Glaucia Mendes Souza
Associate Professor

Signal Transduction Laboratory
Institute of Chemistry
University of São Paulo

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

Participating Institutions	# Projects
UNIVERSIDADE DE SAO PAULO	27
UNIVERSIDADE ESTADUAL DE CAMPINAS	8
SECR EST AGRICULTURA E ABASTECIMENTO DE SAO PAULO	6
MINISTERIO DA CIENCIA E TECNOLOGIA	4
UNIVERSIDADE FEDERAL DE SAO CARLOS	3
SECR EST DESENVOLVIMENTO DE SAO PAULO	2
UNIVERSIDADE FEDERAL DO ABC	2
FUNDACAO GETULIO VARGAS SAO PAULO	1
INST DE ESTUDOS DO COMERCIO E NEGOCIACOES INTERNACIONAIS	1
UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO	1

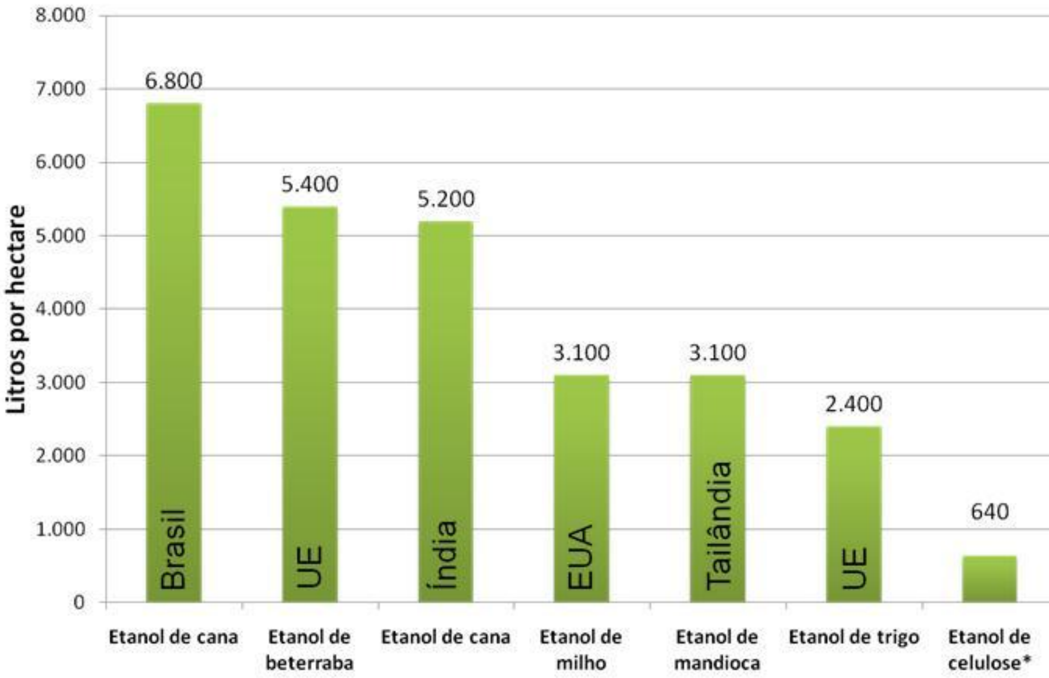
One of the most productive cultivated plants - a large biomass

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

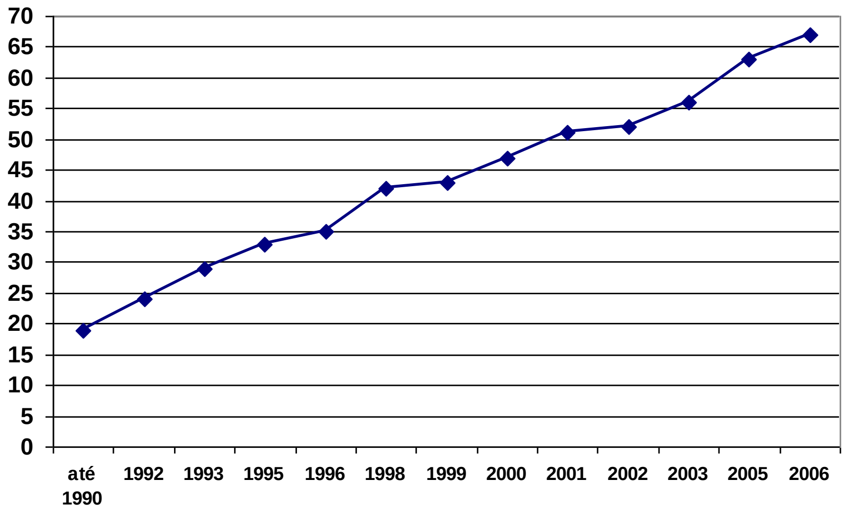
After harvest, underground buds will sprout giving rise to a new crop (6 harvests)

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)

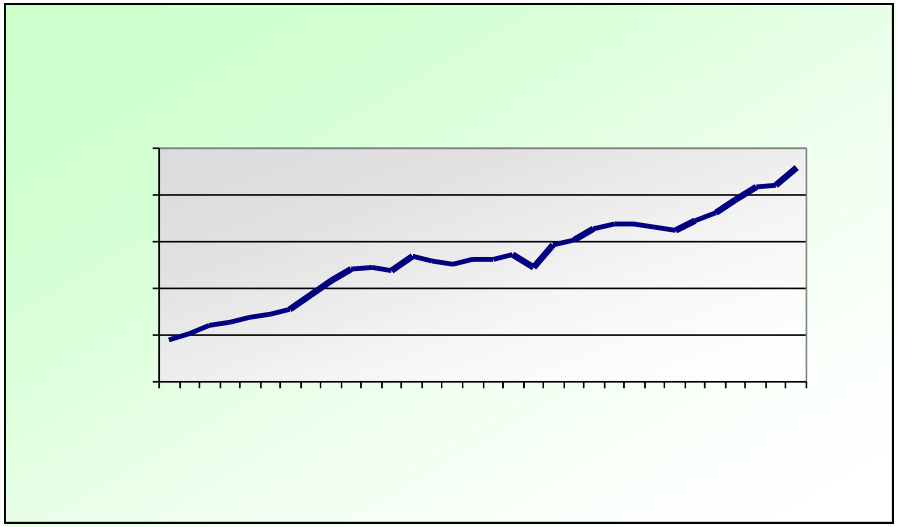


Fonte: International Energy Agencia (2005).
*ISPA

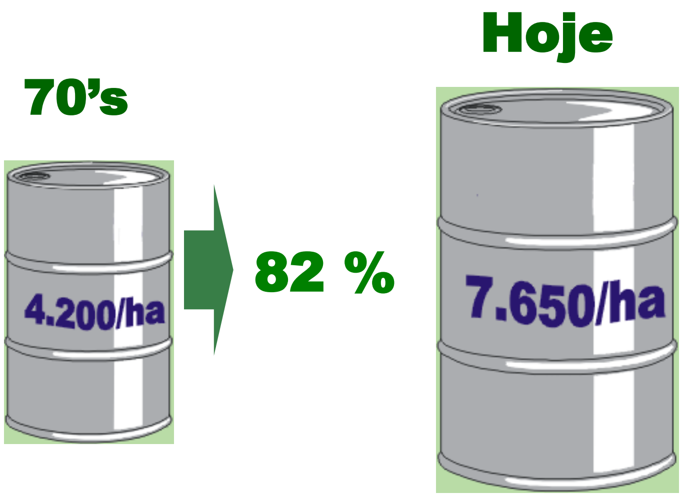
Cultivar Biomass and Ethanol Production in Brazil



Biomass/ha



Sugar



Sugarcane production

Brazil has great tradition in sugarcane cultivation (since 1532)

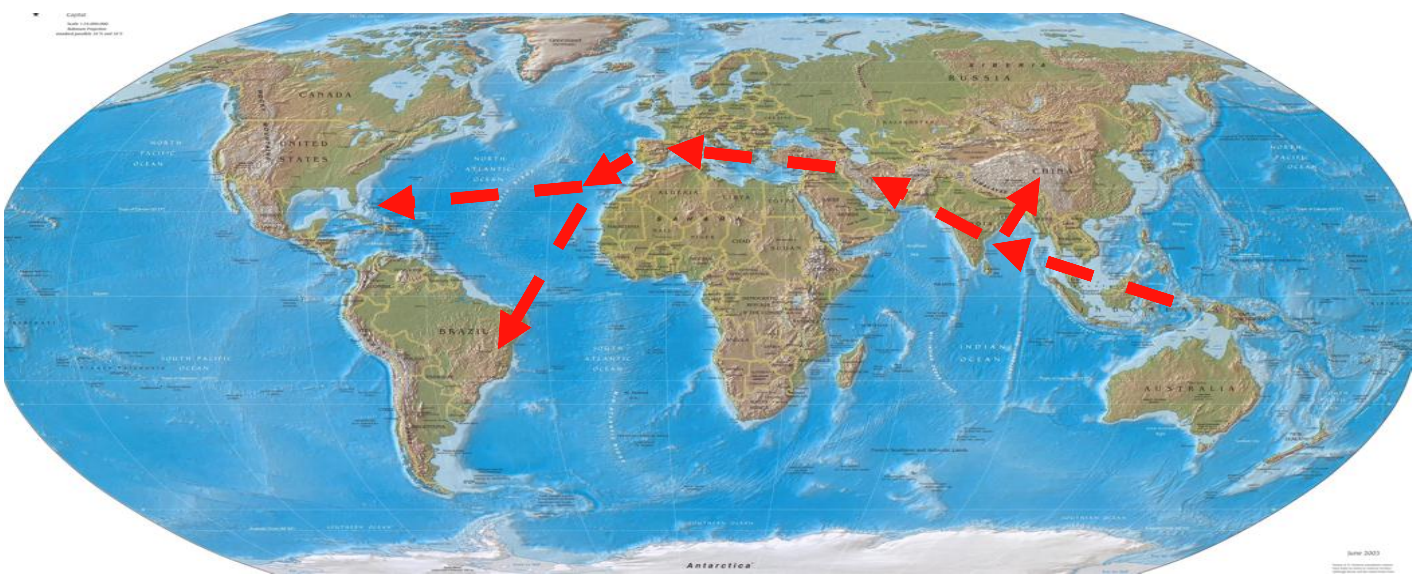
- 8.0 million ha
- World leader
- Several public and private institutions dedicated to P&D
- Very competitive costs and production technology
- Breeding Programs (since 1910)
  IAC (1934)
  Campos (1946-1972)
  CTC (ex Copersucar, 1968)
  Ridesa (1971)
  Canavialis (2003)

Fertilizer experiments. IAC, 1938

Domestication and early evolution of sugarcane

8000 BC	*Saccharum officinarum*	Chewing	SE Asia Pacific Islands
1000-1500 BC	*Saccharum sinence* *Saccharum barberi* (crosses to wild relatives natural hybrids)	Sugar extraction	Intertropics
500 AD		Manufacturing	Persia
6^{th} -8^{th} Century			Mediterranean Spain
15^{th} Century		Cottage industries	Canary Madeira West Africa
16^{th} Century	*Saccharum officinarum* clones	World trade plantation factories	Dominican Republic Brasil
19^{th} Century	*S. officinarum* X *S. spontaneum* (interspecific hybrids)	Modern Breeding	Java India

Modern sugarcane cultivars



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):

Wild species ***Early cultivars*** ***Marginal species***

S. spontaneum (2n=40 to 128) *S. officinarum* (2n= 80) *S. edule* (2n = 60 to 122))

S. robustum (2n= 60, 80 and up to 200) *S. barberi* (2n=81-124)

S. sinense (2n=116-120)

Genome organization of a modern cultivar

Each bar represents a chromosome

Chromosomes in the same column are homologues

S. officinarum

S. spontaneum

Giant Genome (n ≅ 750-930 Mpb) **Polyploid (2n = 70-120 cromossomos)** **~10 Gb**

Breeding

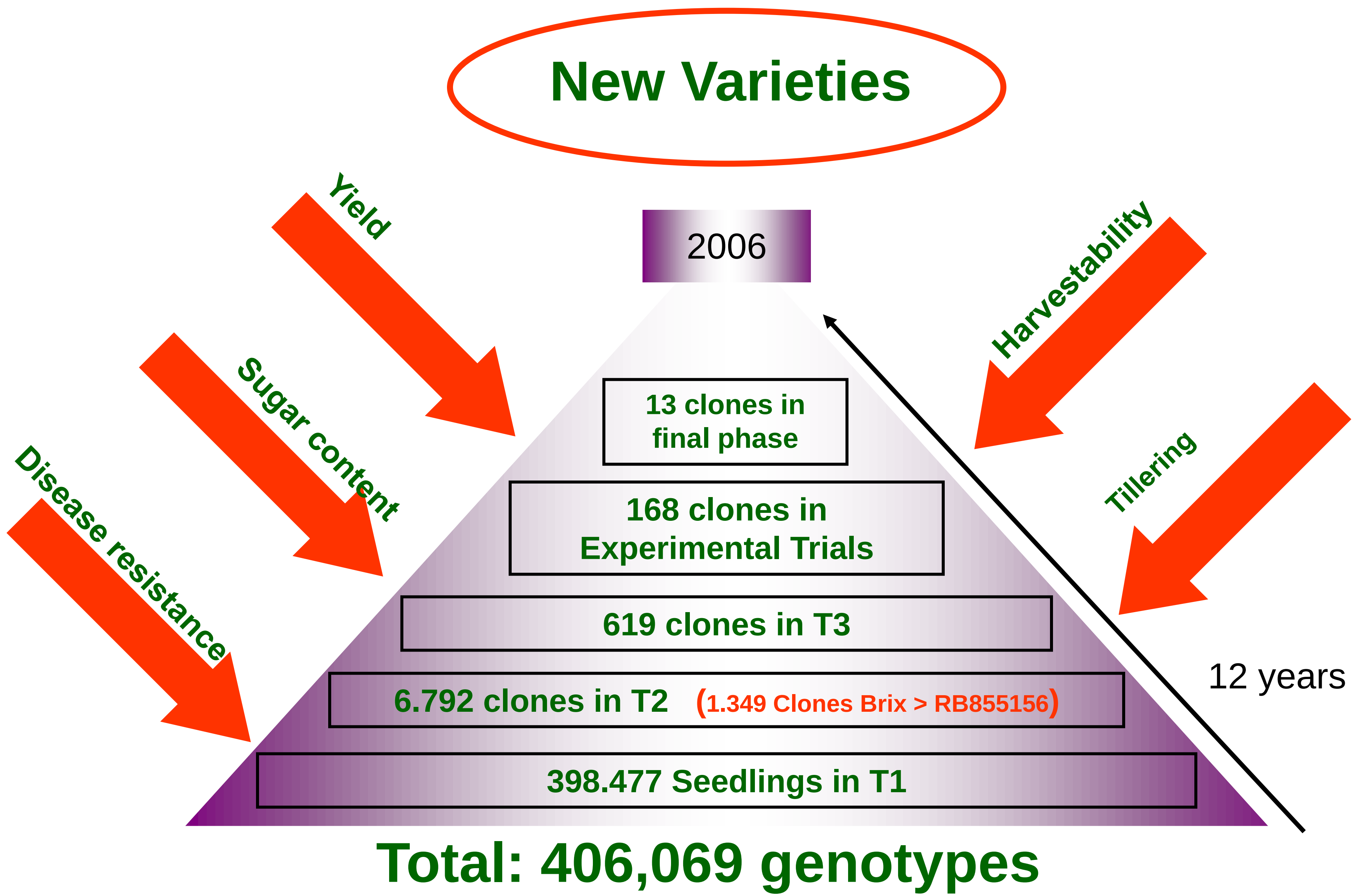

Area Crossings


Bi-parental Crossings


Seedlings


Multiple Crossings

Selection


New Varieties
2006
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
Yield
Sugar content
Disease resistance
Harvestability
Tillering
12 years
Total: 406,069 genotypes

The SUCEST EST Sequencing Project

Resource

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

André L. Vettore,[1,24] Felipe R. da Silva,[1,25] Edson L. Kemper,[1,26] Glaucia M. Souza,[3] Aline M. da Silva,[3] Maria Inês T. Ferro,[6] Flavio Henrique-Silva,[8] Éder A. Giglioti,[9] Manoel V.F. Lemos,[7] Luiz L. Coutinho,[10] Marina P. Nobrega,[11] Helaine Carrer,[10] Suzelei C. França,[12] Maurício Bacci Jr.,[13] Maria Helena S. Goldman,[14] Suely L. Gomes,[3] Luiz R. Nunes,[15] Luis E.A. Camargo,[10] Walter J. Siqueira,[16] Marie-Anne Van Sluys,[4] Otavio H. Thiemann,[17] Eiko E. Kuramae,[18] Roberto V. Santelli,[3] Celso L. Marino,[19] Maria L.P.N. Targon,[20] Jesus A. Ferro,[6,27] Henrique C.S. Silveira,[8] Danyelle C. Marini,[9] Eliana G.M. Lemos,[6] Claudia B. Monteiro-Vitorello,[10] José H.M. Tambor,[11] Dirce M. Carraro,[10,24] Patrícia G. Roberto,[12] Vanderlei G. Martins,[21] Gustavo H. Goldman,[22] Regina C. de Oliveira,[15] Daniela Truffi,[10] Carlos A. Colombo,[16] Magdalena Rossi,[4] Paula G. de Araujo,[4] Susana A. Sculaccio,[17] Aline Angella,[18] Marleide M.A. Lima,[18] Vicente E. de Rosa Jr.,[18] Fábio Siviero,[3] Virginia E. Coscrato,[19] Marcos A. Machado,[20] Laurent Grivet,[23] Sonia M.Z. Di Mauro,[6] Francisco G. Nobrega,[11] Carlos F.M. Menck,[5] Marilia D.V. Braga,[2,28] Guilherme P. Telles,[2] Frank A.A. Cara,[2] Guilherme Pedrosa,[2] João Meidanis,[2] and Paulo Arruda[1,27,29]

13:2725–2735 ©2003 by Cold Spring Harbor Laboratory Press ISSN 1088-9051/03 $5.00; www.genome.org

