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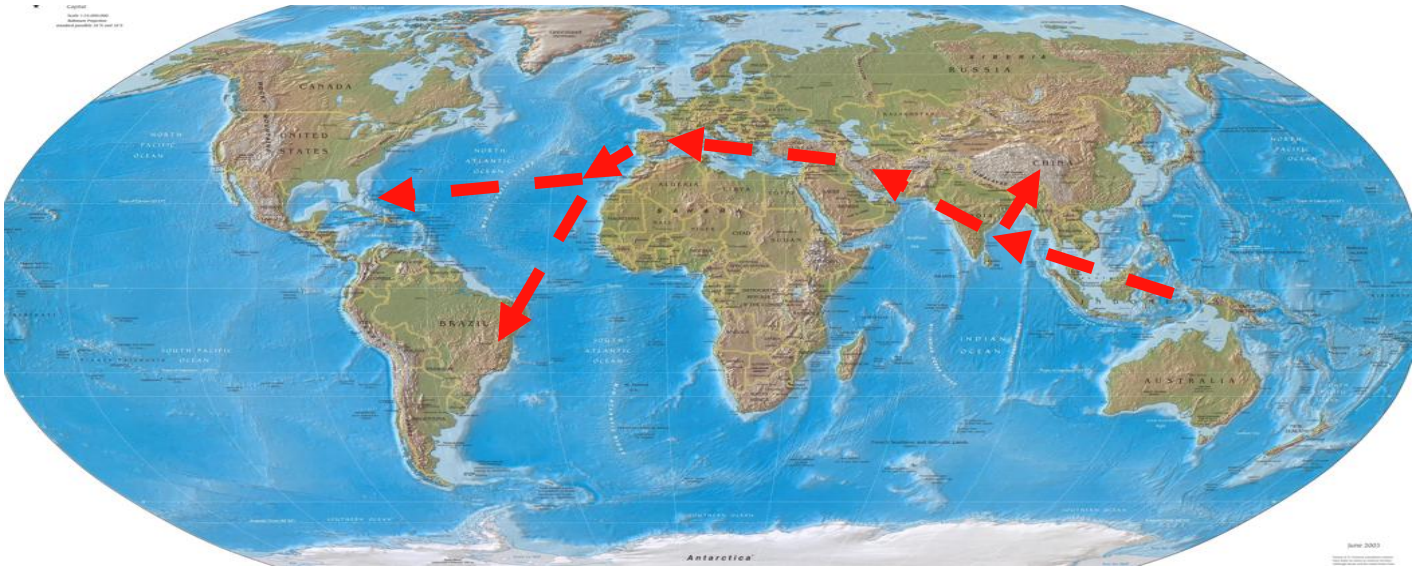
The SUCEST-FUN Project: Using genomic tools for the improvement of biomass in sugarcane

Domestication and early evolution of sugarcane

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

Modern sugarcane cultivars



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)

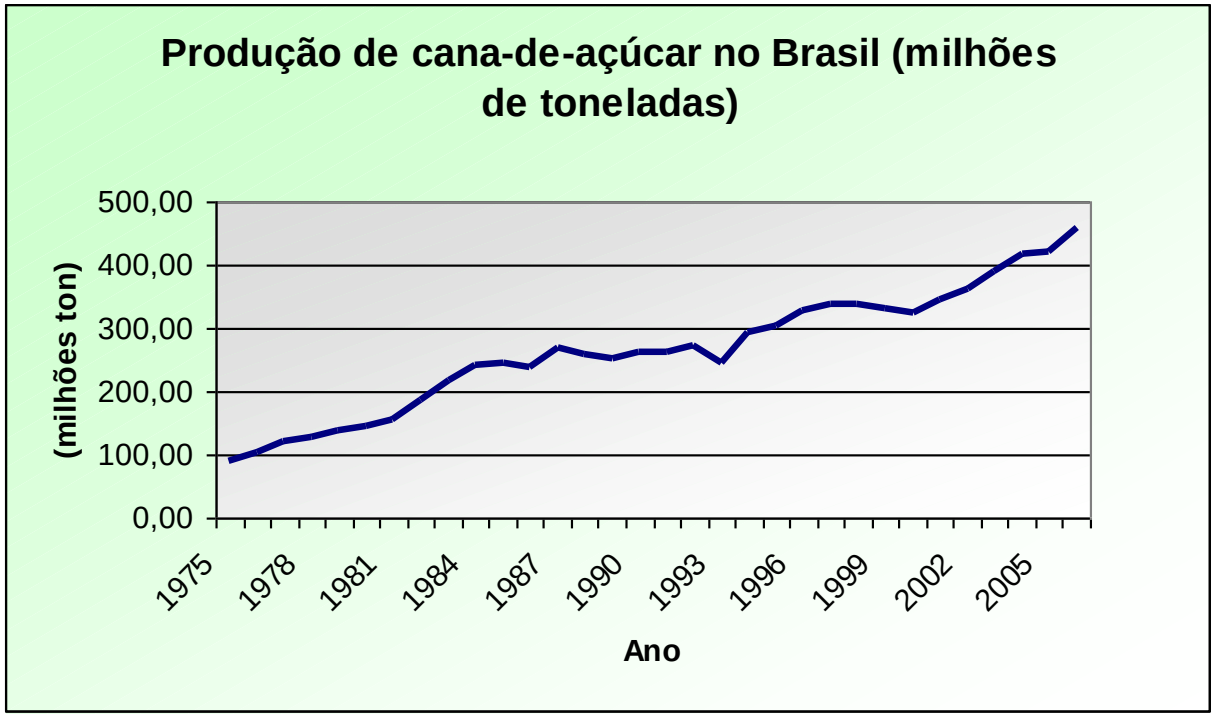
C4 carbohydrate metabolism - large amount of carbon partitioned into sucrose (up to 42% of the stalk dry weight, around 0.7 M in mature internodes)

