Bioenergy Research: Integrating Agronomic Traits, Gene Networks and Carbon Partition for the Development of an Energy-Cane

http://bioenfapesp.org
FAPESP BIOENERGY PROGRAM BIOEN

http://bioenfapesp.org
# BIOEN DIVISIONS

## BIOMASS
Contribute with knowledge and technologies for Sugarcane Improvement
Enable a Systems Biology approach for Biofuel Crops

## PROCESSING AND ETHANOL TECHNOLOGIES
Increasing productivity (amount of ethanol by sugarcane ton), energy saving, water saving and minimizing environmental impacts

## ENGINES
Flex-fuel engines with the same performance, consumption, pollutant emissions and durability as the engines would run on a particular fuel blend

## BIOREFINERIES AND ALCOHOL CHEMISTRY
Complete substitution of fossil fuel derived compounds
Sugarchemistry for intermediate chemical production and alcoholchemistry as a petrochemistry substitute

## IMPACTS
Studies to consolidate sugarcane ethanol as the leading technology path to ethanol and derivatives production
Horizontal themes: Social and Economic Impacts, Environmental studies and Land Use
BIOMASS DIVISION

Improvement of Biomass (Agronomy, Breeding, Biotechnology)
Identify new paths to genetically manipulate the energy metabolism of cultivated plants, creating new biofuel alternatives

- Uncover metabolic networks related to the production of carbohydrates and sucrose through the use of “omics” technologies
- Integrate the results in a single platform and develop bioinformatic tools to assess the information
- Discovery of genes associated with agronomic characteristics of interest
- Development of new sugar cane cultivars
- Signaling, regulation of gene expression and regulatory networks
- Genetic transformation of sugarcane and other grasses
- Molecular markers, statistical-genetics, mapping and breeding
- Sequencing, physical, genetic and molecular mapping of genomes
- Understand cell wall structure, architecture and biological function
- Discover new cellulolytic fungi species capable of degrading biomass
- Refine field practices for enhancing crop production including soil management, fertilization and precision agriculture
- Improve control of weed, pests, and diseases though chemical or biological control, resistant varieties and field practices

Contribute with knowledge and technologies for Sugarcane Improvement
Enable a Systems Biology approach for Biofuel Crops
<table>
<thead>
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<th>Participating Institutions</th>
<th># Projects</th>
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One of the most productive cultivated plants - a large biomass

Commercial sugarcane is vegetatively propagated through stem cuttings.

In 12 months the plant will reach 4-5 meters with the extractable culm measuring 2-3 meters.

After harvest, underground buds will sprout giving rise to a new crop (6 harvests).

C4 carbohydrate metabolism - large amount of carbon partitioned into sucrose (up to 42% of the stalk dry weight, around 0.7 M in mature internodes).


*ISPA
Cultivar Biomass and Ethanol Production in Brazil

Biomass/ha

Sugar

70's

4.200/ha 82 %

Hoje

7.650/ha
Brazil has great tradition in sugarcane cultivation (since 1532)

- 8.0 million ha
- World leader
- Several public and private institutions dedicated to P&D
- Very competitive costs and production technology
- Breeding Programs (since 1910)
  - IAC (1934)
  - Campos (1946-1972)
  - CTC (ex Copersucar, 1968)
  - Ridesa (1971)
  - Canavialis (2003)

Fertilizer experiments. IAC, 1938
Domestication and early evolution of sugarcane

Saccharum officinarum

Saccharum sinense
Saccharum barberi
(crosses to wild relatives
natural hybrids)

Sugar extraction
Manufacturing
Cottage industries

SE Asia
Pacific Islands

Intertropicals
Persia
Mediterranean
Spain

Canary
Madeira
West Africa

Dominican
Republic
Brasil

Java
India

Modern sugarcane cultivars

8000 BC
1000-1500 BC
500 AD
6th - 8th Century
15th Century
16th Century
19th Century

Modern breeding
Interspecific breeding: a major breakthrough in modern sugarcane breeding

Solved some of the disease problems but also provided increased yields, improved ratooning ability and adaptability for growth under various stress conditions

Contributing genera: *Saccharum*, *Erianthus*, *Miscanthus*, *Sclerostachya* and *Narenga*