Genome Researc
www.genome.org

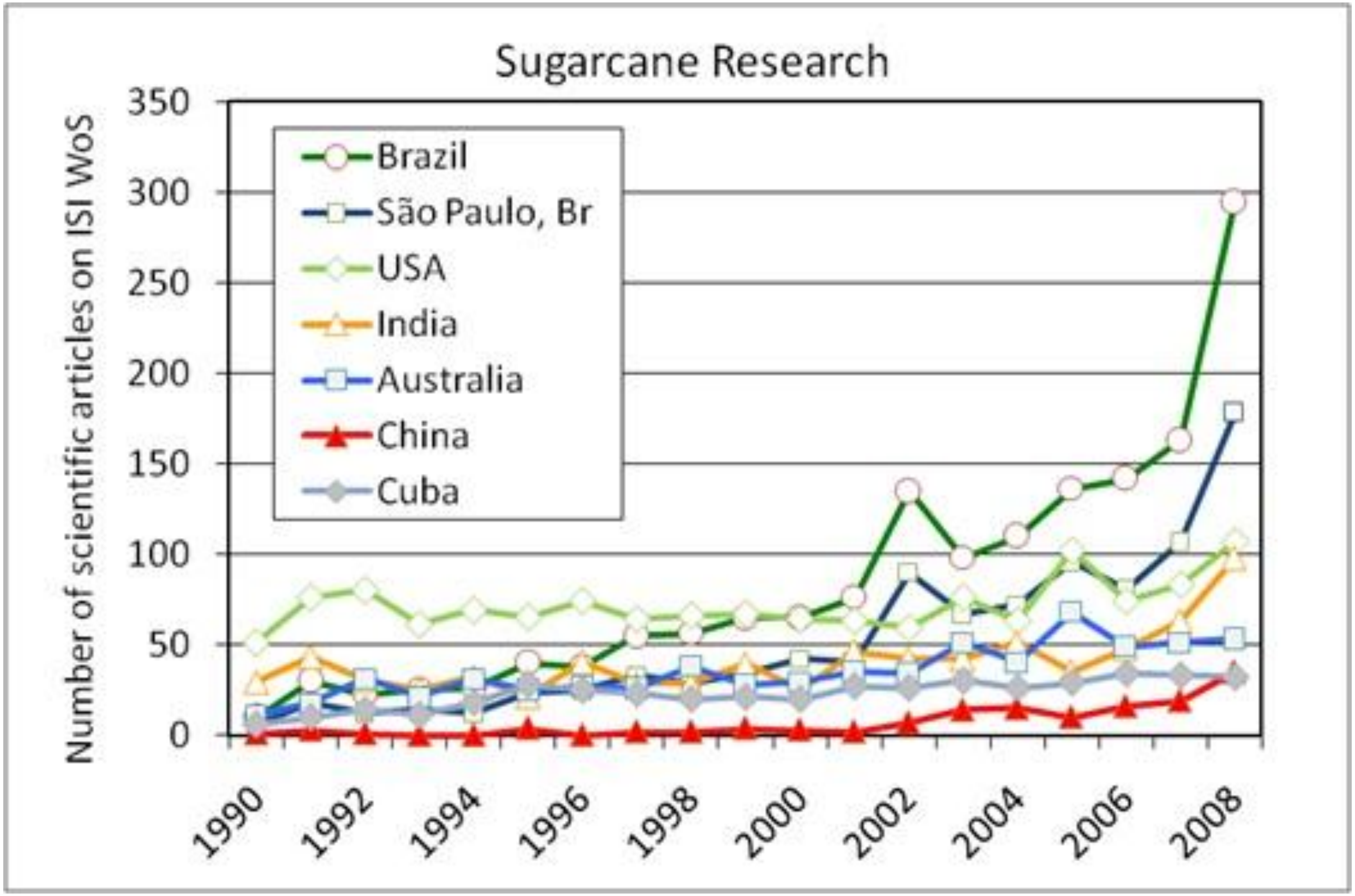
50 labs

200 researchers

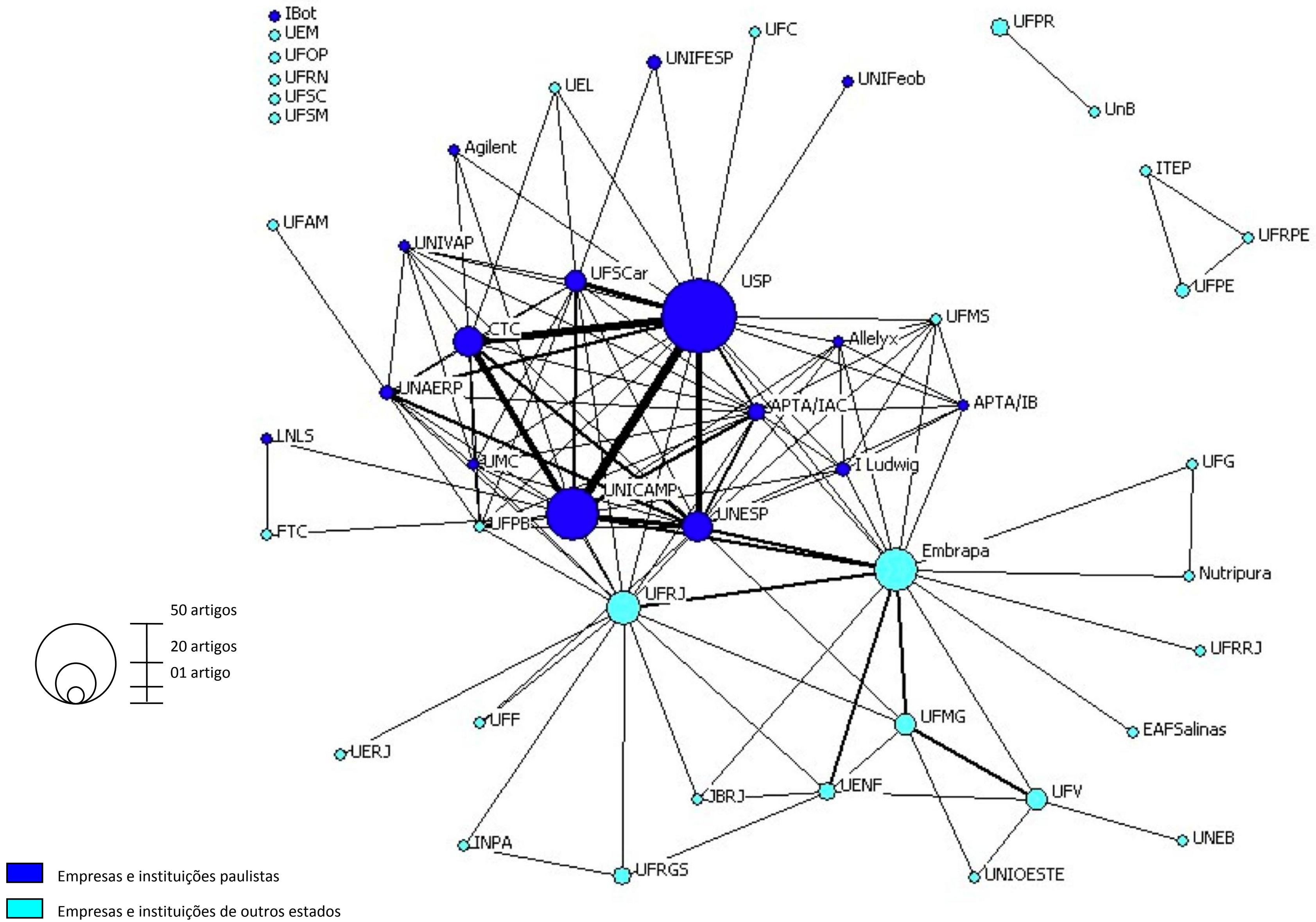
238000 ESTs

43000 Transcripts

26 libraries - 13 cultivars - Over 90% of sugarcane genes tagged


Sugarcane Research
Number of scientific articles on ISI WoS
350
300
250
200
150
100
50
0
Brazil
São Paulo, Br
USA
India
Australia
China
Cuba
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008

Sugarcane Genome Research Network in Brazil



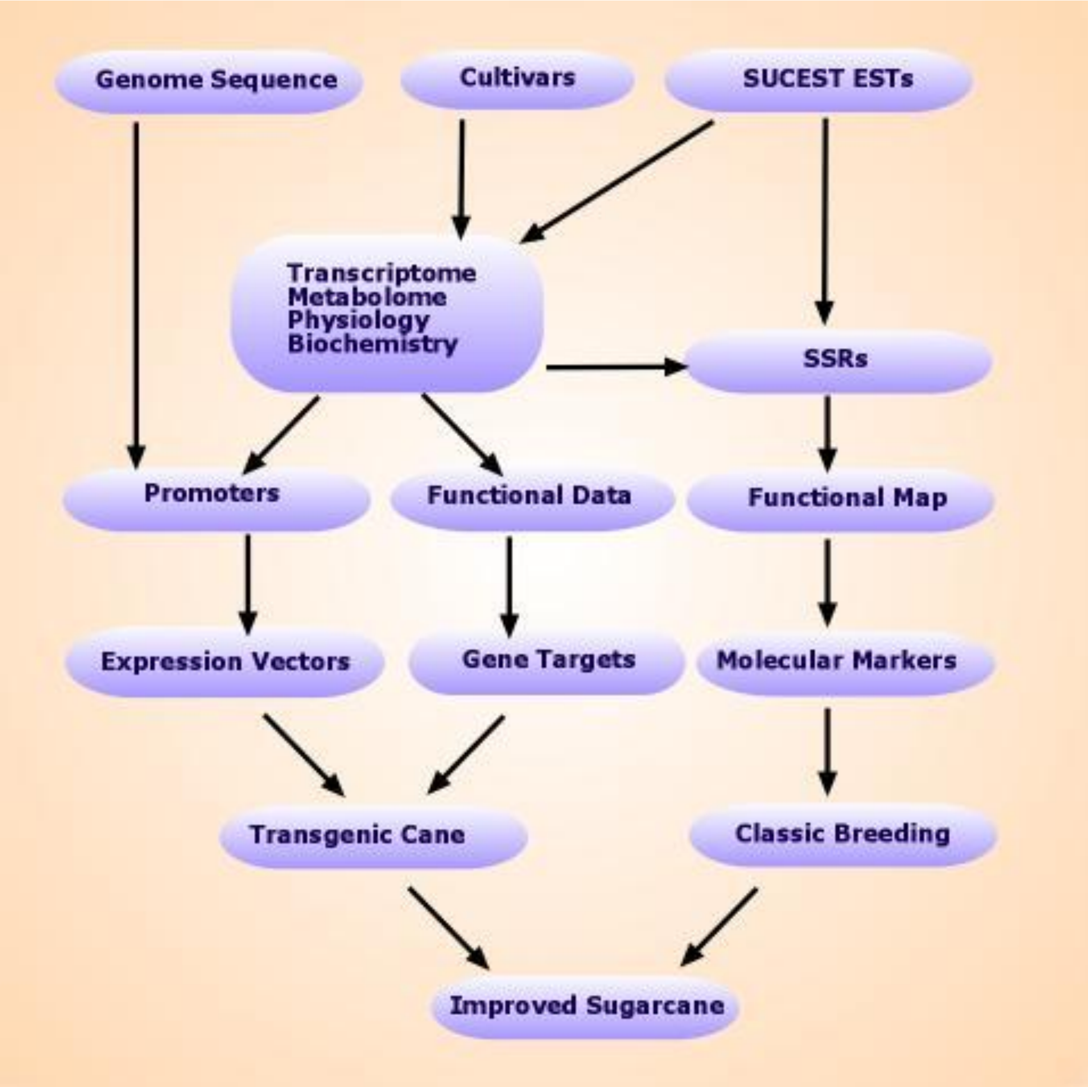
Setting up the priorities

Tropical Plant Biol.
DOI 10.1007/s12042-010-9050-5

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. Buckeridge • Marcelo Menossi • Marie-Anne Van Sluys • Glaucia M. Souza

Biotechnological Roadmap for Sugarcane Improvement



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 mthods

4 - International Bioinformatics
International Consortium

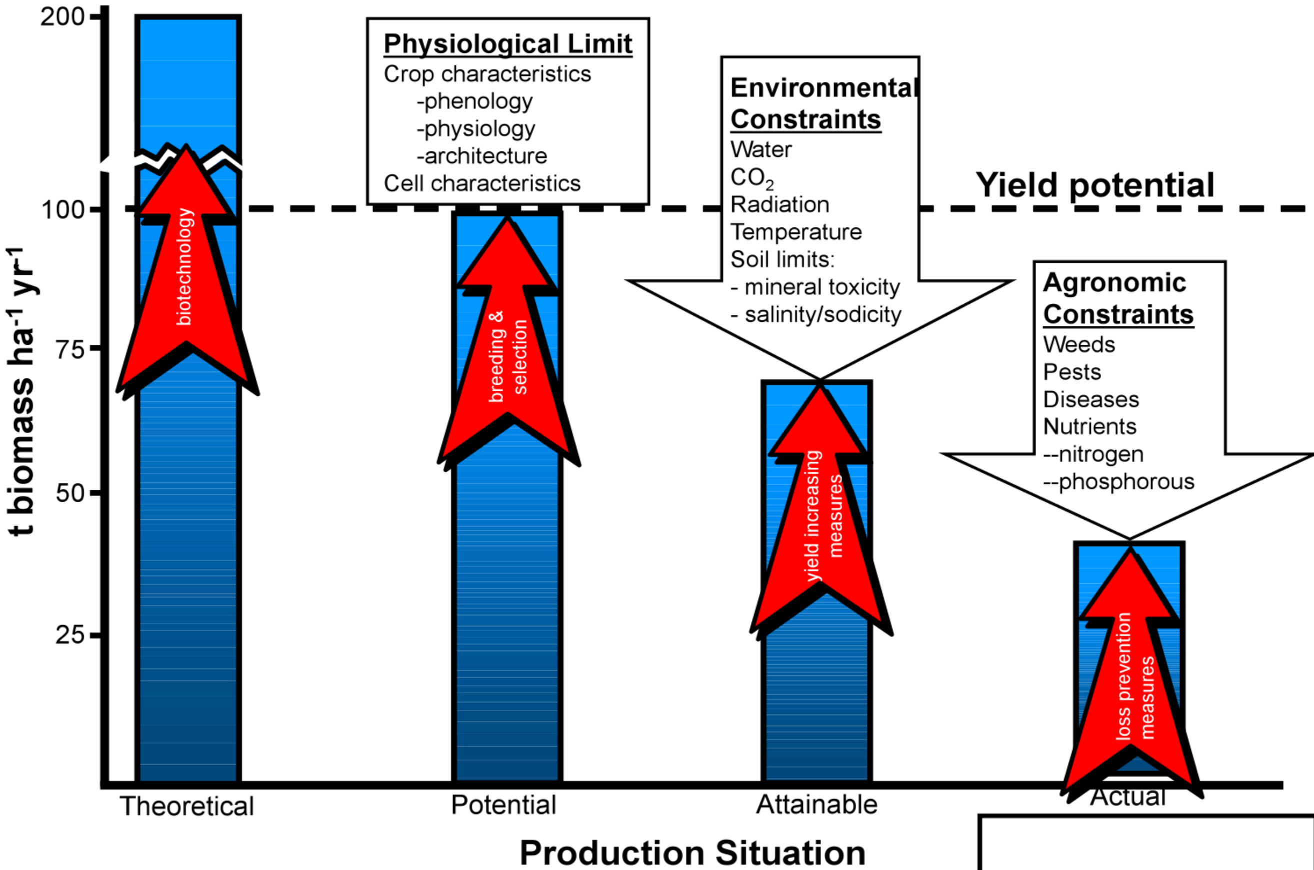
5 – Transgenics
Expression vectors
Complete ORFeome
Promoters
Screening methods for phenotyping (metabolomics, qPCR, biochemical assays, cell wall)

6 - Marker identification
Integrated databases and translation of transcriptome data to marker assays

How far can we go?

Waclawovsky, A. J., Sato, P. M., Lembke, G. M., Moore, P. H. and Souza, G. M. Sugarcane for Bioenergy Production: an assessment of yield and regulation of sucrose content (Plant Biotech. J. *2010)*

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



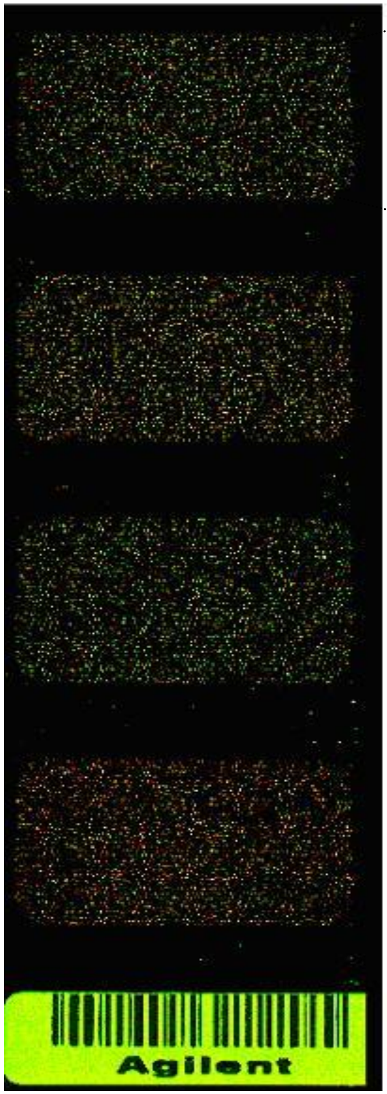
Waclawovsky, A. J., Sato, P. M., Lembke, G. M., Moore, P. H. and Souza, G. M. Sugarcane for Bioenergy Production: an assessment of yield and regulation of sucrose content (Plant Biotech. J. *2010)*

Type of yield	Cane yield ($t\ ha^{-1} yr^{-1}$)	Biomass*	
		($t\ ha^{-1} yr^{-1}$)	($g\ m^{-2} d^{-1}$)
Commercial Average	84	39	10.7
Commercial maximum	148	69	18.8
Experimental maximum	212	98	27.0
Theoretical maximum	381	177	48.5

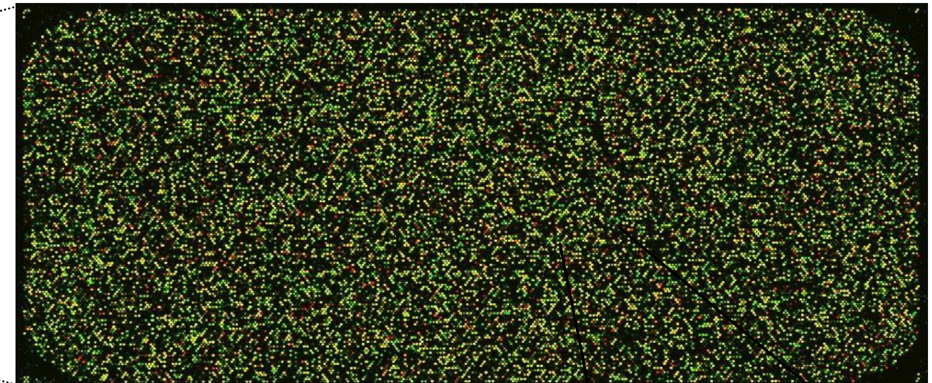
Potential yield of sugarcane

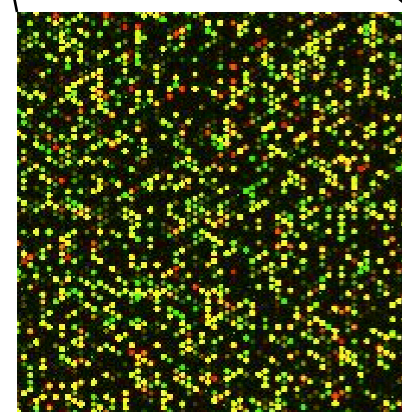
Agilent Technologies : 2 color gene expression
14,000 genes represented

4x44k Chip image

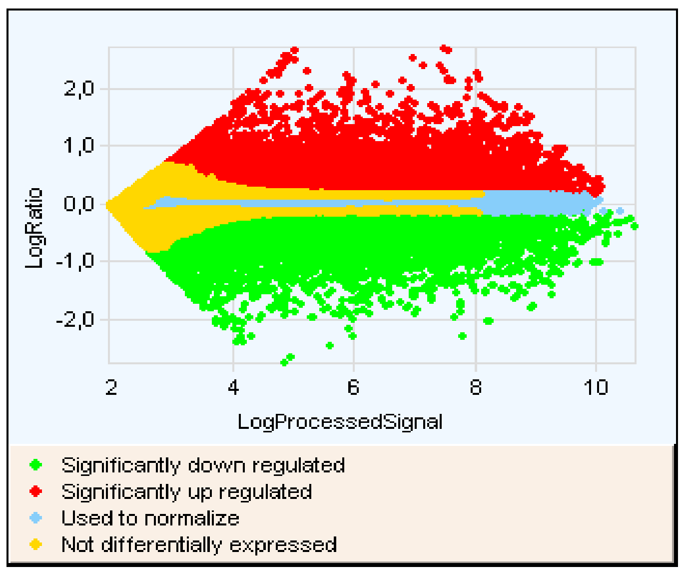


Array 1 (Experiment 1)





LogRatio vs Log Processed Signal



BMC Genomics BioMed Central

Research article Open Access

Signal transduction-related responses to phytohormones and environmental challenges in sugarcane

Flávia R Rocha[1], Flávia S Papini-Terzi[1], Milton Y Nishiyama Jr[1], Ricardo ZN Vêncio[2], Renato Vicentini[3], Rodrigo DC Duarte[3], Vicente E de Rosa Jr[3], Fabiano Vinagre[4], Carla Barsalobres[5], Ane H Medeiros[5], Fabiana A Rodrigues[7], Eugênio C Ulian[6], Sônia M Zingaretti[7], João A Galbiatti[7], Raul S Almeida[8], Antonio VO Figueira[8], Adriana S Hemerly[4], Marcio C Silva-Filho[5], Marcelo Menossi[3] and Gláucia M Souza*[1]

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BMC Genomics IMPACT FACTOR 4.18

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Research article Open Access

Sugarcane genes associated with sucrose content

Flavia S Papini-Terzi, Flavia R Rocha, Ricardo ZN Vencio, Juliana M Felix, Diana S Branco, Alessandro J Waclawovsky, Luiz EV Del Bem, Carolina G Lembke, Maximiller DL Costa, Milton Y Nishiyama Jr, Renato Vicentini, Michel GA Vincentz, Eugenio C Ulian, Marcelo Menossi and Glaucia M Souza

BMC Genomics 2009, **10**:120 doi:10.1186/1471-2164-10-120
Published: 21 March 2009

Int J Plant Genomics. 2008; 2008: 458732. PMCID: PMC2216073
Published online 2007 December 16. doi: 10.1155/2008/458732.
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Sugarcane Functional Genomics: Gene Discovery for Agronomic Trait Development

M. Menossi,[1] M. C. Silva-Filho,[2] M. Vincentz,[1] M.-A. Van-Sluys,[3] and G. M. Souza[4*]

Papini-Terzi, F.S. *et al.* Proc. Int. Soc. Sugar Cane Technol., Vol. 26, 2007

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

By

F.S. PAPINI-TERZI[1*], J.M. FELIX[2*], F.R. ROCHA[1], A.J. WACLAWOVSKY[1], E.C. ULIAN[3], S. M. CHABREGAS[3], M.C. FALCO[3], M.Y. NISHIYAMA-JR[1], R.Z.N. VÊNCIO[4], R. VICENTINI[2], M. MENOSSI[2] and G.M. SOUZA[1]

DNA RESEARCH 12, 27–38 (2005)

Transcription Profiling of Signal Transduction-Related Genes in Sugarcane Tissues

Flávia STAL PAPINI-TERZI,[1,†] Flávia RISO ROCHA,[1,†] Ricardo ZORZETTO NICOLIELLO VÊNCIO,[2] Kátia Cristina OLIVEIRA,[1] Juliana de Maria FELIX,[3,4] Renato VICENTINI,[4] Cristiane de SOUZA ROCHA,[4] Ana Carolina QUIRINO SIMÕES,[1] Eugênio César ULIAN,[5] Sônia Marli ZINGARETTI DI MAURO,[6] Aline Maria DA SILVA,[1] Carlos Alberto de BRAGANÇA PEREIRA,[2] Marcelo MENOSSI,[3,4] and Gláucia MENDES SOUZA[1,*]

Sucrose
Drought
Biomass
ABA
Lignin
Herbivory
Fiber
Phosphate
CO_2
MeJA
Sugars
Endophytes

7000 genes expression profiled

Genetical Genomics of Traits of interest

Progeny 1 Genotypes	Brix	Sucrose % m/m	Glucose % m/m	Fructose % m/m	Progeny 2 Genotypes	Brix
CTC98-241	18.00	7.311	1.322	0.988	C158	18.3
CTC98-242	18.60	9.183	1.430	1.014	C121	18.8
CTC98-243	19.20	10.956	0.649	0.602	C171	16.8
CTC98-244	14.60	11.161	0.633	0.646	C496	17
CTC98-246	18.80	10.974	0.709	0.545	C11	19.2
CTC98-252	18.00	6.370	0.840	0.579	C6	18.2
CTC98-253	19.60	11.120	0.660	0.643	C113	21
CTC98-258	18.00	6.739	1.116	0.865		
CTC98-261	7.00	1.14	0.878	0.755	C436	13.9
CTC98-262	7.40	1.37	0.968	0.823	C292	15
CTC98-265	6.40	0.49	1.200	1.090	C231	13.9
CTC98-268	4.80	0.70	0.342	0.326	C38	12.9
CTC98-271	6.00	0.92	1.098	0.992	C250	11.5
CTC98-272	6.80	1.07	0.725	0.632	C405	15.2
CTC98-277	7.40	1.58	0.774	0.716	C144	13.2
CTC98-279	7.80	1.74	1.318	1.066		

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Research article — Open Access

Sugarcane genes associated with sucrose content

Flavia S Papini-Terzi, Flavia R Rocha, Ricardo ZN Vencio, Juliana M Felix, Diana S Branco, Alessandro J Waclawovsky, Luiz EV Del Bem, Carolina G Lembke, Maximiller DL Costa, Milton Y Nishiyama Jr, Renato Vicentini, Michel GA Vincentz, Eugenio C Ulian, Marcelo Menossi and Glaucia M Souza