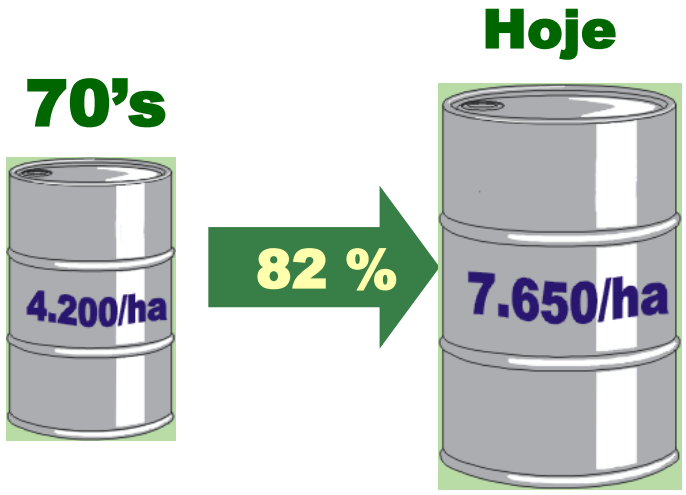
488 million tons of crushed stems (Brazil 2007/2008)
51.1% etanol
48.9% sugar
Up to 90% of the bagasse used for energy co-generation

Sugarcane in Brazil: 1532

Mandated addition of Ethanol to fuel: 1929

Produção e Produtividade





PMGCA –UFV
RIDESA BRASIL

Breeding Program

320 crossings
200 mil plantlets
(genotypes)

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

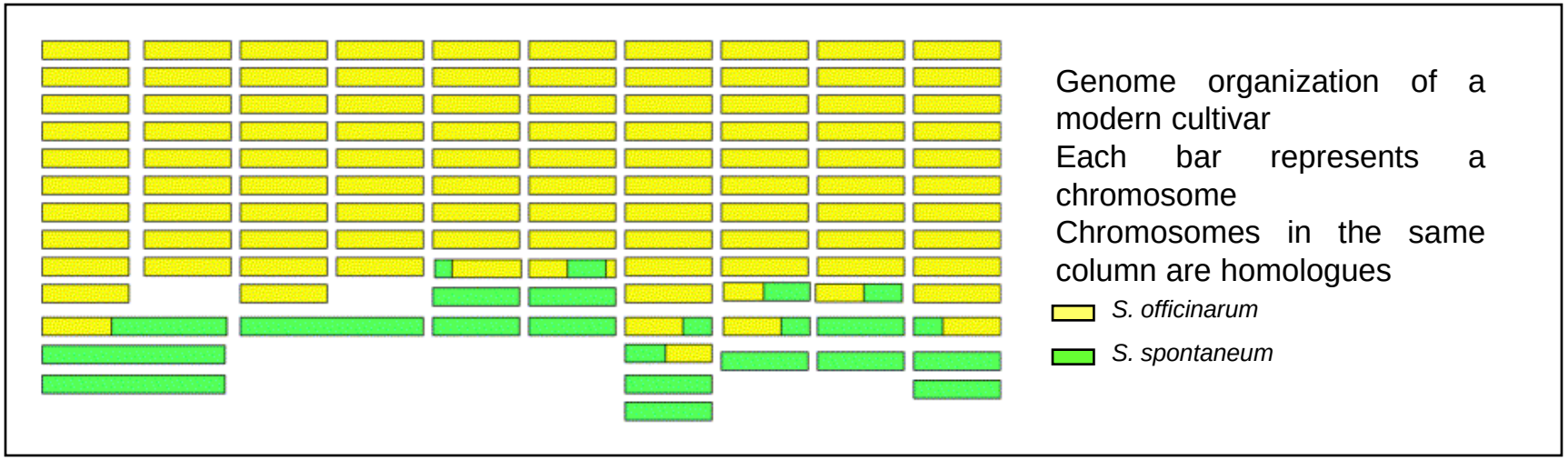
Contributing genera: ***Saccharum*, *Erianthus*, *Miscanthus*, *Sclerostachya* and *Narenga***

