BIOEN

BRAZILIAN RESEARCH ON BIOENERGY

- AGRONOMY
- AGRICULTURAL ENGINEERING
- BOTANY
- ECOLOGY
- ECONOMY

FAPESP

BIOENERGY: THE BRAZILIAN EXPERIENCE

Bioenergy is an important clean energy alternative. The Brazilian experience has shown that sugarcane is a highly sustainable crop for bioenergy production

Brazil is outstanding as the world's most intensive user of bioethanol as an alternative to gasoline for powering transport. Total bioethanol production in 2014/15 corresponds to 28.4 billion liters in 366 plants. In 2015/16 around 58 per cent of the 590 million tons of sugarcane will be used for ethanol and 42 per cent for sugar production. The total sugarcane planted area in Brazil is around 10 million hectares (ha). This accounts for only around 6-7 per cent of total area devoted to agriculture and 1.1% of the country's total area.

CLEAN AND CHEAP ENERGY

Brazil and the USA produce more than 80 per cent of world's ethanol. Brazilian bioethanol production costs are the cheapest in the world. In addition to low production costs, ethanol produced from sugarcane in Brazil has another important advantage: in Central-South Brazil, only 1 unit of fossil energy is consumed for each 9-10 units of energy produced by ethanol from sugarcane. The use of ethanol as a biofuel also reduces carbon emissions: when ethanol is used in substitution to gasoline, there is a 76% reduction in GHG emissions with a simultaneous decrease in SO_2 emission. Since 2003, Brazil's use of sugarcane ethanol has avoided the emission of 242 million tons of carbon dioxide.

THE BRAZILIAN MODEL

Sugarcane was introduced in Brazil in 1532 by the Portuguese and it has been cultivated here ever since. The research that started in the first half of the 20th century has gradually improved this crop, with the development of new varieties of sugarcane that enhanced the yields of both culm and sucrose. During the 1970s, the "Brazilian model" of producing sugar and ethanol together brought important technical improvements and enabled an outstanding increase in competitiveness in the international market for sugar and ethanol.

Ethanol has a great potential to become a worldwide replacement or complement for gasoline. In 2014, 88.2% per cent of the 2.9 million cars and light vehicles sold in Brazil were of the flex-fuel type, which run on any proportion of ethanol and gasoline mixture. Considering the existing opportunities related to biofuels, it is expected that R&D will lead to optimization not only of the sucrose content of the plant, which is relevant for sugar production, but also of the overall energy content (biomass yield).

Traditionally, bioethanol is produced from fermentation of the extracted juice and the molasses resulting from the sugar industry. But, in addition to sucrose, there is also a relevant and extractable amount of energy in the glycosidic linkages of cellulose and hemicelluloses, which account for nearly two thirds of the sugarcane plant biomass (bagasse and leaves).

BIOEN FACTS AND FIGURES

US$ 167 million	**research expenditures by FAPESP and partners**
13	**co-funding partners**
7	**private companies co-funding projects**
467	**scholarships –** 89 ongoing and 378 completed
206	**research projects –** 66 ongoing and 140 completed
300+	**researchers involved**
21	**fields of knowledge**
920+	**scientific publications**
17	**patents filed**

THE ENERGY-CANE

With the possibility of cellulosic ethanol production, scientists envisage a new option, the "energy-cane" and not only the "sugarcane", in which the whole biomass is of interest. The development of hydrolysis and/or gasification processes could be applied to the residual bagasse and trash, transforming the lignocellulosic biomass into ethanol or other liquid fuel, using fermentation of the generated sugar (hydrolysis) or the catalytic synthesis of the generated gas (gasification). It is expected that ethanol output might increase from the current 7,000 to about 12,000 liters per hectare-year, i.e., between 60 and 70 per cent.

In 2014, Brazil burned bagasse to produce energy equivalent to 20,823 GWh. This is enough to meet the internal needs of the mills and the surplus energy can be fed into the country's power grid. Investments in co-generation efficiency and power distribution aim at increasing even further the energy supply from bioelectricity. Also, most of the straw still remains in the field after sugarcane harvest and can be, at least partially, transformed in energy. Bioelectricity from sugarcane can contribute to 18% of the country's energy demand. Additionally, sugarcane offers options in development: alternative routes to produce liquid fuels from lignocellulosic materials for road, maritime and aviation transportation could open new possibilities for an industry that is already energy-efficient.

The growing demand for bioenergy brings new scientific challenges in terms of R&D and assessment of the environmental and social impacts related to the expansion of sugarcane cultivation.

FAPESP'S BIOENERGY PROGRAM – BIOEN

To respond to the increasing need for R&D in the area of bioenergy the São Paulo Research Foundation (FAPESP) created a Bioenergy Program (BIOEN). FAPESP is one of Brazil´s leading public funding agencies for scientific research.

The FAPESP Program for Research on Bioenergy, BIOEN, aims to link public and private R&D, using academic research institutions and industrial laboratories to advance and apply knowledge in fields related to ethanol production. BIOEN is organized in five divisions: Biomass, Biofuel Technologies, Biorefineries, Engines, and Impacts and Sustainability.

Over 300 researchers support BIOEN activities with funds in the order of US$ 167 million. BIOEN is increasingly multidisciplinary including projects from 21 areas of knowledge. This highlights the broad scope of questions addressed. The BIOEN Program consolidated the community in an active network of experts leading 206 research projects and 467 scholarships in 21 institutions in the State of São Paulo, in collaboration with other institutions in Brazil and in 29 countries. Since 2008, BIOEN has generated more than 920 scientific publications. Additionally, more than 300 thesis and dissertations with important contributions for the advancement of science and the industry have been concluded. This represents an important step in generating human resources to increase the potential and the number of qualified professionals working in this field.

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SIMULATING LAND USE AND AGRICULTURE EXPANSION IN BRAZIL: FOOD, ENERGY, AGRO-INDUSTRIAL AND ENVIRONMENTAL IMPACTS

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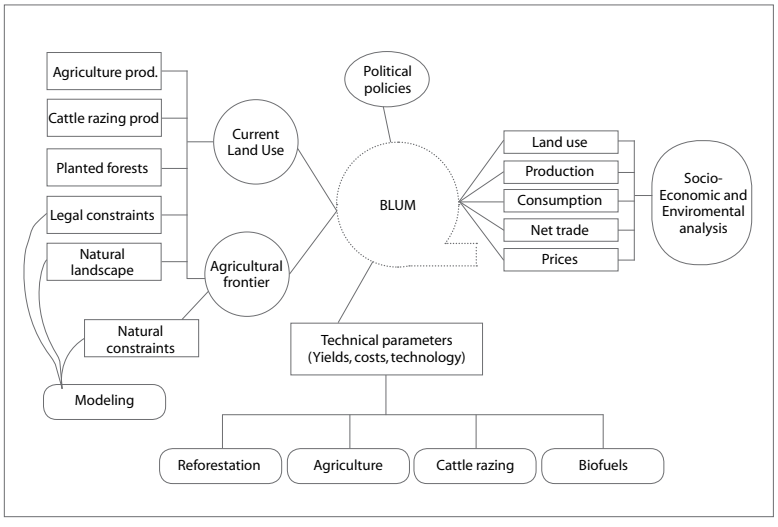
FAPESP Process 2008/56156-0 | Term: Aug 2009 to Dec 2010 | Thematic Project

Brazilian agro-industrial output has experienced a cycle of strong expansion. The awareness that the era of cheap oil has reached an end and the growing energy concerns made the search for viable alternatives to hydrocarbon-based fuels into a global priority. The role of biofuels and bioenergy has shifted from a solution for reducing Green House Gases (GHG) emissions to the cause of rising food prices, environmental degradation and even threatening global food security.

At the same time, increasing income in developing countries has brought millions of people to increase their levels of food consumption, boosting international demand. National demand for agricultural products has also increased, pushed by biofuels (mainly ethanol) and grains for food and feed production. Within this context, Brazilian agricultural industry has responded to the world's need for food, feed, fiber and biofuels by both improving its yields and expanding cultivated area and investments.

To analyze this issues properly, in partnership with FAPRI (Food and Agricultural Policy Research Institute), ICONE developed an economic model called Brazilian Land Use Model (BLUM). The present application proposes the use and development of the BLUM for the food versus fuel debate. The BLUM will be sufficiently general to forecast all the main agricultural products in the entire national territory and, at the same time, detailed enough to deal with the (very) different regional characteristics of national territory.



Methodological diagram of Brazilian Land Use Model

With this project, ICONE wants to answer to the following questions: what will be the growth in sugarcane planted area to respond to a growing demand? How will it affect land use change? What will be the main positive and negative environmental impacts? Is there any policy to be implemented by the government or the private sector to improve the rational use of the land?

The research on Indirect Land Use Change (ILUC) methodology is under continuous development. The BLUM has been improved to generate results on land use change instead of only land allocation. However, the need for additional improvements has been identified, which is the aim of this project.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

ICONE has been working in the BLUM since 2008 and have reached many advances and has established an intelligence network with specialists of several university research institutes in Brazil and abroad. The national network includes the Censoring Remote Center, at Federal Universiy of Minas Gerais (UFMG), various centers of Brazilian Agricultural Research Corporation (EMBRAPA), National Institute for Space Research (INPE), Center for Alternative Energy of Fortaleza (Centro de Energias Alternativas de Fortaleza), Sugarcane Technology Center (Centro de Tecnologia Canavieira – CTC), Luiz de Queiroz Agriculture School (ESALQ/USP), Laboratory of Remote Sensing (Laboratório de Sensoriamente Remoto) of Federal University of Goiás (LAPIG), among others. At the international level, we can mention the Center for Agricultural and Rural Development like the main partner and the World Bank (WB).

Main improvements developed so far in the model:

- ICONE innovated methodologically in, at least, four aspects. First, using satellite images (using GIS – Geographic Information System), which contributes to the incorporation of data of potential area for expansion of agribusiness, considering physical, environmental and legal restrictions – AgLUE-BR model (Esalq-USP). Second, projecting endogenously pasture area, which has not been considered in other land use models. Third, ICONE separates winter crops, which are planted after a primary crop in the same season. Finally, BLUM treats Brazilian agriculture dynamic considering six different regions and its peculiarities, which is essential for more accurate land use change analysis.
- Modification of the structure of the BLUM in order to respect the economic conditions of homogeneity, symmetry and adding up.
- BLUM was integrated to the international model and included into FAPRI's Outlook 2010.

Improvements to be developed in FAPESP project:

- Use deforestation data in the *Cerrado* Biome and land use, in order to estimate more trustworthy parameters (agricultural expansion over vegetation and substitution among different agricultural activities in the *Cerrado* Biome), based on empirical data.
- Estimate parameters using secondary data and satellite images for the different regions in the model.

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REGULATION OF PLANT GROWTH BY THE TARGET OF RAPAMYCIN (TOR) PATHWAY

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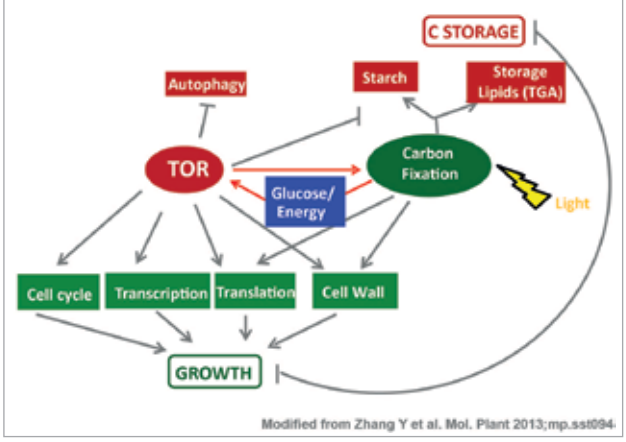

Figure 1. TOR controls several biological processes including transcription, translation and central energy metabolism, which collectively contribute to growth

Rising demand for food and fuels makes crucial to develop breeding strategies for increasing crop yield/biomass. Plant biomass production is closely linked to growth and depends on a tight regulation of a complex signalling network that integrates external and internal factors. One of the key regulators of growth in all eukaryotes is the Target of Rapamycin (TOR) kinase pathway. TOR integrates environmental cues such as energy status or nutrient availability into cell growth and proliferation. In the model plant *Arabidopsis thaliana* the levels of TOR expression are positively correlated with growth. However little is known about the mode of action of TOR controlling growth in photosynthetic organisms. Over the last years, the main goal of our group has been to understand the molecular mechanisms involved in growth and metabolism of plants modulated by TOR. Recently, experimental evidences revealed that the disruption of this essential protein leads to severe growth phenotypes, which are based on the essential reprogramming of the plant metabolism. The identification of plant TOR as a potent metabolic switch offers a novel route for biotechnological optimization of plant carbon partition for bioenergy production. However, there are still many gaps about how TOR controls those metabolic decisions and regulates growth, especially in C_4 plant species used for biofuel production due to their capability to produce biomass at faster rates. Therefore, our aim is to use a systems-oriented approach to dissect TOR signalling network involved in plant growth and metabolism using both C_3 and C_4 model species (Arabidopsis and *Setaria* ssp. respectively). Such approach is expected to reveal how plants adjust their metabolism ratios in order to simplify the conversion of plant biomass into biofuels. Moreover, the comparison between these two photosynthetic systems will allow further comprehension of the growth regulatory network mediated by TOR contributing to the efforts in bioengineering C_4 features in C_3 crops. From a technological standpoint, the proposal includes the establishment of a metabolomic oriented lab that will serve as facility unit for the community and also provide the centerpiece for human resources training.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We first developed a gas chromatography coupled to a mass spectrometry (GC-MS) platform for profiling primary metabolites. This platform allowed us to create a database of polar metabolites from several plant species, including *Arabidopsis thaliana*, *Setaria italica*, *Setaria viridis*, and sugarcane.

In order to investigate the relationships between environmental factors and TOR signaling pathway in carbon allocation, we are performing several analyses in a comparative manner, using model plants with different mechanisms of photosynthesis: *Arabidopsis thaliana* (C_3) and Setaria viridis (C_4). To quantitatively and kinetically control the TOR levels in both species in a similar way, seedlings were grown hydroponically for about 12 days prior to treatment with the ATP-competitive TOR kinase inhibitor AZD-8055(TOR repressor) or DMSO (control). Our data suggested a clear ceased growth phenotype of Setaria and Arabidopsis after 2 days of drug treatment. Biochemical and metabolomics analysis of Setaria and Arabidopsis seedlings treated with AZD revealed differences in carbon allocation mechanism mediated by TOR. Whereas Arabidopsis seedlings treated with AZD presented a starch excess phenotype (elevated rates of starch synthesis independently from the time of drug administration), no differences in starch content were observed in Setaria seedlings. In order to validate these data, we also generated inducible artificial micro RNA transgenic lines targeting the TOR in *S. viridis*.

We are currently working on two hypotheses about TOR mode of action i) directly phosphorylation enzymes of starch synthesis such as SPS and ii) regulation of other proteins (e.g., kinases) that can modulate the activity of enzymes involved in starch metabolism (e.g, SnRK1). These preliminary results will be useful as starting point to characterize the mode of action of TOR on starch metabolism and to dissect its function in plants.

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SOIL SOLUTION DYNAMICS IN THE IRRIGATED SUGARCANE CROP BY SUB-SURFACE DRIP IRRIGATION

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FAPESP Process 2012/21151-4 | Term: Feb 2013 to Jan 2016

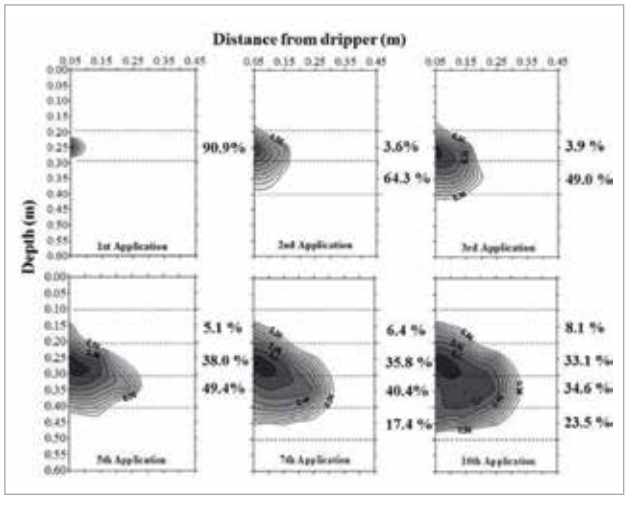

Figure 1. Comparison of the profile electrical conductivity (dS m^{-1}) at the end of each application on soil solution flow of 1.0 L h^{-1}

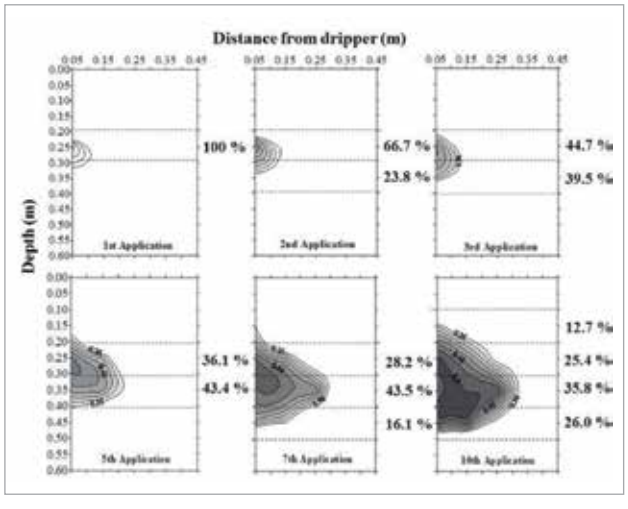

Figure 2. Comparison of the profile electrical conductivity (dS m^{-1}) at the end of each application on soil solution flow of 1.6 L h^{-1}

The inappropriate use of water resources in agriculture, in search of high yield has caused negative impacts on water reserves, decreasing the efficiency of irrigated and fertirrigated productive systems. Due to the potential of subsurface drip irrigation, combined with the need to research with the irrigated sugarcane crop (*Saccarum offinarum* L.), has been increasing demand for information about this technique, especially, with relation to the wet bulb dimension and position in the soil and the water and nutrients distribution available to plants. Thus, this project aims to study, by means of Time Domain Reflectometry (TDR), under controlled conditions, the solution dynamics in a sandy soil with and without the sugarcane crop installation, for two application rates: 1.6 and 3.8 L h^{-1}. To achieve the aims, the project will be divided into three stages: first, it will be studied the we bulb formation, evaluating the effects of the interaction between water-soil-solute in the solution soil distribution and storage; in the second, the estimation of nutrients absorption at each stage of plants development; and in the third, the comparison of results obtained experimentally with those simulated by means of Hydrus 2D program, for the soil solution dynamics. It is hoped this project a better understanding about the water and nutrients behavior in wet bulb and its availability to irrigated sugar cane by subsurface drip irrigation.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In Figures 1 and 2 were observed the electrical conduction profiles, by virtue of the large number of graphics, wetted soil volumes chosen to represent the profiles were 1, 2, 3, 5, 7, 10 L; between the treatments (1.0 to 1.6 L h^{-1}). Each isoline represented by a shade of black, has value of 0.02 dS m^{-1}, it is possible to analyze the formation of the wet bulb by advancing wetting front in the soil.

The results showed that the flow of drippers did not have a differential effect on the formation of the wetted soil volume. According to the values of electrical conductivity readings obtained with the TDR, we analyzed the wetted soil volumes were characteristic forms were described by ZUR (1996), with rounded and elliptical shapes.

Analyzing the wetted soil volumes in both flows were greater horizontal displacement at the beginning, but after the fifth application displacements of the two directions were equal, and getting the last application the average values of 0.50 m (vertical) and 0.38 m (horizontal).

Regarding storage, the highest observed for the two treatments in the 0.30-0.40 m layer, with mean values of 23.3 and 25.3 %, respectively. These monitored for both flows, the largest solute storage began in the third layer (0.20-0.30 m), moving to the fourth layer (0.30-0.40 m) from the third application, noting that there is a relation between the solute storage into the wet bulb, because with the increase of the volume applied, there was a direction of the solute storage to the deeper layers.

Look at a distribution of soil solution, being a higher concentration near the emitter and thus a reduction thereof, when it approaches the wetting front. As can be noted the presence of solute in the wet bulb into average displacement horizontal of 0.37 m vertically from the emitter of 0.18 m.

It was possible to know the dynamics of soil solution with the formation of spherical wet bulbs having the same behavior for the two flows. It was noted that a greater storage of electrical conductivity was coming from the drip zone and possessed a distribution gradient with the highest concentration near the emitter and lowest near of the wetting front.

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CHANGE IN CARBON BALANCE IN GRASSES IN ORDER TO INCREASE SUGAR PRODUCTION

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In recent years, the substitution of fossil fuels by alternative sources of energy has been intensely discussed. Great efforts have been focused on research of renewable fuels and ethanol emerges as a promissory solution for this subject. Together with the idea of substitution of fossil fuels comes in focus the environmental issues and sustainability in our way of living. The increase in areas cultivated with sugarcane could, therefore, results in great environmental impacts, leading us to search alternatives to increase ethanol production. Thus, genetic engineering might contribute significantly to improve productivity, increasing ethanol production without expanding cultivated areas. This project propose the increase in sugar levels in sugarcane and in the model plant *Setaria italica* through transgenic technologies, focusing as strategies changes in redox status of the mitochondrial and changes in the synthesis of other fermentable sugars, as a way to improve ethanol production by area. These strategies can result not only in transgenic events with agronomic potential, but would bring also important information about the relationship of cellular respiration and the use of carbon in sugarcane, bringing new insights of research in this area. Moreover, the characterization of carbon use (such as conversion in fermentable sugars) is still neglected and might contribute to breeding programs. Preliminary results show that varieties, with different harvesting time, present different metabolic profiling in mature and immature internodes, indicating that the carbon dynamics and sugar accumulation differ among genotypes.