**Saccharum genus** (six polyploid taxonomic groups):

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<th>Early cultivars</th>
<th>Marginal species</th>
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<td><em>S. officinarum</em> (2n= 80)</td>
<td><em>S. edule</em> (2n = 60 to 122)</td>
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<tr>
<td><em>S. robustum</em> (2n= 60, 80 and up to 200)</td>
<td><em>S. barberi</em> (2n=81-124)</td>
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<td><em>S. sinense</em> (2n=116-120)</td>
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Genome organization of a modern cultivar
Each bar represents a chromosome
Chromosomes in the same column are homologues

- **Yellow** = *S. officinarum*
- **Green** = *S. spontaneum*

Giant Genome (n ≈ 750-930 Mbp)  Polyploid (2n = 70-120 cromossomos)  ~10 Gb
Breeding

- Area Crossings
- Bi-parental Crossings
- Seedlings
- Multiple Crossings
New Varieties

13 clones in final phase

168 clones in Experimental Trials

619 clones in T3

6.792 clones in T2 (1.349 Clones Brix > RB855156)

398,477 Seedlings in T1

Total: 406,069 genotypes
The SUCEST EST Sequencing Project

Analysis and Functional Annotation of an Expressed Sequence Tag Collection for Tropical Crop Sugarcane


50 labs
200 researchers
238000 ESTs
43000 Transcripts

26 libraries - 13 cultivars - Over 90% of sugarcane genes tagged
Defining Initiatives for Sugarcane Genomics and Biotechnology in Brazil
Sugarcane Genome Research Network in Brazil

- Empresas e instituições paulistas
- Empresas e instituições de outros estados

50 artigos
20 artigos
01 artigo
The Biotechnology Roadmap for Sugarcane Improvement

Carlos T. Hotta • Carolina G. Lembke • Douglas S. Domingues • Edgar A. Ochoa • Guilherme M. Q. Cruz • Danila M. Melotto-Passarin • Thiago G. Marconi • Melissa O. Santos • Marcelo Mollinari • Gabriel R. A. Margarido • Augusto César Crivellari • Wanderley D. dos Santos • Amanda P. de Souza • Andrea A. Hoshino • Helaine Carrer • Anete P. Souza • Antônio A. F. Garcia • Marcos S. Buckridge • Marcelo Menossi • Marie-Anne Van Sluys • Glaucia M. Souza
1 – Gene Discovery
Genes associated to agronomic traits of interest
Gene function evaluation
C4 model plant system

2 – Physiology
Sucrose metabolism, carbon partitioning, photosynthesis, stress responses

3 - Genome Sequence
Full length transcripts
Surveys of several genomes
Gene enrichment methods for regions to be sequenced
BAC library with 10x coverage
BAC screening methods

4 - International Bioinformatics
International Consortium

5 – Transgenics
Expression vectors
Complete ORFeome
Promoters
Screening methods for phenotyping (metabolomics, qPCR, biochemical assays, cell wall)

6 - Marker identification
Integrated databases and translation of transcriptome data to marker assays
How far can we go?


High yield variety: 260 ton/ha in 13 months (commercial at Agrovale, Bahia) and 299 ton/ha (experimental at Fazenda Busato, Bahia)

Potential yield of sugarcane
Agilent Technologies: 2 color gene expression
14,000 genes represented
**Signal transduction-related responses to phytohormones and environmental challenges in sugarcane**


Published: 13 March 2007

**Sugarcane genes associated with sucrose content**


Published: 21 March 2009

**THE SUCEST-FUN PROJECT: IDENTIFYING GENES THAT REGULATE SUCROSE CONTENT IN SUGARCANE PLANTS**

By


DNA Research 12, 27–38 (2005)

Transcription Profiling of Signal Transduction-Related Genes in Sugarcane Tissues

Flávia Stal Papini-Terzi, Flávia Rigo Rocha, Ricardo Zorzetto Nicoliello Vêncio, Kátia Cristina Oliveira, Juliana de Maria Felix, Renato Vicentini, Cristiane de Souza Rocha, Ana Carolina Quirino Simões, Eugênio Cezar Ulian, Sônia Marli Zingaretti de Mauro, Aline Maria Da Silva, Carlos Alberto de Bragança Pereira, Marcelo Menossi, and Gláucia Minder Souza