BMC Genomics 2009, **10**:120 doi:10.1186/1471-2164-10-120

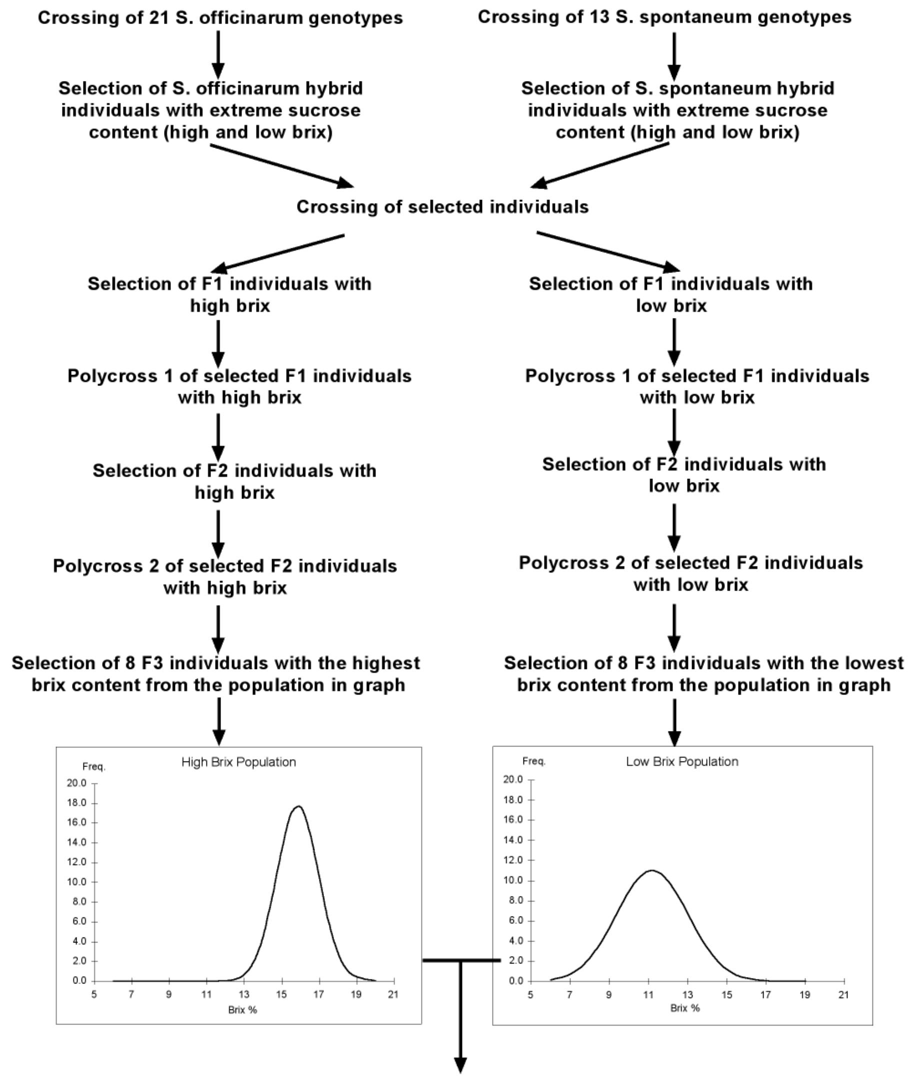
Published: 21 March 2009

Expression Profile of Signal Transduction Components in a Sugarcane Population Segregating for Sugar Content

Journal	Tropical Plant Biology
Publisher	Springer New York
ISSN	1935-9756 (Print) 1935-9764 (Online)
Issue	Volume 2, Number 2 / June, 2009
DOI	10.1007/s12042-009-9031-8
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[2, 5], Flávia Stal Papini-Terzi[3], Flávia Riso Rocha[3], Ricardo Zorzetto Nicoliello Vêncio[3], Renato Vicentini[1, 2], Milton Yutaka Nishiyama Jr[3], Eugênio César Ulian[4], Gláucia Mendes Souza[3] and Marcelo Menossi[2]

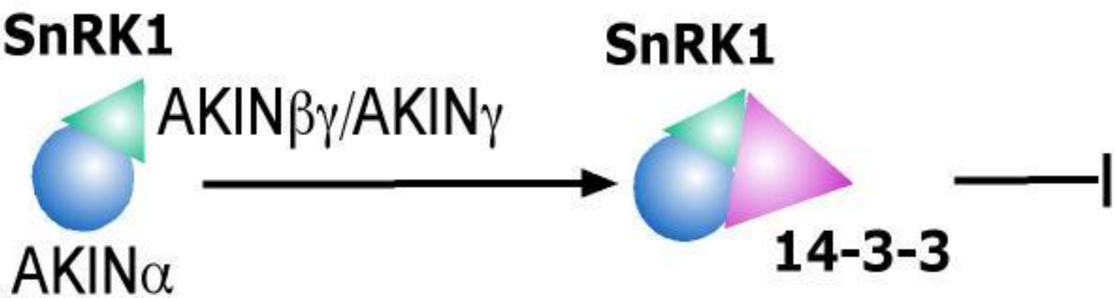


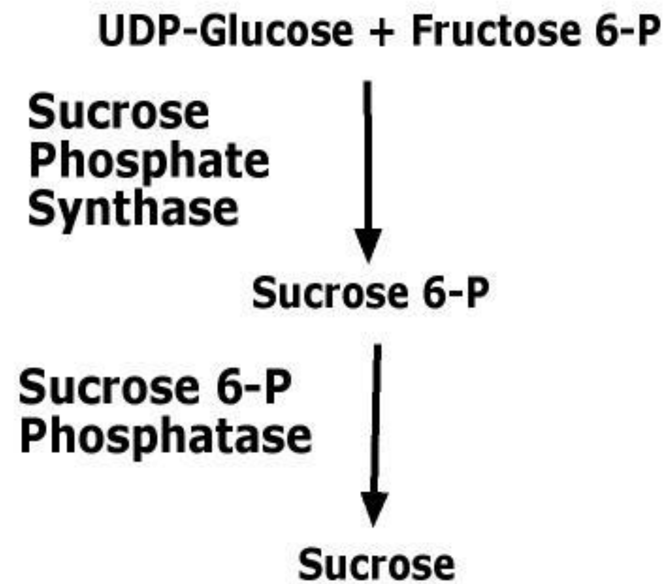
Pool of leaves +1 of the 8 highest brix individuals (LV-HB)
Pool of leaves +1 of the 8 lowest brix individuals (LV-LB)
Pool of internodes 1 of the 8 highest brix individuals (In1-HB)
Pool of internodes 1 of the 8 lowest brix individuals (In1-LB)
Pool of internodes 5 of the 8 highest brix individuals (In5-HB)
Pool of internodes 5 of the 8 lowest brix individuals (In5-LB)
Pool of internodes 9 of the 8 highest brix individuals (In9-HB)
Pool of internodes 9 of the 8 lowest brix individuals (In9-LB)

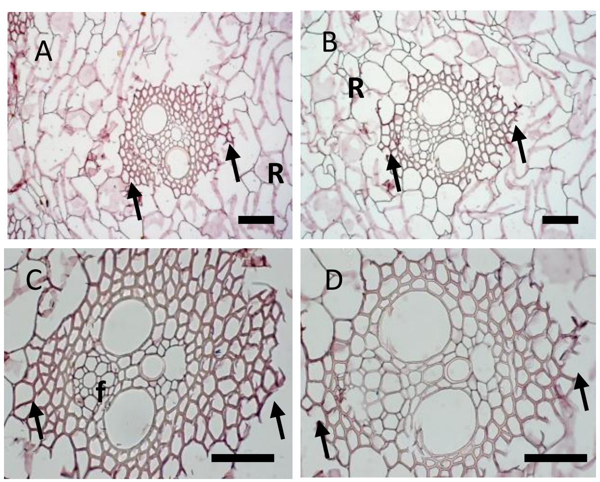
SnRK1

Phosphorylates sucrose phosphate synthase (SPS) and nitrate reductase (NR), which together with binding of 14-3-3 proteins inhibits their activity

category	sub category 1	sub category 2	HB vs LB	MIn vs IIn	Drought	ABA	Sucrose	Glucose
adapter	14-3-3 protein	GF14		1			3	
adapter	14-3-3 protein	GF14		4				
adapter	14-3-3 protein	GF14		2				
adapter	14-3-3 protein	GF14		1				
protein kinase	SNF-like kinase	caneSnRK1-2	1	2			3	

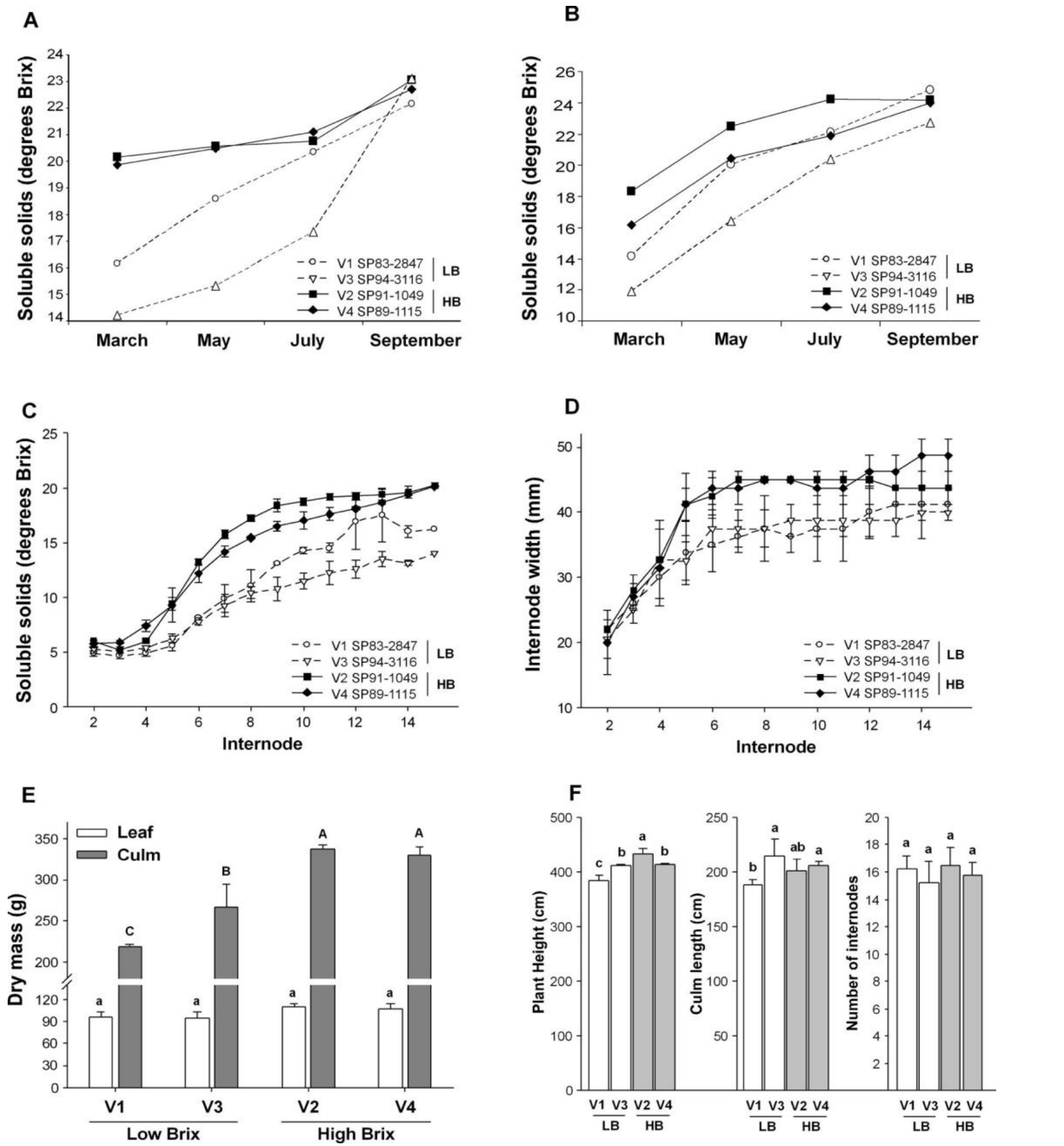






Storage parenchyma (R), Phloem (f), Fiber and bundle parenchymal cells (arrows), Bundle distal cells (ce), Bundle proximal cells (ci)

Physiology of sucrose and biomass accumulation



Waclawovsky, A. J., Sato, P. M., Lembke, C. G., Moore, P. H and **Souza, G. M.** Sugarcane for Bioenergy Production: an assessment of yield and regulation of sucrose content. **Plant Biotechnology Journal** (*2010)*

Regulation of sucrose accumulation and biomass

Hemicellulose and pectin synthesis

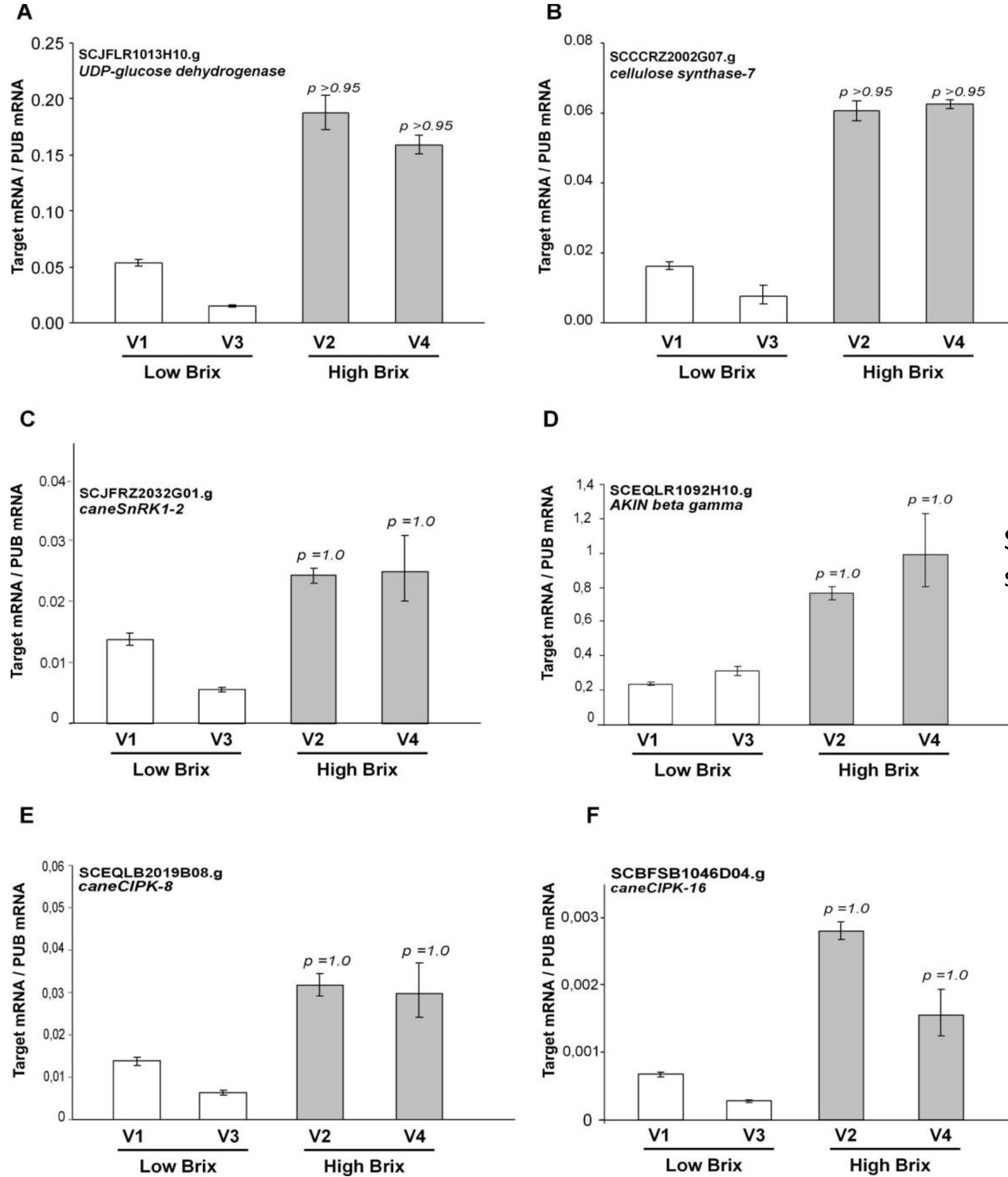
Cellulose synthesis

Sucrose synthesis

Sucrose synthesis

Drought responses

Drought responses



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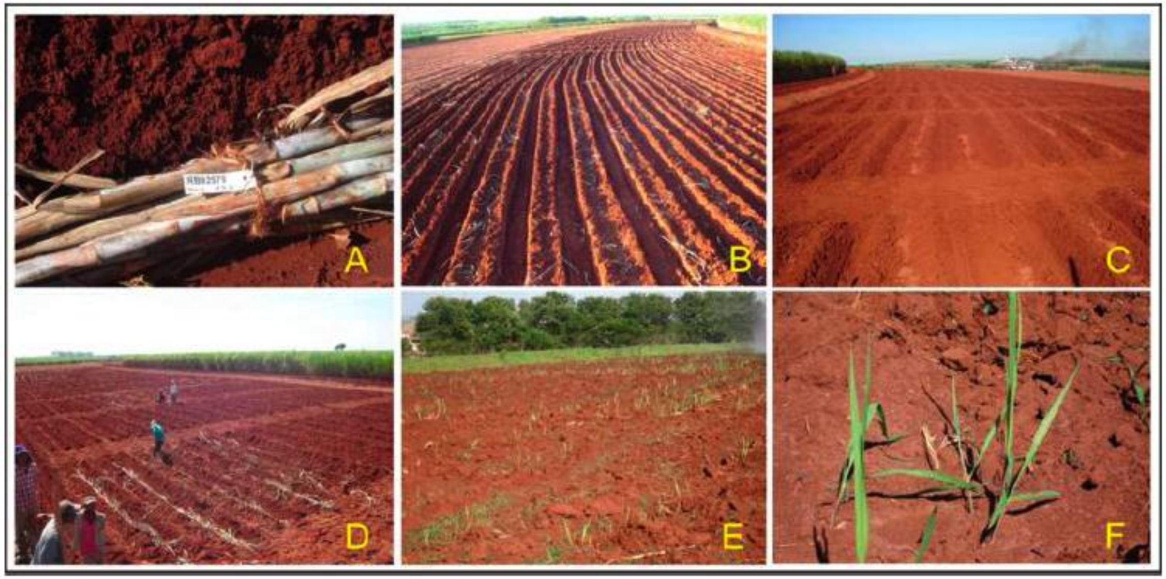
Drought Field Experiments with 6 cultivars: São Paulo, Pernambuco, Alagoas (SE, NE)



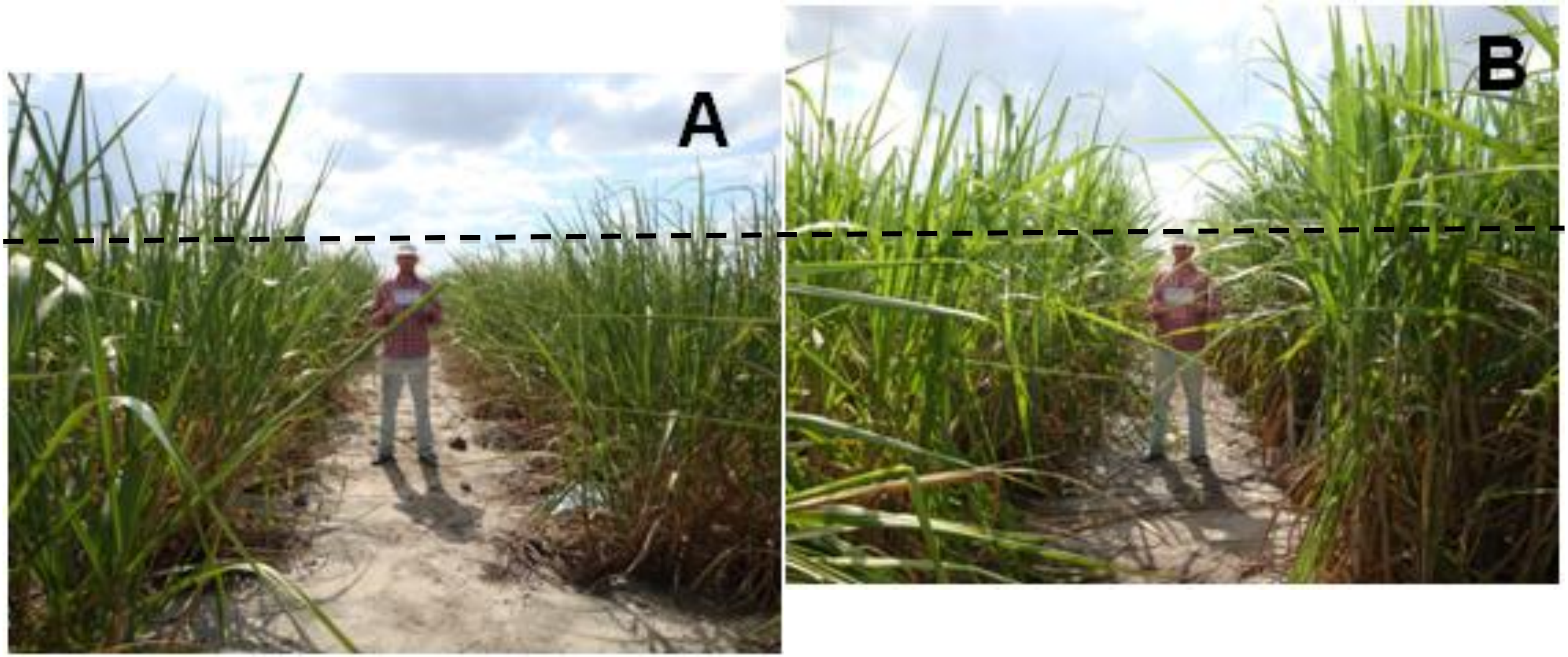
Stomatal conductance
Photosyntesis
Transpiration
Carboxylation Efficiency
Water Use Efficiency
Intrinsic Water Use Efficiency
Hydric Potential 4h
Hydric Potential 13h
Osmotic Potential 5h
Osmotic Pontential 12h
Potential Quantum Yield 5h
Potential Quantum Yield 13h
Effective Quantum Efficiency 10h
Plant Height
Leaf Lenght
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