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INTEGRATING PHYSIOLOGICAL, MORPHOLOGICAL AND ANATOMICAL TRAITS TO UNDERSTAND THE DIFFERENTIAL SUCROSE YIELD IN SUGARCANE GENOTYPES

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Our purpose was to understand some aspects of sucrose yield by addressing physiological, morphological and anatomical traits. This strategy will increase the subjacent knowledge about sucrose yield and let us to a more complex scenario about this important agricultural theme. In fact, improvement in the understanding of ecophysiological aspects related to phytomass production and sucrose yield of sugarcane is an essential condition to the development of the Brazilian sugar-ethanol sector. However, little is known about the relationship between plant traits and sucrose yield in the Brazilian sugarcane genotypes, an important issue for sugarcane breeding programs and modeling. Considering that sugarcane breeding programs have periodically launched many productive cultivars, the following questions are relevant for the Brazilian agriculture: why do sugarcane genotypes accumulate differential sucrose amount in stalks? Is it a physiological and/or morphological and/or anatomical matter? Is it related to the source, sink or source-sink characteristics? Is the high sucrose yield related to differential sensitivity of sugarcane genotypes to stressful conditions found during the winter season? Is the differential sensitivity found in a specific phenological stage or in entire crop cycle? About those questions, an integrated approach for studying sugarcane plants is essential to study plant growth and sucrose accumulation. This challenge was addressed in sugarcane genotypes with differential sucrose yield and canopy architecture growing under field and controlled (greenhouse and growth chamber) conditions. Several physiological, morphological and anatomical traits related to photosynthesis and sugar metabolism were evaluated, with this project being the first step towards an integrative and interdisciplinary approach to understand the ecophysiology of Brazilian sugarcane genotypes.

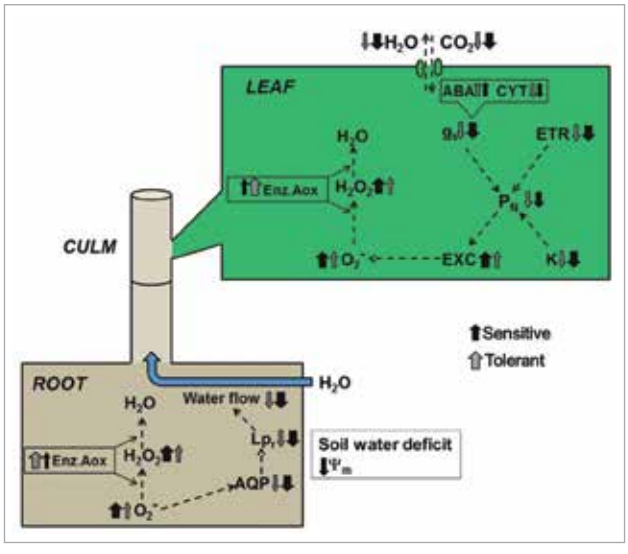

Figure 1. Physiological responses of sugarcane genotypes with differential sensitivity to water deficit. ABA = abscisic acid; CYT = cytokinin; g_s = stomatal conductance; ETR = apparent electron transport rate; P_N = net CO_2 assimilation; K = apparent carboxylation efficiency; EXC= relative excess of light energy; O_2-= superoxide; H_2O_2 = hydrogen peroxide; Enz.Aox = antioxidant enzymes; AQP = aquaporins; Lpr = root hydraulic conductance; Ψm, soil matric potential. Filled arrows indicate increase or decrease in activity or concentration in drought-tolerant (gray) and -sensitive (black) genotypes

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The water deficit is one of the main environmental constrains causing significant reduction of sugarcane yield. In such scenario, drought-tolerant genotypes are fundamental for maintaining sucrose yield and improving resource use efficiency. Herein, we present a comparative analysis of the physiological responses found in sugarcane genotypes that are tolerant or sensitive to water deficit through a general model of reaction to water deficit (*Figure 1*), linking the functioning of roots and shoots. Low soil water availability reduces the soil water potential (Ψm) and then limits water uptake and decreases root water content. Signals of oxidative damage in roots are detected either in tolerant or sensitive genotypes, being more intense in the sensitive ones. Reactive oxygen species (ROS) reduce the aquaporin (AQPs) activity, causing reduction in root hydraulic conductance (Lpr). Such negative impact of water deficit is also higher in the sensitive genotypes (*Figure 1*), which also present low activity of antioxidant enzymes (Enz.Aox) such as superoxide dismutase, catalase and ascorbate peroxidase in roots. As consequence, water flow to shoots is more affected in the sensitive genotypes, with plants presenting reductions in leaf relative water content. Other important responses associated to water deficit were building up of abscisic acid (ABA) concentration and decreases in cytokinin (CYT) concentration in leaves with consequent reduction of stomatal aperture (g_s). Water deficit also affects chloroplasts and the photosynthetic apparatus, with plants showing reduction in the apparent carboxylation efficiency (K). Decreases in g_s and K lead to low net CO_2 assimilation (P_N), being such responses more intense in the drought-sensitive genotypes (*Figure 1*). Low P_N associated with the continuous exposure to solar radiation increases the relative excess of energy at the photosystems II (EXC), increasing the energetic pressure on the photochemical apparatus. Leaf ROS production is then increased, which is accompanied by increases in the activity of antioxidant enzymes and thermal dissipation of energy. Although both processes are present either in sensitive or tolerant genotypes, they are more enhanced in the latter ones (*Figure 1*). As a conclusion about the physiological responses to water deficit, tolerant genotypes present higher water transport than the sensitive ones due to the root ROS detoxifying mechanism. These responses associated with the lower sensitivity of photosynthesis to drought are the physiological bases for maintaining sugarcane growth under low water availability.

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INTERCROP OF OIL SEEDS IN SUGARCANE: AGRICULTURAL PERFORMANCE AND BASIS FOR CROP MODELING

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FAPESP Process 2014/18912-9 | Term: Jan 2015 to Dec 2016

Figure 1. Intercropping sugarcane + crambe (pre-flowering)

Based on the current international high demand, the increasing demand for food, and the environmental issues related to intercropped farming system, this project aims to assess growth, development, and adaptability of canola and crambe, and propose a new farming system for intercroping oil seeds in sugarcane plantations. Experimental plots of oil seeds and sugarcane will be installed, as well as areas of intercropping sugarcane + crambe and sugarcane + canola. Additionally, meteorological measurements and soil conditions in each production system. It is believed to possible generating a new farming system especially useful in the reform areas or those unsuitable for it due to mechanized harvesting, besides adding production of oilseeds in their own sugarcane areas. In this system the cycle of annual crops happen during regrowth of ratoon. The results will be further exploited for studies on crop modeling of crambe and canola and sugarcane in a intercropping system.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Along the first season, the experiment was fully installed ina the area of the Biosystems Engineering Department at Esalq/USP, in Piracicaba/SP. We used the ver. RB867515 as the major sugarcane cultivar in Brazil and sowed the consortium sowing oilseeds crambe (FMS Brilhante) and canola (Hyola 61 and Hyola 401) by April 15th, 2015. In order to evaluate the intercropping, single sugarcane, crambe and canola plots were also sowed. Oilseed plots were not harvested yet and sugarcane plots are still developing to be harvest in 2016. Results are not enough for any preliminary recommendation or specific findings. However, the vigorous growth and development of oilseed and sugarcane in the farming systems pointed out for the feasibility of the proposal.

Figure 2. Crambe plots – flowering

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MICROBIAL DIVERSITY IN SOILS CULTIVATED WITH SUGARCANE IN THE STATE OF SÃO PAULO: A BIOGEOGRAPHIC APPROACH

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FAPESP Process 2011/03487-2 | Term: Jun 2011 to May 2013

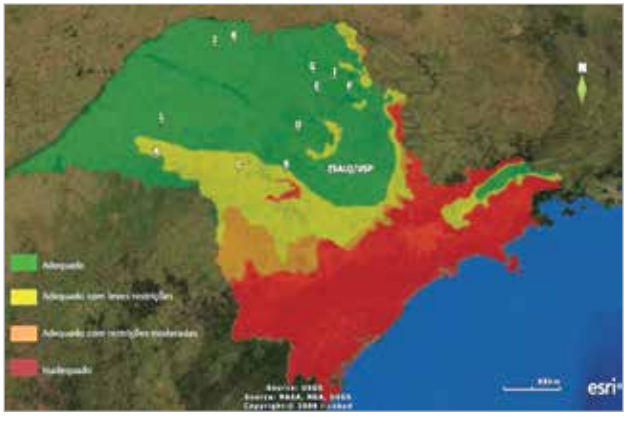

Figure 1. Location of the sugarcane fields (indicated by letters A to K) targeted along the sampling expeditions to compose the soil collection used in the present study

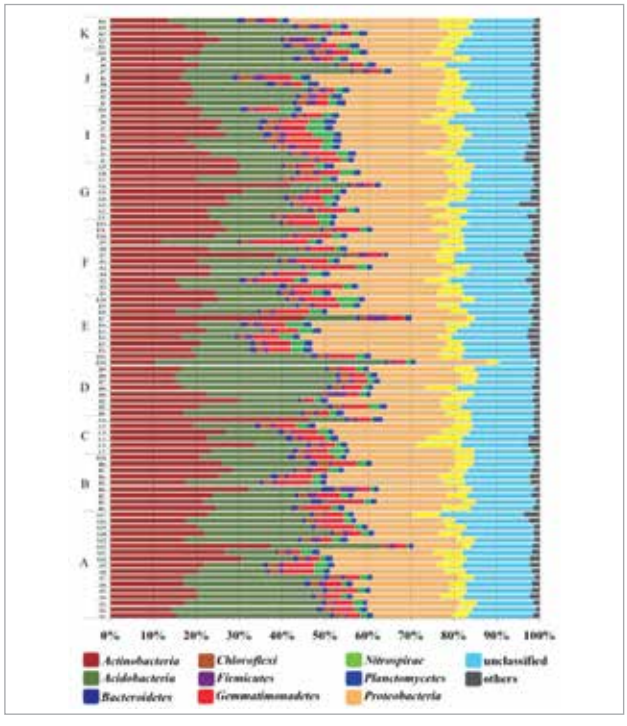

Figure 2. Bacterial diversity observed in 95 samples, by the analysis of the V6 region of the 16S rRNA gene (4,500 sequences per sample). Analyses were performed by affiliation of sequences into bacterial phyla

The sugarcane is the most important agricultural issue in the State of Sao Paulo, where the agro-climatic map describe the existence of two regions with distinct environmental conditions, where this crop can be cultivated, besides a new area, recently occupied by these plantations. However, with the expansion of the agriculture frontiers, a higher productivity and sustainability in the sugarcane production is highly desirable. In this context, among other factors, the role of the microbial community in the sugarcane field soils might be important, helping on the improvement of plant development, supplying it with nutrients or inhibiting the occurrence of diseases and pests. However, little is known about the microbial community residing in soils where sugarcane is cultivated. Then, this project has the aim to describe, in a biogeographic approach, the microbial diversity existing in soils used for sugarcane plantations in the State of Sao Paulo, encompassing the groups of archaea, bacteria and fungi. It is proposed to quantify these communities by quantitative real time PCR (qPCR), to analyze the structure of such communities by terminal restriction fragment length polymorphism (T-RFLP), and to determine the phylogeny of such organisms by pyrosequencing of phylogenetic genes found in targeted organisms. In this purpose, two types of samplings will be carried out aiming: i) to compare the variation on the microbial communities in macro-scale, among different areas describe in the agricultural map; ii) to obtain patterns in micro-scale of these communities, within each area demarked by the sugarcane cultivation map. At last, the microbiological data will be correlated with physico-chemical characteristics of the soils, resulting in a biogeographic approach of the analyzed soils, generating a microbiological map of the soils cultivated with sugarcane in the State of São Paulo.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our data revealed a large variability in soils characteristics. For instance, soil pH varied from 3.7 and 6.6, organic matter contents varied from 4.0 and 37.0 g.dm-3, as well the proportion of sand, clay and silt in the soil mineralogy.

The taxonomical affiliation of bacterial 16S rRNA sequences was performed in 95 samples by high-throughput sequencing using the Ion Torrent platform. After trimming and rarefication, 427,500 high quality sequences (4,500 sequences per sample). An average value of 15.3% of sequences from each sample could not be classified deeper than Bacteria, while others were majorly affiliated with the phyla Proteobacteria (23.1%), Acidobacteria (21.9%), Actinobacteria (19.6%) and Verrucomicrobia (5.1%).

Environmental characteristics (soil properties followed by agricultural practices) are prevalent over the geographical distance on the structuring of bacterial communities. The most explanatory variables within this group of variables were pH and soil granulometry. Among the evaluated management practices, the addition of vinasse, charcoal, filter cake, and the implementation of mechanical harvesting were found to have a determinant role on bacterial assemblages.

The T-RFLP-based profiling of fungal communities detected a total of 742 TRFs. Partial clustering of samples from the same production unit was observed, and the samples from distant locations were mostly segregated. Geographic distance and environmental distance interacted to explain 1.4%, indicating that environmental distance has only a weak correlation with geographic distance (*Figure S2*). Among the measured environmental variables, pH and clay content were the most significant. At larger spatial scales, geographic distance is likely to explain increasingly more of the variance in community composition, possibly due to dispersal limitation. We found a significant correlation between the fungal community similarity and geographic distance ($\alpha = -3.3687$, $p < 0.001$). Thus, the community structure showed a higher relatedness with the geographical distance than the environmental variables.

In combination, these results show that soil microbiomes might be managed, in order to generate better assemblages to improve plant growth and yields, what could lead to a better exploration of microbiomes for agricultural purposes.

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NITROGEN NUTRITION OF SUGARCANE WITH FERTILIZERS OR DIAZOTROPHIC BACTERIA

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Nitrogen is required in large quantities for biomass production. Around 23% of the fertilizer N in Brazil is used in sugarcane. Besides being the most expensive plant nutrient, N fertilizers are an important component of the environmental budget of biofuel production. It is estimated that synthesis of N fertilizer accounts for about 25% of all the fossil energy spent in field operations for ethanol production from sugarcane in Brazil. Emission of nitrous oxide, a potent greenhouse gas associated with fertilizer use, also adds to the environmental costs of ethanol.

There are evidences that biological nitrogen fixation (BNF) is responsible for supplying part of the N required by sugarcane plants because several diazotrophic microorganisms have been isolated in that crop. Besides, the amounts of N fertilizer applied to sugarcane in many cases do not replenish the N removed from the fields with the harvest or lost as part of management practices. However, old sugarcane fields generally do not show signs of soil degradation.

The actual contribution of BNF to sugarcane under field conditions is controversial. Some authors have expressed their view that BNF is of little relevance for sugarcane N nutrition. However, it is generally recognized that BNF presents great potential especially in Brazil where many studies have shown promising results.

BNF is affected by plant variety, bacteria species, and plant-bacteria interactions. An inoculant produced with five strains of N_2-fixing diazotrophs was developed by Embrapa but it has not been extensively tested under field conditions. This project has the objective of studying the contribution of BNF to sugarcane production compared with the use of synthetic N fertilizer under different soils, environments, and sugarcane cultivars, evaluating the emission of N_2O from sugarcane fields fertilized with N, and testing an inoculant produced with endophytic bacteria. At the same time new N_2 fixing organisms are being searched for that are adapted to the sugarcane growing conditions of the State of São Paulo.

Figure 1. Greenhouse gases sampling in a sugarcane field. The photo was the cover of the Journal of Environmental Quality 44(2), 2015

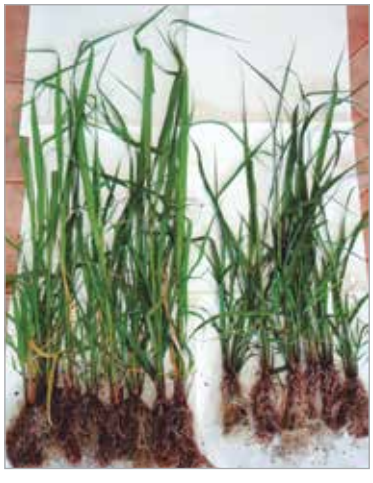

Figure 2. Sugarcane genotype IACSP95-5094 grown in greenhouse for 99 days. Left: plants inoculated with diazotrophic bacteria; Right: control without inoculation. Photo by Silvana A.C.D. Souza

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The GHGs emission, especially that of N_2O, measured as a function of nitrogen fertilization were lower than the default value used by IPCC (1% of the fertilizer N emitted as N_2O), which helps to confirm the high sustainability standards of sugarcane production in Brazil. However, the presence of high amounts of sugarcane straw, derived from unburned harvest, associated with the application of vinasse can significantly increase N_2O emissions. Therefore, adequate management practices are needed to maintain GHGs emission low. The use of nitrification inhibitors with N fertilizers allowed a 90% reduction in N_2O emissions.

A large number of endophytic bacteria isolates were obtained in irrigated and rainfed sugarcane fields showing the great variability of microorganisms that can colonize sugarcane. About 80% of the organisms were isolated with nitrogen-free media, indicating that they are capable of fixing N_2 from the atmosphere. In addition, many isolates showed significant indole production, which is associated with phytohormone production that stimulates plant growth. Selected species (*Azospirillum amazonense*, *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Herbaspirillum rubrisubalbicans* e *Burkholderia tropica*) resulting from long term studies by Embrapa, were inoculated under greenhouse and field conditions to sugarcane varieties. It was clear that the response to inoculation was variety-dependent. Materials such as IACSP-95-5094, IACSP94-2101 and access SES205A of S. spontaneum showed more than 180% and 236% dry matter production of shots and roots in 90 days, whereas some varieties were negatively affected by inoculation.

In field studies comprising 13 site-year of results the inoculation of diazotrophic bacteria failed to increase plant or sugar yields in any experiment. The field studies allowed the conclusions that, at the present stage of knowledge, it is not possible to recommend inoculation of diazotrophic bacteria to increase sugarcane yields or to reduce the use of N fertilizers. However, indirect measurements of BNF using natural abundance techniques (δ 15N) confirmed previous studies indicating that part of the N in the sugarcane plants come from the atmosphere. It is likely that BNF is contributing to sugarcane nutrition through the already existing microbiota. Therefore, new research efforts are needed to understand the complex interactions between sugarcane and diazotrophic bacteria, a promising issue for a sustainable agriculture.

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MICROBIAL NETWORKS IN CONTROL OF GREENHOUSE GASES EMISSIONS IN BIO-BASED AGRICULTURE (MINIBAG)

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Figure 1. Vinasse, produced at a rate of 10-13 liter per liter of ethanol, is recycled in the field adding organic matter and nutrient to soils

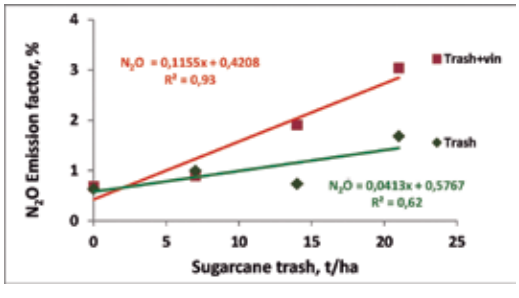

Figure 2. Vinasse and sugarcane straw increase N_2O emission from N fertilization. The N_2O emission factor increases 0.04% per tonne of straw or trash but when vinasse is applied the N_2O emission factor increases three times more. Data adapted from Carmo et al, 2013

Soils are considered principally non-renewable resources. Soil ecosystem services have a large impact on numerous societal demands and are of high economic importance. Within the area of sustainable agriculture, it is expected that agricultural production will increasingly rely on the natural nutrient retention and recycling capabilities of soil. The project described here seeks to provide a fundamental scientific understanding of soil functioning and the resulting ecosystem services in Brazilian and Dutch bio-economies based on innovative microbial ecology and soil science studies. Focus will be in sugarcane production systems by linking soil-borne microbial composition and functioning, waste residues recycling, fertilizers, soil factors and greenhouse gases (GHG) emissions through integrating and complementing the strong expertise of Brazilian and Dutch researchers. In Brazil, we will quantify the microbial functional groups and microbial abundance of C and N cycle genes and measure GHG emissions (CO_2, CH_4 and N_2O) from soils during the productive cycle of the plant under different management practices and verify the temporal and spatial variability of these emissions in the evaluated treatments with different concentrations of sugarcane vinasse residue combined with N mineral fertilizers and with straw additions, and determine the conditions under which such GHG emissions can be counteracted, or minimized. In Netherlands, we will focus on the rules of microbial functional groups, microbial N and C genes, organic matter content/type, GHGs emissions and soil factors on soil quality.

Previous studies of our group (Carmo et al., 2013, Soares et al., 2015) had shown that the emission factor of N fertilizer in ratoon sugarcane is within or more generally below the 1% value suggested by IPCC. However, the presence of sugarcane straw or trash, especially in combination with vinasse, enhances GHGs emissions much above the values of IPCC (Carmo et al., 2013), which contribute to decrease the very good GHGs balance of bioenergy produced with sugarcane.

The strategy behind the present project is to do examine possibilities of reducing GHGs emissions, especially that of N_2O in this sugarcane systems where fertilization is done in fields covered with straw and the application of vinasse is part of the regular crop management due to the need to recycle this by-product in the soil.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

So far three field experiments were conducted in Brazil in which GHGs emissions were evaluated as a function of N fertilization, vinasse – both regular or concentrated application. In all sites straw derived from unburned harvesting was preserved on the soil.

The largest N_2O emission occurred when concentrated vinasse (CV) was applied together with N fertilizer, reaching 2.5% of the N applied. This emission factor was above the default emission factor of 1 % of the IPCC. However, N_2O emissions from the fertilized plots without vinasse or when vinasse application was anticipated was 0.25% of the N applied and were, therefore, well below the default value, regardless of the addition of mineral N. As a result of the atypical (much dryer) weather conditions in the 2013/2014 season, the application of regular vinasse (RV), regardless of the time of application, showed low N_2O emission. The application of N along with CV increased GHG's emissions, possibly due to the presence of both readily available C and N for the soil microorganisms. Therefore, a 1-month interval between application of CV and N seems to be a good strategy to reduce the N_2O emission.

The effects of fertilization and straw on N_2O emissions have being previously reported; however they did not take into account the microbial community. This project is showing novel information about microorganisms involved in GHGs emissions and how the microbial community varies in short time, depending on the prevailing substrates. Besides the common Proteobacteria model organisms for denitrification, we have found taxa recently described as potential drivers of N_2O production and consumption. Additionally, we have identified taxa with potential biotechnological properties that might improve the sustainability of bioethanol by increasing C yields and improving N efficiency in sugarcane fields. Preliminary results also point to the important role of nitrification as a driver of N_2O emission in sugarcane fields fertilized with N.