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

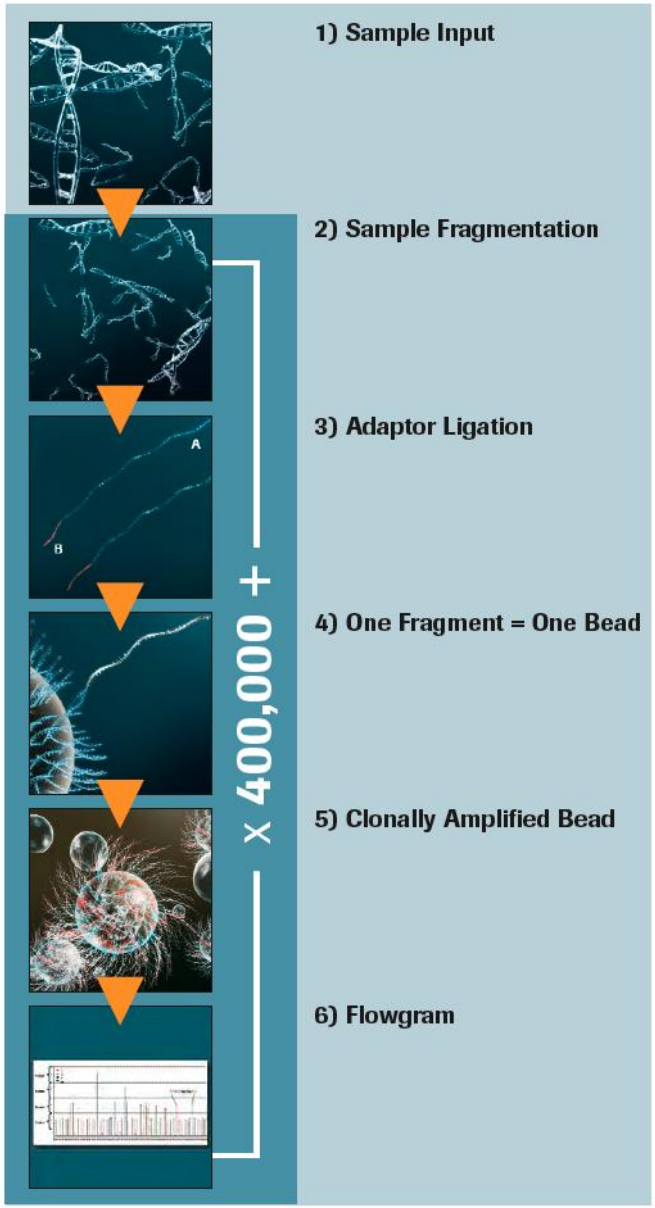
Wild species
S. spontaneum (2n=40 to 128)
S. robustum (2n= 60, 80 and up to 200)

Early cultivars
S. officinarum (2n= 80)
S. barberi (2n=81-124)
S. sinense (2n=116-120)

Marginal species
S. edule (2n = 60 to 122))



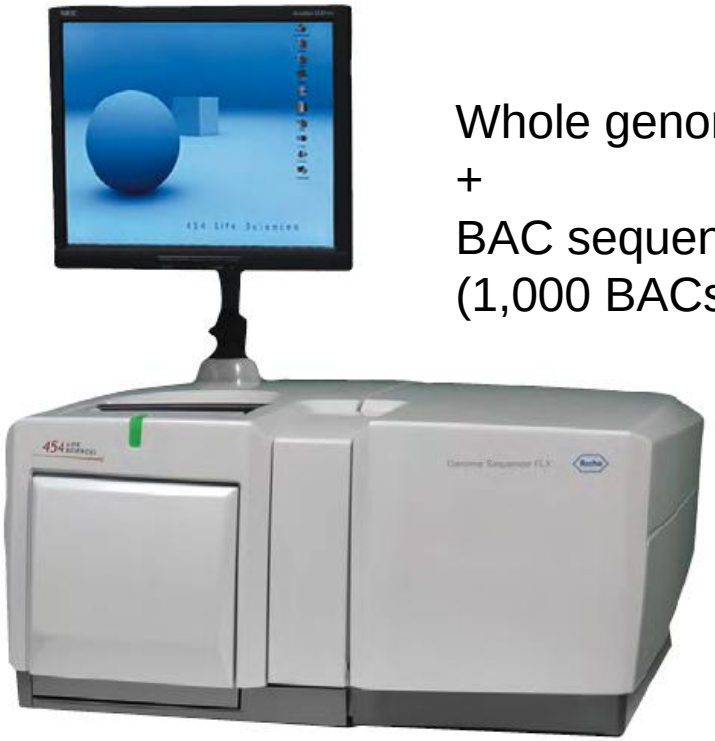
Giant Genome (n ≅ 750-930 Mpb) **Polyploid (2n = 70-120 cromossomos)** **~10 Gb**