Non-irrigated **Irrigated**

Drought Field Experiments with RB855536 in Alagoas

Non-irrigated

Irrigated

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

Sensors (30, 60, 90 cm)

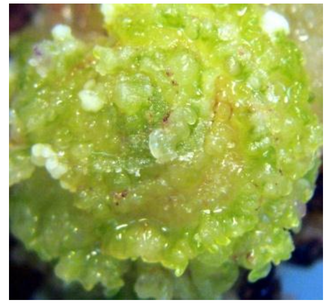
10 genotypes
(pre-selected based
on performance
over 2009 from a
group of 100 clones)

Irrigation

IAC

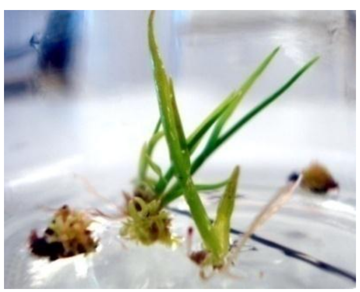
Production of transgenic sugarcane plants

Explants:
Immature Leaves

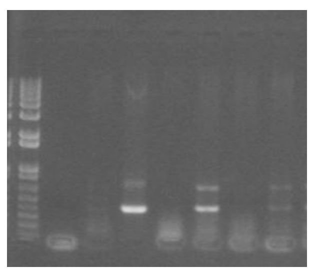


Callus
Induction

Regeneration Selective
Medium

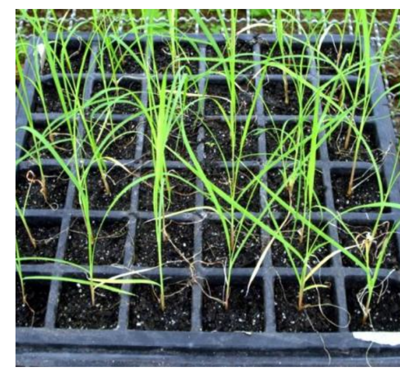


Rooting



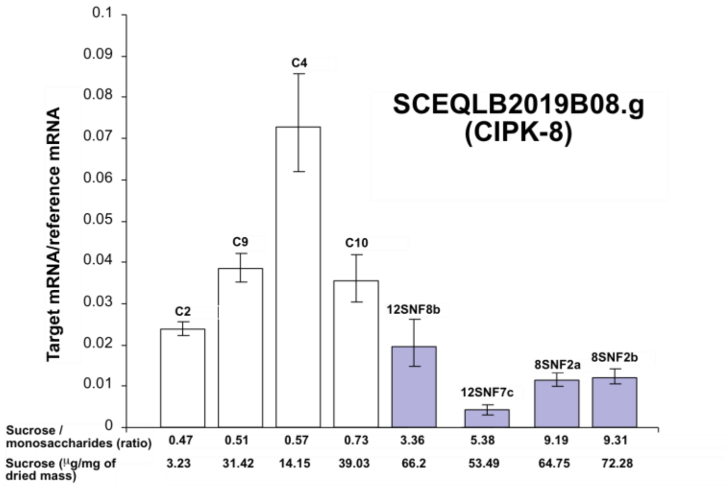
PCR

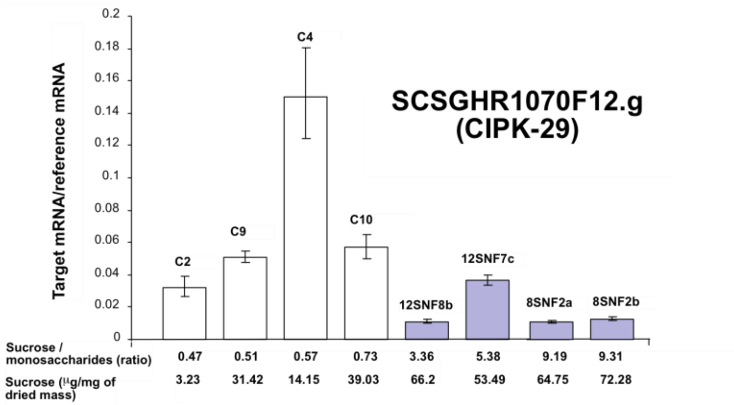
Shoot Growth

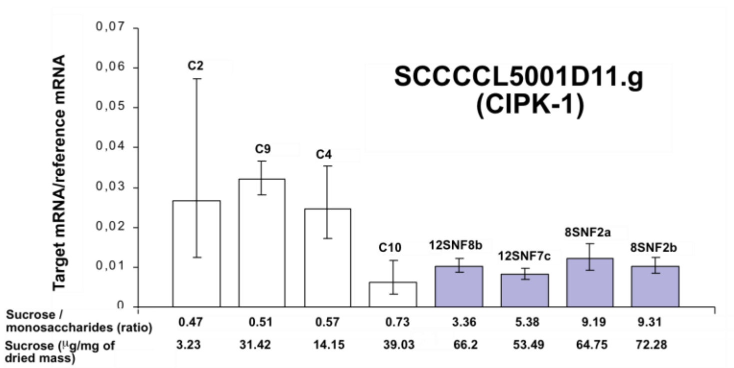


Greenhouse

Sugarcane transgenic plants with increased sucrose content:
3 CIPKs gene silencing using RNAi



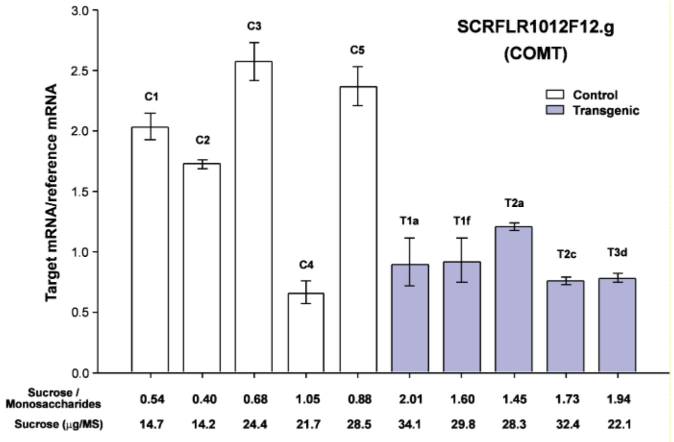




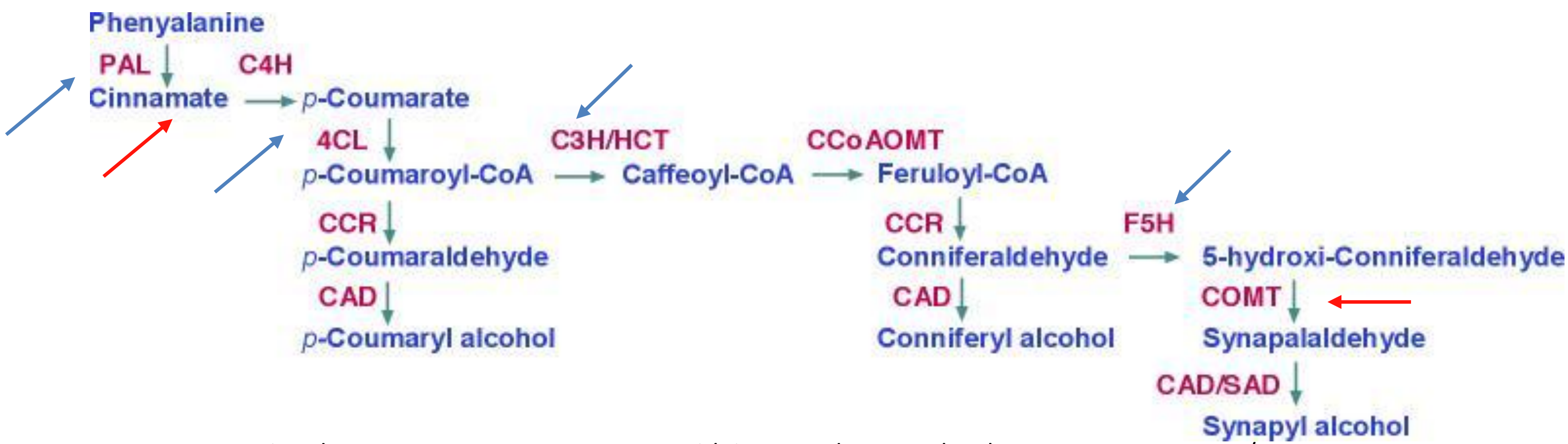
Papini-Terzi, F. S., Rocha, F. R., Vêncio, R. Z. N., Felix, J. M., Branco, D., Waclawovsky, A. J., Del-Bem, L. E. V., Lembke, C. G., Costa, M. D-B. L., Nishiyama-Jr, M. Y., Vicentini, R., Vincentz, M., Ulian, E. C., Menossi, M., **Souza, G. M.** (2009). Genes associated with sucrose content. **BMC Genomics** 10, 120. doi:10.1186/1471-2164-10-120

Genes associated to sucrose content, sugarcane with increased sucrose levels
USPTO Patent US 11/716,262.
PCT/BR2007/000282.
Gláucia Mendes Souza, Flávia Stal Papini-Terzi, Flávia Riso Rocha, Alessandro Jaquiel Waclawovsky, Ricardo Zorzetto Nicollielo Vêncio, Josélia Oliveira Marques, Juliana de Maria Felix, Marcelo Menossi Teixeira, Marcos Buckeridge, Amanda Pereira de Souza, Eugênio César Ulian.
Universidade de São Paulo, Unicamp, Centralcool, CTC and FAPESP

Lignin Biosynthesis is associated to sucrose content



category	sub category 1	sub category 2	HB vs LB	MIn vs IIn	Drought
cell wall metabolism	expansin	EXPA11	1	2	
cell wall metabolism	expansin	OsEXPA23	1		
cell wall metabolism	cytochrome P450	P-coumaroyl shikimate 3'-hydroxylase	2		
cell wall metabolism	cytochrome P450	Ferulate-5-hydroxylase	1	2	
cell wall metabolism	cytochrome P450	Cinnamic acid 4-hydroxylase	1		
cell wall metabolism	.	Caffeic acid 3-O-methyltransferase	2	2	
cell wall metabolism	polysaccharide metabolism	Xyloglucan endotransglycosylase		4	
cell wall metabolism	salicylic acid/lignin	Phenylalanine ammonia-lyase	1		
cell wall metabolism	salicylic acid/lignin	Phenylalanine ammonia-lyase	1	1	
cell wall metabolism	salicylic acid/lignin	Phenylalanine ammonia-lyase	1	1	



Genes associated to sucrose content, sugarcane with increased sucrose levels USPTO Patent US 11/716,262. PCT/BR2007/000282. **<u>Gláucia Mendes Souza,</u>** Flávia Stal Papini-Terzi, Flávia Riso Rocha, Alessandro Jaquiel Waclawovsky, Ricardo Zorzetto Nicollielo Vêncio, Josélia Oliveira Marques, Juliana de Maria Felix, Marcelo Menossi Teixeira, Marcos Buckeridge, Amanda Pereira de Souza, Eugênio César Ulian.

Universidade de São Paulo, Unicamp, Centralcool, CTC and FAPESP

Energy Cane

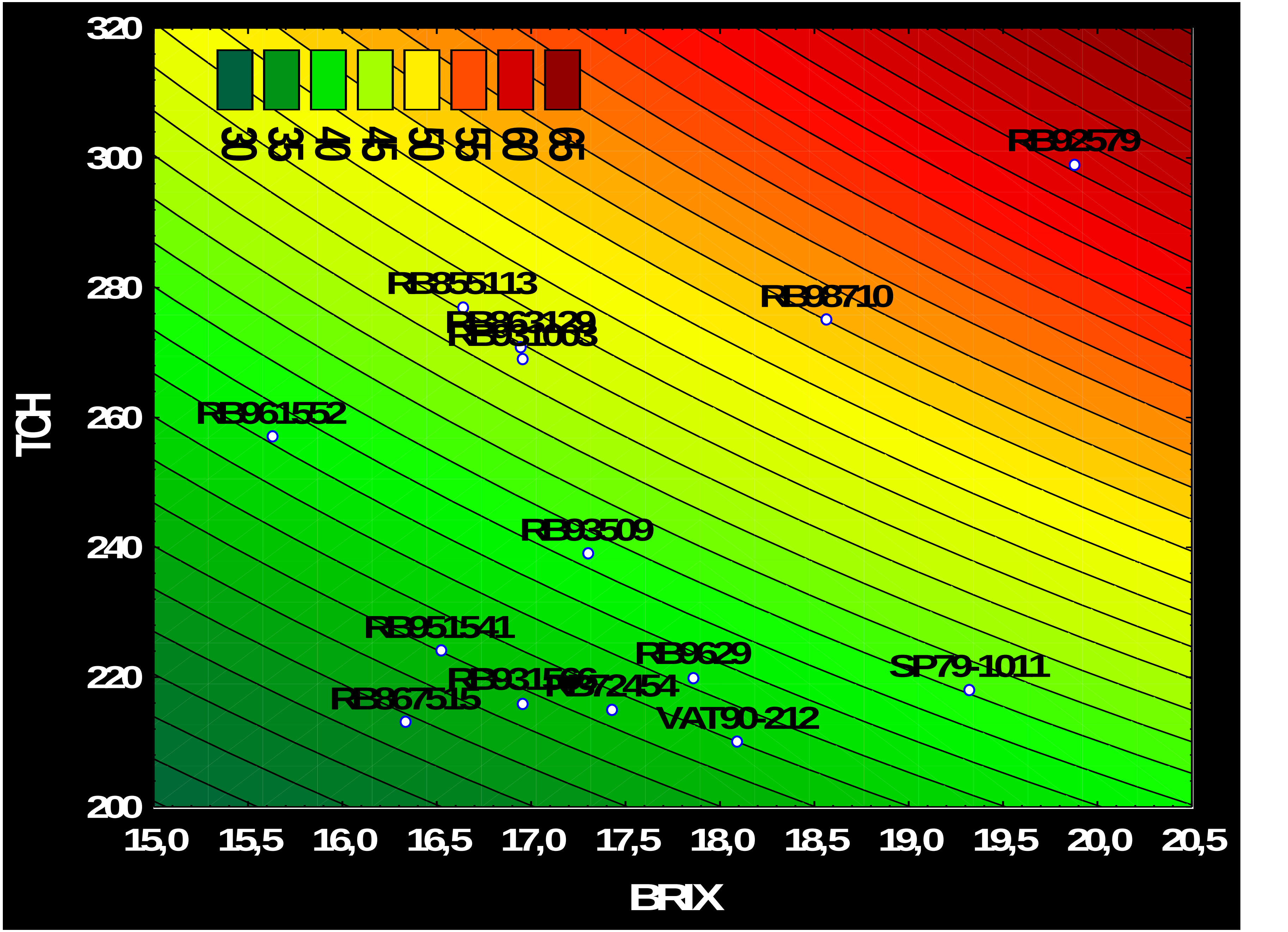
Sugarcane Mill

Industrial aspects: research in all aspects of the production

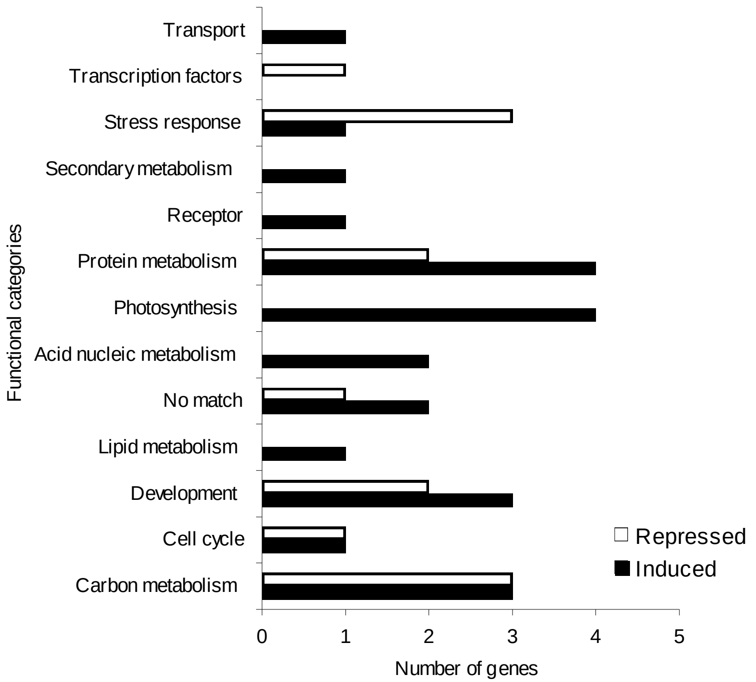
Total sugarcane production is estimated to be 664,33 ton/ha for 2010/2011
Total bioethanol production for 2010/11 is projected at 28.5 billion L
54,6% (362,8 million tons) for ethanol (20,14 billlion L hydrated and 8,4 billion L anyhidride)
45,4% (301,6 million tons) for sugar (38,7 million tons)
405 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

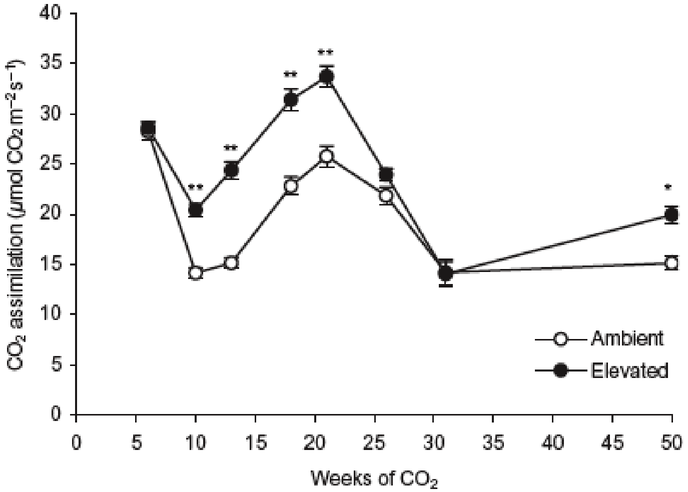


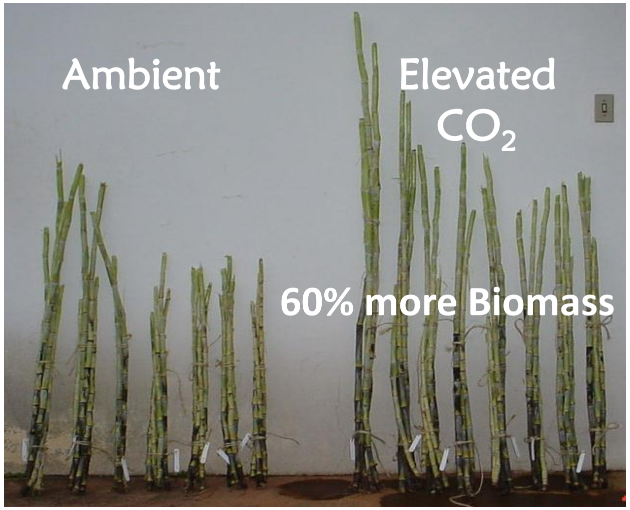
Climate Change: +60% biomass +35% photosyntesis