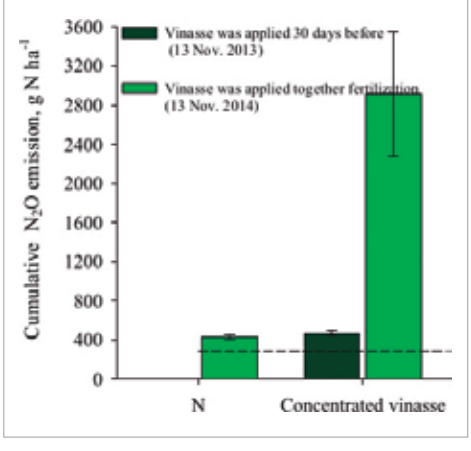

Figure 3. Separating concentrated vinasse and fertilizer application by one month sharply decreases N_2O emission

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N_2O, CO_2 E CH_4 EMISSIONS FROM SOIL DURING AGRO-BIOFUEL PRODUCTION IN SÃO PAULO STATE, BRAZIL

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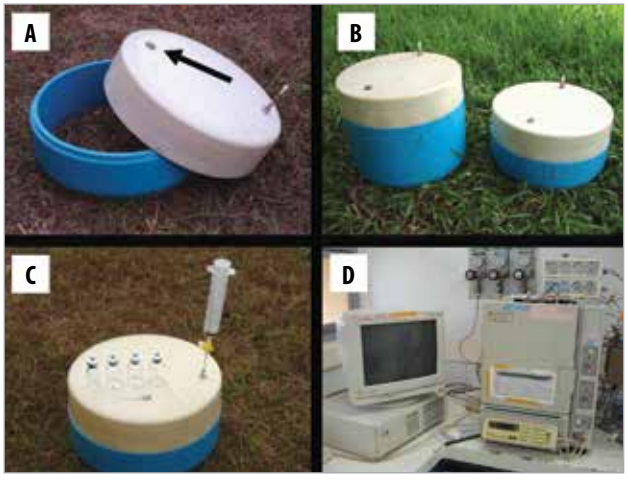

Figure 1. Chambers used for the collection of N_2O, CO_2 and CH_4 (A,B) showing flasks utilized for the storing the samples (C), details of the orifice that equilibrates the internal and external pressures of the chamber (A - arrow) and collection of the sample utilizing a 60 ml syringe (C) and gas chromatograph equipment (D)

Brazil is the world's largest producer of sugarcane, with an annual crop yield of over 470 x 106 metric tons in 2006-2007, planted in approximately 7 million ha. About half of sugarcane in Brazil is planted in the state of São Paulo, where sugarcane is the main agricultural product and contribute to about 27% of the state's GDP.

With about half of the global ethanol production, Brazil is already the largest contributor in the international ethanol trade. Yet, production is predicted to continue to expand due to geopolitical instability in oil producing countries and an increasing commitment from developed countries to the Kyoto Protocol to reduce emissions of carbon dioxide and other green house gases.

According to estimates from models and numerical analyses, Brazilian ethanol ranks among the best biofuels in terms of net energy produced for the amount of fossil fuel used in the production and, consequently, of CO_2 emitted. Also, sugarcane crops in Brazil grow with less nitrogen fertilizers than other biofuel crops, such as corn, which results in lower levels of nitrous oxide, a potent green house gas, during the production of Brazilian ethanol. However, the lack of real measurements and actual data about emissions of green house gases (GHG: N_2O, CO_2, CH_4) associated with the production ethanol in Brazil hinders our capacity to properly quantify its effectiveness at reducing emissions of GHG. Studies estimates that soil emissions of GHG, which are not associated with the consumption of fossil fuels, account for more than 50% of the total emissions. Meanwhile, *in situ* estimates of nitrous oxide ($N\text{-}N_2O$) emissions from fertilizer application in sugarcane fields in Brazil are in the order of 1%. If confirmed with further *in situ* measurements in a more comprehensive study, these low GHG emission can have important implications for the sugarcane industry in Brazil. In this project, we propose to determine *in situ* emission of GHG from soils, according methodology presented in *Figure 1*, planted with sugarcane in the state of São Paulo during its productive cycle to improve and expand existent estimates. *In situ* measurements of GHG in Brazilian sugarcane fields are practically non-existent, probably because N losses from fertilizers as N_2O ($N\text{-}N_2O$) are assumed to be insignificant in comparison to other losses, and because fossil fuel use during sugarcane production is low because much of the management practices in Brazil rely on manual labor. With eminent changes about to occur in the sugarcane ethanol industry in Brazil, these assumptions need to be revised and new data collected to guarantee the low emission.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

According to estimates of GHG emissions generated from the burning of agricultural residues in Brazil since 1994, sugarcane accounted for about 97% of the emissions. However, the lack of field data and measurements from different systems of agricultural production create large uncertainties in emission calculations.

In this project, we expect to produce a complete assessment of GHG emissions from soils in sugarcane crops in the state of São Paulo. By evaluating the variability of emissions as a function of management practices and climatic variation during measurements, we also expect to determine the hot spots and hot times for GHG emission during the sugarcane crop cycle so that strategic plans can be targeted to minimize these emissions.

Overall, we plan to produce reliable and realistic data on GHG emissions from sugarcane soils in São Paulo in order to calibrate and validate soil emission models that can be used to estimate emissions of GHGs from sugarcane plantations. By improving present estimates, predictions of the GWP of ethanol produced in Brazil can be properly assessed and compared to other forms of biofuels. We understand the complexity of representing the wide range of conditions for GHG emissions from the approximately 140 sugarcane mills in the state of São Paulo, where sugarcane grows under different management practices, climatic conditions, and soil types. However, by including different experimental treatments with a wide range of management practices and rates of fertilizer application and use of agricultural waste, we should be able to address the complexity of the system.

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UNRAVELING THE SOURCES AND SINKS OF NITROUS OXIDE (N_2O) IN SUSTAINABLE BIO-BASED AGRICULTURE

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FAPESP Process 2013/50940-0 | Term: Aug 2004 to Jul 2016

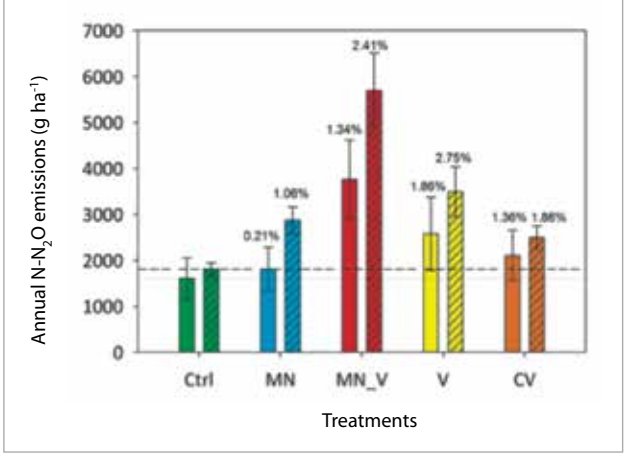

Figure 1. Annual nitrous oxide emissions from soils with sugarcane and respective emission factors (%). Bars are the standard errors. Reference lines are the calculated emissions from the control (Ctrl) treatments without straw (dotted line) and with straw (dashed line). Ctrl: no nitrogen addition; MN: mineral nitrogen fertilization; MN_V: mineral nitrogen plus vinasse fertilization; V: vinasse; CV: concentrated vinasse. Transversal lines: treatments with straw left on soil

One of the main concerns of biomass production for bio-energy is the potential positive feedbacks of crop production to global change, particularly in the form of greenhouse gas emissions (CH_4, CO_2, N_2O – GHGs). Nitrous oxide (N_2O) is a significant by-product of agricultural intensification, primarily due to the application and transformation of inorganic Nitrogen (N) fertilizers. In our previous and ongoing studies with sugarcane crop we have observed that not only nitrogen fertilizer in soil increases N_2O emissions but also the combination of N and vinasse (by-product of ethanol) (Carmo et al. 2013; Pitombo et al, 2015). Soil microorganisms are central to these transformations and thus regulate the loss or retention of inorganic N, including N_2O. Biological emissions of N_2O are mainly controlled by two microbial processes: nitrification and denitrification. However, we have limited understanding of how these processes are regulated in complex systems such as soils under crops for bio-energy. Prior work has often focused on individual microbial species that contribute to each process and on ecosystem scale parameters such as organic matter content, soil texture, pH, soil N status and precipitation. What is lacking is an approach that combines fine scale mechanistic details on the physiology of key functional groups of the N cycling microbial community and their interaction with their environment and each other. Our goal is to combine advanced omic technologies (meta-genomics, -transcriptomics and -proteomics) to determine the key players in the biogeochemical cycling of N, with a specific focus on organisms involved in denitrification in a model of sustainable sugarcane biomass production system.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

A series of field experiments measuring GHGs to provide data for life cycle analyses are in the scope of other FAPESP projects coordinated by the members of the same research group. The use of molecular techniques in these field experiments has stated that the microorganisms used as models for nitrification and denitrification are not representing the ones present in the field (Pitombo et al. 2015; *Fig. 1*). Specifically in sugarcane fields it has being suggested that the microorganisms present in the vinasse may increase the release of N_2O from soil (*Figure 2*). On the other hand, potential N_2O reducers were highlighted by linking N_2O release with microbial abundance in soil. Contemporary GHG gas models presume that N_2O to N_2 reduction (i.e., the final step of the denitrification pathway) is the major attenuation process controlling N_2O flux to the atmosphere. Then, it is in what the main aim of this research is focused on.

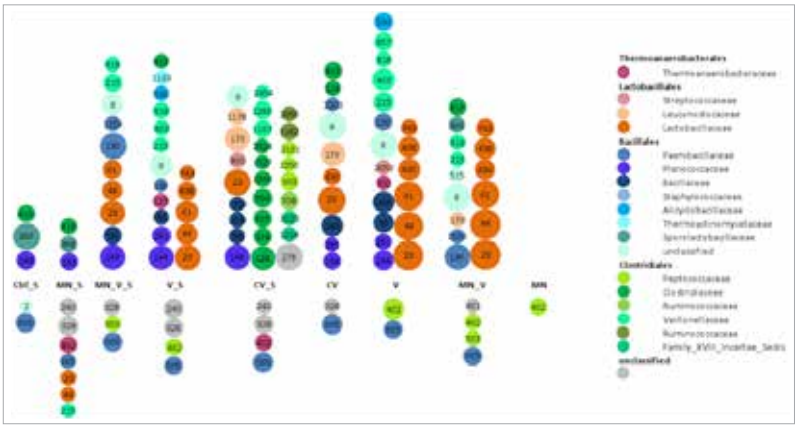

Figure 2. Effect of different treatments on OTUs belonging to Firmicutes other phylum. Numbers represent the OTU identification. Color groups at Family. Circle size indicates the fold-change of the respective OTU when compared to the control treatment (Ctrl). Overrepresented OTUs are in the upper part of the plot and underrepresented OTU are in the below part of the plot. Ctrl_S: no N with straw; MN_S: mineral nitrogen with straw; MN_V_S: mineral nitrogen plus vinasse with straw; V_S: vinasse with straw; CV_S: concentrated vinasse with straw; CV: concentrated vinasse; V: vinasse; MN_V: mineral nitrogen plus vinasse; MN: mineral nitrogen.

For a more detailed understanding of the processes related with N_2O release, we brought to the lab the microorganisms to date used as models and the ones that are being pointed out as important drivers of nitrogen redox processes (e.g. *Pseudomonas sp.*, *Lactobacillus sp.*, *Nitrosospira sp.*, *Nitrososphaera sp.*, *Anaeromyxobacter sp.*). Most of the strains have the genome already sequenced and they will be used as template to verify which ones are active and which steps are they developing in the soil to contribute to lower or higher N_2O emissions. A microorganism which requires high ammonium concentrations and uses oxalic acid as carbon source was present in all models explaining N_2O fluxes from the soil under different management practices in the work of Pitombo et al. (2015) and now it was included in the mock community under study.

The mock community has being introduced in microcosms and submitted to variation of conditions simulating the environmental and management factors which most likely control the N_2O releases. Among these factors are soil moisture; nitrogen availability and speciation; straw; vinasse; and copper availability in soil. To date, no study considering soil copper content and N_2O reduction has being published despite it has being pointed out as determining factor for the syntheses of the most studied nitrous oxide reductase.

The deep understanding of the interactions between the microorganisms and their roles on nitrogen cycle at genomic and postgenomics level will provide knowledge to promote a more efficient fertilizer use with lower N_2O emissions. Recycling vinasse in the fields as fertilizer is essential to keep the ethanol lifecycle as close as possible. As vinasse holds a huge biotechnological potential, the perspectives are that it might be used as media to develop probiotic products addressed to lower N_2O emissions and N fixation in the fields.

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ADOPTION OF AGRO-ENVIRONMENTAL PARADIGM: TECHNOLOGICAL CHANGES IN THE SUGARCANE CROP AND IMPACTS ON SUGARCANE OCCUPATION AND LIVESTOCK IN SÃO PAULO STATE FROM 2007 TO 2014

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FAPESP Process 2013/09490-0 | Term: Aug 2014 to Jul 2016

Figure 1 and 2. Mechanical harvesting of sugarcane

In 2007 was signed the Agro-Environmental Protocol between Sugarcane Industry Union, supplier associations and State Departments of Environment and Agriculture of São Paulo stating that the burning of sugarcane straw to facilitate harvesting should be eliminated by 2014 in mechanized areas and by 2017 in non-mechanized areas or smaller than 150 hectares. The aim was to accelerate the adoption of mechanical harvesting of unburned sugarcane to replace the manual harvesting of burnt cane, reinforcing the good environmental image of ethanol. In a previous project, it was found that between 2007 and 2010, increased crop and mechanical planting resulted in a significant decrease in the number of unskilled sugarcane workers, particularly cane cutters. At the same time, with the growth of sugarcane production, the number of employed people with higher professional qualifications raised. This project aims to verify, by 2014, the intensity, economic, social and administrative consequences of the process of expansion of the sugarcane mechanical harvesting in São Paulo. With the Ministry of Labor and Employment data we intended to calculate the variation in the number of sugarcane workers and the other sugarcane occupations in management activities or with higher professional qualification in sugarcane plantations, in the factories and distilleries and in administrative and support functions. Also, social characteristics of sugarcane occupational categories, gender composition, education level and age group will be analyzed. From consultations to documents and official data and interviews with their technicians, we intend to check how public institutions are following this process. Based on specific studies and interviews with staff from sugarcane companies we intend to analyze the effects of this process in the production and sectorial productivity and in people management issues. For four São Paulo regions, we intend to check local socioeconomic consequences of sugarcane mechanization. In addition, there will be econometric study based on several variables raised.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In the period 2007-14 there was a growth close to 27% in area and 8% in sugarcane production in São Paulo, less expressive dynamism than that observed between 2003 and 2007. The decrease in production per hectare of cane, or cultural performance, was little more than 15% between 2007 and 2014, may be considered that, in part, this result was associated with how quickly grew planting and mechanical harvesting of sugarcane, characterizing the learning cost of new processes. In 2009, it was estimated that 33% of the plantations in the State were mechanized, a figure that jumped to 57% in 2012, while mechanical harvesting of sugarcane passed from 42% to 85% between 2007 and 2013. Such technological change was accompanied by a very positive environmental effect, registering a reduction in the percentage of burnt cane area in order to facilitate their harvest, from 53% to 16%. At the same time, it was found marked reduction in the number of people formally occupied in São Paulo sugarcane companies in activities with low demand for professional qualification. In this sense, the average monthly number of sugarcane workers, which includes the cane cutters, reduced from 178,510 people in 2007 to 73,271 people in 2014, decreasing more than 100K people, therefore, or 59%. Other professional groups in the sugarcane plantations, machine operators, people in management and technical position in agricultural sciences, recorded an increase in their number, but without compensating the drop in sugarcane workers, so that the average monthly occupation in sugarcane production fell from 209.700 to 124.256 people between 2007 and 2014. In other professional groups observed in the whole period, the number of employed persons increased, as in the case of those engaged in industrial activities, in the mills and distilleries, or in administrative and support activities. Making up a balance sheet, it's seen that the total number of sugarcane occupation in São Paulo dropped from a monthly average of 316.122 in 2007 to 264.157 in 2014, and this reduction was followed by change in the composition of sectorial occupation, in favor of those activities with higher requirements of professional qualification. Among the sugarcane workers is observed an increase in the average level of education, a fact consistent with the recent growth of this indicator in the entire Brazilian population. However, unlike other social situations and other sugarcane professional categories, increased the percentage of women and participation of older in this area. Apparently, the younger, more educated, have managed to find other job opportunities, avoiding cutting cane, known to be very exhausting. Other social evidence in São Paulo sugarcane region is the reduction in the presence of seasonal workers who once came, in much more significant number, from states of the Semi-Arid Region to work temporarily in cutting sugarcane.

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ORGANIZATIONAL DESIGN OF BIOEN PROGRAM: INTELLECTUAL PROPERTY, INCENTIVE MECHANISM AND IMPACT EVALUATION

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Photo by Eduardo Cesar

The project aims to attend one of the components of the call for the BIOEN Program related to intellectual property and technology transfer. It consists on a multidisciplinary project that articulates economic issues, assets management and social network formation together with scientific and technical components of the researches that are being selected to join the program of studies on Bioenergy.

The project's general goal is the formulation of an organizational design that involves the projects of BIOEN considering three basic approaches: a) analysis of the demands due to distinct forms of intellectual property of technologies, supplies and genetic material that can block or create risk situations for the continuity of specific projects or even of the program itself; b) preparatory analysis of business plans based on the construction of economic exploration 'models' of BIOEN research results in different levels, from intermediate products and supplies to final products, as, for example, new improved variety. It also includes the relation between different projects and an incentive system, and scenarios of partnership formation for products development.

The models must be built up from the formation of networks that identify patent families, networks of quotations and case studies on intellectual property attribution in vegetal biotechnology, focusing the bio energy field; c) a block of ex-ante impact evaluation that provides subsidies for the formulation of business plans based on the research results.

The project strategy consists in establishing a training program with four advanced undergraduate or graduate students and a trainee in computer sciences. Under de supervision of the project, they have to prepare training material and the collect subsidies from the Workshops to perform multicriteria analysis and generate scenarios in the three main areas of the project.

About ten Workshops are programmed for the 4 years project schedule, half of them with BIOEN project participants (scientists, technicians and managers) and 5 with external researchers and BIOEN staff looking forward building scenarios of property rights and evaluation of impacts.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The project started in September, 2009. Three meetings was hold by the group to the initial definition of the research tasks for the next four years and the strategy to keep the three groups connect alongside the research schedule.

The team, headed by the Institute of Economics of UNICAMP comprised: a) a team responsible of the ex-ante evaluation of the impacts of BIOEN Program in selected sectors of Brazilian Economy. It has 3 senior researchers, faculty at FEA/USP – Dr Abraham Yu has the role of coordinator of one research area – and a group of graduate students; b) a team in charge of FTO and MTA analysis, headed by Prof. Maria Beatriz Bonacelli, faculty of the Department of Technology and Science Policy of the Geosciences Institute, UNICAMP. It comprises three more senior researches from the Public Policy Institute/UNICAMP; Federal University of Grande ABC and a Pós-Doctor Researcher placed in the Geosciences Institute, sponsored by a CAPES program. A group of training students will be contacted to help development of the research; c) a Contract Design and Patent Evaluation and Scientific foresight group is located at the Institute of Economics. It is coordinated by Prof. José Maria F.J da Silveira, coordinator of the Project and Prof. Maria Ester Soares Dal Poz, faculty at Applied Sciences Institute of UNICAMP (FCA-Limeira), with the assistance of the Prof. Jacques Weiner, Institute of Computer Sciences, UNICAMP. A grant for training a graduate in Computer Sciences is planned by the research and the results are expected for the end of the year 2010. Undergraduate student with a grant for initial research sponsored by National Council for the Development of Research (CNPQ) is reviewing literature related to patent valuation.

Photo by Eduardo Cesar

Some contacts with research groups abroad, like University of Limburg University, Adelaide University (in Australia), and Wageningen University (at Haia, The Netherlands) and University of California at Berkeley are planned to be established in the year 2010, in the aim of gathering methodologies to sustain mostly the areas "b and c" mentioned above.

MAIN PUBLICATIONS

As mentioned above, the project is its very beginnings. Up till now there is no papers published based by the research team. It worth mentioned a that a paper related to Intellectual Property in Agriculture was presented by Adriana Pinto Vieira in the XIII Latin American Meeting in Technological Management, ALTEC, in November, 2009, in Cartegena das Indias, Colombia.