7000 genes expression profiled
## Genetical Genomics of Traits of interest

### Progeny 1 Genotypes

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<th>Genotype</th>
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### Progeny 2 Genotypes

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### Sugarcane genes associated with sucrose content


Published: 21 March 2009

### Genetical Genomics of Traits of interest

- **Genotype**: Selection of S. officinarum hybrid individuals with extreme sucrose content (high and low brix)
- **Crossing of selected individuals**: Polycross 1 of selected F1 individuals with high brix
- **Selection of F2 individuals with high brix**: Polycross 2 of selected F2 individuals with high brix
- **Selection of F3 individuals with the highest brix content from the population in graph**: Polycross of selected F3 individuals with the lowest brix content from the population in graph

### Sugarcane genes associated with sucrose content


Published: 21 March 2009

- **Expression Profile of Signal Transduction Components in a Sugarcane Population Segregating for Sugar Content**: Tropical Plant Biology
- **Publisher**: Springer New York
- **ISSN**: 1935-9756 (Print); 1935-9764 (Online)
- **Issue**: Volume 2, Number 2 / June, 2009
- **DOI**: 10.1007/b12042-009-0031-9
- **Pages**: 98-109
- **Subject Collection**: Biomedical and Life Sciences
- **SpringerLink Date**: Wednesday, July 15, 2009

**PDF (394.7 KB)** | **HTML** | **Supplemental Material**

**Juliana de Maria Felix**, Flávia Stai Papini-Terzi, Flávia Riso Rocha, Ricardo Zorzetto Nicoliello Venclo, Renato Vicentini, Milton Yuuta Nishiyama Jr, Eugênio César Uillian, Glâucia Mendes Souza and Marcelo Menossi
Phosphorylates sucrose phosphate synthase (SPS) and nitrate reductase (NR), which together with binding of 14-3-3 proteins inhibits their activity.

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Storage parenchyma (R), Phloem (f), Fiber and bundle parenchymal cells (arrows), Bundle distal cells (ce), Bundle proximal cells (ci)
Physiology of sucrose and biomass accumulation

Drought Field Experiments with 6 cultivars: São Paulo, Pernambuco, Alagoas (SE, NE)

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- Plant Height
- Leaf Length
- Leaf Width
- Expanded Leaves
- Green Leaves
- Plants in Central Line (NPLC)
- Visible Sky
- Leaf area index (IAF)

- Carotenoids
- Chlorophyll a
- Chlorophyll b
- Total Chlorophyll
- Chlorophyll a/b

- Soluble Sugar
- Proline
- Soluble Proteins
- Soluble Amino acids
- MDA
- CAT
- APX

irrigated

Non-irrigated

USP, UNICAMP, UFV, UFAL, UFPE, UFRPE
Drought Field Experiments with SP79-1011 in Alagoas

A

Non-irrigated

B

Irrigated
Drought Field Experiments with RB855536 in Alagoas

Non-irrigated

Irrigated

RIDESA
Drought Field Experiments with 10 genotypes: Goianésia, Goiás (CW)

Sensors (30, 60, 90 cm)

10 genotypes (pre-selected based on performance over 2009 from a group of 100 clones)

Irrigation
Production of transgenic sugarcane plants

Explants: Immature Leaves

Callus Induction

Regeneration Selective Medium

Rooting

PCR

Shoot Growth

Greenhouse

CEBTEC - Esalq/Usp
Sugarcane transgenic plants with increased sucrose content: 3 CIPKs gene silencing using RNAi


Genes associated to sucrose content, sugarcane with increased sucrose levels
PCT/BR2007/000282.


