of this fuel. First place is held by the United States, which makes ethanol from corn and is heavily subsidized by the government. São Paulo accounts for 60% of Brazilian domestic production. The productivity gains have exceeded 3% in the past 40 years as a result of genetic improvements to sugarcane. Thanks to ethanol, Brazil is the only country that has replaced the use of gasoline on a large scale. In the state of São Paulo, 56% of energy comes from renewable sources, of which 38% is sugarcane.

One result of Bioen was the establishment of the São Paulo Bioenergy Research Center (CPPB) in 2010. Its purpose is to encourage interdisciplinary research and increase the contingent of researchers involved with this topic, led by FAPESP, the state government of São Paulo and the three state-run universities of São Paulo. According to this agreement, the state government has already invested and will continue to invest resources to create research infrastructure at USP, Unicamp and Unesp. In turn, the universities will hire professors and researchers, and FAPESP will be responsible for investments to finance the research. USP created the Center for Research on Bioenergy and Sustainability (NAPBS); Unicamp created the Bioenergy Laboratory (Labioen); and Unesp created the Bioenergy Research Center, (Bioen-Unesp). "With the funds released by the state government in 2010 and 2011, the three universities are already building the infrastructure at their respective campuses. The process of hiring new professors and researchers is already well underway," says Luis Cortez, a professor at Unicamp and center coordinator. Another initiative linked to the CPPB is the Synthetic Biology and Systemic Biomass Center at USP, envisioned in 2008 by Glaucia Souza, Marie-Anne Van Sluys and Marcos Buckeridge. This center will bring together researchers from the institutes of Chemistry, Mathematics and Statistics, Biosciences, Biomedical Sciences and the Polytechnic School. Synthetic biology combines biology and engineering to construct new biological functions and systems. "The intent is to invest in an area in which Brazil does not yet have a great deal of expertise and involve researchers from different areas," Glaucia Souza said. ■

EDUARDO CESAR

BIOEN PROGRAM NUMBERS

49 research projects underway
11 research projects concluded
Total concession: R$64.170.267,56
108 grants in Brazil underway
102 grants in Brazil concluded
Total concession: R$14.008.313,89

SCIENTIFIC ARTICLES

1. WACLAWOVSKY, A. J. *et al.* Sugarcane for bioenergy production: an assessment of yield and regulation of sucrose content. **Plant Biotechnology Journal**. v. 8, 263-76. Published on-line 19 Feb. 2010.

2. BASSO, T. O. *et al.* Engineering topology and kinetics of sucrose metabolism in Saccharomyces cerevisiae for improved ethanol yield. **Metabolic Engineering**. v. 13, 694-703, 2011.

FROM OUR ARCHIVES

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