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MAPPING AREAS FOR SPECIFIC MANAGEMENT IN SUGARCANE PRODUCTION AREAS USING PEDOMETRICS

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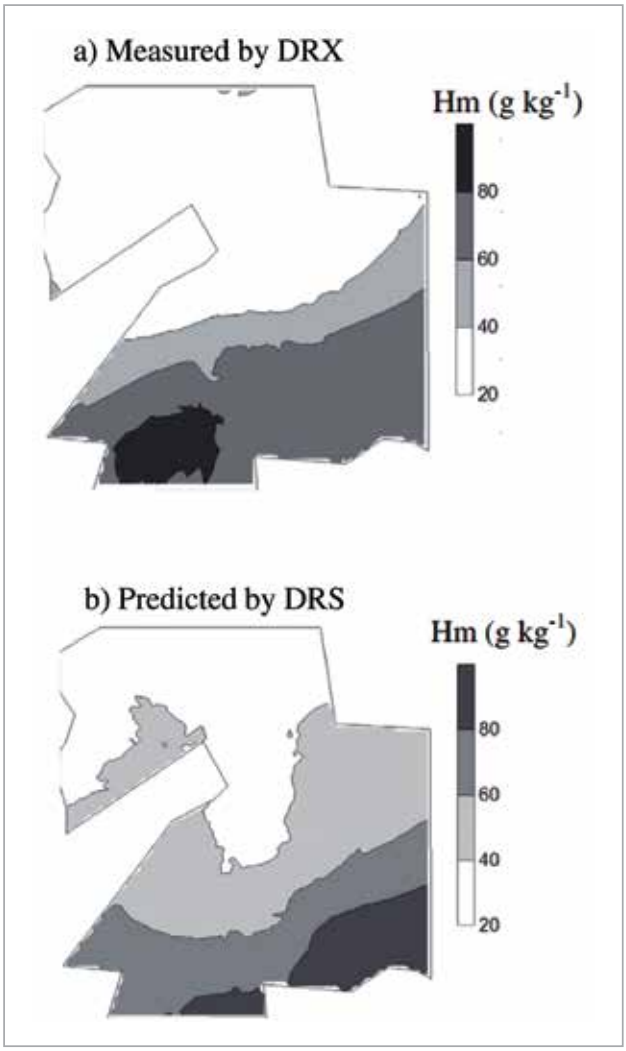

Figure 1. Spatial distribution maps of the hematite (Hm) measured by X-ray diffraction (DRX) (a) and Hm predicted by diffuse reflectance spectroscopy (DRS) (b) in an area of 770 hectares cultivated with sugarcane (n= 110) (Camargo et al., 2015)

The demand for detailed information on soils to assist agricultural and politic decision making is increasing. Despite it is well known that detailed soil attributes information could be used to support sustainable production, current study reports the requirement for new and detailed indexes on the global impacts of land use and management. In general, the greater is the number of data points, the greater the accuracy, pushing into the way of spatial variability studies with a lot a soil samples, unless an alternative soil analysis method helps into the modelling . The magnetic susceptibility (MS) and diffuse reflectance spectroscopy (DRS) are rapid, convenient, less expensive, non-destructive, and sometimes more accurate methods than the conventional soil laboratory analysis. In addition, this technique allows the simultaneous characterization of many soil attributes with agronomic and environmental relevance, besides being adaptable for field use. The MS and DRS are covariate of mineralogical attributes sensitive the soil formation factors and processes. Spatial variability studies of mineralogical attributes at different scales (1 – 100.000 ha) and different landscape models have proved spatial dependence of these minerals and spatial correlation with physical, chemical, environmental attributes (erosion and greenhouse gas emissions) and plant response. The results indicate that MS and DRE (pedometrics techniques) can be used as alternative techniques to identify areas in the "boundaries", between two or more environments of contrasting soil minerals (hematite, goethite, maghemite, ferrihydrite, kaolinite and gibbsite) (*Figure 1*). This allows, by using pedometric mapping, to predict values of some soil attributes at unobserved locations and to access the uncertainty of estimations using statistical inference and sensors.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The use of pedometrics techniques based on MS and DRS have enabled the conversion of basic knowledge in mineralogy to better understanding tropical soils, especially in a new strategy applied to improve yield in sugarcane areas. These results have helped the innovation of the agricultural sector improving the planning and recommendation of vinasse application in sugarcane areas (*Figure 2*). In addition, we have results that have pointed to the identification of areas with potential CO_2 emission , erosion or even having higher potential for production total reducing sugars.

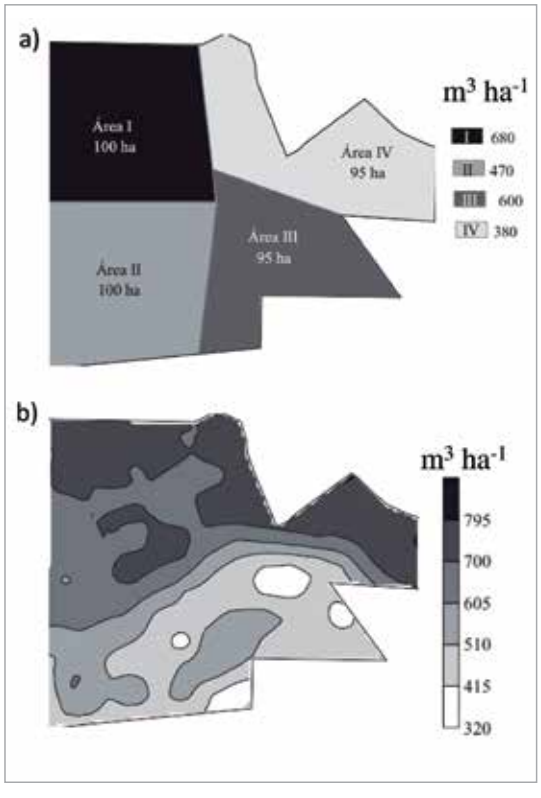

Figure 2. Vinasse[1] application recommendation map, according to the Company's sampling of Environmental Sanitation Technology (CETESB) (a) and the soil bearing capacity to the application of vinasse, estimated according to the magnetic susceptibility of the soil (b) in an area of 380 hectares cultivated with sugarcane (n= 241) (1 - residue of ethanol production) (Peluco et al., 2013)

The future prospects are the production of those thematic maps to other regions of Brazil, based on BigDatas, integrating useful information to different areas, allowing the creation of new hypotheses and generate results based on the update allowed information on soils (eScience).

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USE OF CHITOSAN IN THE ADSORPTION OF METALS IN SEDIMENTS FROM CONTAMINATED STREAMS: BIOREMEDIATION OF IMPACTED AREAS AND EFFECTS ON *CHIRONOMUS XANTHUS* LARVAE

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FAPESP Process 2013/24268-2 | Term: May 2014 to Apr 2016

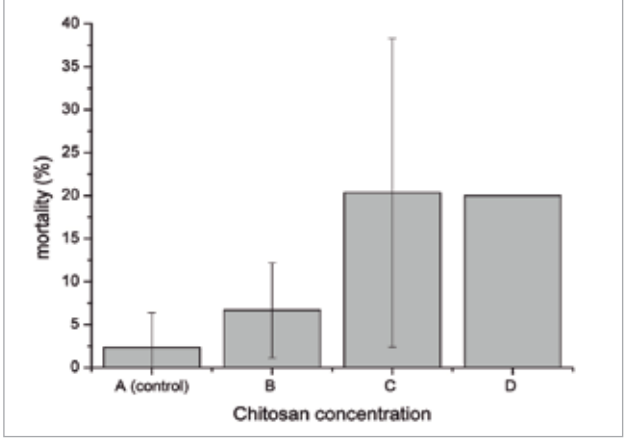

Figure 1. Acute toxicity test with different Chitosan concentration using sand sediments (A: without Chitosan; B: 0.1mg.L^{-1}; C: 0.5 mg.L^{-1}; D: 1.0 mg.L^{-1})

Streams located in areas near sugarcane cultivation receive high concentrations of fertilizers, containing metal ions, causing severe in contamination in the aquatic sediment and accumulation in food chains. Chitosan, a biopolymer used in cosmetic formulations, as a food additive and in wastewater treatment, exhibits high affinity for metal ions. Is this study was to evaluate the effects of solid chitosan on the larvae of *Chironomus xanthus* by carrying out chronic and acute toxicity tests, in order to evaluate its potential for use in remediation activities. The chitosan will be used in toxicity tests on larvae of *Chironomus xanthus* in order to adsorb the metals present in the streams sediment. Thus, knowing the amount of metals incorporated into larvae, as well as that found in the sediment, in experiments without the presence of chitosan and also in the presence of this biopolymer, can contribute to the knowledge on reducing the impacts of metals from the sugarcane cultivation to aquatic biota and the environment around you. Thus, this data could form the basis for establishing strategies for the restoration of degraded areas for sugarcane activity, devoid of riparian vegetation.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The acute toxicity tests, using sand sediments, pointed to low mortality of larvae of *C. xanthus*, especially when the chitosan concentration was 0.1 $g.L^{-1}$. At this concentration, the percentage of larval mortality was lower than the value considered being non-toxic and it was similar to mortality displayed in the control experiment (without chitosan). When the concentrations of chitosan were 0.5 $g.L^{-1}$ and 1.0 $g.L^{-1}$, the larval mortality was approximately 20%, showing evidence of little toxicity to aquatic biota. The results of chronic toxicity tests, using sand sediments, indicated low toxicity for two chitosan dilutions: 0.1 $g.L^{-1}$; 0.5 $g.L^{-1}$, especially by sand substrate, without organic matter content. Nevertheless, concentration of 1.0 $g.L^{-1}$ showed evidence of toxicity for aquatic biota. The analysis of chitosan metals complexation in larvae, using sand sediment, pointed to significant differences between the concentrations of metals in the experiment using 0.1 $g.L^{-1}$ and 1.0 $g.L^{-1}$ of chitosan (*Figure 5*). Especially with the concentration of 1.0 $g.L^{-1}$, values of Cd, Cu, Mn, Zn and Fe were significantly lower in larvae. Our study, using sterilized sand sediments, indicated the potential for the use of chitosan (in solid phase) in water systems, without causing the mortality and development of *C. xanthus* larvae. From the results obtained in the present study, we can infer that the use of chitosan in the concentration of 0.5 $mg.L^{-1}$ has also potential to be used in future in experiments on adsorption of metals in larvae of insects in contaminated environments.

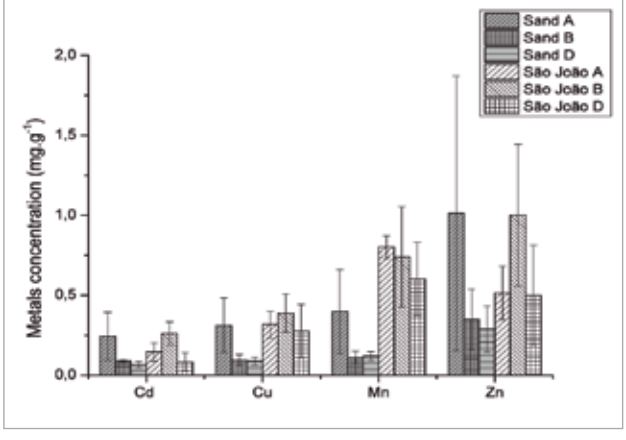

Figure 2. Mean values and standard deviations of metal concentrations detected in larvae (10 days test) using different chitosan concentrations with sand and contaminated sediments (São João stream) (A: without Chitosan; B: 0.1 $g.L^{-1}$; D: 1.0 $g.L^{-1}$)

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RESISTANCE MECHANISMS OF SUGARCANE CULTIVARS TO THE SPITTLEBUG MAHANARVA FIMBRIOLATA

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FAPESP Process 2011/08458-0 | Term: Aug 2011 to Jul 2013

Figure 1. A tolerant cultivar IACSP94-2094 infested (right) and non-infested (left) with spittlebug nynphs (Dinardo-Miranda LL)

The spittlebug *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) is one of the most significant sugarcane pests found in Brazil, causing yield reduction up to 50 % and affecting sugarcane quality due to the reduction of stalk sugar levels and the increase of fiber content. Furthermore, sugarcane industrial processes are also affected by *M. fimbriolata*, as damaged stalks can reduce milling capacity. In particular, cracked and deteriorated stalks lead to increased levels of contaminants, making sugar recovery difficult and inhibiting fermentation.

Due to the importance of this insect to sugarcane growers, a number of studies have been conducted to establish parameters for integrated pest-management programs. However, information on the resistance of sugarcane cultivars to *M. fimbriolata* is very limited and is crucial for use in integrated pest-management strategies. In addition, resistant genotypes will be invaluable for breeding programs aimed at developing new resistant cultivars. Therefore, the objective of this study was to evaluate the resistance mechanisms of various sugarcane genotypes (obtained from the IAC Sugarcane Breeding Program) to *M. fimbriolata* attack. For that, isolated buds of each cultivar were planted in pots and maintained in a greenhouse for approximately three months. The pots were then moved to climate-controlled chambers (26 ± 1ºC; 70 ± 10 % RH; 12-h photoperiod) to carry out laboratory tests to evaluate adult feeding and female oviposition preferences (using both free-choice and no-choice tests) as well as the effects of the cultivars on nymph development and the cultivars tolerance to the pest attack.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The least attractive cultivars for adult feeding were IACSP96-2042 and IAC91-1099. In free-choice oviposition test, the cultivars IACSP95-1218, IACSP94-2094, SP91-3250, IACSP94-4004, IACSP95-3028, IACSP93-3046, IACSP95-5000 and IAC87-3396 appeared to be preferred for oviposition, whereas cultivars IACSP96-3060 and IACSP96-2042 received the least eggs. Cultivars IACSP96-2042, IACSP96-3060 and IACSP94-2101 also received the fewest eggs in the no-choice oviposition tests and exhibit some level of antixenosis resistance. It was observed that nymph survival was significantly greater for cultivars IACSP94-2101, IACSP95-5000, IACSP95-3028 and SP81-3250 (about 80 % nymph survival) compared with IACSP96-2042 (33 %) and IAC91-1099 (20 % nymph survival). Cultivars IACSP96-2042 and IAC91-1099 showed the highest level of antixenosis resistance. Finally, cultivar IACSP94-2094 appears to be tolerant to M. fimbriolata, as it did not show significant reductions in aboveground biomass weight, despite showing reduced leaf chlorophyll levels following pest attack.

Figure 2. A susceptible cultivar IAC87-3396 infested (right) and non-infested (left) with spittlebug nymphs (Dinardo-Miranda LL)

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DEVELOPMENT OF A DATABASE FOR MOLECULAR PROFILE MANAGEMENT OF COMMERCIAL CULTIVARS, PARENTS AND WILD ACCESSIONS OF SUGARCANE

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FAPESP Process 2013/22500-5 | Term: Mar 2014 to Feb 2016

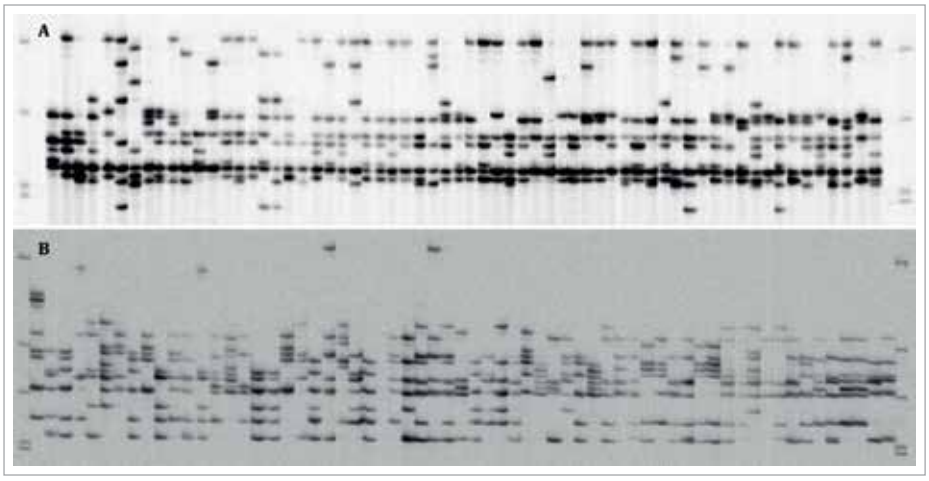

Figure 1. Molecular profile obtained by using SSRs genotyped at the 4300 DNA Analyser (LiCor) System. A: Sugarcane elite clones from the IAC Active Germplasm Bank. B: Saccharum officinarum *clones*

The efficient management and utilization of the germplasm of a breeding program is critical to the development of new cultivars. In Brazil, the sugarcane breeding programs have used molecular markers to characterize the molecular profile of their genotypes. However, there is a lack of a consolidated bank with the molecular profile of cultivars, elite clones and wild accessions. This project has as main objective start the construction of a molecular profile database of sugarcane cultivars, wild accessions and elite clones used as parents in crosses. In addition, we intend to create a database of genomic DNA of these genotypes; identify a set of SSR primer pairs with high discriminatory power; estimate the genetic variability within and between the groups assessed and develop SCAR markers (Sequence Characterized Amplified Regions) derived from AFLP (Amplified Fragment Length Polymorphism) species-specific to *S. spontaneum*. The development of SCAR markers will allow the rapid identification of hybrids derived from crosses between sugarcane commercial cultivars and accessions of *S. spontaneum* assisting the Genetic Introgression Bioenergy Programs. We emphasize that, to our knowledge, until the present moment, in the case of public sugarcane breeding programs, in Brazil does not exist, a molecular profile database of such nature to sugarcane.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

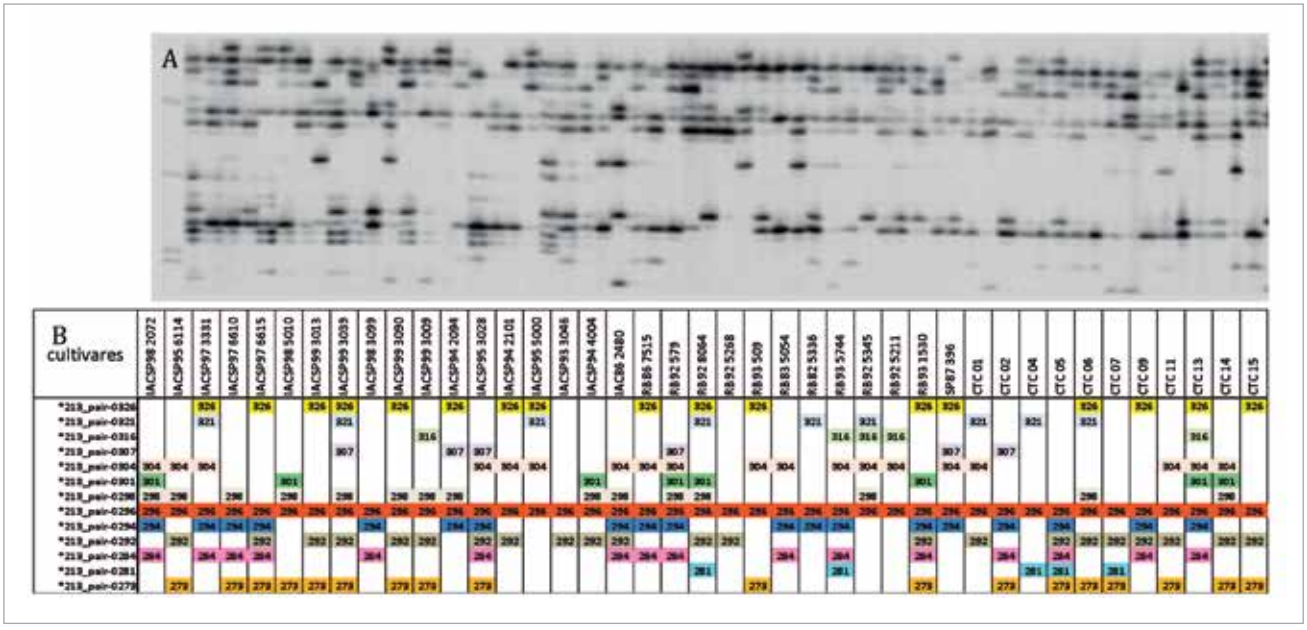

Figure 2. A: Molecular profile of some sugarcane cultivars from different Brazilian sugarcane breeding programs obtained by using SSRs genotyped at the 4300 DNA Analyser (LiCor). B: Example of a graphic representation of the cultivars based on their allele types and sizes represented by different colors, stored at the allele panel of the Molecular Profile Data Bank

The molecular profile based on microsatellite markers of the sugarcane cultivars and elite clones have already been established. In addition, the molecular profile of the majority of the accessions from the Saccharum species (*S. officinarum*, *S. spontaneum*, *S. baberi*, *S. robustum*) and related genera (*Erianthus* and *Miscanthus*) which compose the IAC Sugarcane Germplasm Collection is near to be completed. All these molecular profiles are being deposited in a molecular profile data bank which will be used in the management of the germplasm collection and also in a genetic diversity study to define a core collection that can be used for association mapping and breeding purposes. The SSR primer set showed high PIC values (0.734 up to 0.926) and was suitable either to establish the molecular profiles or capture the genetic variability assessed through SSRs. Initial analysis conducted for each genotype category, i.e., varieties and elite clones, revealed a high degree of average genetic diversity, respectively, 0.887 and 0.860. Only 3.22% of the total SSR alleles were common among the variety category. Some alleles were variety exclusive (private), showing potential application for variety identification. In general, the dendrograms were in agreement with the pedigree information. In relation to the SCARs development, a preliminary screening for species-specific AFLP markers at the Saccharum Complex was conducted by using AFLP markers. This search allowed the identification of candidate species-specific and genera-specific AFLP derived markers. These candidate markers are being cloned and sequenced and will be validated to be used as SCARs.

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IMPACTS OF THE EXPANSION OF THE SUGARCANE AGROINDUSTRY ON FRESHWATER COMMUNITIES

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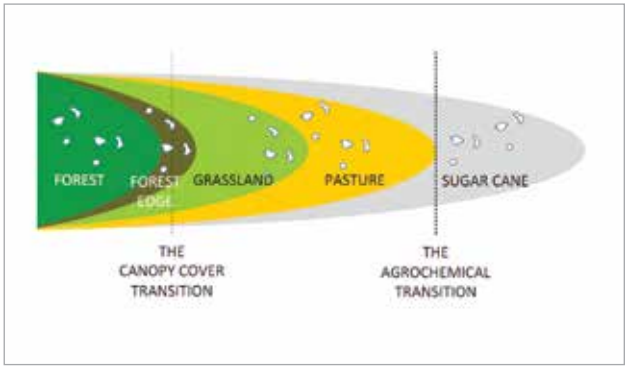

Figure 1. The gradient of environmental degradation in landscapes of sugarcane expansion ranges from native habitats (various cerrado physiognomies) to pastures and sugarcane plantations and is here conceptualized as presenting two steep transitions in physico-chemical properties, with important consequences for the organization of freshwater communities: the canopy cover transition, where most of the changes in the community are expected to be mediated by the presence or absence of a canopy and its influence on pond hydroperiod, temperature and primary production, and the agrochemical transition, where most of the changes in the community are expected to be mediated by the employment of fertilizers and pesticides and their influence on water quality

The dawn of a new paradigm in energy supply – biofuels – points to the continued expansion of agriculture in Brazil in the near future. The country is in a favorable position to assume the global leadership in biofuel production for possessing both ideal geographic and environmental conditions and the already most efficient ethanol industry worldwide. Not surprisingly however, agriculture involves both benefits and costs to society. Industrial agriculture is one of the most environmentally harmful human activities, being directly involved in habitat destruction and in the contamination of water resources. It is unacceptable that Brazil, entering the XXI century with the largest share of the world´s biodiversity and native tropical habitats, and with adequate technical and scientific human resources, misses the historical opportunity to assume, in addition, a model role in reconciling economic growth with environmental preservation.