**Universidade de São Paulo, Unicamp, Centralcool, CTC and FAPESP**
Lignin Biosynthesis is associated to sucrose content

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<td>Phenylalanine</td>
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Energy Cane
Sugarcane Mill

**Energy Cane**

**Sugarcane Mill**

- Ethanol storage tanks
- Distillery
- Sugar plant
- Bagasse
Industrial aspects: research in all aspects of the production

Total sugarcane production is estimated to be 664.33 ton/ha for 2010/2011
Total bioethanol production for 2010/11 is projected at 28.5 billion L
54.6% (362.8 million tons) for ethanol (20.14 billion L hydrated and 8.4 billion L anyhydride)
45.4% (301.6 million tons) for sugar (38.7 million tons)
405 plants of which 157 are exclusive to ethanol

Brazilian Bioethanol production costs are the cheapest in the world
Industry estimates the cost of producing ethanol from sugarcane at approximately US$ 0.29/L (1 gallon = US$ 1.00).
Co-generation in 2008 = 1.400 MW
In 2020 = 14.000 MW (equals 1 Itaipu)
Fazenda Busato – Bom Jesus da Lapa - BA
Climate Change: +60% biomass  +35% photosynthesis

- 370 ppm CO₂
- 720 ppm CO₂

- Carbon metabolism
- Cell cycle
- Development
- Lipid metabolism
- No match
- Acid nucleic metabolism
- Receptor
- Lipid metabolism
- Development
- Cell cycle
- Carbon metabolism

Functional categories

Number of genes

Repressed
Induced

CO₂ assimilation (μmol CO₂ m⁻² s⁻¹)

- Ambient
- Elevated CO₂

60% more Biomass

Weeks of CO₂
Cellulosic Ethanol

70's

4.200/ha

82%

Today

7.650/ha

40-60%

In 10 years?

12.000/ha

a
Breeding and Genetics Workgroup

Challenge

To understand the genetic architecture of quantitative traits in sugarcane, in order to implement marker assisted selection

Why is this a challenge

- Marker systems that are informative in other scenarios (e.g. Microsatellites) provide less information in polyploids, having a dominant action
- Commonly, only markers that have a single copy (dosage) on the genome have been used
- Single Nucleotide Polymorphisms (SNP) are useful (codominant), but the data provided by current approaches and technologies cannot be readily used
- Good genetic maps and QTL (quantitative trait loci) results are not available to date

State of the Art

- Up to 400 SNPs were developed and used to genotype a biparental Brazilian population
- Methods to interpret this data were developed and are ready to use
- Statistical methods to build genetic maps and to map QTL using markers with higher doses have been developed

Group Leaders: Anete P Souza (UNICAMP) and Augusto Garcia (USP)
Sugarcane Map incorporating double and triple dose markers (SSR, EST-SSR, RFLP)

Mollinari, M; Silva, RR; Margarido, GRA; Marconi, TG; Souza, AP; Garcia, AAF

SP80-180 x SP80-4966, 200 individuals

934 markers

Final map with 347 linked markers

329 single dose (239 1:1 and 90 3:1)

16 double dose

2 triple dose

Assembled in 102 linkage groups

The total map length 2,880.3 cM (4,361.3) with a density of 7.6 cM (11.6)
1 – BAC Sequencing Strategies
R570 BAC selection (3-D pools, PCR, membrane hybridization)
Construction of new BAC libraries (SP80-3280)
Sequence 1000 BACs
BAC assembly from pyrosequencing and Sanger (454 and ABI)
Anchoring to sorghum
BAC-end sequencing and BAC homeologues sequencing

2 – Whole Genome Sequencing
WGS pilot experiments: gene-rich enrichment, methylation filtration chip hybridization, preliminary surveys, ChIP-Seq

3 – SSR, SNP discovery
Sequenon

4 - Bioinfo and database development
http://sucest-fun.org

5 – Sugarcane Gene Nomenclature
Transcription factor recommendation
Which Genotype should we sequence?

Percentage of area with main varieties of sugarcane

Source: Datagro (from 1991 to 2005 – estimated)
Which Genotype should we sequence?

Sugarcane is a collection of alleles: ideally one needs to sequence a hybrid cultivar and ancestor genotypes (relatively pure autoploidics).