center for advanced technologies in genomics

CATG

centro avançado de tecnologias em genômica

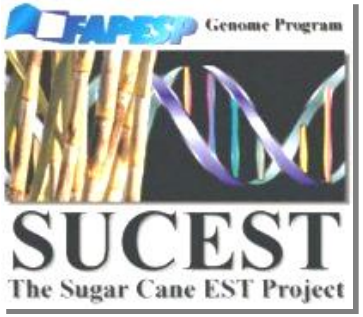


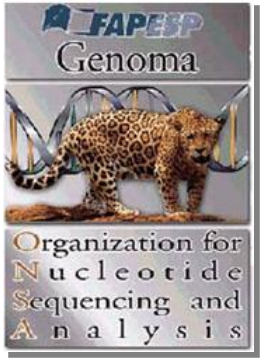
Whole genome Shot-gun
+
BAC sequencing
(1,000 BACs - 10,000 genes)
alleles

One SUCEST in 2 days...

SUCEST Project

33,620 genes





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 Research
www.genome.org

50 labs

200 researchers

238000 ESTs

43000 Transcripts

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

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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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*]

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

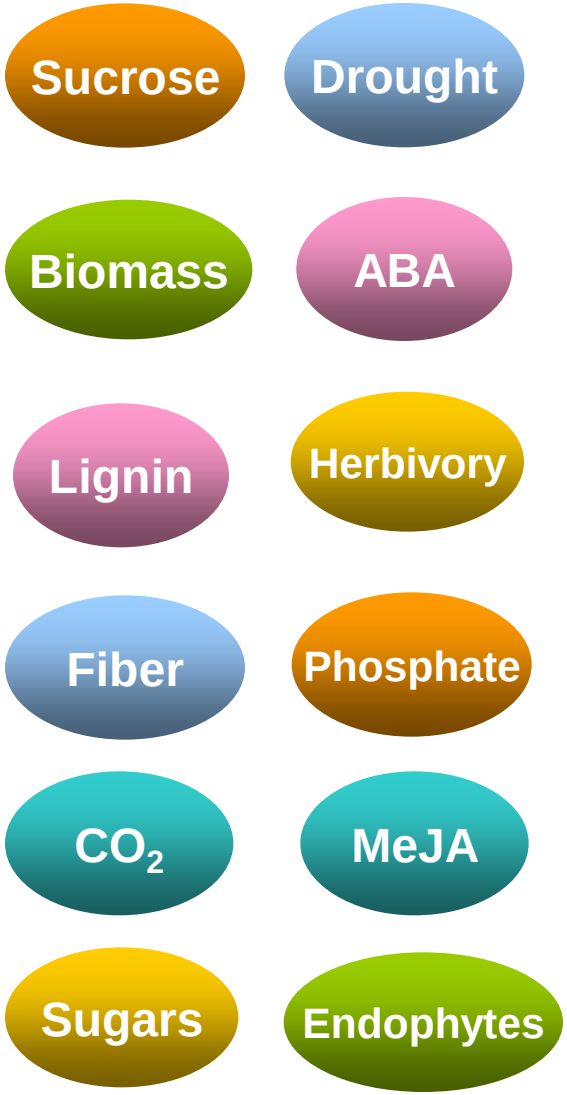
By

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]