This project proposes to test the hypothesis that the expansion of sugarcane and soybean – the most important feedstocks of ethanol and biodiesel in Brazil – has substantial impacts on freshwater communities, a significant part of which can be directly or indirectly attributed to agrochemicals such as fertilizers and pesticides. More than documenting impacts, it proposes to understand the mechanisms through which these impacts are generated. This project proposes in addition to validate, for tropical systems, methodologies employed in ecological and ecotoxicological studies in temperate systems, as well as to establish the foundations for the development of a bioindication concept for the contamination of water bodies. These objectives will be achieved in a broad research programme involving sampling and experimentation in laboratory, mesocosms and field. Sampling surveys of temporary pond communities – including algae, tadpoles and predatory insects – across a gradient of environmental degradation (*Figure 1*) will reveal patterns of association among land use, environmental physico-chemical properties, and community composition and structure. In turn, experiments will test the importance of agrochemicals in generating the observed patterns. Through studies conducted in multiple experimental scenarios, we aim at generating a line of extrapolation from lab to field, and to establish clear cause-and-effect relationships between hypothesized processes and observed impacts. Knowledge derived from this project will be important in the development of better agroindustrial practices, towards sustainability in biofuel production and a larger acceptance of Brazilian biofuels in international markets.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Land use and land management had strong effects on water quality and community composition. In general, ponds embedded in agricultural fields had higher temperature, pH, turbidity, and nutrient concentrations than reference ponds. We also detected residues of pesticides and elevated concentrations of some metals. Overall, there was an impoverishment of amphibian (*Figure 2*) and predator faunas as one moved from forests to pastures to plantations. However, patterns are complex, particularly in Mato Grosso where land management actually increases habitat available to fast developing amphibian species, at the same time that insecticide application wipes out much of the predator fauna.

Regarding the experimental component, short term bioassays and laboratory experiments indicate that inorganic N can have lethal and sublethal effects to anuran larvae, and that the magnitude of these effects is modulated by species identity, developmental stage, and duration of exposure. Field concentrations of N do not appear to reach toxic levels to anuran larvae with the possible exception of early developmental stages and at higher pH. Mesocosm experiments manipulating simple food webs and N, P, and N:P scaled to field-measured concentrations indicate significant effects on primary production and consumer performance.

As a first step towards the risk assessment of pesticides in biofuel crop production, we reviewed the toxicity of all pesticides registered for use in sugarcane and soybean in Brazil, and their potential to cause effects of concern for humans or the environment. We are now crossing this information with quantitative on-site application data.

Increased nutrient levels and phytoplankton standing crop, predatory insect depletion, and tadpole die-offs in ponds embedded in agricultural fields are consistent with a role for agrochemicals in influencing community composition and structure across gradients of environmental degradation in agroindustrial landscapes.

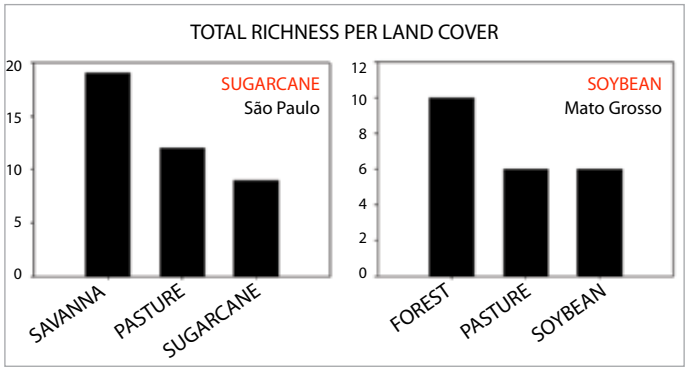

Figure 2. Total species richness per land cover for amphibian larvae. In both sugarcane- and soybean-dominated landscapes, land use for production is associated with an overall impoverishment of the fauna

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FUNCTIONAL´OMICS OF THE RATOON STUNTING DISEASE OF SUGARCANE

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FAPESP Process | Term: Jun 2009 to Jun 2014 | Thematic Project

Figure 1. The disease causes significant reduction of biomass in susceptible cultivars of sugarcane. The "stunting" symptom reflects a shortening of the internodes of infected canes (left cane) compared to healthy ones (right cane)

The ratoon stunting disease (RSD) of sugarcane is caused by the fastidious xylem-limited gram-positive bacterium *Leifsonia xyli* subsp. *xyli* (Lxx). RSD is one of the most important diseases of sugarcane worldwide. Although control of the bacterium relies primarily on using healthy heat-treated stalks as planting material, this approach is not 100% effective and, given the perennial nature of sugarcane plants and the prevalent mechanical mode of transmission of the bacterium, the disease can reach epidemic levels during successive ratoon crops starting from a small amount of infected planting material. In Brazil, losses in biomass of sugarcane due to RSD are estimated to be around 3.3 million tons/yr or R$ 107 million/yr given the price of R$ 32/ton practiced in 2009.

The objectives of our study are a) to establish a time course of colonization of sugarcane by Lxx using the quantitative real time (q)PCR approach in order to identify time points that encompass the onset of the plant reaction to infection; b) to identify sugarcane genes and proteins differentially expressed in a resistant and a susceptible cultivar infected or not with Lxx based on microarray technology at the time points previously defined; c) to characterize the biological effects on sugarcane plantlets of a presumed toxin-like compound secreted by Lxx and study its effects on gene expression in plants cultivated *in vitro*. In addition, genes thought to be involved in the production of this toxin will be characterized by heterologous expression, purification and analysis by mass-spectrometry.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The lack of information regarding the time course of colonization of sugarcane by Lxx prompted us to focus on the establishment of protocols for the inoculation and quantification of Lxx in plant tissue by qPCR. With this information, we were able to define time points for gene and protein expression analyses. We then used 2D-DIGE to compare changes in protein profiles of plants colonized with low and high titers of Lxx (*Figure 2*). Plantlets of the variety SP80-3280 naturally infected with Lxx were either mock inoculated (low titer treatment) or inoculated with a suspension of Lxx CTCB07 (high titer treatment). Proteins were extracted 30 and 60 days after inoculation (DAI) from leaf whorls and profiles were compared within treatments over time. Quantitative PCR of plant tissue at 30 and 60 DAI indicated that the bacterial population remained low in the first treatment, whereas in the second it increased tenfold. Thirteen and 68 differentially expressed proteins were uniquely detected in the low and high titer treatments, respectively. Protein identification by MS indicated that, in the first case, they were mainly categorized as involved in stress responses. In the second case, however, proteins were functionally more diverse. Noteworthy were markedly downregulated proteins involved in plant growth such as calreticulin and cyclin, and upregulated proteins involved in hormone reception (ABA and JA) and in responses to osmotic stress. These results are consistent with the main symptom of the disease and indicate that temporal changes in protein expression associated with increased bacterial titers could result from altered hormonal balance (*Figure 2*).

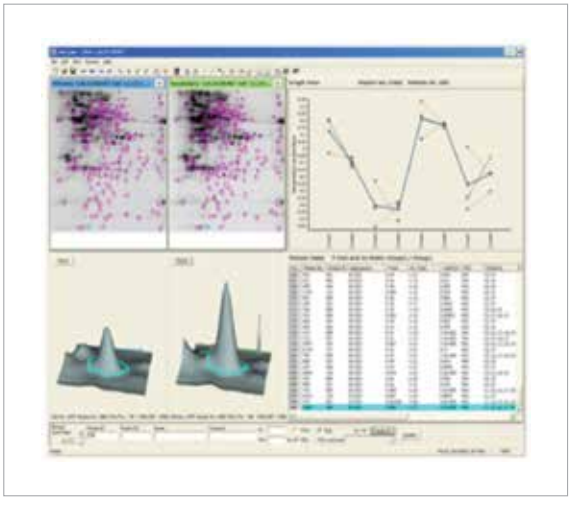

Figure 2. 2D-DIGE profile and quantification analysis using DeCyder software

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SPATIO-TEMPORAL DYNAMICS OF SOIL CARBON STOCKS AND NITROUS OXIDE EMISSIONS UNDER SUGARCANE IN BRAZIL – CONVERGENCE BETWEEN SPECIFIC MODELS FOR TIME AND SPACE

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FAPESP Process 2012/06933-6 | Term: Mar 2013 to Feb 2017 | Young Investigator

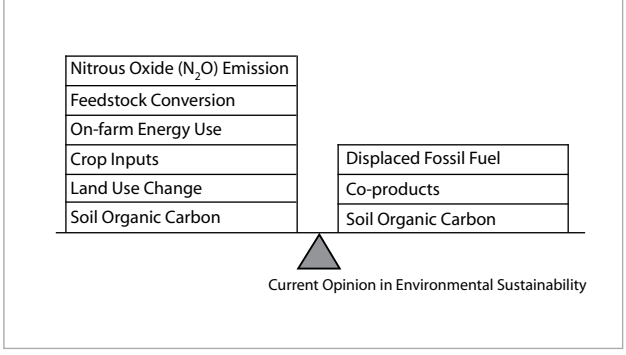

Figure 1. Greenhouse Gas (GHG) balance for bioenergy production systems. Soil nitrous oxide (N_2O emissions) and land use change are important, but uncertain, components that often determine if a particular system is a net GHG source or sink. Soil organic carbon appears on both the left (sources) and right (sinks) because, depending on previous land use and current management, soils used for feedstock production can lose or sequester carbon. Del Grosso et al. 2014

Both land use change and crop management changes under sugarcane are intensively occurring on a large scale in Brazil. It is essential to estimate the impacts of those changes in greenhouse gas emissions and soil carbon stock changes, taking into account soil, climate land use and management aspects, in order to ensure the sustainability of those processes. The goal of this project is to develop research focusing on mathematical modeling and geoprocessing applied to the assessment of soil carbon stocks and soil nitrous oxide emissions under sugarcane production in Brazil. The use of modeling and geographic information systems will enable the evaluation of the environmental impact of land use changes and crop management associated with sugarcane production, contributing to the definition of public and sectoral policies aiming at sustainable sugarcane production (*Figure 1*). This environmental modeling system will allow the comparison of different practices such as no-till, minimum cultivation and conventional tillage; replacing burned harvest by mechanized unburned harvest with maintenance of different amounts of straw on the field; the application of nitrogen fertilizers, limestone and agroindustrial residues such as stillage and filter cake; and the expansion of sugarcane over pastures, annual and perennial crops and native vegetation. The main computer models capable of simulating soil carbon stocks and soil nitrous oxide emissions will be used: CENTURY, Daycent, APSIM and RothC. Furthermore, the GEFSOC (Global Environmental Facility – Soil Organic Carbon) system, integrating modeling and geoprocessing, will be used. GEFSOC will be used to spatially and temporally explicitly map soil carbon stocks under sugarcane in Brazil in two periods: recent past (2002-2012) and future scenario (2012-2022), taking into account changes in land use and agricultural practices. Environmental modeling is an emerging research area in Brazil, and will enable scientific collaboration between research groups both in the country and abroad.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Results so far have been focused on a case study in Piracicaba, an important sugarcane growing region in Brazil. A database has been compiled including input data for the process-based models used in this project. Climate data on long-term averages for precipitation and temperature were retrieved from a global gridded database. Soils data were obtained from a global product covering physical, chemical and biological parameters organized in a gridded database in a high spatial resolution. The land use dynamics was assessed through the use of remote sensing. The main land uses in the studied region were classified and mapped (*Figure 2*) in order to build a land use history to be used in the model runs. The climate, soil and land use data was organized in a gridded dataset with a common resolution, in order to allow geoprocessing operations be performed. A large number of unique combinations was generated, and the storage, processing and visualization operations is computationally demanding. These operations are being performed in virtual machines located in a cloud service, using the Microsoft Azure platform through a research grant. The Century model was applied in sugarcane experiments in São Paulo State including a) fertilizer management – rates and nitrogen fertilizer types; b) application of filter cake and vinasse; c) soil tillage comparing no till, minimum tillage and conventional tillage; d) use of green manure in the reform period of sugarcane. The model was also applied to simulate conditions prior to sugarcane production, such as cerrado vegetation and pastures. The next steps will include running the process-based models in each unique combination of climate, soil and land use history for the studied area, and mapping the results expressed as soil carbon stock changes. Once the model simulations are performed for this region, the same methodology will be applied in other important sugarcane growing regions in São Paulo state.

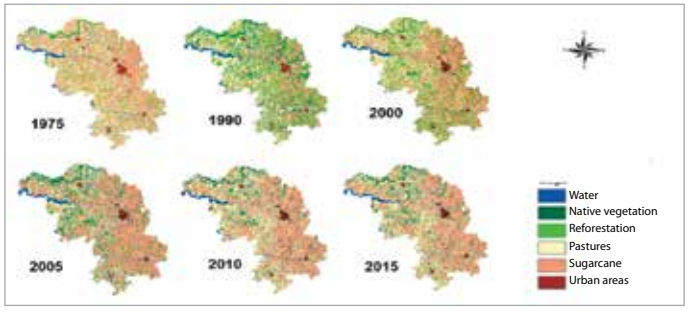

Figure 2. Temporal dynamics of land use in the Piracicaba region

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SUSTAINABLE BIOENERGY SUGARCANE BREEDING AND CULTIVARS DEVELOPMENT

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Figure 1. A. Sugarcane germplasm collection (Sugarcane Cross Station, Uruçuca, BA). B. Photoperiod Facility. C. Sugarcane wild accessions. D. Sugarcane transition genotypes for biomass

This Project proposed a reorientation of the current selection processes in the sugarcane genetic breeding focused on the development of cultivars designed to fill the new bioenergetic demand by acting on four main research topics. In the first topic, clones fitting the definition of primary energy production potential already at the advanced stages of the IAC Sugarcane breeding Program Experimental Network were characterized and selected. In the second topic, applying the combined selection process at the early stages of the breeding process, the most efficient parents in generating progenies with high potential for bioenergy was identified and selected. In the third topic, a genetic introgression program involving crosses between commercial cultivars and *Saccharum spontaneum* accessions was initiated to promote the incorporation of new genes for sucrose accumulation and biomass production. Finally, in the last topic, the genetic diversity for the major sugarcane pathogens (*Puccinia melanocephala* – brown rust; *Ceratocystis paradoxa* – pineapple rot; *Xanthomonas albilineans* – leaf scald, *Ustilago scitaminea* – sugarcane smut) accessed in the different producing regions of Brazil, was investigated to establish a more efficient system of resistant/tolerant genotypes. The results will contribute to sugarcane cropping, as well as to keep Brazil in the leading position in the production of sugar and ethanol.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Crosses were made between sugarcane wild accessions and cultivars and/or elite clones in order to obtain families to select clones with agro-energetic potential. This process was made through hybridization, introduction and selection of clones that are used as parents in the IAC Energy Cane Project, totalizing 207 genotypes. These genotypes have high biomass associated with higher fiber content (around 20%) in the stalk compared to conventional sugarcane populations. The chemical characterization of the fiber revealed for these genotypes a very heterogeneous structural composition of its components. This fact gave rise to a proposal of classification model based on the fiber composition (cellulose, hemicellulose and lignin). This information can be applied to the planting schedule of the cultivars in the future biorefineries, once the cultivars, by itself, could inform the average percentage of 6 carbon sugars (cellulose), 5-carbon sugars (hemicellulose) and non-fiber sugars (lignin).

In order to introgress *S. spontaneum* genes into commercial sugarcane cultivars to meet the new bioenergy market demand, wild accessions of the Saccharum Complex have been selected, imported, quarantined and released to compose the IAC Germplasm Bank. According to this dynamic, the IAC germplasm collection holds, at present, more than 400 genotypes imported from USA and Australia coming from various projects and partnerships established by the IAC Sugarcane Breeding Program.

Thus, this work encompassed the introgression practice as from technical criteria, evidencing a concern, not only restricted to the germplasm acquisition, but also to their maintenance and characterization to be used in the hybridization process. These actions will allow, in short and medium term, meet the demands of the Research and Development areas, especially the sugarcane production sector, transforming science into technology innovation.

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NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY OF BIOETHANOL

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FAPESP Process 2008/57908-6 | Term: Mar 2009 to Feb 2015 | Thematic Project

In the 70s, Brazil started a program to substitute gasoline by ethanol in order to decrease dependence from politically and economically variable periods. The plant species chosen was sugarcane and as a consequence, agricultural and technological studies were greatly intensified, leading Brazil to a very favorable position in terms of energy security. Nowadays, Brazil has more than 80% of its cars running with bioethanol and even airplane engines are now being developed. With the increasing political instability in the Middle East, since 2001, the USA has also decided to direct its energy policy towards the use of biofuels. This is now being followed by Europe and Japan and it is likely to be followed by several other countries in the world. This imposes an enormous pressure on the production of crops that can supply bioethanol.

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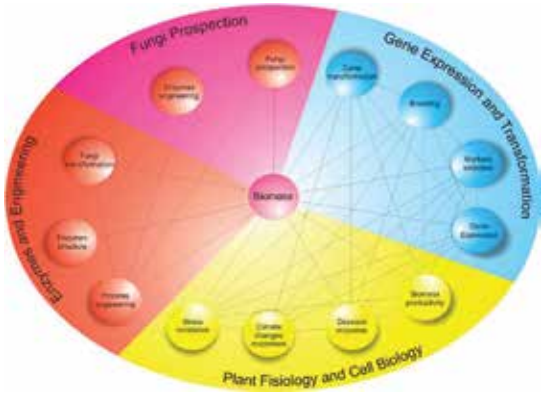

Figure 1. Structure of the INCT do Bioetanol, which is divided in 4 centers that congregate 30 laboratories in 6 states of Brazil

Brazilian sugarcane is probably the most efficient extant bioethanol producing system. However, only part of the biomass produced is used for bioenergy production, 1/3 of the plant being used for sucrose production, 1/3 is bagasse, which is burnt for production of electricity and the last third is left in the field and latter on decomposed by microorganisms. Therefore, in order to supply wider needs, a significant increase in production of ethanol is possible if we can provide the basic knowledge necessary for development of technologies that will be capable to obtain energy from the polysaccharides of the cell wall, which makes 70% of the biomass burnt inefficiently and left in the field. The availability of such processes within the distillery and the higher marketing value of liquid fuel provide additional economical advantages to its conversion instead of simply burning bagasse. Although the chemical hydrolysis of biomass is a consolidated methodology under laboratory conditions, its large-scale application is not economically viable, yet. The necessary use of acids reduces the life-time of equipments, produces toxic wastes and produces non-fermentable sugars, increasing the costs of the products. One alternative is the enzymatic hydrolysis of the cell wall. Such a process requires the use of a complex machinery of specific enzymes that are produced either by the plant itself or by microorganism able to degrade plant cell walls. On the other hand, relatively little is known about the structure and architecture of the cell wall. One of the goals of the INCT do Bioetanol (*Figure 1*) is understand the fine structure of the principal hemicelluloses of sugarcane and other possible sources of biomass. We intend to find patterns of gene expression that could be useful to find ways to induce the plants to degrade their own wall and become prepared for subsequent hydrolysis. In parallel, we intend to prospect microorganisms, enzymes and genes both in microorganisms and sugarcane, that are capable to efficiently hydrolyze the walls. Such enzymes will be designed to have the highest possible performance to degrade plant cell walls, especially the walls of sugarcane. A group of researchers screen existent varieties of sugarcane to find gene markers that could guide the groups to quickly identify plant materials that would be more suitable for use in industrial processes. From the latter viewpoint, our group intends to perform tests of mechanical preparation of sugarcane for further acid and/or enzymatic hydrolysis. With this data in hands, we expect to be able to provide the fundamental knowledge of biotechnology that is necessary for scaling up studies and further increase in efficient of bioethanol production in Brazil.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

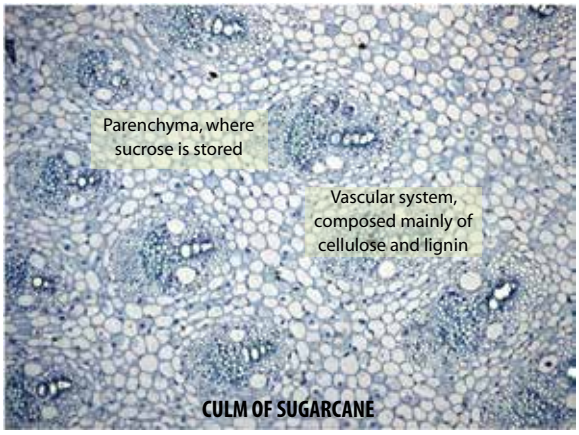

Figure 2. Section of the culm ("stem") of sugarcane showing where sucrose and walls are in the tissue. This picture shows the general structure of what has to be degraded by enzymes to produce free sugars for fermentation and production of bioethanol

UNDERSTANDING HOW SUGARCANE PLANTS FUNCTION

1) Sugarcane has been deeply studied regarding its physiological traits so that the latter could be related with the genetic markers under development by breeders and help to find superior sugarcane varieties in many senses. To do that, plant physiologists started to construct a databank that will be available to other researches;

2) To understand how sugarcane will respond to the global climatic changes, plants have been grown in elevated CO_2 and it was found that its photosynthesis and biomass increased considerably. Researchers discovered that plants capture more light to compensate elevated CO_2 and activate genes related to the electron transport system. Now researchers will try to increase gene expression of photosynthesis to see whether growth is positively affected in the same way, but without the extra CO_2;

3) With the development of molecular markers, it has been possible to map the genome so that researchers are starting to find genes that can indicate important features related to the agronomic features of sugarcane, such as higher productivity, resistance to drought, higher sugar and fiber contents;

4) The expression of important genes and proteins, related to photosynthesis, drought resistance, sugar content and cell wall metabolism are being studied. This information, together with the physiological data, will be important to design strategies to understand how sugarcane plants function. This information can be of great help to breeders as they could produce varieties (or modify plants genetically) that will be more productive and better adapted to different environmental conditions throughout the country.

PRODUCING THE BASIC SCIENCE FOR THE CELLULOSIC ETHANOL

1) The sugarcane cell wall had its chemical structure determined and the polysaccharides have been subjected to hydrolysis with fungal enzymes to understand their mode of action;

2) Sugarcane tissues were sliced and analyzed anatomically (*Figure 2*). We have now enough data and are producing an Atlas that will permit researchers to understand plant better structure of the tissues that have to be degraded to produce bioethanol;

3) Sugarcane bagasse has been characterized and pre-treatment systems are under intensive focus, especially the use of acid hydrolysis and steam explosion;

4) The chemical structure of the trash left in the field has been followed for over a year and the quality of this material, important for use as raw material for second generation bioethanol, is now established;

5) Several fungi species have been found to produce enzyme cocktails capable to degrade sugarcane cell walls. Many enzymes of these cocktails have been purified, their genes cloned and heterologously expressed in bacteria;

6) Yeast species were found that are capable to metabolize pentoses such as xylose and arabinose.