Initial Candidates:
- SP80-3280 (most ESTs)
- R570 (BAC library and dense genetic map)
- Q165 (genetic map)
- LA Purple (S. officinarum)
- SES208 (S. spontaneum)
- Mol6081 (S. robustum)

There are no homozigous diploid genotypes.
Syntheny with Sorghum

Saccharum and Sorghum diverged between 5 and 9 million years ago

Some genotypes can still be crossed to one another

Grivet et al, 1996; Dufour et al, 1997
D’Hont et Paulet (Personnal Comm.)
**Adh1 region**

**Gene rich region:**
1 gene/9 kb

**Divergence time:**
- 8-9 Mya
- 1.5-2 Mya

**Exon sequence identity**
- 94.5%
- 97%

**Perfect colinearity**
**High gene structure conservation**
**High gene sequence conservation**

Jannoo et al, Plant Journal, 2007
Transposable elements on Adh1 region

Very little collinearity among TEs between sugarcane homoeologous haplotypes

Jannoo et al, Plant Journal, 2007
Prediction of Ortholog Groups
Sugarcane and Sorghum promoter alignments

Segment 724bp (81% identity)

Fragment of 936bp

Fragment of 2169bp

5 Segments

<table>
<thead>
<tr>
<th>Length</th>
<th>Identity</th>
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<tr>
<td>22bp</td>
<td>97%</td>
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<tr>
<td>52bp</td>
<td>94%</td>
</tr>
<tr>
<td>126bp</td>
<td>89%</td>
</tr>
<tr>
<td>240bp</td>
<td>94%</td>
</tr>
<tr>
<td>751bp</td>
<td>80%</td>
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Genome Walking: A putative promoter that does not align to sorghum

Fragment 5 (SASGMS09692)
Length: 1298bp
Total aligned: 110bp in 3 segments

Alignment of the SASGMS09692 (walking: Fragment 5)
ChIP-Seq: 7 Abs raised against TFs associated to drought and sucrose
Alignment to Sorghum: Coverage and Identity of UTR

- Coverage of 5' UTR
- Coverage of 3' UTR
- Identity of 3' UTR
We are using RNA polymerase II repeat YSPTSPS antibody to generate a promoter and active genes database. This antibody reacts with the non-phosphorylated heptapeptide repeat of the largest subunit of eukaryotic RNA polymerase II.
R570 BAC Library

R570 BAC library (HindIII) = 103,296 clones (Tomkins et al, 1999, TAG)
1.3x total genome equivalent
14x basic genome equivalent

BAC-end sequences (Paterson)
Overgo screen (Paterson)

---

R570 map I
77 individuals (Grivet et al 1996)
-> 408 RFLP markers from 128 RFLP probes
+ 138 SSR from 55 SSR loci
+ 120 AFLP
+ 458 RFLP (unpublished data)
= 1124 markers on 164 cosegregation groups (CG) and 8 homology groups (HG)

---

R570 map II
300 individuals (Hoarau et al 2001)
-> 887 AFLP markers
Sub-set of 112 individuals (Rossi et al 2003):
+ 134 SSR from 55 SSR loci
+ 148 RFLP from 50 RGA loci
= 1123 markers on 128 CG and 7 HG
+ 890 DArT markers (some redundant)

around 2500 markers in total
BAC mapping on sorghum gene-rich regions

- Overgo hybridization against sugarcane BACs
- http://bacman.sourceforge.net
- 6.021 overgo sequences (40 nt) BLASTed against the sorghum and rice genomes and 438 sugarcane SAS of interest (genes associated to sucrose content and drought)
  - Select only stringent hits (38 nt aligned with no gaps)
  - Select only if presented 10 copies or less against the BAC library
  - Select only if correspond to unique locus in both sorghum and rice

- Select only stringent hits (38 nt aligned with no gaps)
- Select only if presented 10 copies or less against the BAC library
- Select only if correspond to unique locus in both sorghum and rice

- gene-rich BACs
- Genome walking

Collaboration: Glaucia Souza, Roberta Campos
Andrew Paterson and Changsoo Kim - University of Georgia USA
BAC library 3D pools

CNRGV – France
Helene Berges (INRA-CNRS)
Marie-Anne Van Sluys (IB-USP)

269 plates (whole BAC library)
11 blocks (line x column)
11 SuperPool Samples
plate pool, line pool, column pool (Hamilton Microlab Star)
Global DNA amplification with Phi29 enzyme (rolling circle amplification)

Membranes 7x7 (2 membranes contains the whole library)
A draft of the sugarcane monoploid genome (1 Gb)