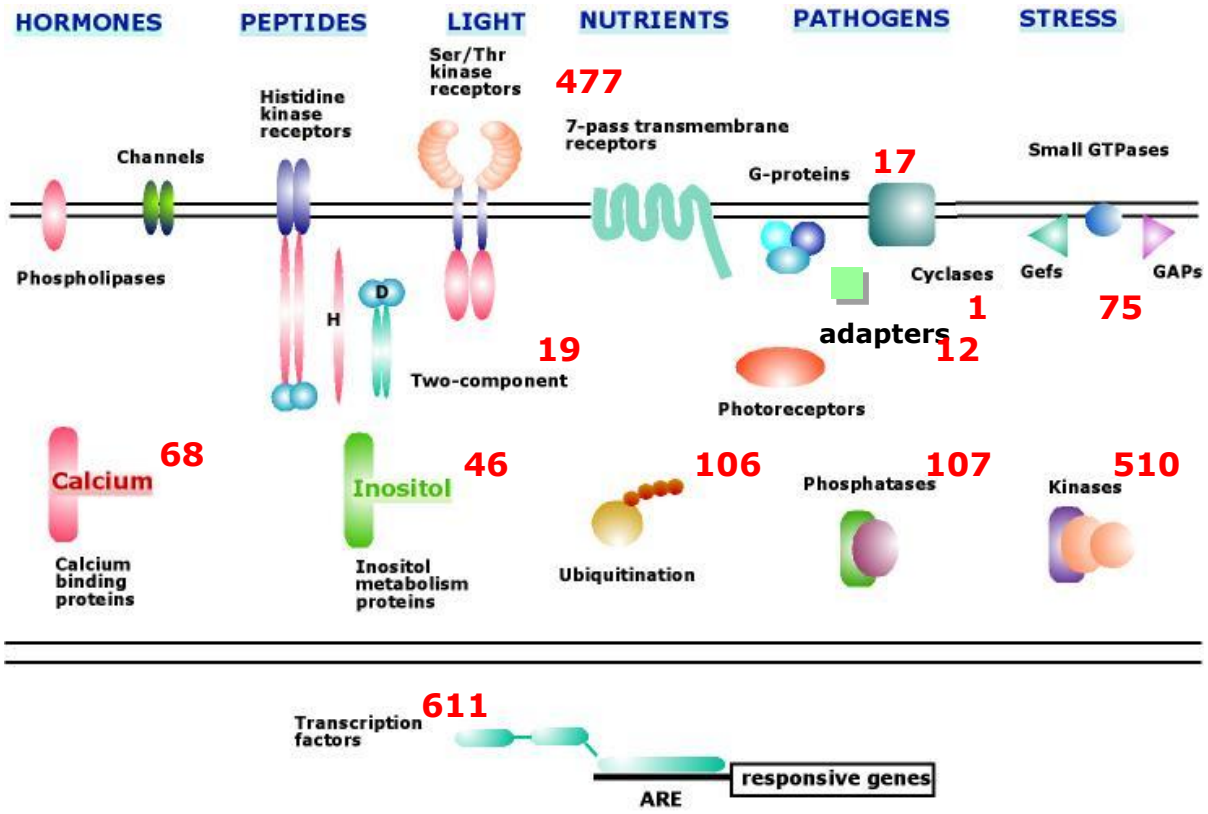
DNA Research 12, 27–38 (2005)

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,*]