7) Enzymes have been crystallized and some were engineered by artificially introducing catalytic sites from laccase and xylanase in the same protein. These enzymes are being tested with different substrates.

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INTERNATIONAL BIOENERGY MARKET: ASSESSING INSTITUTIONAL STRUCTURES

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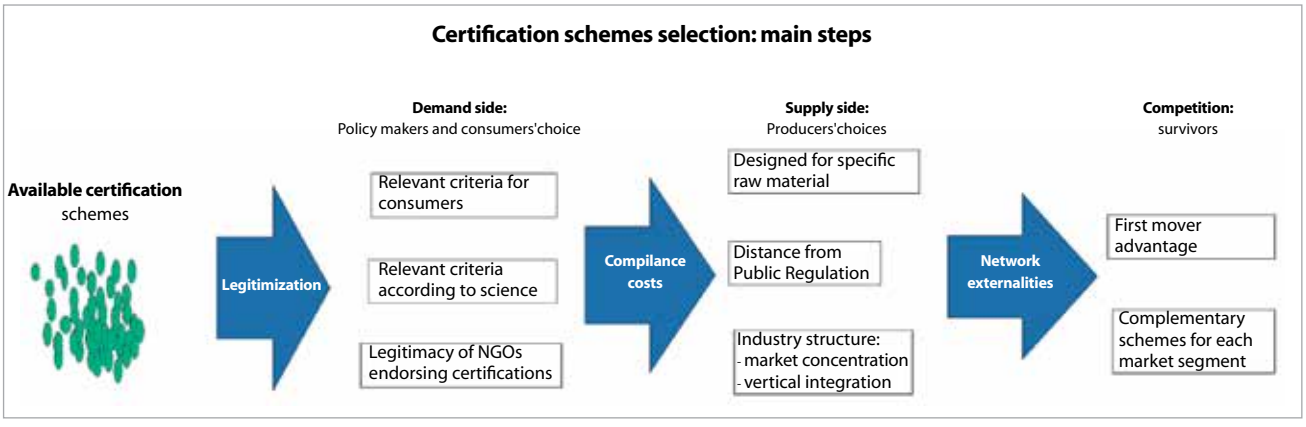

Source: Perosa and Azevedo (2015)

The fast-growing global bioenergy market and the particular features of its demand bring into question the production and trade of renewable products and how they should be regulated in the years to come. Institutional structures governing renewable energy trade are costly and still vaguely defined. Different from regular commodities markets, the emergence of market institutions in the international trade of bioenergy products requires mechanisms that transmit information about socio-environmental sustainability, inasmuch as this is an essential feature of its growing demand.

We investigate the current institutional features of the emerging – and still incipient – international bioenergy market, in particular the capability of those institutions to encompass the production of a broader range of third world countries, so as to increase the number of suppliers and to mitigate risks associated to regional supply shocks, such as draughts or political instability. A more reliable supply of bioenergy products seems a necessary condition for fostering the international demand by energy consumers, particularly for the adoption of mandates and other mandatory consumer measures.

This task is complex because bioenergy products are by large credence goods, i.e., they must carry the information of environment sustainability, which drives the demand for bioenergy products. As a consequence, specific coordination is required in order to establish international standards, and to build institutional arrangements to transact information about the credence aspects of those products, both oriented to the reduction of transaction costs in this emerging market.

This research work aims to understand how institutions of international bioenergy markets will develop in the next years, and what the most relevant impacts will be on the competitiveness and production sustainability in Brazil and other potential producers, particularly in Latin America and Africa. By understanding better the factors involved in the development of bioenergy market institutions, we expect to support public policies oriented to the sustainable development of third world countries, as well as to stabilize the geopolitics tensions derived from the regional concentration of oil- based energy.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The received literature states that the international ethanol trade is quite prosperous. Our research disputes this conclusion. The relevance of international trade is actually diminishing and the majority of the ethanol currently traded does not carry the required information about sustainability. As a consequence, although ethanol is a promising world commodity, it is still mainly a domestic one, and the international trade is yet to flourish.

Cointegration analysis of time series of ethanol prices indicates that there are no strong and direct connections between to two most important domestic markets for ethanol, Brazil and the U.S . This finding is consistent with the anecdotal evidence that there is not yet a well-developed international market for this biofuel. As a result, due to the lack of appropriate socioenvironmental governance, the international ethanol market is yet not able to coordinate production and consumption decisions across countries.

These results have the following implications: a) eliminating trade barriers is not a sufficient condition to enhance trade of sustainable biofuels; b) multi-market models should take into account important frictions in the international market; and c) information is an important constraint to the development of bioenergy as an alternative to fossil fuels. Important constraints for the emergence of appropriate market institutions are: the lack of an established and dominant certification system; complex intergovernmental coordination; and the harmonization between socioenvironmental requirements and WTO norms.

Our analysis indicates that the development of certification systems is driven by three pre-existent variables: public regulation, industry competition and organization, and legitimation mechanisms, whose interaction determines the evolution of socioenvironmental governance. Competition among certification systems and network externalities result in the convergence of public and private environmental regulations. As a normative implication, the efficacy of socioenvironmental governance depends on the compliance costs and, as a consequence, on the prevailing public regulation. The latter must be designed not only taking into account its direct effects, but also its indirect effects on the development of private certification systems.

Technological and organizational features of biofuels production also play an important role on the development of certification systems, affecting compliance and coordination costs. This may limit the emergence of new players from Africa and Central America.

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CONTROL OF LIGNIN BIOSYNTHESIS IN SUGARCANE: MANY GAPS STILL TO BE FILLED

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Although significant knowledge on lignin in plants has been obtained, we still do not know to which extent plants can survive without this polymer. Lignin content may vary in response to several biotic and abiotic stresses and understanding how this occurs may help to understand the control of lignin biosynthesis. We know "almost nothing" about lignin in sugarcane. However, taking in account the information accumulated for other plants and the agronomical practices and problems in sugarcane cultivation, we may have enough hints to plan several studies on how sugarcane modulates lignin composition and content. Therefore, the aim of this project is 1) to cultivate contrasting sugarcane genetic material for lignin content in 5 locations well characterized for temperature, water availability and irradiance and analyze lignin, sucrose and cellulose, and then, based on these results to study gene expression and perform a more detailed study of lignin composition; 2) to search the SUCEST database for ESTs coding transcription factors known to be involved in lignin metabolism in model plants and use this information in controlled studies (on water supply, nitrogen fertilization, light intensity and low temperatures under field and greenhouse conditions, and growth chamber) to establish correlations between transcription factors regulation and lignin content; 3) search the SUCEST database for ESTs coding ortologs to peroxidases and laccases and use this information in the controlled studies to evaluate the involvement of these enzymes in lignin biosynthesis; 4) to perform a system biology study of regulatory network involved in lignin biosynthesis. With this information we may get some valuable knowledge on the lignin biosynthesis in the complex sugarcane genome.

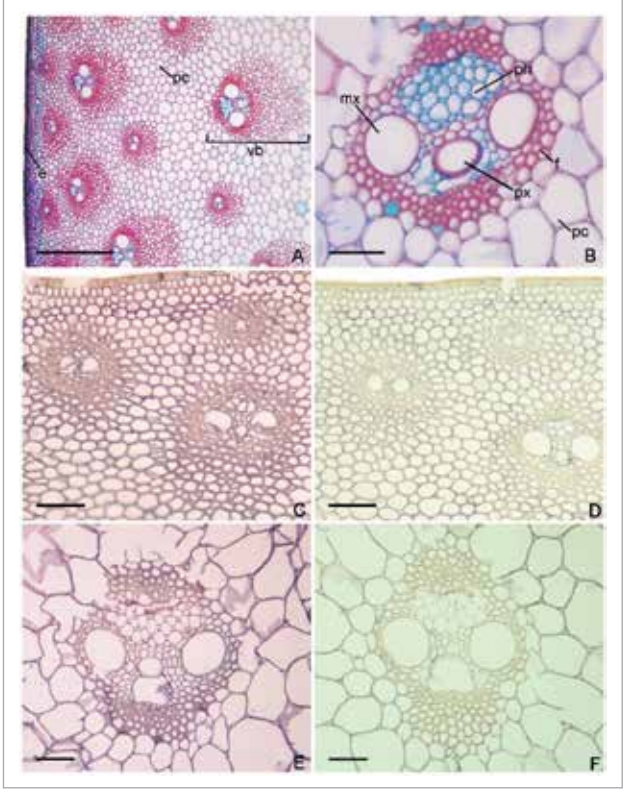

Figure 1. Anatomic cuts of a sugarcane stalk showing stained vessels for lignin (A and B) and in situ expression of dirigent proteins (C-F).

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The main result of this BIOEN project was the identification of 6 genes that can be used to produce genetic modified sugarcane for lower or modified lignin. They were two of the lignin biosynthetic pathway – ferulate 5-hydroxylase (F5H), hydroxycinnamoyl transferase (HCT) – a dirigent protein – DIR1 – and two transcription factors – NHS and MYB58. Other very important result was the realization that there is variation in the ratio S/G in the pith and rind f the sugarcane stem during its development. This was initially observed in two contrasting genotypes for lignin growing in the greenhouse and then in four genotypes grown in the field. This shows that during cane ripening S are mainly synthesized in the cortical cells, which is desirable, since lignin with S units is more easily removed by chemical treatments, increasing the efficiency to obtain cellulose. This result defined the choice of the F5H gene, which is in the unit S biosynthetic branch of lignin pathway, as well as transcription factor MYB58, which showed strong correlation with the expression of F5H. It is worth mentioning that the control F5H by MYB58 bypasses the control or interaction with other transcription factors. In the rind, either on new stems or mature culms, S/G ratio remains low. Thus, as a consequence the following questions become very important: In relative terms, how important is the rind and pith lignin in the total lignin content of the sugarcane? What is the amount found in both tissues and this would affect sacharification?
The control of gene expression in the pith is certainly different of the cortex, since they have different S/G ratio, but would be feasible to manipulate gene expression in a way that S/G was also high in the rind? Transformed sugarcane for F5H and MYB58 gene could provide an answer to this. The project also allowed developing methods for determination of lignin oligomers and S/G ratio.

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IDENTIFICATION OF SOIL AND PLANT ATTRIBUTES RELEVANT FOR A SUSTAINABLE SUGARCANE MANAGEMENT USING PRECISION AGRICULTURE TECHNIQUES

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FAPESP Process 2011/02817-9 | Term: Feb 2012 to Mar 2014

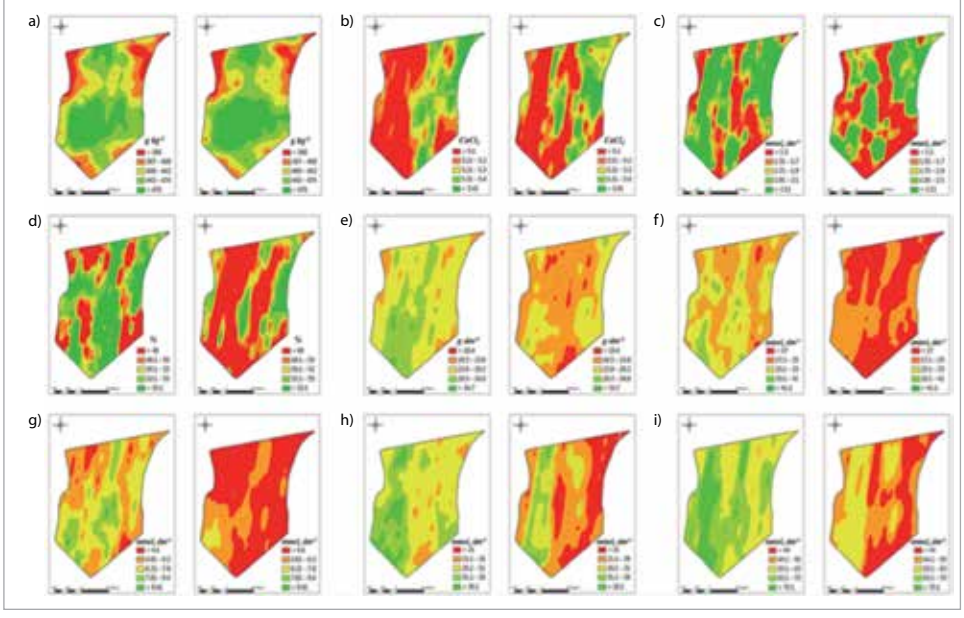

Figure 1. Soil chemical attributes determined based on a regular dense grid (left) and on oriented and reduced soil sampling using soil electrical conductivity (ECa) as external drift in Kriging (KED) (right)

The production of biofuels can only be justified if their economic and environmental impacts are favourable, compared to similar energy sources, and if there are real benefits to all segments of society directly involved. One of the innovations of science and technology that should be incorporated into the production of sugarcane is the Precision Agriculture (PA), which allows the producer to understand and control more precisely the inherent spatial variability found in the fields in order to obtain higher productivity, economic return and environmental protection. In this context, this project has as main objective the evaluation of the causes of spatial and temporal variability of sugarcane productivity in order to optimize the agricultural management through AP technique. The specific objective are identify the factors, and their interactions that influence the spatial variability of yield and quality of sugarcane. The project aim to improve agronomic diagnostic models and guide the development of sensors that allow a determination "on-the-go" of soil parameters to complement the scientific knowledge needed to extend the full potential of AP as an effective tool of crop management on a commercial scale. For this, two areas cultivated with sugarcane, each with 50 ha, one area applying PA concepts, such as fertilizer application at varied rate and one area with standard management, were evaluated regarding their soil attributes, chemical and physical, and plant nutritional status. This research project, seeks to contribute to the technical/scientific knowledge about the spatial variability of sugarcane productivity through: survey of more detailed data, to assist in understanding the crop response to variations in soil and climate conditions within the cane fields. Then, contribute to the development of new management strategies for sugarcane, aiming a vertical integration of productivity and reduction of environmental and economic impacts.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The experiment comprised a diversity of technologies to measure soil and plant attributes within a 50 ha sugarcane area. In our data analysis workflow, the diversity of measured attributes was treated on a common ground, providing a platform to compare data acquisition technologies and to eventually select those technologies suited for large-scale PA practice. We are working to expand the sugarcane PA database, adding data from other finished and ongoing experiments. With the expanded dataset, we expect to recognize relevant patterns that are reproduced consistently across distinct experiments. For instance, data evidenced annual change in spatial distribution of sugarcane yield as well as a correlation structure relating soil pH with concentrations of organic matter and lime elements. This type of information is valuable for future PA practice. The proposed analysis workflow applied systematically to an ever-growing dataset will help consolidating the knowledge required for effective sugarcane PA.

Through an optimised grid guided by ECa, it is possible to predict the spatial distribution of soil physical and some chemical properties using Kriging with external drift (KDE). We conclude that KDE (using dynamic secondary information) presents advantages when using sparsely sampled points, providing more reliable estimates of the attributes. To improve prediction, more information could be added from other soil sensors in the trend model. These results offer a perspective for obtaining other attributes that must be estimated over large areas based on sparse soil samples. This method could assist farmers with crop management and ensure higher economic returns and a sustainable production system.

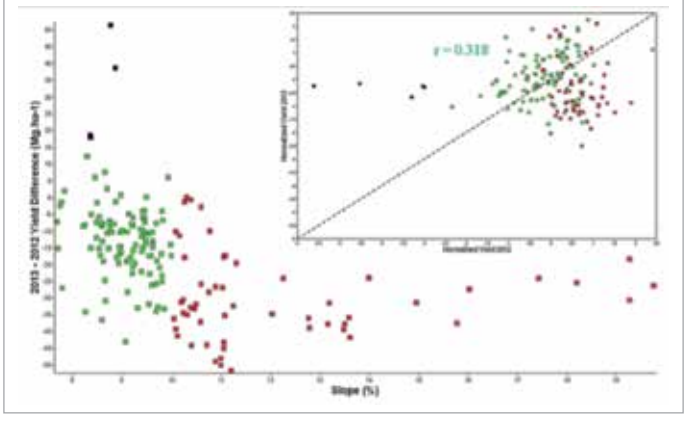

Figure 2. Yield decrease between successive years as function of the field slope and the Person´s correlation between the successive years yield normalized data

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IMPROVING PHOSPHORUS EFFICIENCY IN SUGARCANE

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FAPESP Process 2013/21604-1 | Term: Sep 2014 to Aug 2016

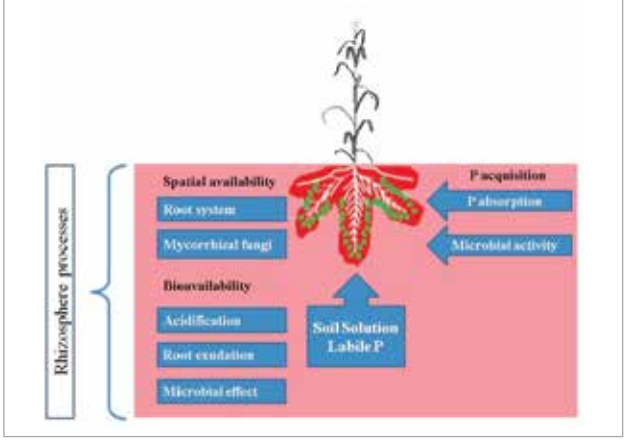

Figure 1. Dynamics of phosphorus in the rhizosphere by the interaction between plant/soil/microorganisms. Adapted from Shen et al. (2011)

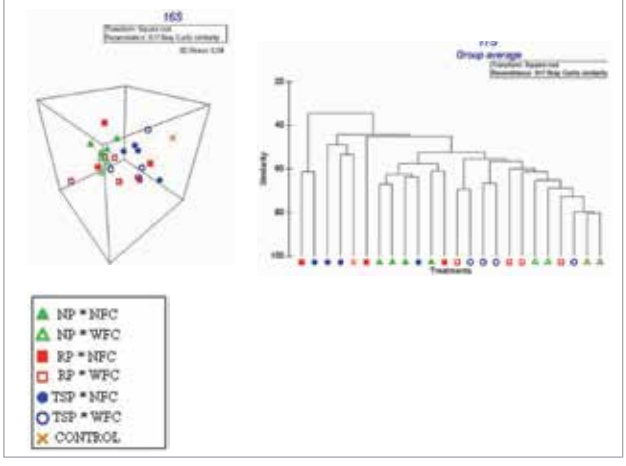

Figure 2. A) NMS (Bray Curtis similarity) plots from bacterial 16S TRFLP results and B) clusters from fungal ITS TRFLP results. Points represent samples. NP – no phosphate; RP – rock phosphate; TSP – triple superphosphate (Both at rate of 180 mg soluble P_2O_5 kg^{-1}); NFC – no filter cake; and WFC – with filter cake at rate of 10 t DM ha^{-1}

The sugarcane cultivated area in Brazil is around nine million hectares and still expanding, in order to supply the improving demand for ethanol and sugar. However, the productivity is limited by several factors, including the low soil phosphorus (P) availability and the adsorption of this P by oxy-hydroxides of Fe and Al in the soil, especially in tropical conditions. According to the rising costs of fertilizers and concerns about reduction of finite reserves of phosphate rock, the use of P by crops in general must become more sustainable. The objective of this research is to evaluate the use efficiency of P by sugarcane fertilized with organic (filter cake) and inorganic (reactive phosphate and superphosphate) sources of P applied at planting, in order to develop strategies to decrease the adsorption of this nutrient in soils and increase the recovery of P from fertilizer. Four experiments were established in field conditions in São Paulo State, as well as three experiments in controlled conditions (greenhouse). Field experiments were established in plant cane and conducted throughout the crop cycle, until the next replanting. The evaluation includes: i) production of stalks; ii) technological attributes; iii) P accumulation by the plant; iv) use efficiency of P; v) fractionation of P in the soil. Under controlled conditions, the experiments aimed to assess the soil-rhizosphere interaction in the availability and utilization of P from soil and fertilizer by plants. Thus, the main goal of the research is to develop management practices to maximize the efficiency of P use from the soil and fertilizer by sugarcane, improving the sustainability of phosphate fertilization.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The microbial activity and nutrient content in soil microorganisms is normally affected by the crop and/or genotype used, since the rhizosphere interaction is important to solubilize nutrients (*Figure 1*). In our study, the microbial P level was not affected by sugarcane genotypes, this was also observed under different phosphate rates, with low effect of rates in the microbial P levels in the soil. In addition, changes in the rhizosphere can be improved by microorganisms, because in our case the presence of fertilizer has altered the microbial community composition compared to no fertilizer. Under no fertilizer the microbial groups are closer to each other, indicating low diversity, which may be due to the absence of available P, reducing the interaction between microorganisms and plant. Otherwise, under phosphate application the microbial groups are more disperse, indicating more diversity for all sugarcane genotypes, indicating that the presence of phosphate has provided adequate environmental conditions for the development of a larger diversity of microorganisms, interacting each other and increasing also the plant development.

The genotype RB96-6928 was the best one to adapt under low soil P availability since it presented the largest volume of root and better plant development. The microbial community of all genotypes was changed by phosphate fertilization; more available P in soil has promoted exchanges in microbial communities (*Figure 2*). The dose of around 90 mg kg^{-1} of P_2O_5 as TSP was the most adequate to promote a good seedling development and also better soil rhizosphere parameters, like pH and acid phosphatase activity. Filter cake improved P uptake and availability of labile Pi in the rhizosphere and also modified the fungal microbial community. In addition, the combination between filter cake with rock phosphate was efficient to improve sugarcane growth, such as shoot dry matter and number of tillers per plant.

In the field, the sugarcane yield was higher with TSP as compared to RP, both superior to the control, and there was also a great productivity response when applied filter cake associated to phosphate sources at planting. This behavior was observed for both years evaluated at a sandy soil. However, in a clayey soil there was little response to fertilization, not differing between phosphate sources (TSP or RP) and application ways (broadcast incorporated or at furrow bottom). More research is necessary under field conditions to establish good parameters to get better plant results under soil P restrictions, in this way, we plan to keep this experiments at least for 6-7 years.

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HYPERSPECTRAL DATA FOR PREDICTION OF LEAF NITROGEN CONTENT IN SUGARCANE

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FAPESP Process 2013/21604-1 | Term: Sep 2014 to Aug 2016

Nitrogen is an essential nutrient for crop production, interacting in a very complex system with environment, so its monitoring is therefore important, both economically as well as environmentally.