Genome organization of a modern cultivar
Each bar represents a chromosome
Chromosomes in the same column are homologues

Giant Genome \((n \approx 750-930 \text{ Mpb})\)  Polyploid \((2n = 70-120 \text{ cromossomos})\)  \(~10 \text{ Gb}\)
The Sugarcane Transcriptome Project & CaneRegNet

Sub-Project 1
Resources

Sub-Project 2
CREs and Promoters
- Search TF orthologs in grasses
- Search promoters in BACs/WGS seqs
- Search promoters in grass genomes
- Identify CREs

Sub-Project 3
Transcriptome
- Sugarcane Exps
  - Bnx
  - Biomass,
  - Sugar
  - Drought
  - Hormones
  - Insect,
  - Pathogen
  - Endophyte
  - Climate changes

Sub-Project 4
Transgenic Plants

Sub-Project 5
Database and Bioinfo
- GRASSIUS db
  - TF Promoter Motif
- canePROMDB
  - Promoters
- caneGENEXPRESSDB
  - Expression data
- canePKDB/PPaseDB
  - PKs PPases Catalogue
- caneTFDB
  - TFs Catalogue
- caneREGNET
  - CREs, networks

ChIP-Seq
- Phenotyping
  - agronomic evaluation
  - data

Sequencing
- WGS
- BAC sequencing

PKs PPases
- Orfeome

TFs
- SE constructs
- SI constructs
- Expression constructs E. coli

Abs
- Reporter constructs

Phenotyping
- Agronomic traits of interest

Plant physiology and Biochemistry
- Progenies
  - Genotypes
  - Cultivars

Hybridization
- Data analysis
  - Oligos arrays
  - cDNA microarray
  - NGS technology
Bioinformatics Workgroup

Challenge

Create and maintain a Database, tools and resources for the community for a grass with a giant genome, hundreds of cultivars and not enough hands

Why is this a challenge

• Sequencing of the sugarcane genome is one of the most challenging projects in genomics nowadays
• We want to develop an evolving database that can grow and host heterogenous data as projects progress
• We want to advance data collection and storage to a Systems Biology approach integrating genomics, functional genomics, molecular markers, statistical-genetics tools, physiological and agronomical data
• Large multigene families and polyploidy makes allele identification difficult

State of the Art

• The SUCEST-FUN Database has been created
• It currently hosts data on ESTs, BACs, shot-gun sequencing (caneGenome)
• ESTs and SAS can be related to results of over 300 hybridizations (caneGeneExpress)
• We started developing a sorghum vs. sugarcane ortholog dataset
• We started identifying gene promoters
• We are creating a database dictionary

Group Leader: Glaucia Souza (Instituto de Química – USP)
The SUCEST-FUN DB is based on five main topics: Gene Annotation, Gene Expression, Public Resources, Sequencing Projects and Functional Genomics.
SUCEST-FUN DB (http://sucest-fun.org)

1 – Genome (CaneGenomeDB)
   - WGS
   - Promoters
   - ChIP-Seq
   - BACs
   - Methylation

2 – Transcriptome (CaneGeneExpress)
   - genes/ESTs/proteins
   - probes
   - Microarray
   - experiments
   - RT-PCR

3 – Proteome
   - proteins/domains
   - Ontology/Nomenclature
   - Structure PDB

4 – Metabolome
   - Metabolic Pathways
   - Mass Spectrometry
   - Ontology

5 – Protein Catalogue (CaneCatalogueDB)
   - Transcription Factors (CaneTFDB)
   - Kinases (CanePKDB)
   - Phosphatases (CanePPaseDB)
   - Cell Wall (CaneCellWallDB)

6 – Genotypes and Transgenics (CaneTransgenicDB)

7 – Regulatory Networks (CaneRegNetDB)

8 – Comparative (CaneSimDB)

DB Architecture designed to facilitate autonomous and heterogenous DB correlations
- Data Dictionary (descriptions, attributes and domains)
- Import/export scheme (XML)
- Software
  JAVA
  João Eduardo Ferreira (IME-USP)