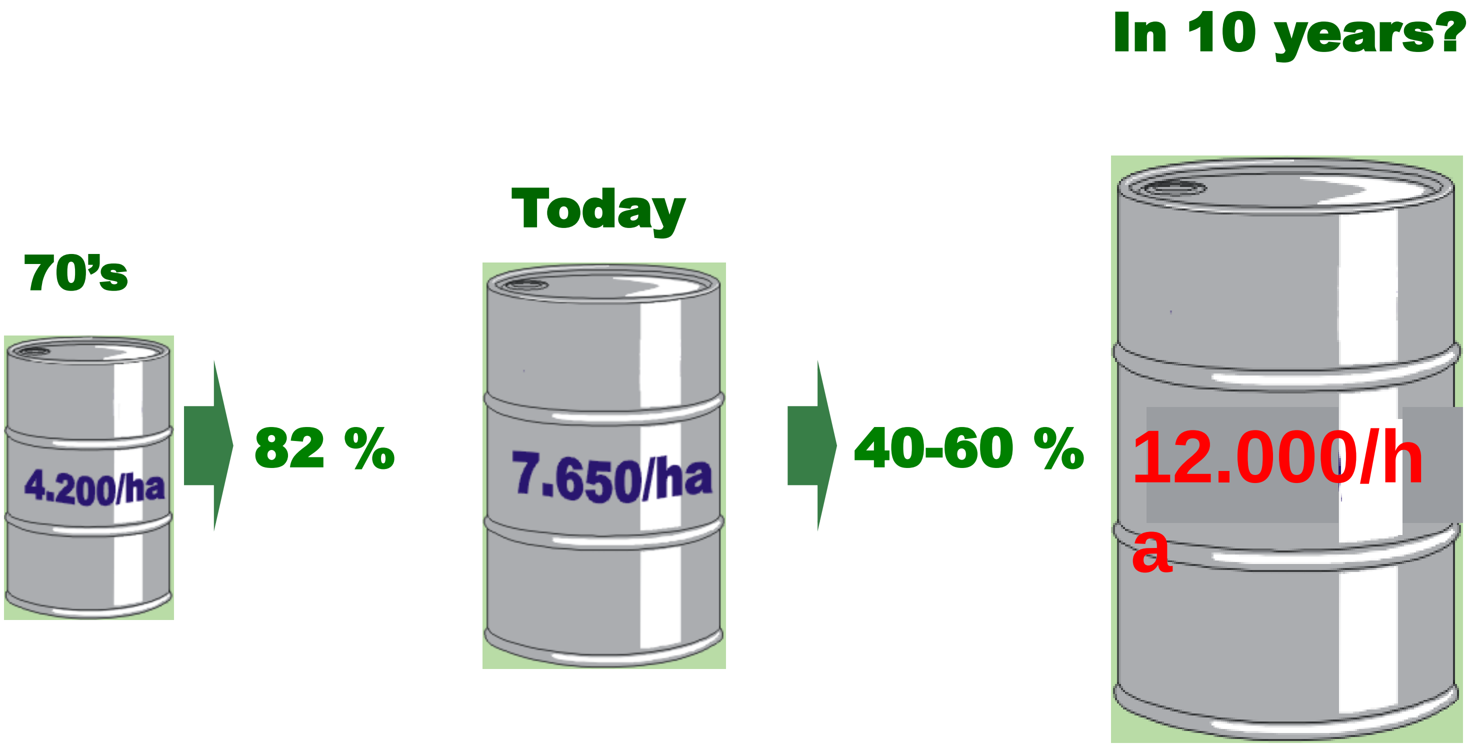
370 ppm CO_2

720 ppm CO_2

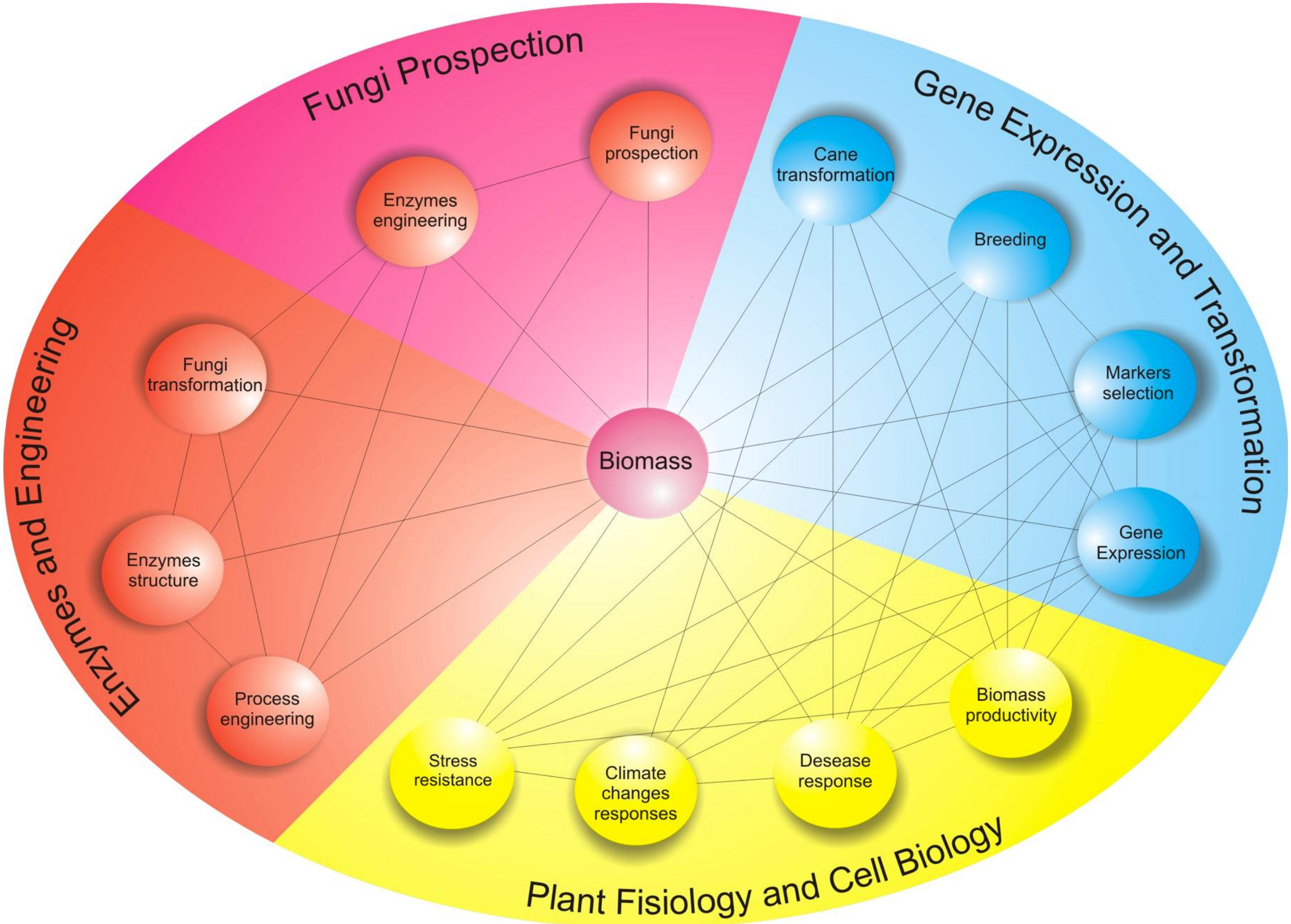




Cellulosic Ethanol



National Institute of Science and Technology of Bioethanol
CNPq, FAPESP



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

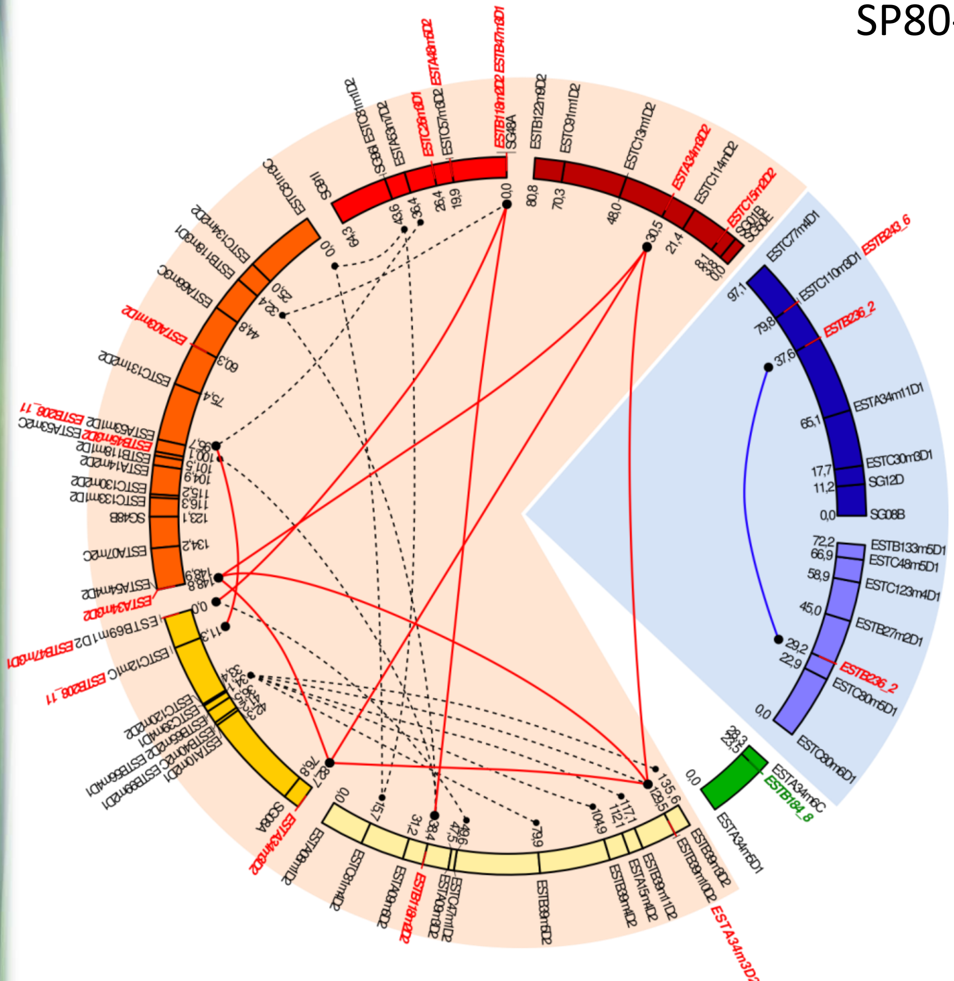
- Marker systems that are informative in other scenarios (e. g. Microssatelites) provide less information in polyploids, having a dominant action
- Commonly, only markers that have a single copy (dosage) on the genome have been used
- Single Nucleotide Polymorphisms (SNP) are usefull (codominant), but 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)



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)

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

Sequencing Workgroup

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

BIOEN FAPESP

SUGESI Partners

Instituto de Química

Instituto de Biociências

BIOEN
FAPESP

CATG
center for advanced technologies in genomics
centro avançado de tecnologias em genômica

ESALQ

IB
Instituto de Biologia

Southern Cross UNIVERSITY
A new way to think

CSIRO

INTERSECT

SIIB
CRC Sugar Industry Innovation through Biotechnology

UNSW
THE UNIVERSITY OF NEW SOUTH WALES
SYDNEY • CANBERRA • AUSTRALIA

cirad

1785
UGA

Energy Biosciences Institute

sasri
South African Sugarcane Research Institute

University of KwaZulu-Natal

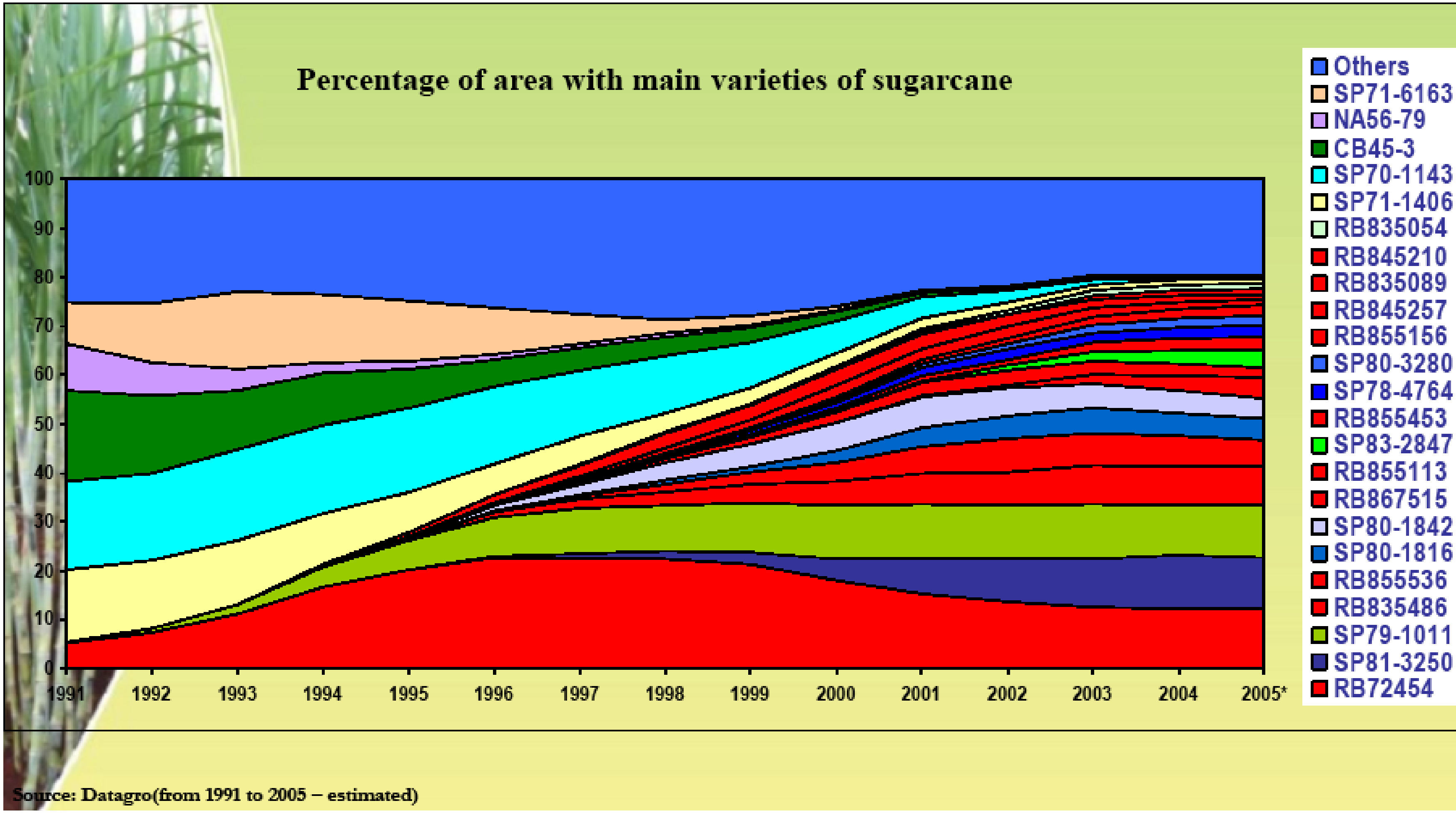
SMRI
Sugar Milling Research Institute

Centre National de Séquençage
GENOSCOPE

UIUC

International Consortium for Sugarcane Biotechnology

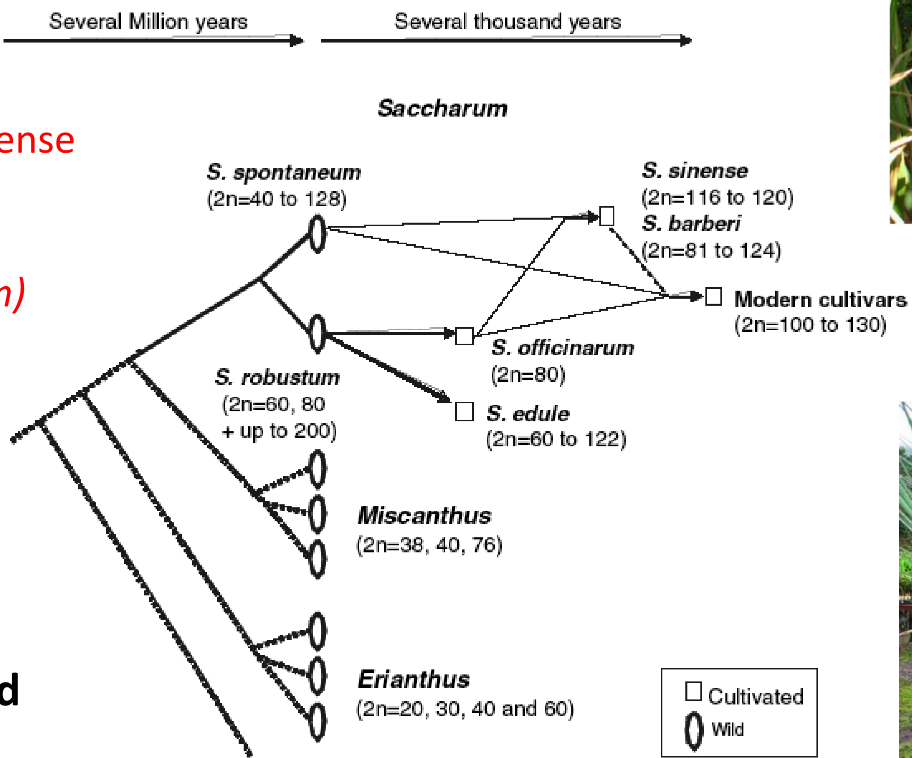
Which Genotype should we sequence?



Which Genotype should we sequece?

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

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)*



LA Purple
S. officinarum

SES208
S. spontaneum

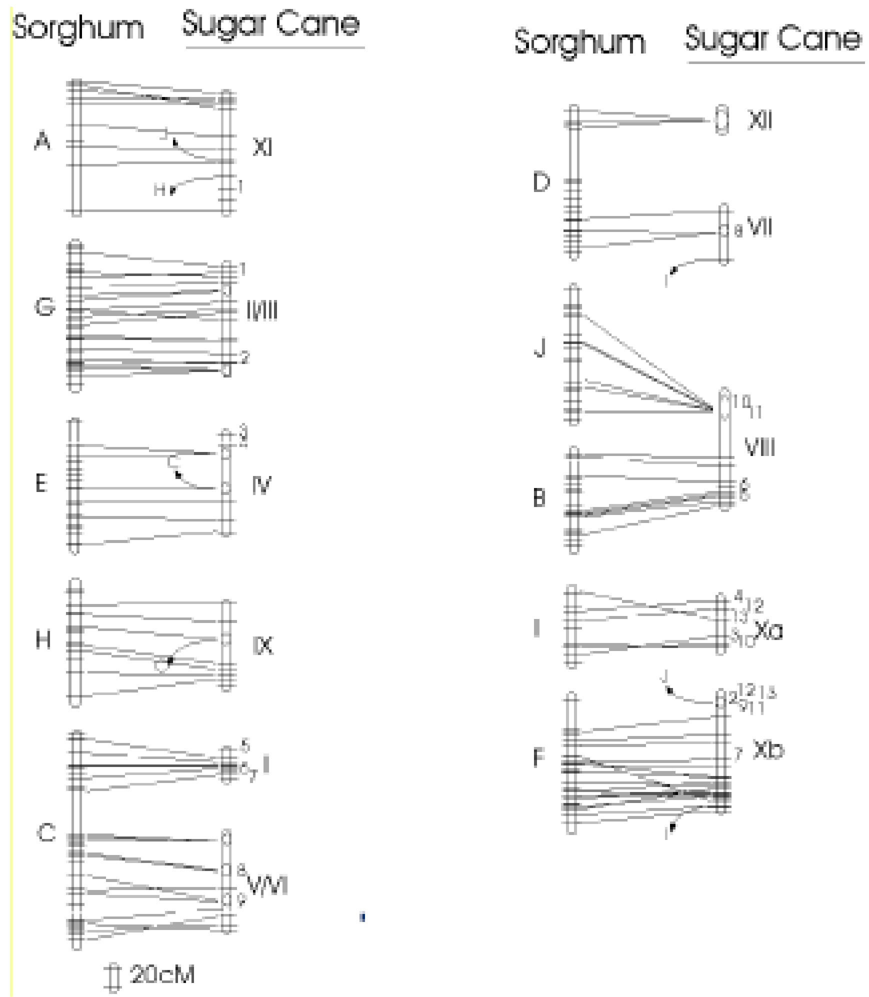
There are no homozigous diploid genotypes

Angelique D'Hont, Glaucia Mendes Souza,Marcelo Menossi, Michel Vincentz, Marie-Anne Van-Sluys, Jean Christophe Glaszmann and Eug�io Ulian

Syntheny with Sorghum

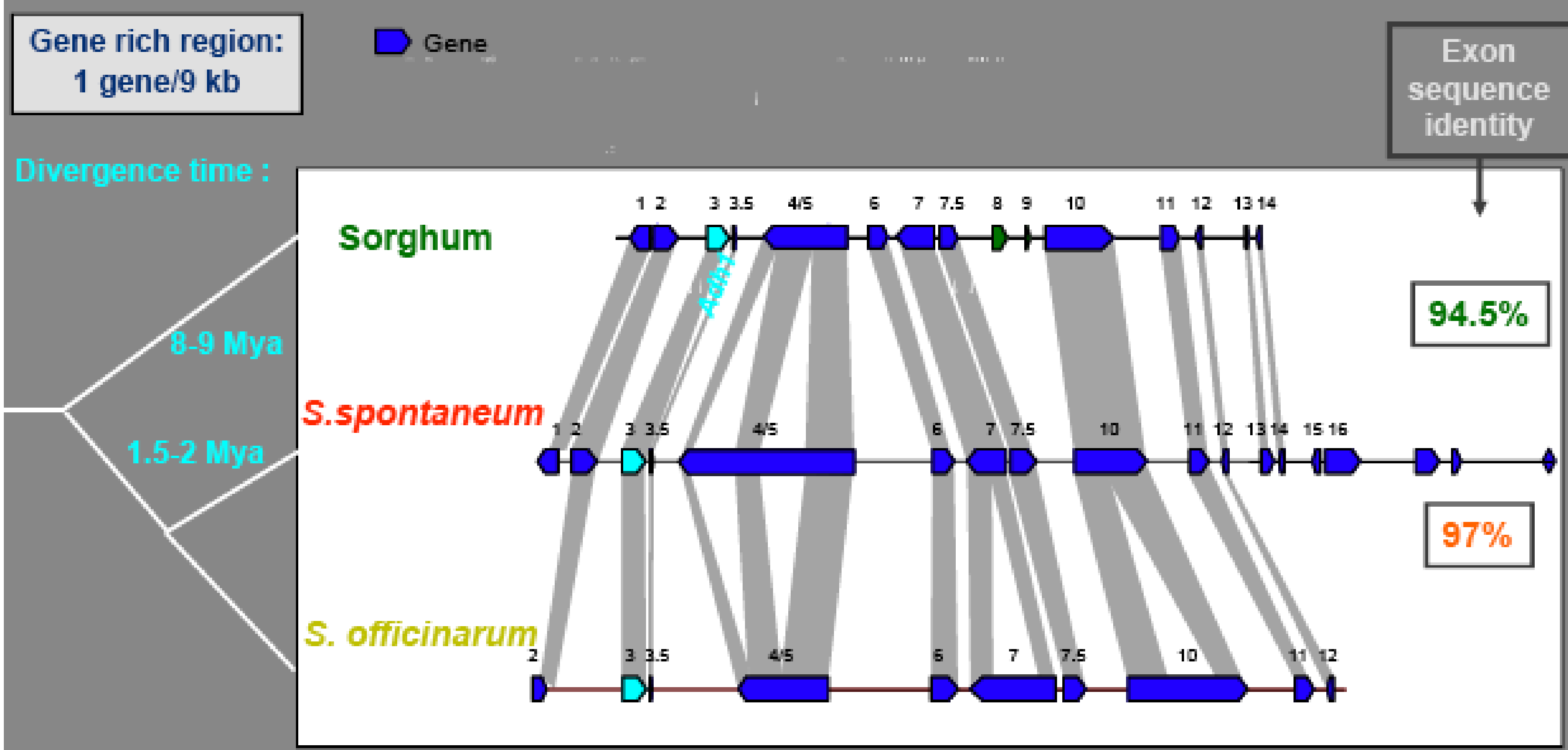
Saccharum and Sorghum diverged between 5 and 9 million years ago