There is wide variation in the literature regarding the best dose to be applied in sugarcane, and these differences are directly related to the variety, soil type, and area and cropping system. Thus, the need to develop a tool to determine the nutritional status of sugarcane in different growing conditions is evident, in order to minimize costs, maintain or increase productivity without impacting environmental integrity.

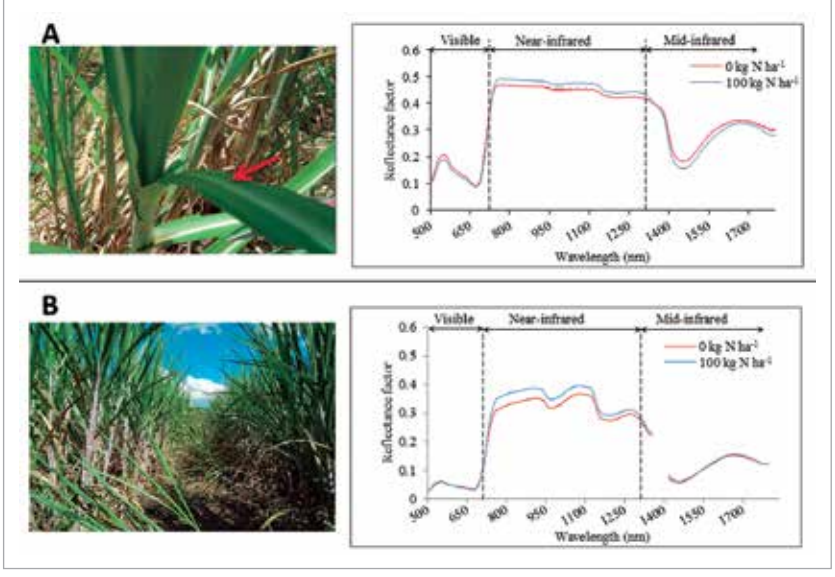

Figure 1. Responses of spectral reflectance of sugarcane at leaf (A) and canopy (B) level, grown on sandy soil, 2013-2014 harvest, using different doses of Nitrogen: stressed (0 kg N ha^{-1}) and unstressed (100 kg N ha^{-1})

As has been noted, the use of remote sensing has expanded in agricultural sciences, providing a useful tool to monitor and manage such activities, including the nitrogen fertilizer administration on crops.

The spectral behavior of sugarcane, through the reflectance curves, makes it possible to estimate various parameters based on the overall condition of the plant. Each range of wavelength yields a different spectral response curve that allows us to predict some physiological states. Therefore, a study on monitoring nitrogen in the culture of sugarcane using hyperspectral sensors is necessary.

Evaluations are performed at different growth stages of sugarcane in a permanent plot of land used for field experiments of São Paulo Agency for Agribusiness Technology, which studies effects of different nitrogen doses in some sugarcane varieties. Spectral readings are held of leaf and canopy; leaves (+1) area collected and sent to laboratory for leaf analysis to determine nitrogen content. These data will be used to generate spectral models of multiple linear regression to predict the leaf nitrogen content, thus detecting the wavelengths that are more related to the plant nitrogen status.

This study aims to show the results that may guide future applications of spectral data to monitoring sugarcane nitrogen fertilization, as new research and development of dedicated sensors for the culture.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The project consists in the temporal analysis of the sugarcane (*Saccharum spp. L.*) leaf and canopy spectral behavior in different production environments, under the effect of different nitrogen doses. In 2013-2014 harvesting, the precipitation of several months was lower than the historical average recorded which may influence the crop growth, and consequently the reduction of leaf area, due to the provision of smaller amounts of water during the early growth stages, increasing stress in plants in all treatments with different nitrogen doses.

Preliminary results have shown a difference in the spectral behavior of sugarcane leaves, when average curves were compared between the stressed and unstressed treatments in the different environments. The visible spectral region (400 to 700 nm) of unstressed leaves curves has showed lower reflectance values with opposite behavior near-infrared region (750 to 950 nm).

As the concentration of pigments such as carotenoids, xanthophylls, and anthocyanins cause variations in the visible spectral region, the stressed plants tend to turn clear yellowish-green foliage, while vigorous plants leaves turn to shades of dark green.

For canopy spectral behavior analysis, biometric variables are compared to the average of spectral curves, showing that it is possible to establish meaningful relationships between agronomic variables of culture and the spectral variables obtained by remote sensing; whose spectral parameters can describe the conditions of vigor and development of sugarcane.

Although the canopy spectral curves are more susceptible to interferences from external factors (climate, plant architecture, soil and others) during the collection of data, these curves showed the same pattern of leaves spectral curves measured in a controlled environment.

For both curves, the same tendency regarding the leaf nitrogen content was observed, wherein the stressed plants (0 kg ha^{-1}) have showed a higher reflectance intensity in the red region (660nm) and lower reflectance in the near infrared region (840nm); and unstressed plants (100 kg N ha^{-1}) had the typical curve of healthy vegetation, with lower reflectance intensity in the red region and higher in the near infrared region.

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CONCENTRATED VINASSE USE ON SUGARCANE PLANTS: SOIL CHEMICAL ATTRIBUTES, ION LIXIVIATION AND AGRONOMIC EFFICIENCY MONITORING

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FAPESP Process 2008/58029-6 | Term: Jun 2009 to Aug 2011

Figure 1. Vinasse application in the plot experiment

The increasing ethanol production represents many advantages to Brazil as a less pollutant renewable fuel, but it will generate higher quantities of vinasse, the residue that is applied as a fertilizer for sugarcane. On the other hand, it is already known that there is a potential risk of ion lixiviation to the underground waters when high rates of vinasse are applied to the same soil during several years. In this way, other uses for vinasse shall be studied, but the high vinasse value as an organic fertilizer turns indispensable to find out new viable procedures for its use on sugarcane cropping. In this sense, the concentrated vinasse might be one alternative to take the residue to more distant soils, contributing to the sugarcane nutrition with significant savings for the country on imported fertilizers. However, further investigation is urgently required to find out whether the concentrated vinasse is a potentially higher pollutant residue than the natural vinasse. In order to evaluate concentrated vinasse effects on soils and its potential risk of ion lixiviation to underground waters, some trials are being carried out. The specific aims are: (1) Compare the normal and concentrated vinasse physical-chemical characteristics from several samplings from the same factory, and also, to compare their characteristics with the parameters established by the legislation for agriculture use. Besides, to evaluate the concentrated vinasse residue effects on soils, in order to obtain knowledge to its sustainable use in crop ferti-irrigation mainly to sugarcane cropping; (2) Evaluate the concentrated vinasse biodegradation and mineralization compared to the normal vinasse; (3) Evaluate the ion lixiviation potential of concentrated vinasse compared to the normal vinasse and to the mineral potassium fertilization; (4) Evaluate the concentrated vinasse agronomic potential as a source of nutrients to sugarcane crop.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

This project started in November 2009 by plotting the field experiment. The area used was located in Batatais region, São Paulo State, and the sugarcane was in the first ratton stage. The results reported at the present are still preliminary being the conclusions expected in September – October of 2010 by harvesting the plants and analyzing all the samples for monitoring vinasse uses.

In the experiment, samples of two kinds of vinasse were applied, one is the vinasse that is normally obtained in the ethanol production (normal vinhasse), and the second is a concentrated vinasse, both proceeding of the same sugarcane mill. A scheme for sampling and analyzing vinasse samples periodically was established so the different vinasses were compared (*Table 1*). The results showed that chemicals properties of both vinasses are similar, however macro-nutrients concentrations, especially potassium (K), are higher in concentrated vinasse. There were variations on macro-nutrients contents in vinasse through the months, but in general, K concentrations have been 20 to 30 times higher in concentrated vinasse.

Soil samples, obtained from the field experiment, were taken to the laboratory to evaluate carbon (C) and nitrogen (N) mineralization after normal vinasse and concentrated vinasse application. The preliminary results of these tests showed that C and N mineralization occurs faster with normal vinasse when compared to concentrated vinasse.

Table 1. Macronutrients and pH in concentrated vinasse compared with normal vinasse

	pH	N	PO_4I	K_2O	Ca	Mg	SO_4
				gL^{-1}			
Vinasse	4,1	0,35	0,08	2,18	0,19	0,35	1,1
Concentrated vinasse	4,1	3,7	1,3	52,8	3,1	4,4	18,2

N – Kjeldahl, K – flame photometry, P, Ca, Mg, SO4 – ICP-AES

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ENVIRONMENTAL MONITORING AND MODELING OF THE GENETIC POTENTIAL OF SUGARCANE CULTIVARS UNDER APPROPRIATE SOIL WATER AVAILABILITY

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FAPESP Process 2010/52139-4 | Term: Apr 2011 to Mar 2014

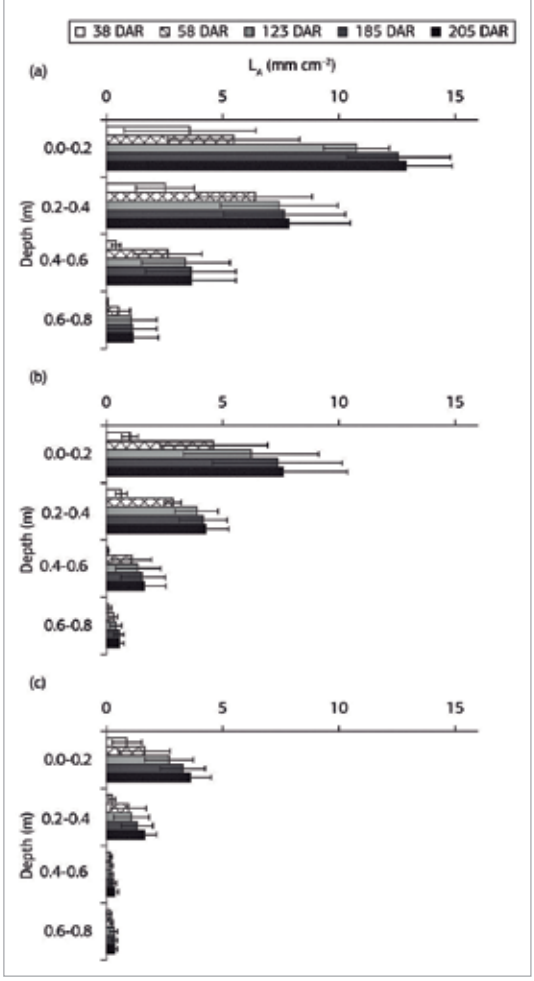

Figure 1. Cumulative root density (L_A) in sugarcane cultivars IACSP94-2101 (a), SP79-1011 (b) and IACSP94-2094 (c) in five assessments up to 0.8 m depth. Each bar represents the mean value of three replications (± s.e.). DAR = days after ratoon (Source: Ohashi et al., 2015)

The need for bioenergy production is a growing concern on modern society due to the sustainability of the activities associated with the growing economic demand. The average yield achieved in Brazil is less than 80 tons per hectare. This value is low when compared to the crop biological potential value, which is more than 300 tons per hectare. The use of irrigation practices in sugarcane can improve the crop yield and enhance the nutrient efficiency, especially when a subsuperfical drip fertirrigation is adopted.

Concerning the sugarcane crop irrigation in Brazil, it is mainly used for vinasse application. Systemically irrigated areas are still just few if compared to the potential for the use of this technique. In this context it is important to point out that the yield response to water differs according to variety, environment and cultural practices. It is also necessary studies related to water use efficiency in irrigated crops. Therefore, it is important determine parameters to promote water use efficiency and evaluate the response of different varieties to water supply.

In order to understand the plant water dynamic in relation to the environment, the use of sensors to monitoring the climate, soil water, soil fertility and plant growth becomes necessary. Thus, the objective of this research was to evaluate the crop evapotranspiration, crop development, yield, water use efficiency, root system growth and distribution, and calibration and use of models to yield forecast in four different sugarcane cultivars. These cultivars have different characteristics related to the water availability and canopy architecture. It was used a subsurface drip irrigation system and the irrigations were done every day, except on rainy days.

The field experiment was conducted during two sugarcane ratoons. The meteorological parameters, (agricultural weather station), soil water and soil electrical conductivity (capacitance probes), root growth (rootscanner -minirhizotron) and distribution (auger method), crop growth, yield and qualitative attributes were monitored during the field experiment. The crop evapotranspiration was estimated using a water balance.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Root growth varied during the crop cycle, with the highest values being found between 38 and 58 days after ratoon (DAR). There was a genotypic variation in root growth, with IACSP94-2101 showing the highest La (cumulative root density) of 12.9 mm cm^{-2}. It was possible to observe variation in root growth rates among cultivars and among the sampling time. Considering the sampling time, higher rates were observed between 38 and 58 DAR in all cultivars (*Figure 1*). This information could be useful when using saving irrigation. The lateral root distribution varied between IACSP94-2101 and IACSP95-5000 cultivars and the effective rooting depth was respectively 0.6 and 0.8 m depth (*Figure 2*). Understanding the root distribution along the crop cycle could provide useful information for water and nutrient management and modeling purposes.

The highest yield and water use efficiency (WUE) were observed in IACSP94-2101 and IACSP95-5000 cultivars. The obtained yields were 241,9, 236,5, 197,8, and 183,5 Mg ha^{-1} for IACSP 94-2101, IACSP95-5000, SP791011 and IACSP 94-2094 cultivars, respectively. Considering yield and actual evapotranspiration, the WUE varied from 20,85 to 14,4 kg m^{-3}. Higher WUE values were obtained for IACSP 94-2101, IACSP95-5000. The obtained results showed different responses in yield and WUE according to the sugarcane cultivar when irrigated by SDI. Concerning the second sugarcane ratoon,the achieved yields were more than two times higher in all cultivars evaluated when compared to the average yield in the country. In the first ratoon there was good calibration for AQUACROP and CANEGRO the DSSAT system model. The potential production (PP) cultivars with erect leaves as IACSP94-2101 and SP79-1011 were simulated with a small decrease in precision (R2) in relation to the other cultivars when used CANEGRO model from DSSAT system. In the second ratoon, the AQUACROP model did not presented good yield estimate as observed for the cultivars with sprung leaves (IACSP95-5000 and IACSP94-2094). However, CANEGRO model DSSAT system showed better calibration for second ratoon than the observed for the first one.

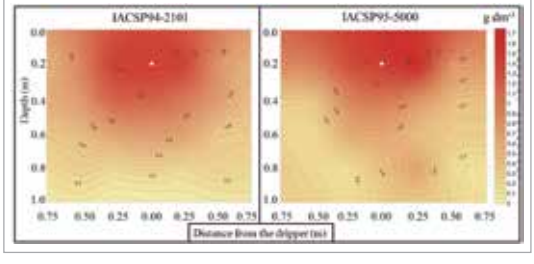

Figure 2. IACSP94-2101 and IACSP95-5000 root distribution (MDR; g dm^{-3}) in the soil profile up to 1.0 m depth and 0.75 m apart from the planting row from both sides. The white points represent the dripper position, at 0.2 m depth (Source: Ohashi et al., 2014)

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ASSOCIATION ANALYSIS USING SSR AND SNP LOCI TO FIND QTL FOR SEED OIL CONTENT IN SOYBEAN

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FAPESP Process 2008/56249-9 | Term: Feb 2009 to Jan 2014 | Young Investigator

Brazil is known by its great variability in seed yields that may be used to extract vegetable oil. Nowadays, soybean is the best alternative among all oilseed crops, because of the large cultivated area throughout the country, the adequate levels of productivity and quality of oil (viscosity and cetane ratio), the short-term cycle (nearly four months from seeding to harvesting) and the network of crushing industries available nationwide. The objective of this research was to identify molecular markers associated with QTL regions related soybean oil content and composition of its main fatty acids.

Soybean accessions from different regions of the world were evaluated in field conditions of ESALQ/USP for years 2010 and 2011. Oil and oleic content of seeds were estimated by gas-chromatography. Genomic DNA samples were prepared from a lyophilized bulk of leaf tissue of each accession and polymorphism of SSR loci (Simple Sequence Repeat) and some genes (SNP loci- Single Nucleotide Polymorphism) involved in oleic biosynthesis were detected in DNA Analyzer.

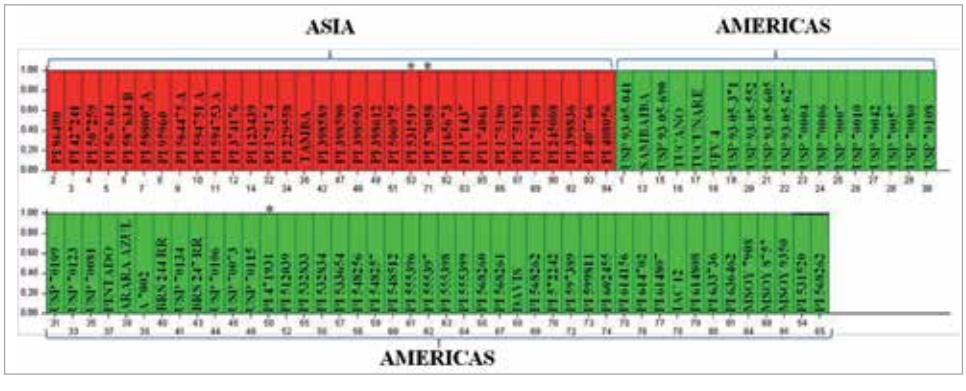

*Figure 1. Two germplasm clusters, red and green, based on Bayesian analysis for the 94 soybean accessions analyzed by using 142 SSR markers. Details of identification of accessions and their geographical origins are indicated. * Marks refer to the accessions present in a cluster that not correspond to their origins*

Accession	Palmitic	Oleic	Linoleic	Linolenic	Satt294		Satt386		Satt586		Satt571		Satt270		Sat_224		Sat_144		Satt487	
					268	286	184	217	199	220	149	179	240	243	134	221	148	196	202	226
PI 568261	5.61	50.96	34.08	4.07		+	+			+				+				+		+
PI 531520	8.65	54.05	28.80	2.41		+								+					+	
PI 578058	9.57	45.95	36.51	2.47		+		+		+	+			+	+		+			+
PI 599811	3.24	42.73	43.76		+			+			+			+			+		+	
PI 568260	5.40	56.18	28.16			+	+							+			+			+
PI 512039	9.37		44.00					+			+			+			+		+	
USP 70109	9.09			4.80	+			+		+	+			+		+	+			
USP 70106	9.19			4.31	+			+		+			+			+	+			
PI 602455	3.32				+			+		+					+		+			
PI 532833	7.41				+						+			+						
PI 614702		38.82	42.04	2.96			+				+		+			+	+			
PI 533654		38.26	38.93	4.06	+		+			+							+			
PI 555396		37.64	40.73				+			+	+			+			+			
USP 70081		37.35	42.57				+				+			+				+		
PI 633736		35.30		4.17	+			+			+						+			
PI 597389				4.50	+			+			+			+	+		+			
USP 70108				4.80	+			+		+	+			+		+	+			

Figure 2. Molecular profiles and fatty acid content soybean accessions based on the best alleles from each of the 8 SSR loci that were significantly associated with four fatty acids. (+) Symbol indicates presence of allele

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Based on polymorphisms at SSR loci, the soybean accessions were divided into two clusters corresponding to accessions from Asian and American gene pools (*Figure 1*). Linkage disequilibrium (LD) among the SSR loci suggested a structure across the soybean genome (LD decay) of approximately 12 cM. Pairwise kinship estimates among the accessions showed low values. Association analysis was performed by a mixed linear model (MLM) that identified 110 significant associated marker-traits. Oleic acid content had the highest number of associated SSR loci (33), followed by linoleic acid (26), palmitic acid (24), oil (16) and linolenic acid (14).

Modifying the fatty acid content of soybean oil for improved functionality is the objective of many soybean breeders. Increasing oleic acid levels and decreasing linoleic and linolenic acid levels makes soybean oil healthier for human consumption. Moreover, to optimize the fuel characteristics of soybean oil for use in biodiesel, it has been suggested that oils that are high in oleic acid and low in palmitic acid should be developed. We identified specific alleles at eight loci that yielded high performing soybeans with respect to balance of fatty acid content and these are presented in a crossing matrix (*Figure 2*).

We also used to candidate gene approach for association analysis. We observed single nucleotide polymorphisms (SNP) in genes FAD2-1 and FAD2-1B after sequencing of 800 bp of 20 soybean accessions that presented high and low oleic acids content. The sequences were highly similars to sequences present in gene bank (96 and 95%, to FAD2-1 and FAD-1B gene, respectively) but they had not any polymorphism significantly linked to fatty acids.

In conclusion, our analyses of SSR loci and fatty acid content were consistent with previous findings and also identified many new markers, which may be useful for quickly improving the fatty acid composition of soybean oil. By using the favorable individuals identified in this study, it may be possible to exploit the soybean germplasm using the suggested SSR loci and allele matrix to allow breeders to strategically plan fatty acid breeding programs.