-DB
  MySQL, Postgree
- Hibernate Framework
http://sucest-fun.org

The SUCEST-FUN Database Project

The SUCEST-FUN Project aims to associate function to the genes identified by the Sugarcane EST Project (SUCEST)

The Sugarcane Regulatory Network Database

This Project aims to study gene expression regulation and to generate tools that will allow us to employ a Systems Biology approach in sugarcane to identify regulatory networks
Horizontal themes: Social and Economic Impacts, Environmental studies and Land Use

**Ethanol as a global strategic fuel**

- Certification Methodology for ethanol produced in a sustainable environmentally friendly manner
- Research on new agronomical practices (precision agriculture, mecanization, no-till farming, low input practices, new crop protection systems) and their impact on soil loss, management and efficiency in different production environments
- Improve recycling plant nutrients of crop and industry residues in the sugarcane farm and industry system
- Define changes in carbon sequestration, greenhouse gases emission gains, carbon and energy balances impacted through the use of Bioenergy
- Evaluate the environmental impact of GM sugarcane and biosafety
- Risk assessment of effects on environment, on social relations and other economic activities (competition with food supply, energy supply and local materials)

**Studies to consolidate sugarcane ethanol as the leading technology path to ethanol and derivatives production**
Expansion of sugarcane to new land areas
8.1 million hectares 2010/11 = 9.2% increase over the last cycle

In Brasil:
Total arable land 355 mi ha
Total cropland 76.7 mi ha
Total pasture land 172 mi ha
Total available land 105 mi ha
Sugarcane 2-3%

65% of expansion land is pasture

Source: Horta Nogueira e Seabra (2008)


São Paulo 4.4 million ha; Minas Gerais 648 thousand ha; Parana 608 thousand ha; Goiás 601 thousand ha; Alagoas, 464 thousand ha.
Total = 0.95% national territory
Expansion of sugarcane to new land areas

Southwest: dry winter
Marginal land, pastureland, and poor soils

Research:

- Drought resistance
- Crop breeding to new environments
- Soil/chemical management for deep rooting (addition of calcium)
- Chemical/fertilizer supply to compensate for deficiencies in new land areas
- Revise nutritional needs: (inorganic nutrients are 5% of plant dry matter)
- Optimize the use of fertilizers and chemicals (Sugarcane: 13% of fertilizer used in Brazil)
- Recycle nutrients of crop and industry residues
**Green cane:**
Thick mulch of plant residues (8-14 t/ha DM)
better soil protection and nutrient cycling (C, N)

**Challenges:**
Problems with some insects
Difficult to incorporate fertilizers (lower efficiency, nutrient losses)
Some varieties have reduced sprouting

**Research needs:**
Assess environmental gains due to cycling, soil protection, C accumulation in soil
Create varieties adapted to green cane
Adequate management practices to green cane

Burning phasing out in 2014/2017 in São Paulo

**Pests and Diseases**
may compromise cane production

Disease resistant varieties
Biological Control (viable for several pests in sugarcane)
Chemical control and crop management: combination of best management practices to minimize use of pesticides
Environmental challenges

- Pollution of soil and water with chemicals and residues
- Fossil fuel use to produce ethanol and GHG (CO₂, N₂O, CH₄) must be low to justify production of biofuels
- N fertilizer: 25% of fossil energy used to produce and transport cane (Production of N fertilizer requires lots of energy: 53.8 MJ/kg N or 1400 m³ natural gas per ton N)
- N₂O release after N fertilization (1-4%, N₂O has a GWP 300 greater than that of CO₂)

Research:
- Good management, precision agriculture, efficient tool to monitor pests and diseases
- Management practices to reduce GHG in agricultural processes
- Optimize nutrient use. Biological fixation of N
- Improve recycling of residues (vinasse, filter cake, ashes, plant residues etc.)

Vinasse channel

Sugarcane industry is in a privileged position: only C, O and H are exported (all mineral nutrients can be recycled in the farm-industry system)
Leaching losses that may affect deep water quality has not been a problem associated with sugarcane cultivation
For each ton of ethanol used as fuel 2.3t of CO₂ are not emitted to the atmosphere with a simultaneous reduction in SO₂ emission
**FAPESP BIOENERGY PROGRAM BIOEN**

http://bioenfapesp.org

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Thank You!