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



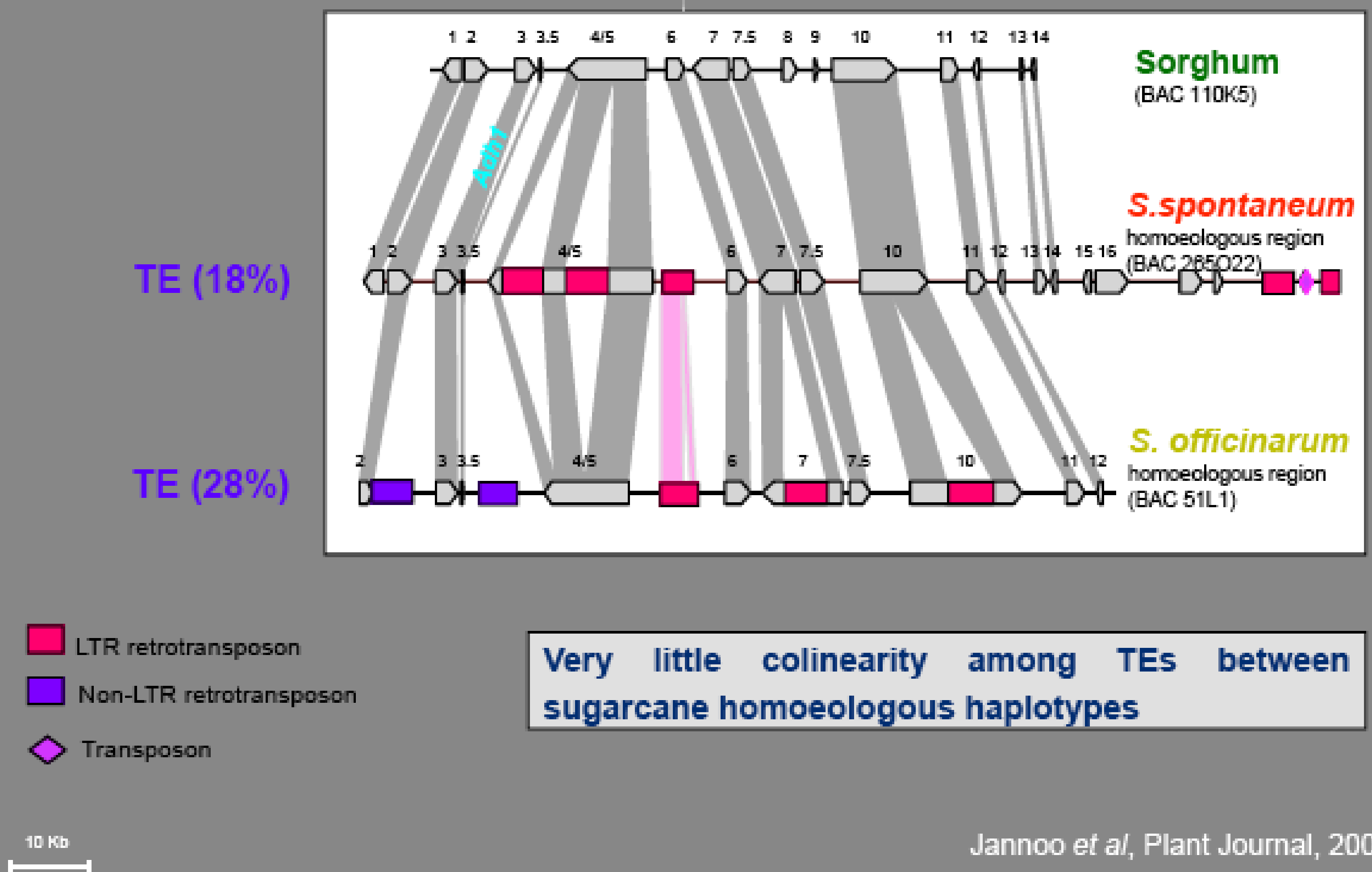
Perfect colinearity

High gene structure conservation

High gene sequence conservation

Jannoo *et al*, Plant Journal, 2007

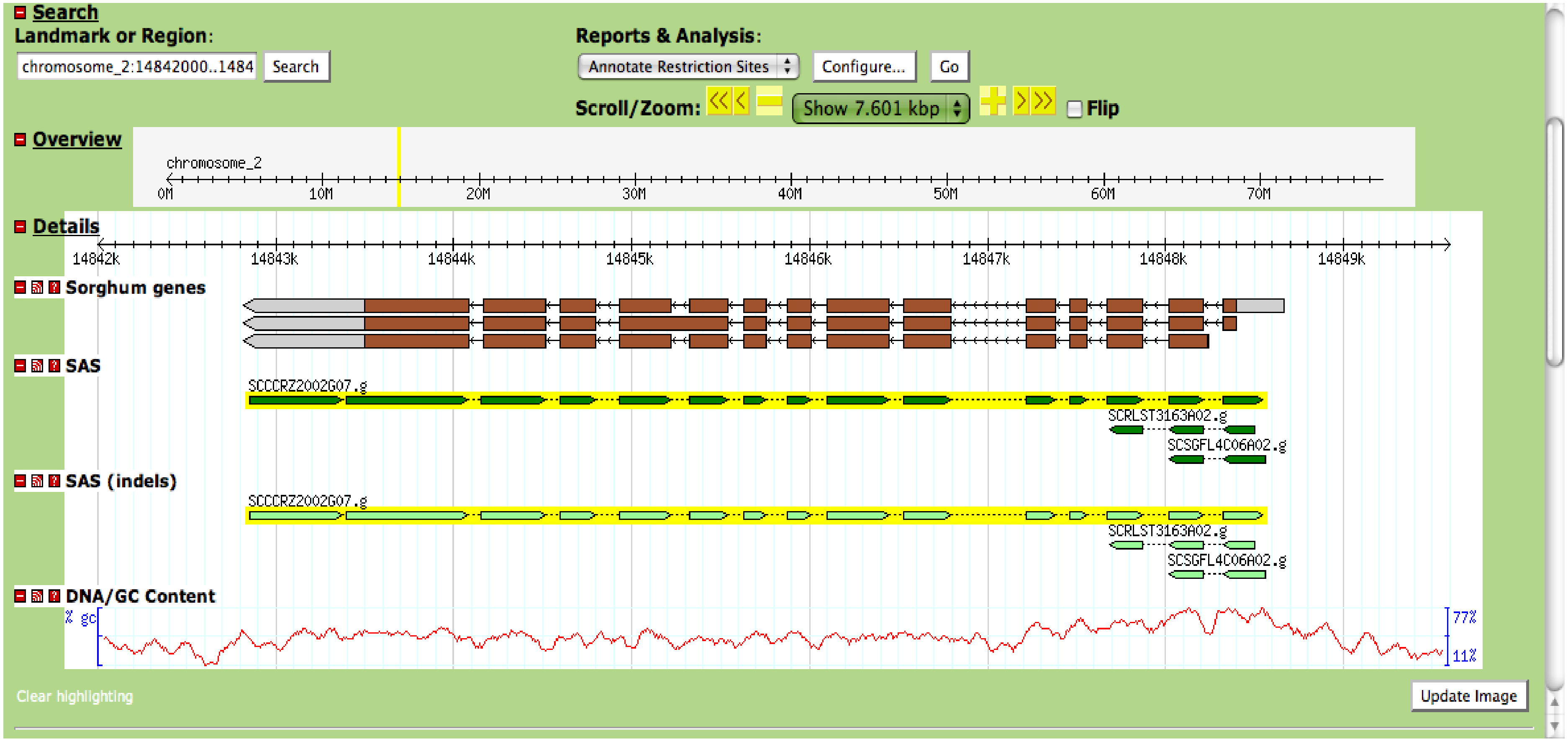
Transposable elements on Adh1 region



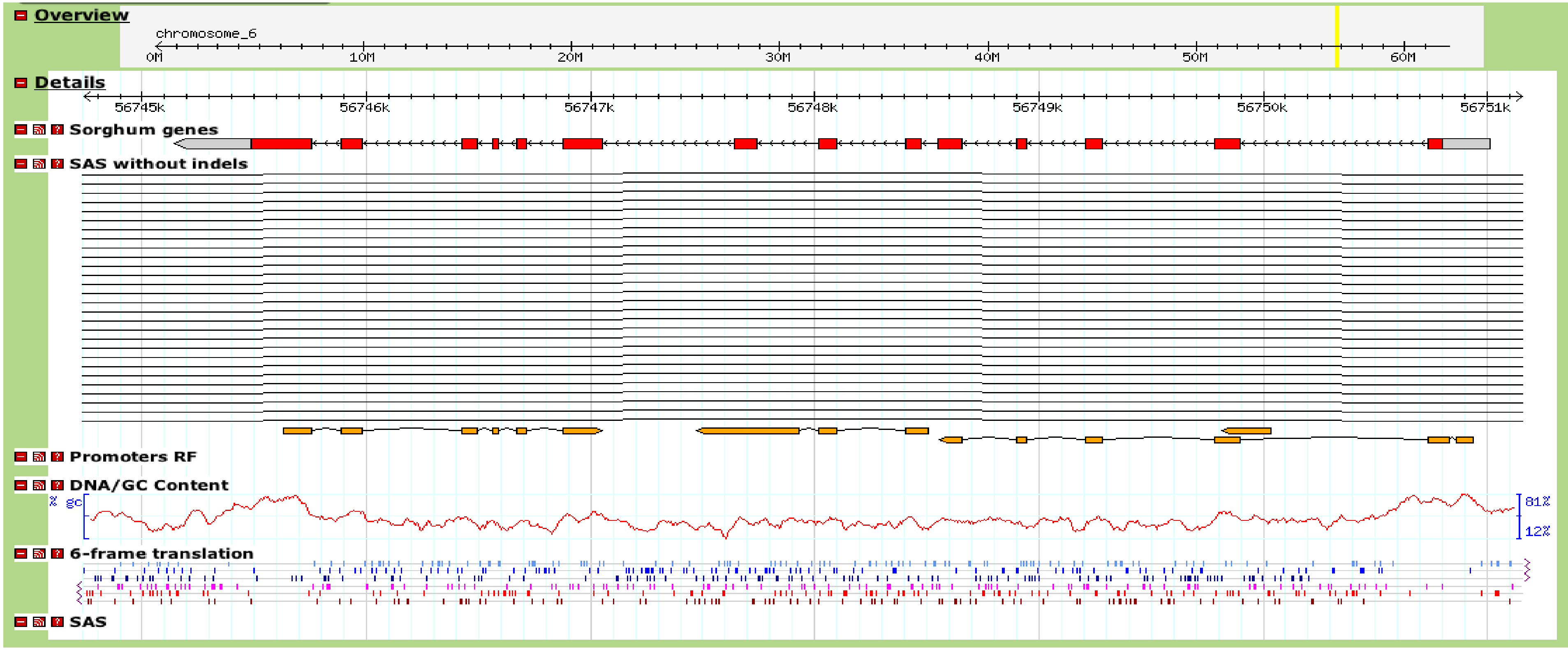
Very little colinearity among TEs between sugarcane homoeologous haplotypes