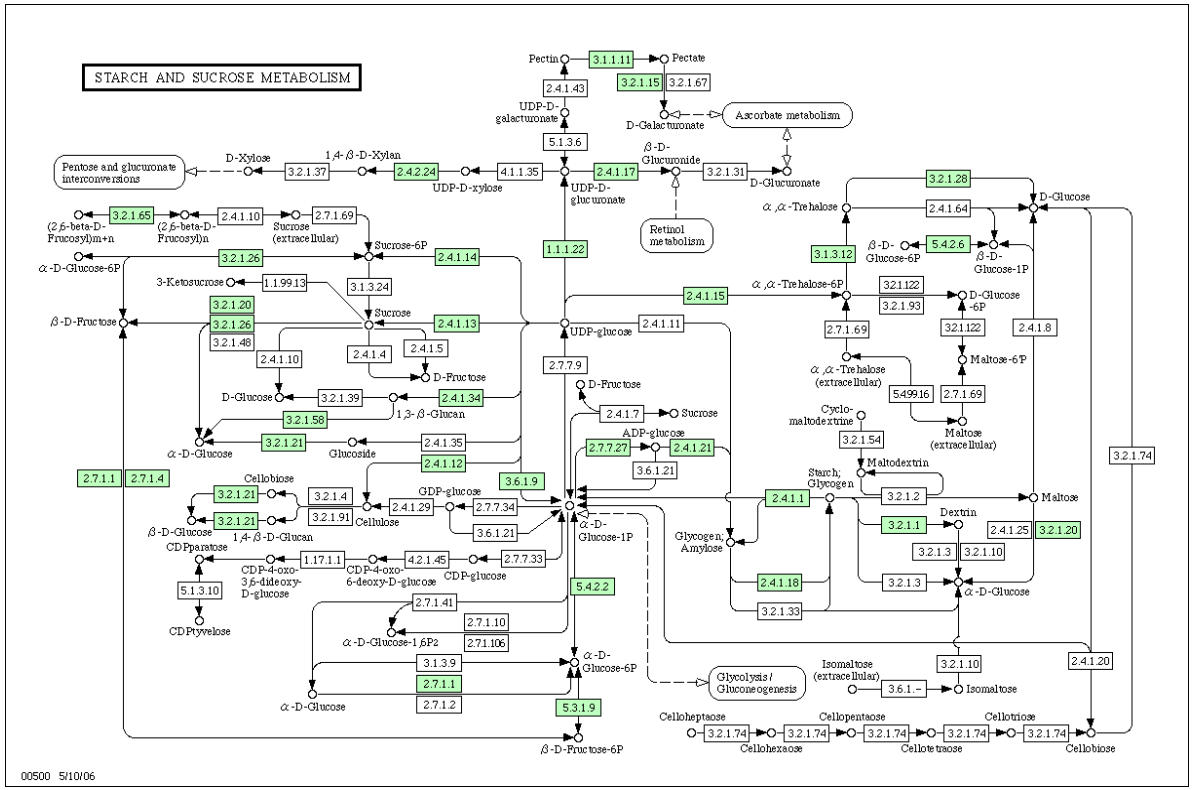
7000 genes expression profiled



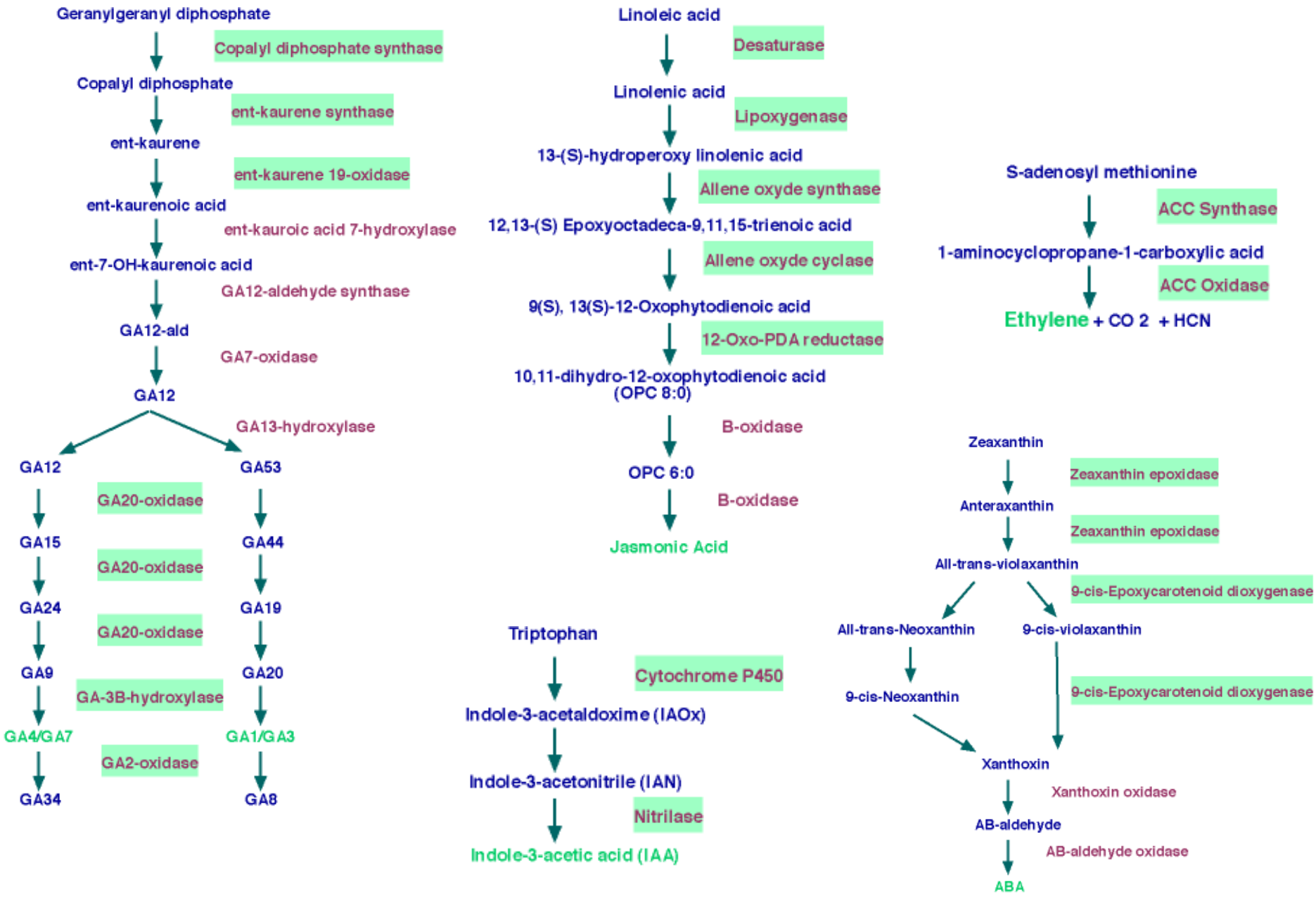
SUCAST Catalogue

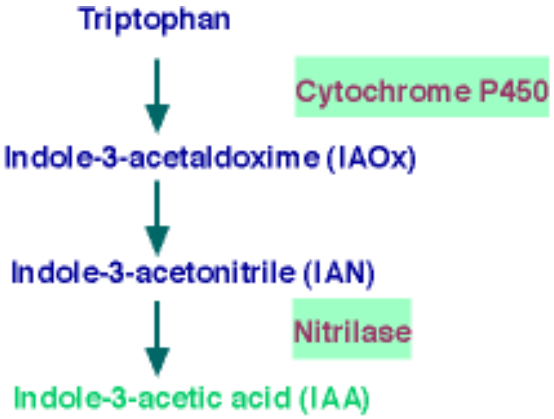
Souza et al., (2001) Genet Mol Biol 24, 25-34.