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SUGARCANE ENERGETIC BALANCE: A SYSTEMS APPROACH TOWARDS UNDERSTANDING REGULATION OF SUCROSE METABOLISM AND SUGAR SIGNALING

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Figure 1. Bayesian network based on the relationships between gene expression, lignin composition, and lignin content data from internodes at three developmental stages and different tissue types of high- and low-lignin content sugarcane genotypes (Bottcher A. et al. 2013)

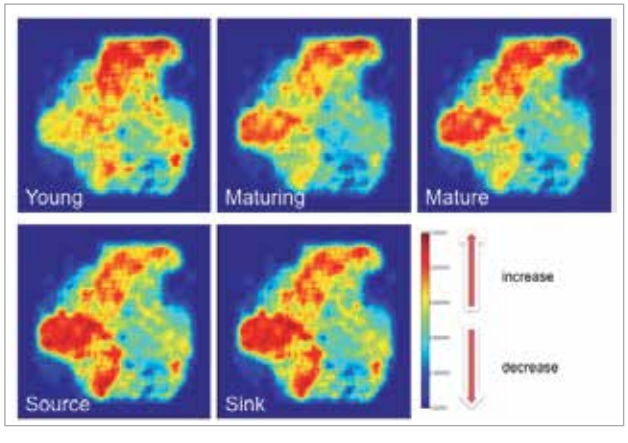

Figure 2. Gene regulatory network of source-sink relationship for sugarcane and its transcriptional activity

Our laboratory has dedicated in developing Systems Biology approach for understanding molecular and physiological aspect of plants species. Sugarcane research has identified and characterized a suite of proteins involved in carbon biosynthesis and sugar sensing. However, current results towards understanding sucrose biosynthesis and accumulation have fallen short of expectations. The molecular mechanisms responsible for the cross talk between these different regulatory and signaling pathways and their diversification in plants still need to be further elucidated to better understand plant growth patterns and biomass production. We are only beginning to produce the detailed gene expression data needed for understanding the network of interactions at a molecular level. To address the rate of gene discovery, high-throughput approaches have being developed for biological experimentation and relevant biological questions regarding gene, protein interactions or networks of biological process can now be addressed. Here, we developed a research approach that integrates molecular and systems biology to improve the knowledge about carbohydrates biosynthesis and sugar regulatory signaling in sugarcane. In this research project we elaborated ways to apply analyses of regulatory network and dynamic metabolic models in molecular and genetic data of sugarcane related with sucrose biosynthesis. We expected that the models captures the regulation of many sugarcane genetic components and anticipate that the data will improve our view of sugar signaling in plants. Simulations of our models will provide an efficient tool for the identification of candidate to genetic manipulations that have the best chance to promote increase in sucrose content and for the prioritization of future analyzes.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

A long-term goal of sugarcane growth modeling is to be able to do analysis based on gene expression data organized into genetic and metabolic pathways that are modeled for interactions. In this study we developed ways to apply analyses of gene regulatory network in molecular, physiological and genetic data of two contrasting sugarcane genotypes submitted to changes in the sink:source ratio. The lowest sucrose content genotype shows the highest levels of chlorophylls and a highest efficiency in the photosystem II, mainly in the middle of the day. This genotype also shows high levels of sucrose in leaves but do not accumulate high levels of sucrose in the stems. The de novo transcriptome analyses results in a total of 191,871 transcripts, where the most of transcripts have at least one hit with viridiplantae known proteins, which almost half of the transcripts are related to sorghum. Alignment against Sorghum genome showed that ~13.3% of transcripts could be ncRNAs. With the comparison of gene networks against different plant species was possible to reduce measurement noise and to reinforce the common signal present in the networks. Using the differential expressed genes identified in the source-sink experiments we can detect more than 50% genes inside regulatory complex conserved across sugarcane and rice. We also detected several gene clusters, including many hubs, which incorporate different regulatory genes (ncRNAs, siRNAs, and miRNAs). In source-sink experiment was possible to detect that 1% of transcripts change its expression to response to the sink manipulation and 5% of transcripts show differential expression between the contrasting sugarcane genotypes. The sugarcane source-sink gene regulatory network shows a strong negative correlation between photosynthetically genes and genes involved in the cell wall metabolism. This study brings new knowledge on the diversification of the regulatory network that controls sucrose metabolism and source-sink balance in sugarcane. The results should contribute to improve our ability to manipulate the biomass production through classical breeding or biotechnological approaches.

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IMPROVED SPACE-BASED REMOTE SENSING FOR LAND USE MAPPING: TOWARDS A SUSTAINABLE EXPANSION OF THE BIOETHANOL SECTOR IN BRAZIL

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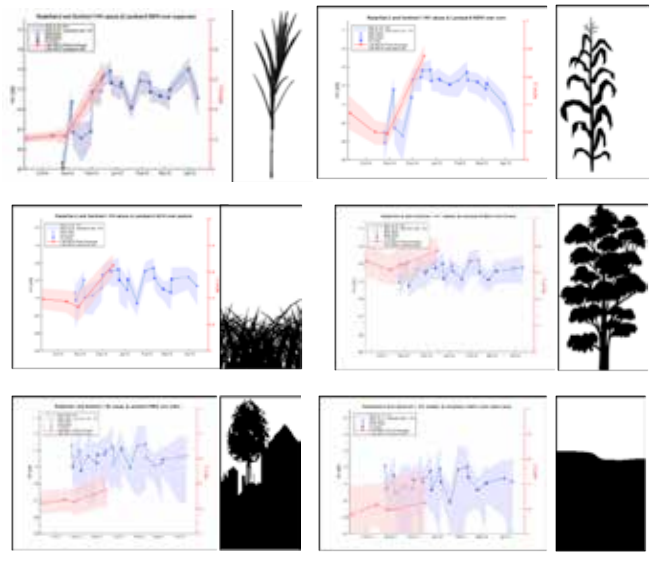

Figure 1. Radar and optical profiles

It is projected that ethanol production in Brazil will increase from 22 billion liters (2011) to 46-65 billion liters in 2020. However, an increased deployment of biomass for energy and materials could have significant adverse socio-economic and environmental impacts. Many initiatives for sustainability frameworks for biomass for energy have addressed the importance of the monitoring of direct and indirect land use (LU) change in respect to enable the identification and quantification of their impacts such as GHG emission, food security, biodiversity and competition for water etc. The objective of this project is to develop a methodology for the accurate and systematic monitoring of land use change dynamics as a result of the expansion of the cultivation area of sugar cane for ethanol by the assimilation of remotely sensed data. The development of the methodology aims at combining optical and radar data in order to allow for the development of reliable, high resolution, discriminative land use maps in a time and cost effective way. For two case study areas optical, radar and ground truthing data is collected. For both the optical and the radar data, the signatures of the different land uses are identified and an effective methodology will be designed to assimilate the radar and optical information for the production of land use maps in robust and effective way. The methodology will be validated for the two selected regions, after which it will be scaled to enable land use change mapping at a larger area (micro region / state level). Based on sequential maps of land use for the two larger areas, a first order environmental and socio-economic impact analysis of the expansion of sugar cane is made. The data developed in this project will be implemented in a database management system which will allow for interactive exchange and analysis of the data and will ensure access for potential users.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The results of the project can be reduced to Preliminary data. The majority of time had been spent in the field data collecting, storage and calibration of the classification algorithms for generating the Land Use Maps. Remote Sensing applied to crops monitoring need a huge amount of field data to understand completely their cycles.

The preliminary data have shown some profile built from Radar and Optical Remote Sensing data for some crops as sugarcane, pasture, corn, forest, water and urban area. These data will help in the identification the differences among the profiles in time per target. In the *Figure 1* are shown some profiles until now processed from SAR data and OLI/Landsat 8 data as well. This procedure is being followed for a small area where it is possible collect data. Two areas are being used as sampling areas: around Capivari Municipality/SP and Mogi Guaçú Municipality/SP considering that in the first one are found areas with sugarcane and pasture while in the latter area is very common to find annual crops, citrus and forestry.

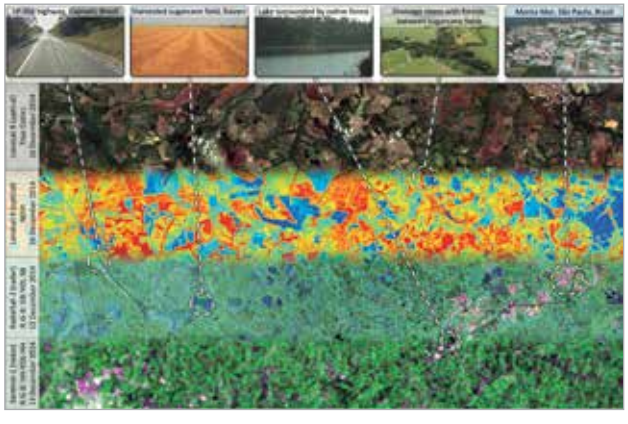

Figure 2. From: Iannini L, Molijn R, Mousivand A, Hanssen R. Sugarcane identification through time-series of landsat and ERS/ ENVISAT Data. SBSR 2015, Brasilian remote sensing symposium, April 2015, João Pessoa, Brazil

For the second part of the project, the sustainability study will be used two maps for starting the analyses. Both are being processed from the maps available by Brazilian Environment Ministry until the new Land Use maps, described previously, to be generated.

Radar profiles

The radar profiles and optical data (*Figure 1*) from specific fields per crop type, only now for qualitative and illustrative means.

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WATER USE EFFICIENCY FOR BIOMASS AND ENERGY IN SUGARCANE CROP

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Sugar cane crop stands out as one of the main crops in Brazil. In the new areas of sugarcane expansion industry (SP west, MS, MT, GO, MG, BA, AM, MA and PI) it is observed periods of more intensive drought in the soil throughout the year, being of fundamental importance to consider the water-plant relations to obtain economic productivity. This project under development in the Biosystems Engineering Department at the University of Sao Paulo (ESALQ / USP), aims to present to the sugar cane breeding programs in Brazil, the current rates of water productivity into Biomass conversion (biomass kg / m evapotranspired H_2O) and Energy conversion (MJ of energy / m H_2O evapotranspired) for the main varieties under different maturation strategies and water levels in the soil. The development of this project will also identify the maximum sucrose content for different varieties and parameterized the physiological model CANEGRO, aiming its application to the production environments of ETH Bioenergy Clusters. This research project is based on a research platform called "PAC-CANA" where it is possible to quantify precisely and continuously the water use efficiency of plants under controlled conditions.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The sugarcane industry is currently facing an industrial and agricultural crisis. The use of irrigation systems is imposed as an alternative of higher productivity, but requires considerable investments and water resources. Based on the hypothesis that there is for each variety of sugar sugarcane, a more appropriate combination between irrigation depth and intensity water deficit during ripening, in order to maximize productivity, the aim of this work was to evaluate the effect of four irrigation depths and four levels of water stress during ripening, for eight drip irrigated sugarcane varieties, analyzing the variables related to quality and productivity per area unit, as well as quantifying productivity per unit of evapotranspired water (water productivity). Treatments were arranged in a factorial design (4x4x8) with split-split-plot with three replicates, totaling 128 treatments and 384 plots, and the plots consist of a box with about 330 liters of soil containing two plants. The evaluated irrigation depths (L50, L75 and L100 L125) were variations in the fraction applied over time, relative to the treatment L100, where soil moisture remained close to field capacity (θcc) throughout the experiment. Each tested variety had its own reference L100. The intensities of water deficit during maturation were evaluated for M1 (moderate water deficit of long duration), M2 (intensive water deficit of long duration), M3 (no water deficit) and M4 (severe water deficit of short duration). The sugarcane yield (TCH) was favored by the combination L100 and M3, regardless of the studied variety, with an estimated average value of 232.2 t ha^{-1}. Among the varieties, V4 had the highest TCH, with average of averaging 250.4 t ha-1 for L100. Sugar yield (RBA) is directly linked to sugarcane yield and is also favored by the combination of L100 and M3, with an average value of 23.4 t ha^{-1}. The V1, V2, V4 and V6 varieties, for irrigation depth L100, did not differ significantly for RBA and had the highest average, 23.9 t ha^{-1}. The water productivity in sugar and biomass differed in 4 and 2 variety groups, respectively, and was independent of the adopted irrigation depth and maturation management. Breedings techiniques in sugarcane related to water use efficiency (WUE) focused on 1G ethanol were successful, but new traits needs to be considered for WUE in 2G ethanol.

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CHARACTERIZATION OF SUGARCANE TRANSCRIPTS RESPONSIVE TO WATER STRESS AIMING THE DEVELOPMENT OF TRANSGENIC DROUGHT-TOLERANT PLANTS

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FAPESP Process 2011/50661-8 | Term: Dec 2011 to May 2014

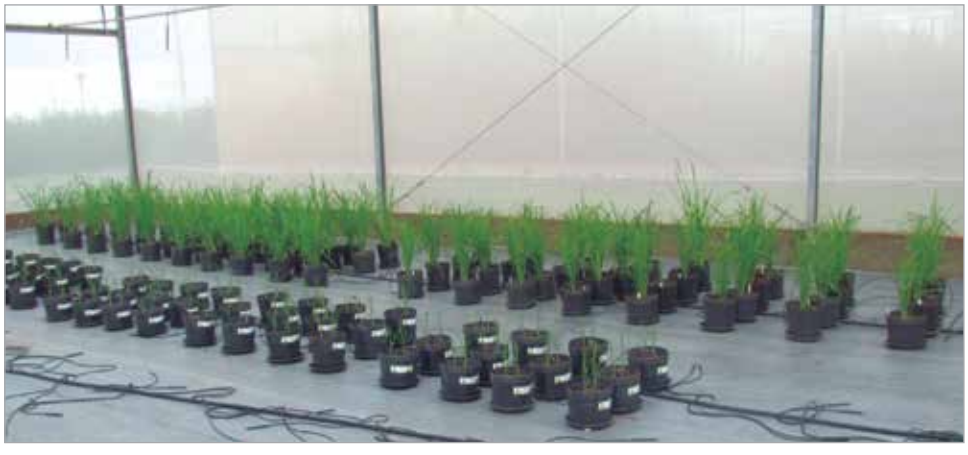

Figure 1. Rice transgenic plants at greenhouse overexpressing genes related to drought stress from sugarcane

Sugarcane (*Saccharum spp.*) is a promising source of renewable energy, and Brazil is the world leader of this crop. Significant yield losses have been observed in the last years, mostly because of drought conditions. For this reason, drought tolerance represents one of the most important targets in the development of transgenic sugarcane cultivars. However, despite of the limitations of the process in sugarcane transformation, mainly related to the low efficiency of the used methods, high dependence on genotype and gene silencing, the lack of well-characterized target genes to be used represents the main bottleneck in the development of transgenic sugarcane from a commercial point of view. Previous studies focused on global gene expression analysis (microarray and RNA-seq) using sugarcane genotypes contrasting for drought tolerance were conduced independently with experiments on field and at greenhouse, aiming to identify and characterize genes involved in drought tolerance. A set of differentially expressed genes identified and categorized as transcription factors (TFs) and miscellaneous classes were confirmed by qPCR. Two TF (bZIP_1 and MYB_2) and two miscellaneous were chosen for functional analysis through rice transformation (*Oryza sativa*), a grass model, evolutionarily close to sugarcane, but with well-established transformation procedures. Therefore, the full-length sequences of the coded sequences were accessed in SUCEST (Sugarcane Functional Genomics Database), Phytozome and NCBI database. The cloning was performed using the vector pGEM-T Easy (Promega) and E. coli DH10B lineage, and sequences subcloned into pDONR 211 Gateway vector. Overexpression vector construction, including Ubiquitin promoter (pEN-L4-UBIL-R1) and Hygromicin (*hpt*II) as selectable marker (pHb7m24GW), was used for *Agrobacterium tumefaciens*-mediated transformation of rice seeds. The rice transformation efficiency ranged from 50-60%, and the relative expression of transgenes was evaluated in transgenic plants for each target gene, using the $2^{-\Delta\Delta Ct}$ method. The transgene copy number was evaluated by a Taqman® assay based on *hpt*II gene. Analyses focusing on phenotyping and physiological evaluations were conduced in the second (T1) generation in four independent events for each target gene exhibiting only one copy number. Data analyses revealed that bZIP_1 has a role in drought tolerance in rice plants, whereas one gene categorized as miscellaneous resulted in an increase on biomass. Once confirmed the contribution of the genes here studied, these genes will be used in sugarcane genetic transformation aiming the development of cultivars with higher performance under restrictive water availability conditions.

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FUNCTIONAL BIO-INDICATORS FOR SOIL QUALITY MONITORING FOR SUSTAINABLE MANAGEMENT OF SUGARCANE BIOMASS PRODUCTION (BIOSQ)

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FAPESP Process 2011/51749-6 | Term: Jul 2012 to Jun 2014

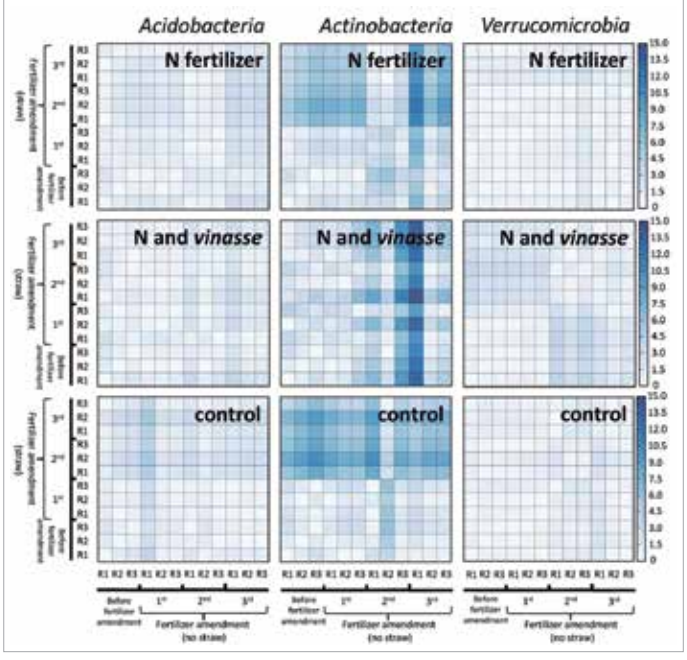

Figure 1. Taxonomical heatmap based on group-specific bacterial communities in sugarcane cultivated-soils. The actinobacterial community revealed a greater response to straw blankets compared with the response of acidobacterial and verrucomicrobial communities

Here, we propose to find bio-indicators that are responsive to sugarcane production under different sustainable management regimes (no-tillage, cover trash and diazotroph bacteria inoculation) as well as conventional managements. The aim is the identification of numerous taxa and genes that hold potential for use as bio-indicators of specific attributes of soil status, such as nutrient availability, level of disturbance and soil fertility, and provide proof-of-principle platform for soil quality assessment.

In order to achieve this aim, we propose to (1) determine the genes in key functions (nitrification, nitrogen fixation, denitrification, methane oxidation) most indicative of specific soil status; (2) determine functional bio-indicators involved in the emission/sequestration of greenhouse gasses (CO_2, CH_4 and N_2O); (3) develop high throughput platform (BIOSQ) to assess and monitor bio-indicators; (4) apply bioinformatics tools and appropriate basic and multivariate statistical analyses in order to integrate 'omics' data and soil physicochemical data for monitoring microbes and gene function under sugarcane biomass production; (5) test and validate the platform BIOSQ in different sites with sugarcane in São Paulo State.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The activities of the project have involved greenhouse and field experiments. A greenhouse mesocosm experiment monitored for gas emissions and chemical factors focused on the effects of organic and inorganic amendments and straw retention on the microbial biomass (MB) and taxonomic groups of bacteria in sugarcane-cultivated soils. Taken together, the results showed that MB carbon and MB nitrogen responded to changes in soil chemical factors and CO_2-C and N_2O-N emissions, especially for nitrogen (N) + vinasse (V)-amended soils. The results also indicated that several taxonomic groups of bacteria, such as *Acidobacteria*, *Actinobacteria* and *Verrucomicrobia*, and their subgroups acted as early-warning indicators of N+V amendments and straw retention in sugarcane-cultivated soils, which can alter the soil chemical factors.

In another study, disparate patterns were revealed for verrucomicrobial community in sugarcane rhizosphere sampled on optimal and deficient soil fertility for sugarcane. Verrucomicrobial community abundance in sugarcane rhizosphere was negatively correlated with soil fertility, accounting for 2 and 5 % of the total bacterial signal, under optimal and deficient soil fertility conditions for sugarcane, respectively. In nutrient-enriched soils, verrucomicrobial community structures were related to soil factors linked to soil fertility, such as total nitrogen, phosphorus, potassium and sum of bases, i.e., the sum of calcium, magnesium and potassium contents. This case study showed that community structure and abundance represent important ecological aspects in soil verrucomicrobial communities for tracking the changes in chemical factors linked to soil fertility under tropical environmental conditions, including management practices for sugarcane.

An ongoing study has applied GeoChip 5.0, a functional gene-array-based high-throughput environmental technology for microbial community analysis, in order to identify genes that hold potential for use as bioindicators of disturbance in sugarcane soils under zinc applications in sandy-textured soil with natural low fertility. Preliminary results have revealed that zinc applications at the doses of 10 and 20 kg ha^{-1} negatively affected the abundance of functional microbial genes associated to virulence. The abundance of specific genes associated to carbon, nitrogen, phosphorus cycling, zinc homeostasis, secondary metabolism and stress was also affected by zinc applications at the same doses (10 and 20 kg ha^{-1}) in comparison to 0 and 5 kg ha^{-1}.

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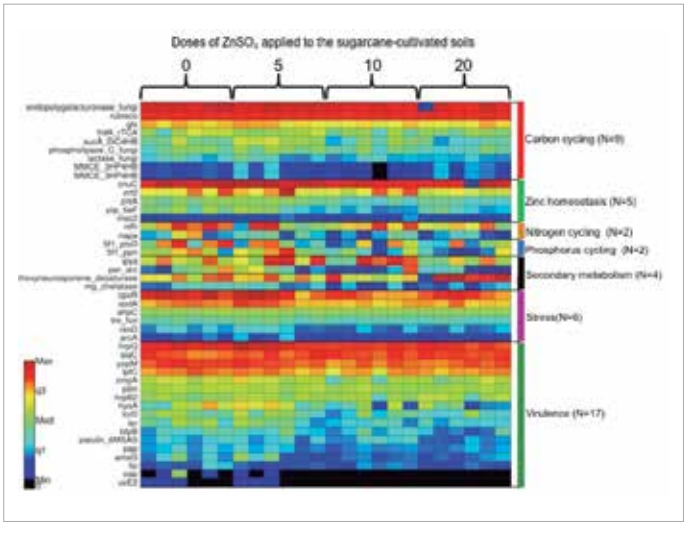

Figure 2. Functional heatmap based on GeoChip 5.0 data. Microbial functional genes associated to virulence decrease in abundance after applications of 10 and 20 kg ha-1 of $ZnSO_4$ to sugarcane-cultivated soils

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In addition to advancing fundamental and applied knowledge on Bioenergy, the FAPESP Bioenergy Research Program, BIOEN, aims to contribute decisively to the training of scientific and technical personnel in the field of Bioenergy R&D.
Grant applications s hould be presented to the FAPESP programs for Regular Projects Awards and Young Investigators Awards.
Applications for Regular Awards must be submitted by researchers from public or non-profit private higher education and research institutions in the State of São Paulo, Brazil.
FAPESP also encourages young researchers to apply for awards. For the Young Investigators Awards FAPESP will accept proposals of researchers who may not as yet have a professional association with a higher education or research institutions in São Paulo.
Laboratories conducting research under the BIOEN Program are continuously offering Post-doctoral, PhD and Master positions.
Visit the Program website at http://bioenfapesp.org and http://www.fapesp.br/en/bioen for further details.

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