Jannoo *et al*, Plant Journal, 2007

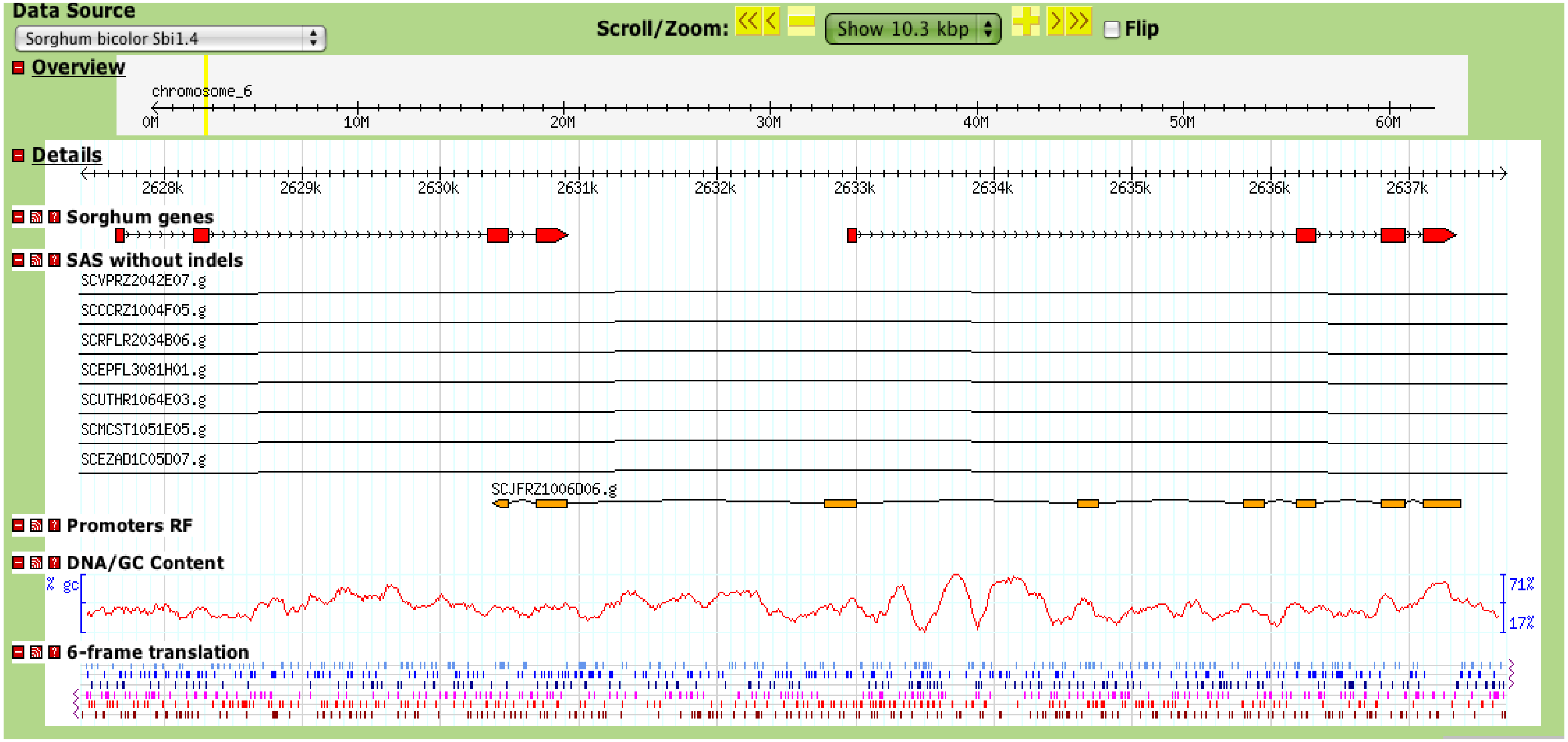
Prediction of Ortholog Groups



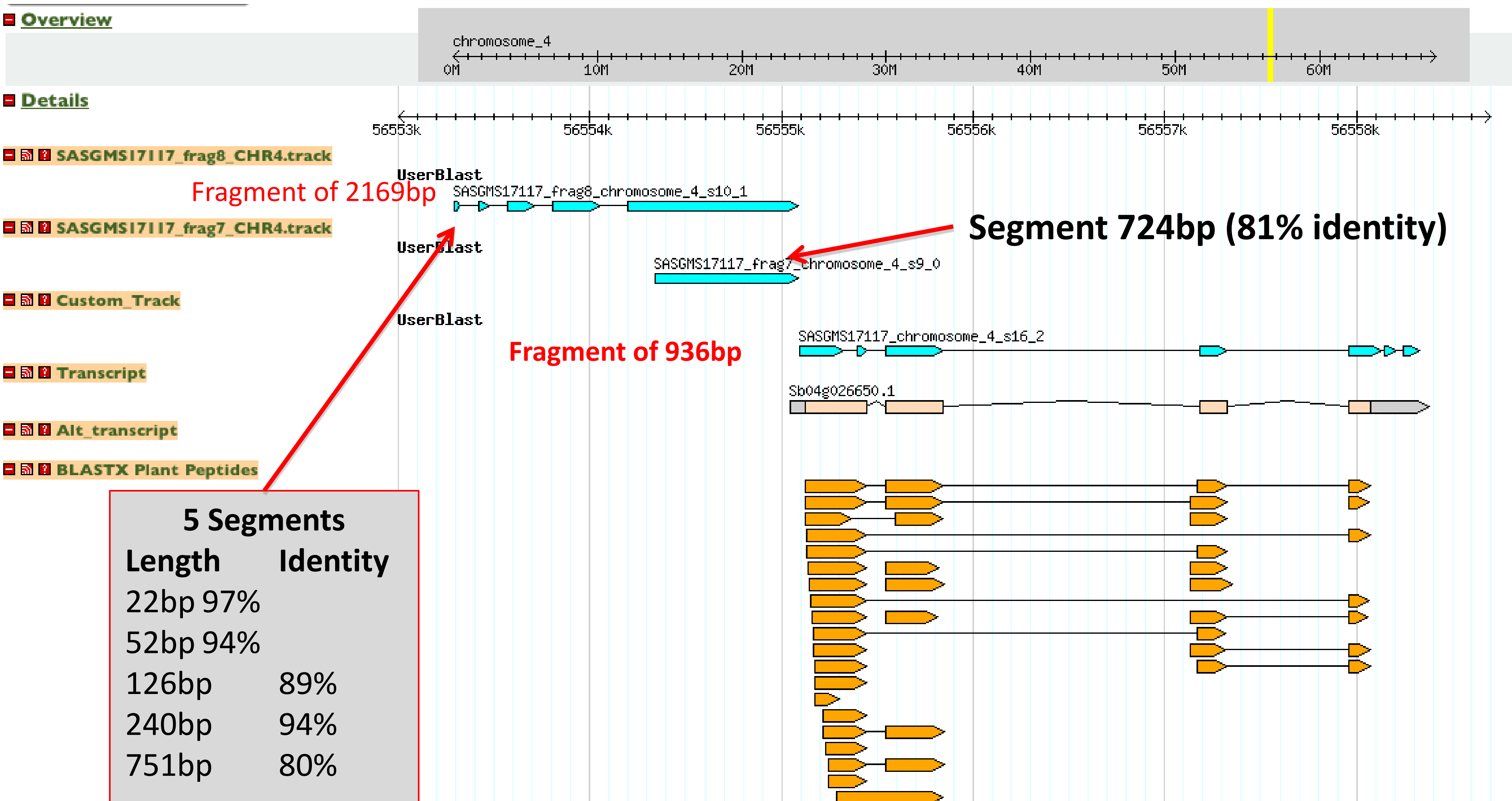
Prediction of Ortholog Groups



Prediction of Ortholog Groups



Sugarcane and Sorghum promoter alignments

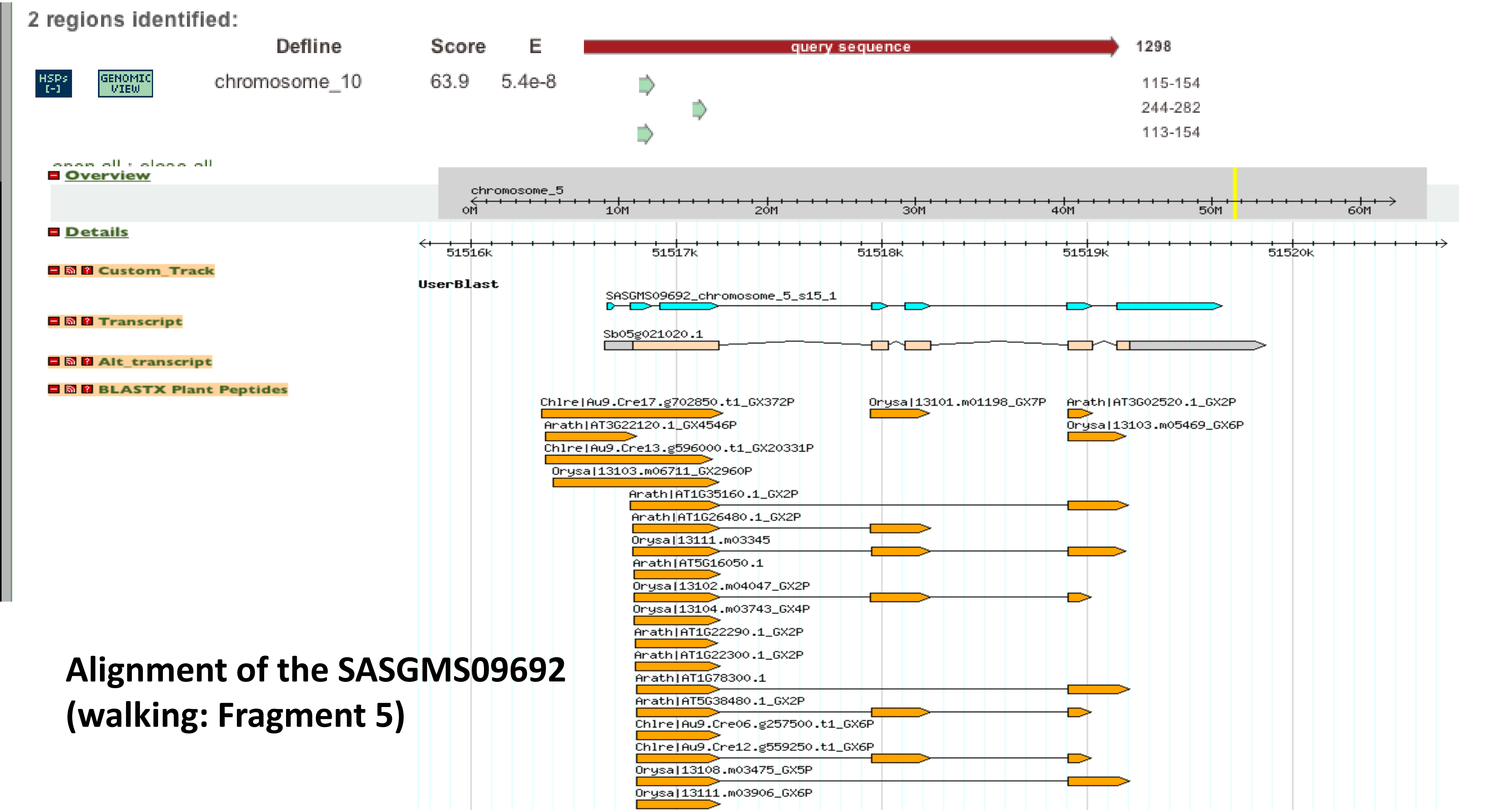


5 Segments

Length	Identity
22bp	97%
52bp	94%
126bp	89%
240bp	94%
751bp	80%

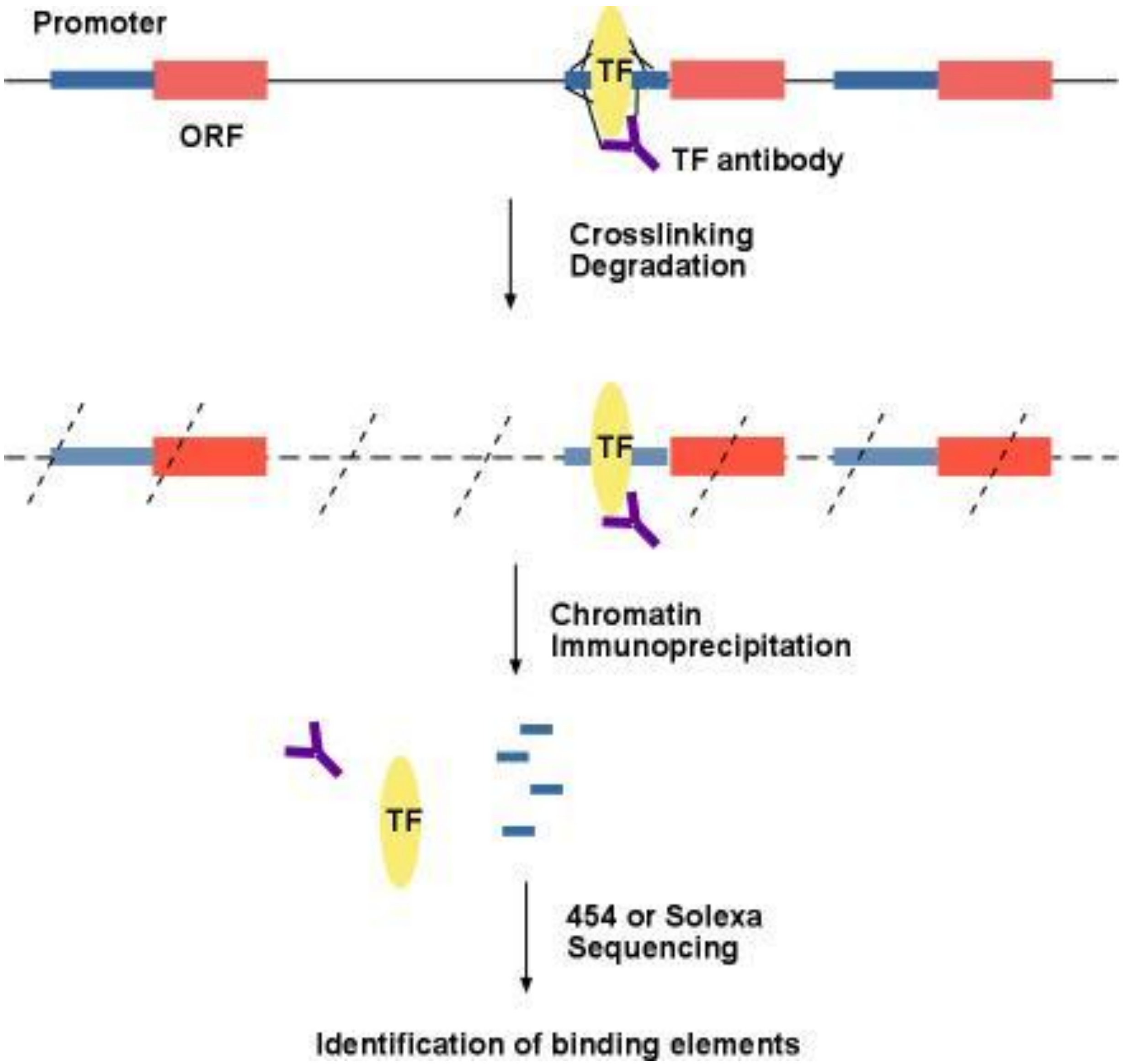
Genome Walking: A putative promoter that does not align to sorghum

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

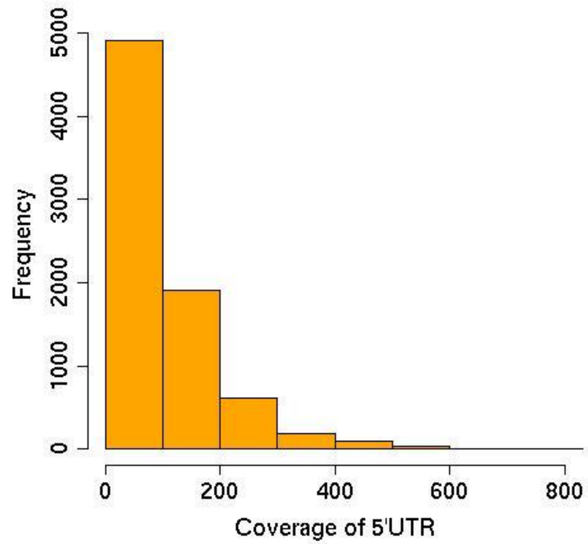


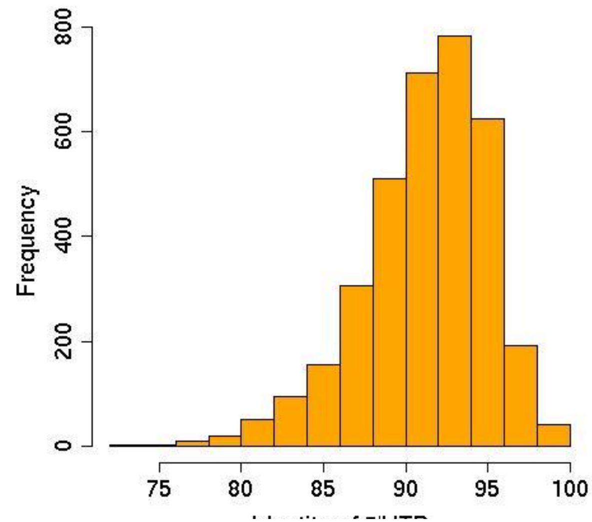
Alignment of the SASGMS09692 (walking: Fragment 5)

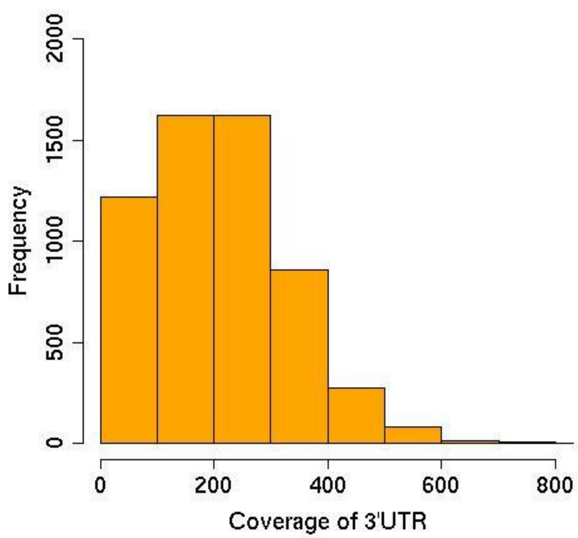
ChIP-Seq: 7 Abs raised against TFs associated to drought and sucrose

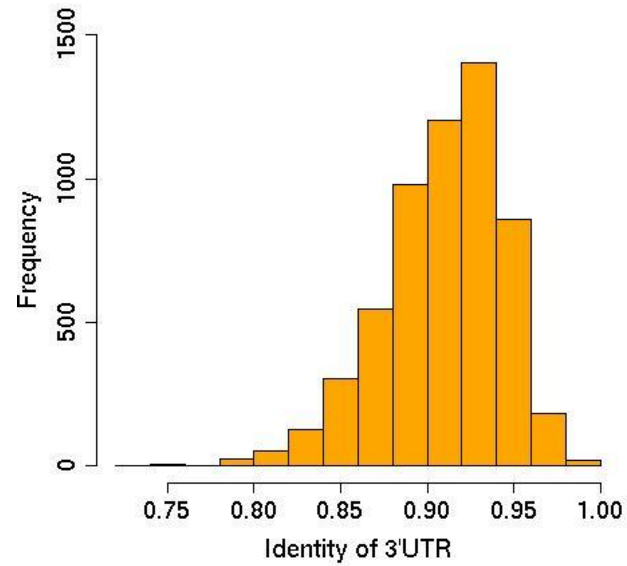


Alignment to Sorghum: Coverage and Identity of UTR

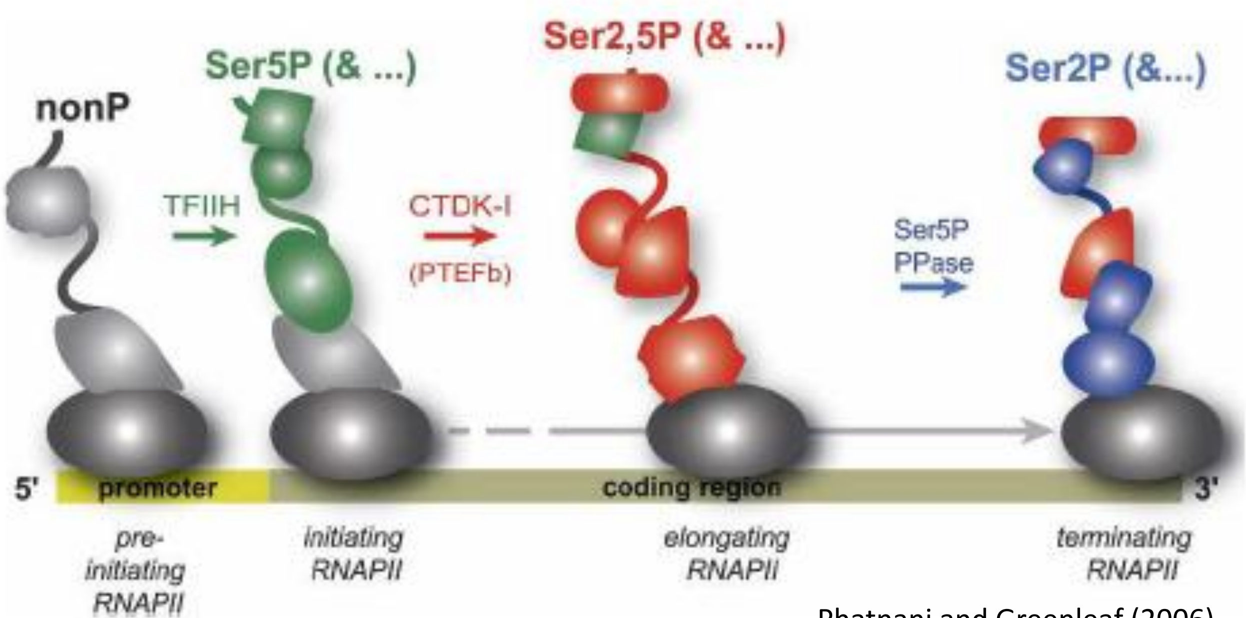








ChIP-Seq Experiments: RNApolII ChIP to construct a promoter database



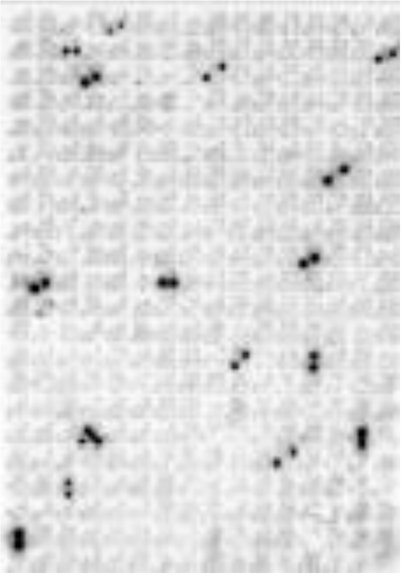
Phatnani and Greenleaf (2006)

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 redondant)

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)

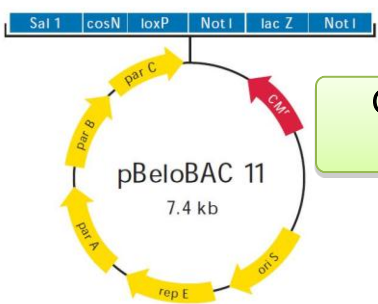
↓

- 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

▼

gene-rich BACs

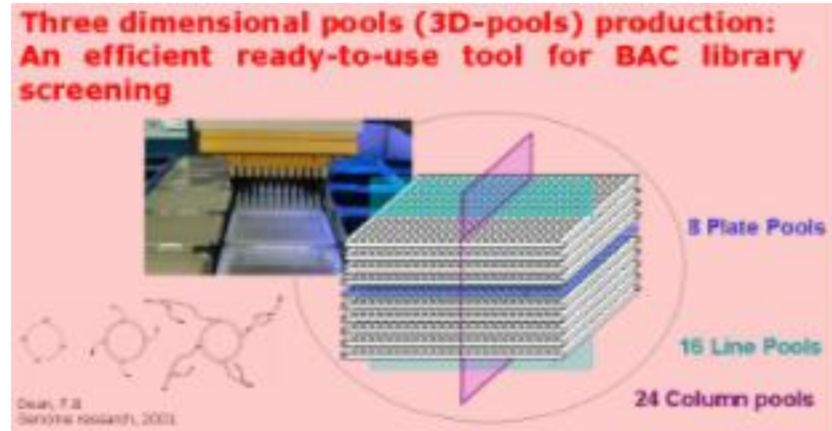
← Genome walking



Collaboration: Glaucia Souza, Roberta Campos

Andrew Paterson and Changsoo Kim - University of Georgia USA

BAC library 3D pools



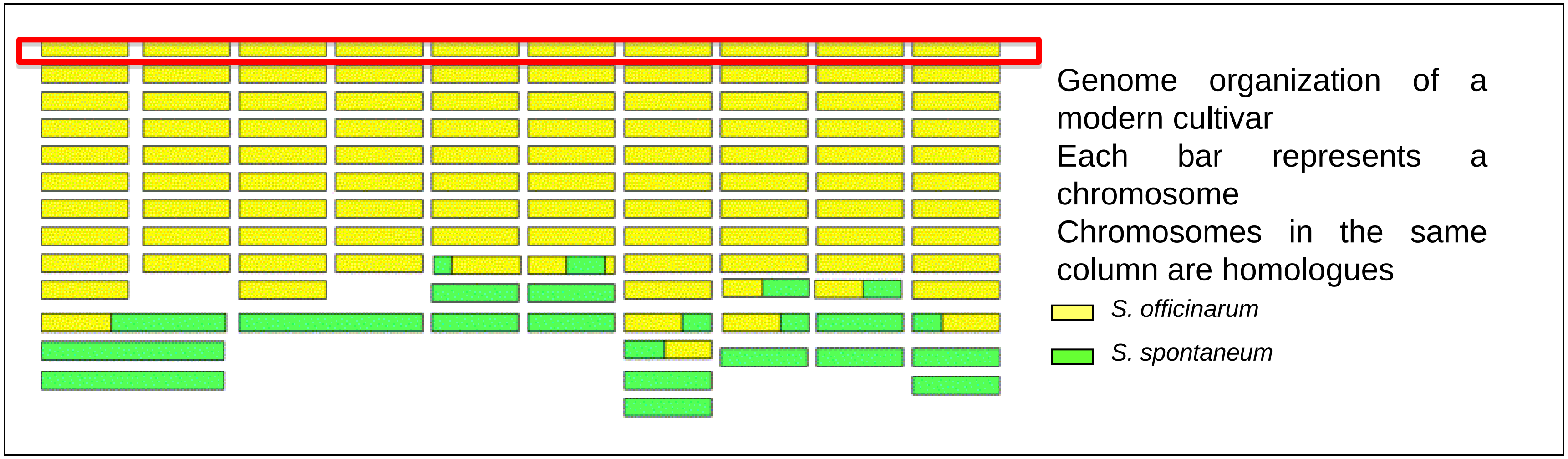
CNRGV – France
Helene Berges (INRA-CNRS)
Marie-Anne Van Sluys (IB-USP)

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)

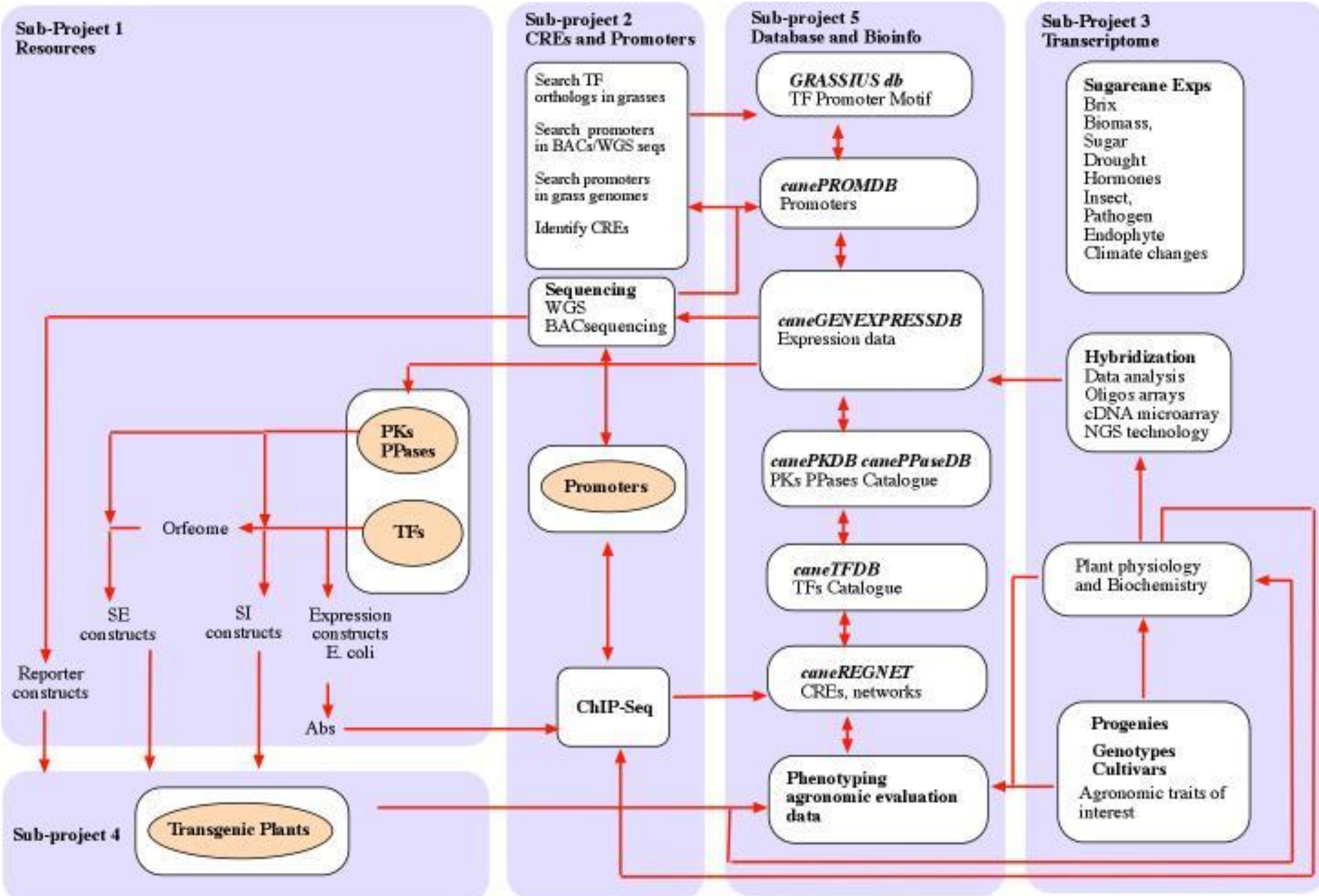
The SUGESI Project

A draft of the sugarcane monoploid genome (1 Gb)



Giant Genome (n ≅ 750-930 Mpb) **Polyploid (2n = 70-120 cromossomos)** **~10 Gb**

The Sugarcane Transcriptome Project & CaneRegNet



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 polyplody makes allele identification difficult

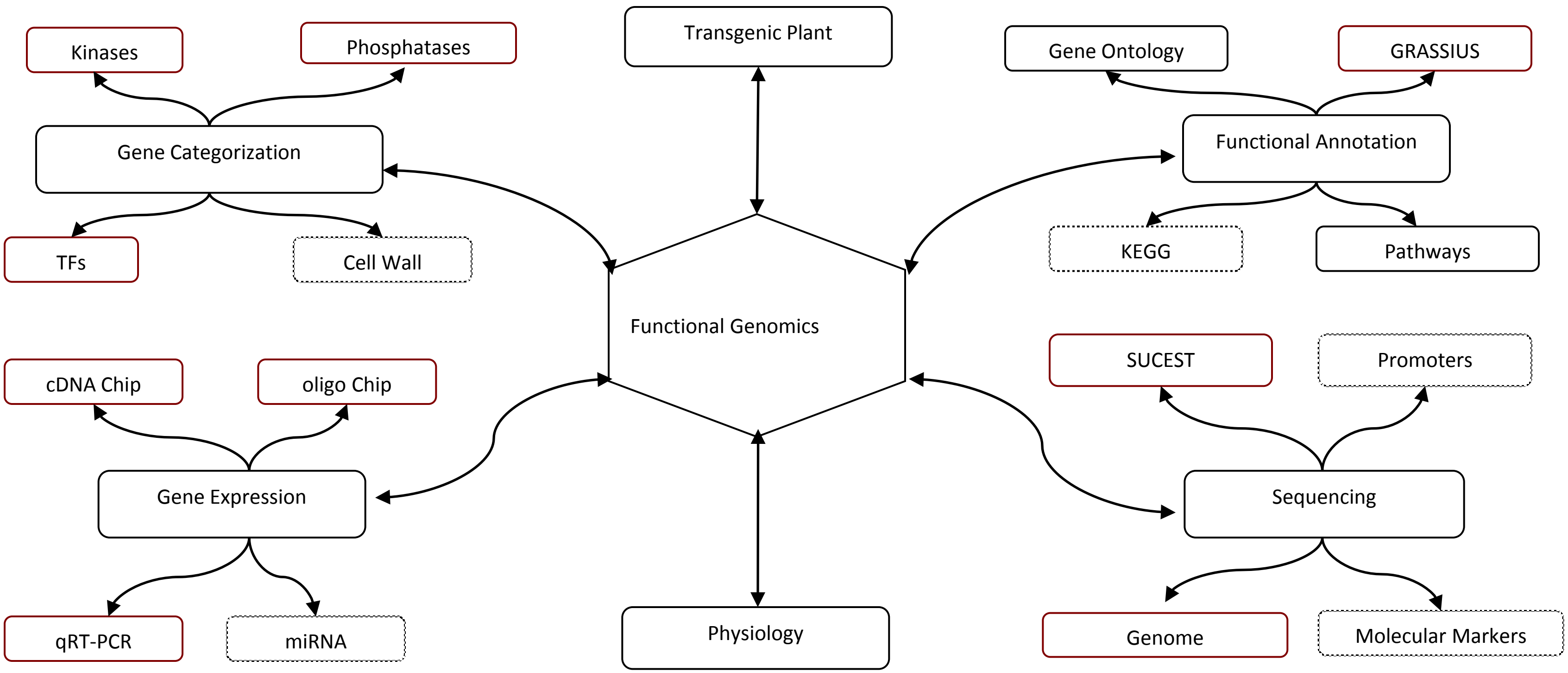
State of the Art

- 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)