SUCAST classification	# SAS
Protein categories:	
Receptors	477
Adapters	12
G proteins	17
Small GTPases	75
Two component relay	19
Cyclase	1
Calcium metabolism	68
Inositol metabolism	46
Protein Phosphatases	107
Protein kinases	510
Ubiquitination	106
Transcription factors	611
Hormone biosynthesis	75
Hormone related	22
Functional categories:	
Development	30
Cell cycle	34
Stress	305
Pathogenicity	382
'No matches' and unknown proteins	548
Others	118
TOTAL	3563

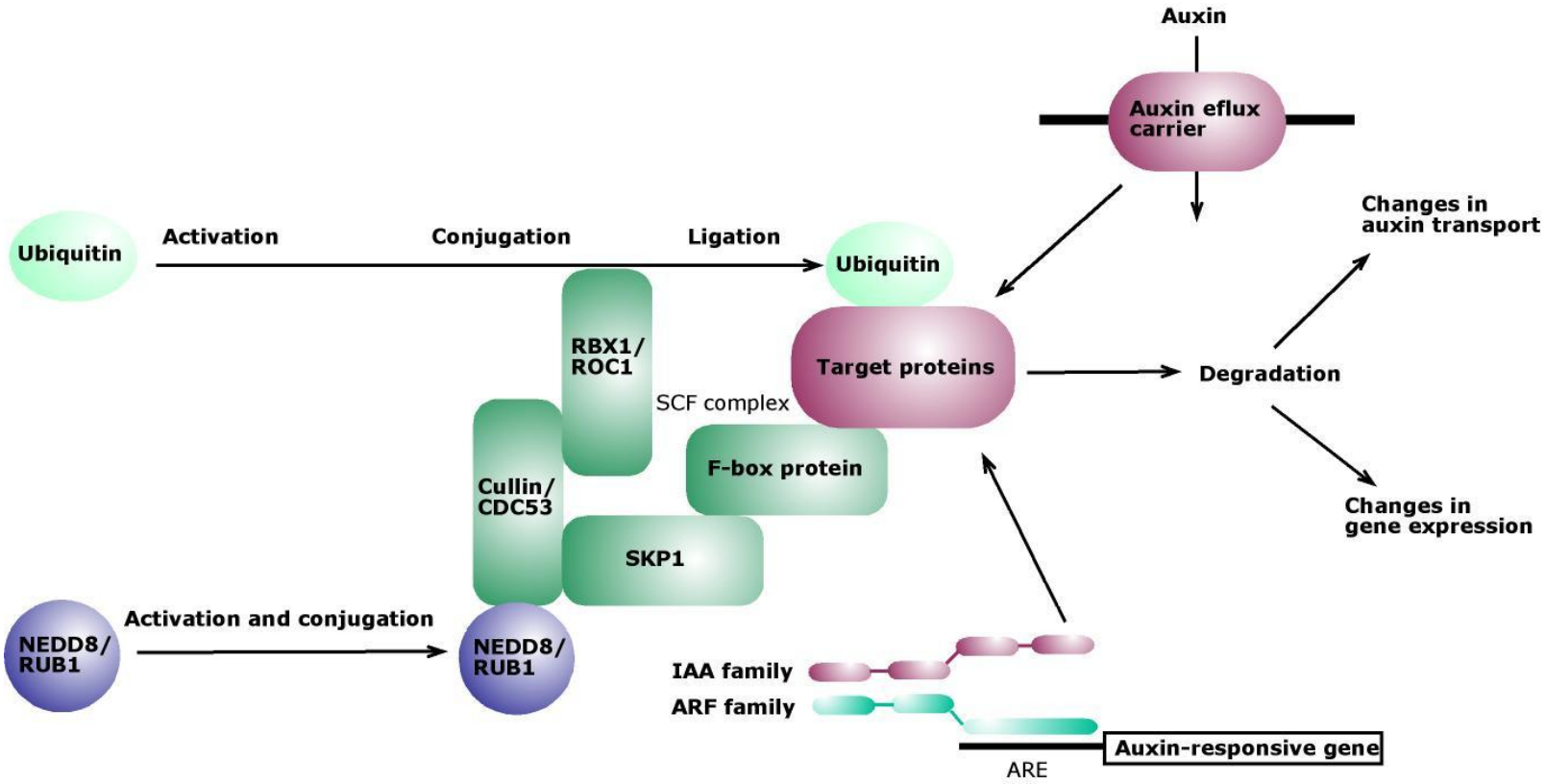


SUCAMET Catalogue	# SAS
Adapters	2
Amino acid metabolism	217
Bioenergetics	125
Calcium metabolism	8
Carbohydrate metabolism	688
Cell cycle	257
Cellular dynamics	2
Chromatin regulation	18
Development	151
G-proteins	3
Hormone related	50
Inositol	19
Lipid, fatty-acid and isoprenoid metab.	165
Nitrogen metabolism	12
No matches	297
Nucleic acid metabolism	351
Pathogenicity	138
Phosphatases	46
Photosynthesis	48
Protein Kinases	189
Protein metabolism	457
Receptors	132
Secondary metabolism	171
Small GTPases	29
Stress response	438
Sulfur metabolism	3
Transcription	321
Transporters	202
Two component	7
Ubiquitination	33
Unknown protein	29
Total	4608