SUCEST-FUN DB (http://sucest-fun.org)

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 (**CaneGeneExpressI**
- genes/ESTs/proteins
- probes
- Microarray
- experiments
- RT-PCR

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

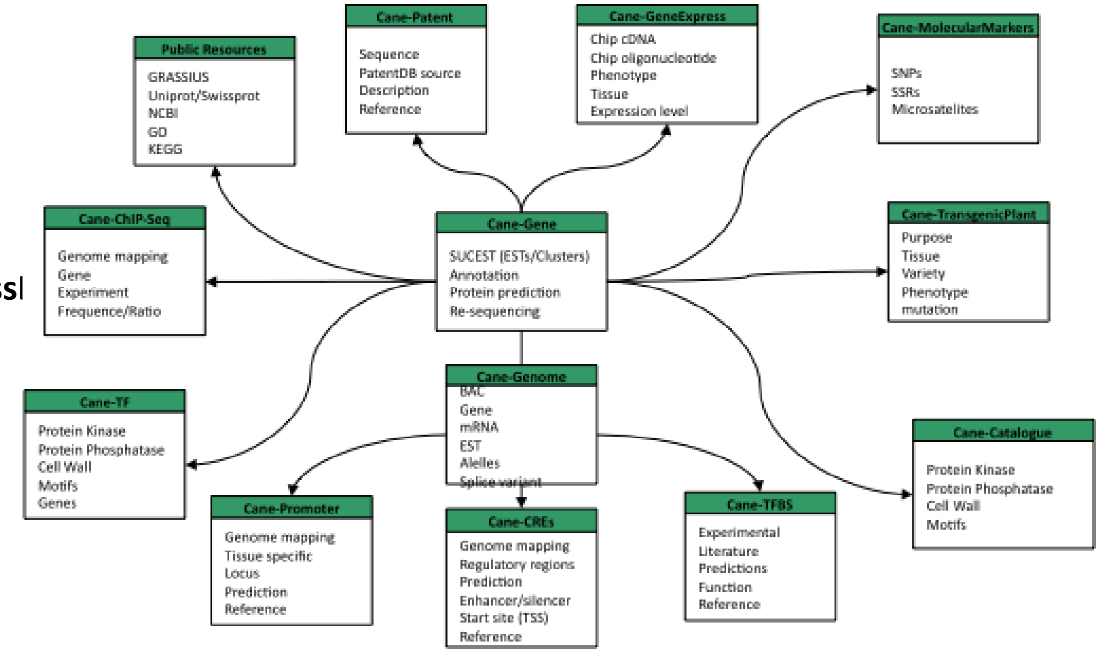
4 – Metabolome
- Metabolic Pathways
- Mass Spectrometry
- Ontology

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

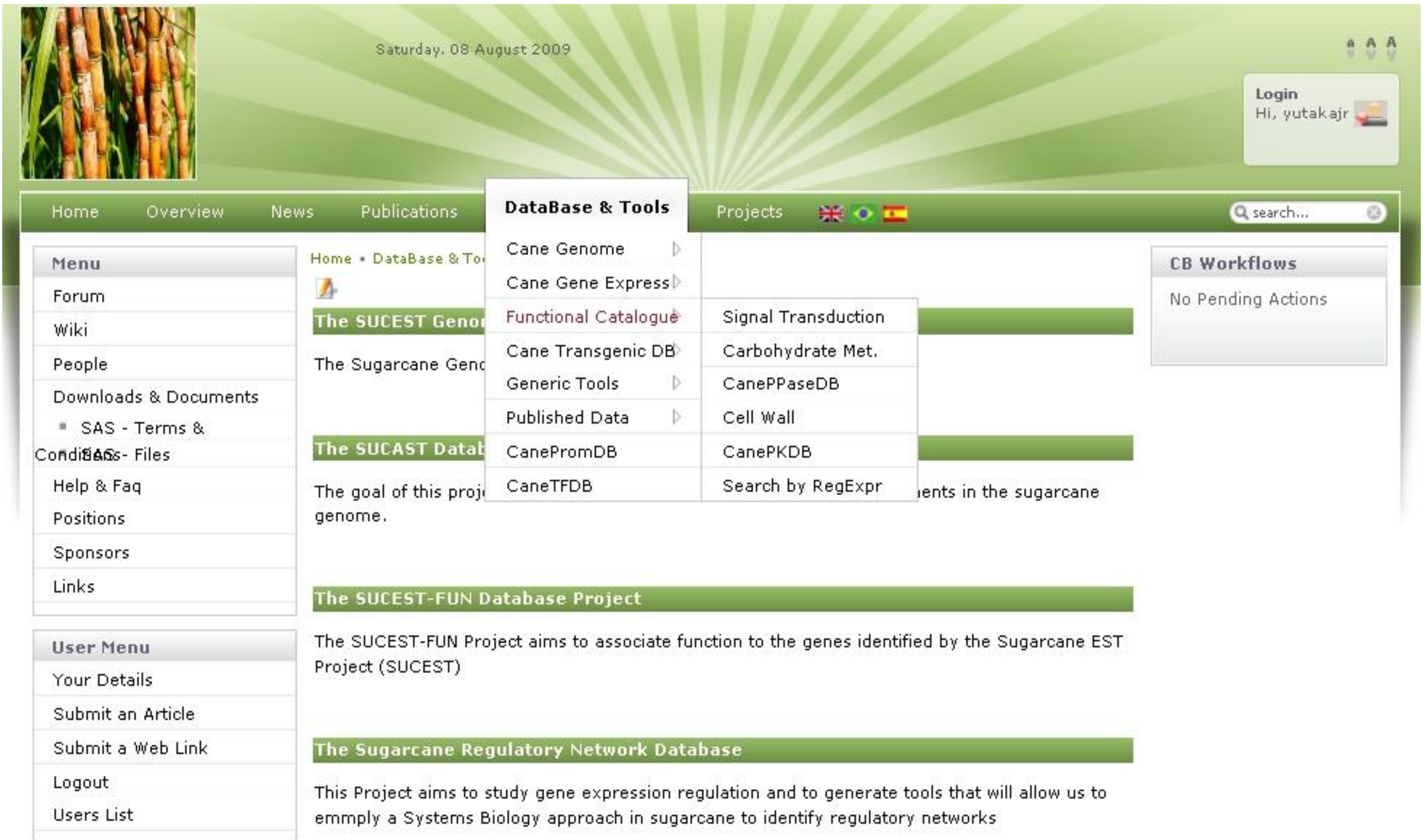
-DB

MySQL, Postgree

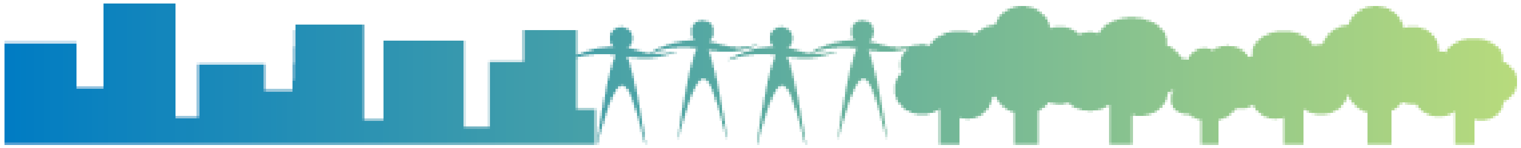
- Hibernate Framework

João Eduardo Ferreira (IME-USP)

http://sucest-fun.org



IMPACTS DIVISION



Horizontal themes: Social and Economic Impacts, Environmental studies and Land Use

Ethanol as a global strategic fuel

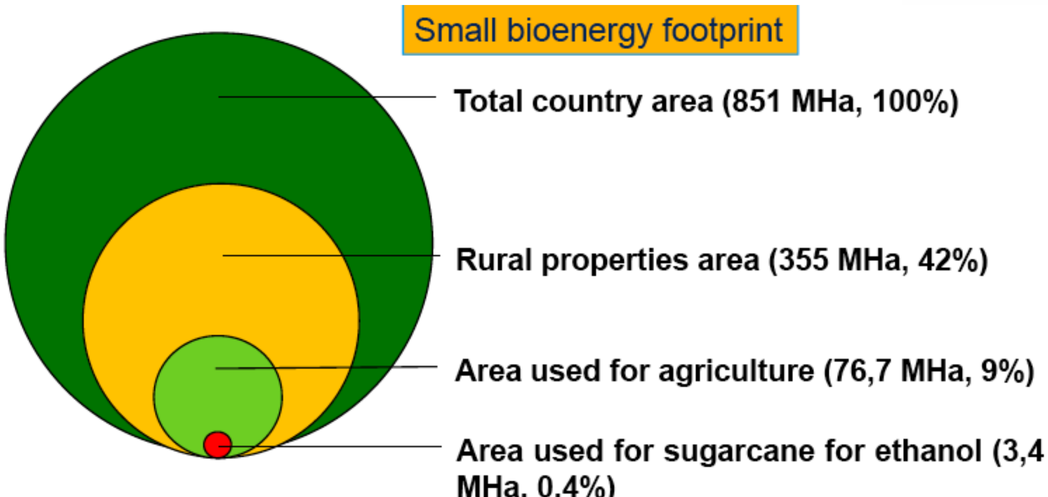
- Certification Methodology for ethanol produced in a sustainable environmentally friendly manner
- Research on new agronomical practices (precision agriculture, mecanization, no-till farming, low input practices, new crop protection systems) and their impact on soil loss, management and efficiency in 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

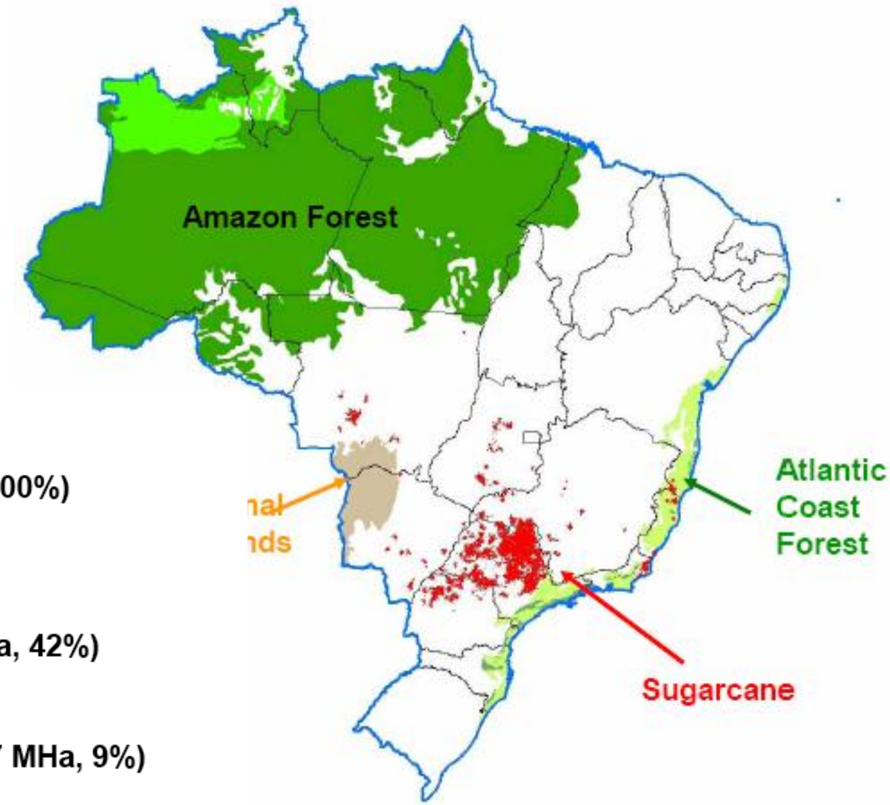
Expansion of sugarcane to new land areas

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%



Source: Horta Nogueira e Seabra (2008)



65% of expansion land is pasture

CONAB, 2008; J. Goldemberg (2008) Biotechnology for Biofuels 1:6. – USDA 2007

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

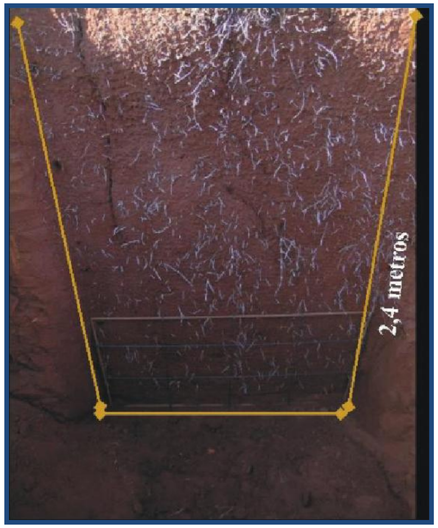
Expansion of sugarcane to new land areas

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



Burned X Green Cane

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 (CO_2, N_2O, CH_4) 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:

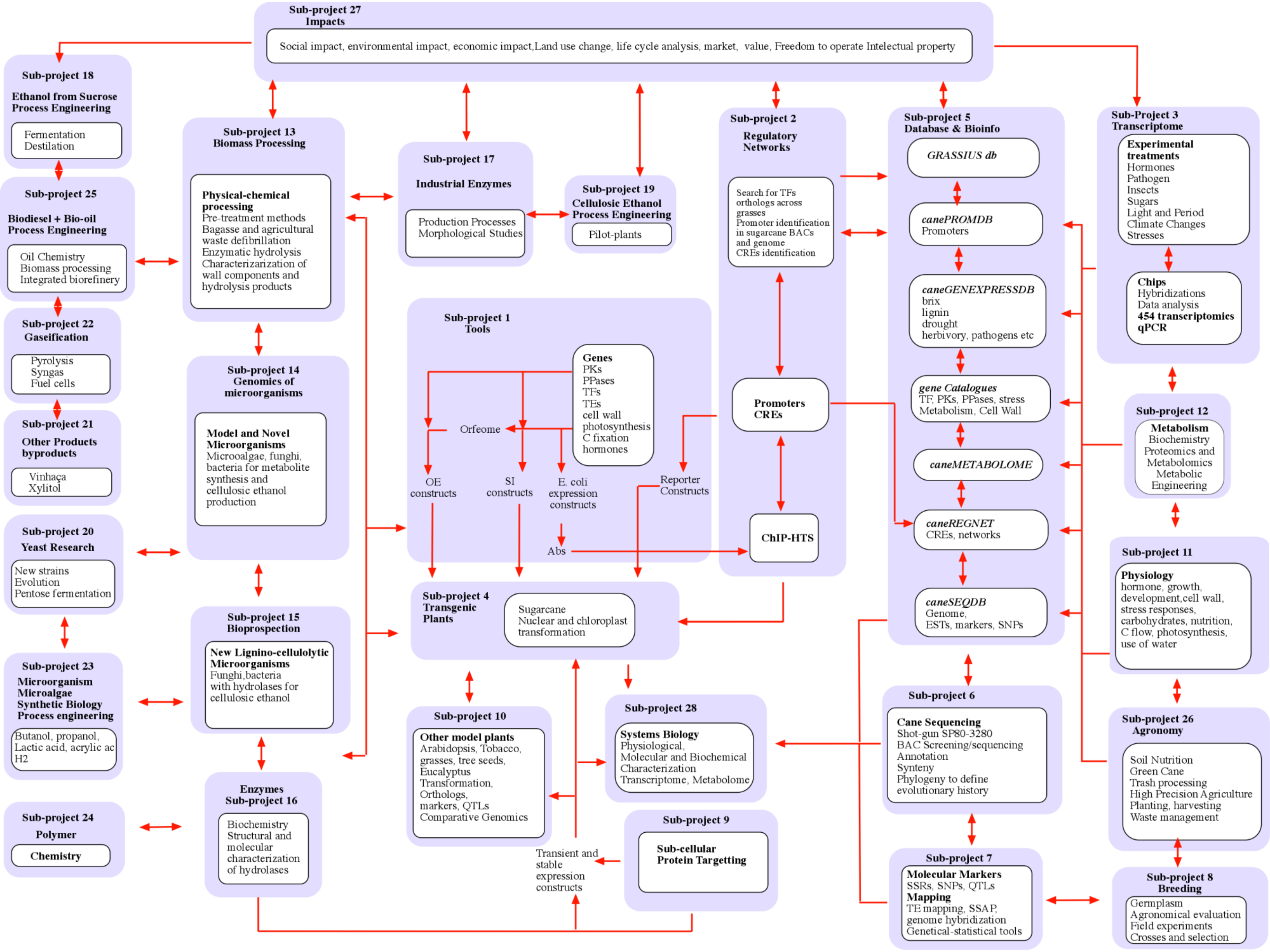
- Good management, precision agriculture, efficient tool to monitor pests and diseases
- Management practices to reduce GHG in agricultural processes
- Optimize nutrient use. Biological fixation of N
- Improve recycling of residues (vinasse, filter cake, ashes, plant residues etc)

Vinasse chanel

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 CO_2 are not emitted to the atmosphere with a simultaneous reduction in SO_2 emission


Sub-project 27
Impacts
Social impact, environmental impact, economic impact,Land use change, life cycle analysis, market, value, Freedom to operate Intelectual property
Sub-project 18
Ethanol from Sucrose Process Engineering
Fermentation
Destilation
Sub-project 25
Biodiesel + Bio-oil Process Engineering
Oil Chemistry
Biomass processing
Integrated biorefinery
Sub-project 22
Gaseification
Pyrolysis
Syngas
Fuel cells
Sub-project 21
Other Products byproducts
Vinhaça
Xylitol
Sub-project 20
Yeast Research
New strains
Evolution
Pentose fermentation
Sub-project 23
Microorganism
Microalgae
Synthetic Biology
Process engineering
Butanol, propanol,
Lactic acid, acrylic ac
H2
Sub-project 24
Polymer
Chemistry
Sub-project 13
Biomass Processing
Physical-chemical processing
Pre-treatment methods
Bagasse and agricultural waste defibrillation
Enzymatic hydrolysis
Characterization of wall components and hydrolysis products
Sub-project 14
Genomics of microorganisms
Model and Novel Microorganisms
Microalgae, funghi, bacteria for metabolite synthesis and cellulosic ethanol production
Sub-project 15
Bioprospection
New Lignino-cellulolytic Microorganisms
Funghi,bacteria with hydrolases for cellulosic ethanol
Enzymes
Sub-project 16
Biochemistry
Structural and molecular characterization of hydrolases
Sub-project 17
Industrial Enzymes
Production Processes
Morphological Studies
Sub-project 19
Cellulosic Ethanol Process Engineering
Pilot-plants
Sub-project 1
Tools
Genes
PKs
PPases
TFs
TEs
cell wall
photosynthesis
C fixation
hormones
Orfeome
OE constructs
SI constructs
E. coli expression constructs
Reporter Constructs
Abs
Sub-project 4
Transgenic Plants
Sugarcane
Nuclear and chloroplast transformation
Sub-project 10
Other model plants
Arabidopsis, Tobacco, grasses, tree seeds, Eucalyptus
Transformation,
Orthologs,
markers, QTLs
Comparative Genomics
Sub-project 28
Systems Biology
Physiological,
Molecular and Biochemical Characterization
Transcriptome, Metabolome
Sub-project 9
Sub-cellular Protein Targetting
Transient and stable expression constructs
Sub-project 2
Regulatory Networks
Search for TFs orthologs across grasses
Promoter identification in sugarcane BACs and genome
CREs identification
Promoters
CREs
ChIP-HTS
Sub-project 5
Database & Bioinfo
GRASSIUS db
canePROMDB
Promoters
caneGENEXPRESSDB
brix
lignin
drought
herbivory, pathogens etc
gene Catalogues
TF, PKs, PPases, stress
Metabolism, Cell Wall
caneMETABOLOME
caneREGNET
CREs, networks
caneSEQDB
Genome,
ESTs, markers, SNPs
Sub-project 6
Cane Sequencing
Shot-gun SP80-3280
BAC Screening/sequencing
Annotation
Synteny
Phylogeny to define evolutionary history
Sub-project 7
Molecular Markers
SSRs, SNPs, QTLs
Mapping
TE mapping, SSAP, genome hybridization
Genetical-statistical tools
Sub-Project 3
Transcriptome
Experimental treatments
Hormones
Pathogen
Insects
Sugars
Light and Period
Climate Changes
Stresses
Chips
Hybridizations
Data analysis
454 transcriptomics
qPCR
Sub-project 12
Metabolism
Biochemistry
Proteomics and Metabolomics
Metabolic Engineering
Sub-project 11
Physiology
hormone, growth, development,cell wall, stress responses, carbohydrates, nutrition, C flow, photosynthesis, use of water
Sub-project 26
Agronomy
Soil Nutrition
Green Cane
Trash processing
High Precision Agriculture
Planting, harvesting
Waste management
Sub-project 8
Breeding
Germplasm
Agronomical evaluation
Field experiments
Crosses and selection

FAPESP BIOENERGY PROGRAM BIOEN

http://bioenfapesp.org

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Thank You!