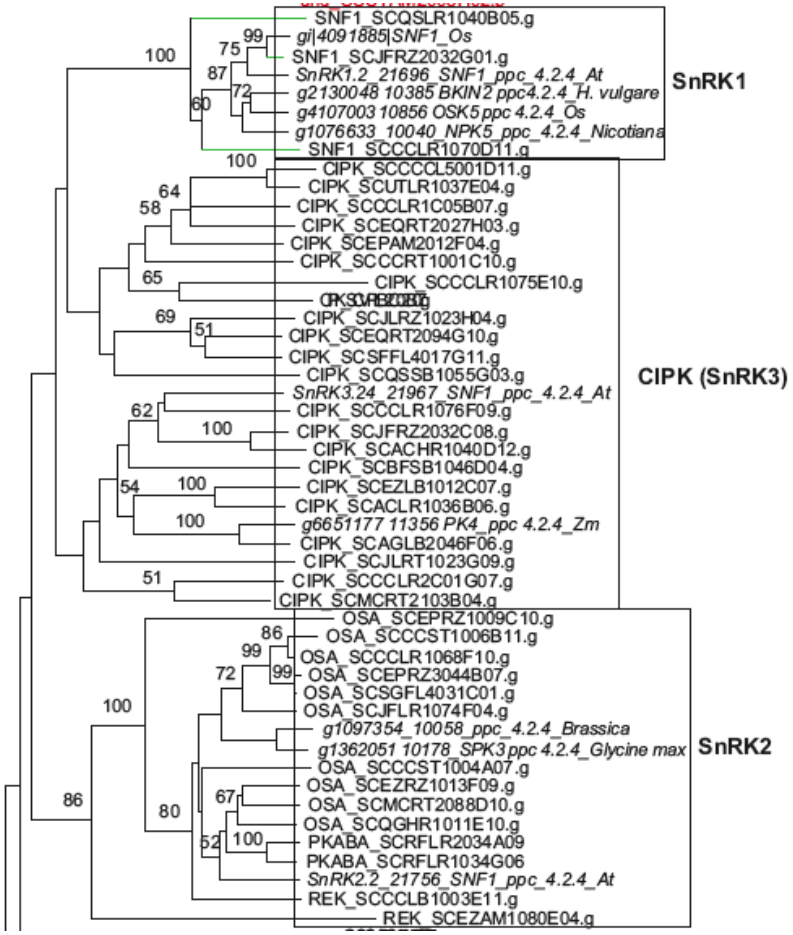

Geranylgeranyl diphosphate
Copalyl diphosphate synthase
Copalyl diphosphate
ent-kaurene synthase
ent-kaurene
ent-kaurene 19-oxidase
ent-kaurenoic acid
ent-kauroic acid 7-hydroxylase
ent-7-OH-kaurenoic acid
GA12-aldehyde synthase
GA12-ald
GA7-oxidase
GA12
GA13-hydroxylase
GA12
GA53
GA20-oxidase
GA15
GA44
GA20-oxidase
GA24
GA19
GA20-oxidase
GA9
GA20
GA-3B-hydroxylase
GA4/GA7
GA1/GA3
GA2-oxidase
GA34
GA8
Linoleic acid
Desaturase
Linolenic acid
Lipoxygenase
13-(S)-hydroperoxy linolenic acid
Allene oxyde synthase
12,13-(S) Epoxyoctadeca-9,11,15-trienoic acid
Allene oxyde cyclase
9(S), 13(S)-12-Oxophytodienoic acid
12-Oxo-PDA reductase
10,11-dihydro-12-oxophytodienoic acid
(OPC 8:0)
B-oxidase
OPC 6:0
B-oxidase
Jasmonic Acid
S-adenosyl methionine
ACC Synthase
1-aminocyclopropane-1-carboxylic acid
ACC Oxidase
Ethylene + CO 2 + HCN
Zeaxanthin
Zeaxanthin epoxidase
Anteraxanthin
Zeaxanthin epoxidase
All-trans-violaxanthin
9-cis-Epoxycarotenoid dioxygenase
All-trans-Neoxanthin
9-cis-violaxanthin
9-cis-Neoxanthin
9-cis-Epoxycarotenoid dioxygenase
Xanthoxin
Xanthoxin oxidase
AB-aldehyde
AB-aldehyde oxidase
ABA
Triptophan
Cytochrome P450
Indole-3-acetaldoxime (IAOx)
Indole-3-acetonitrile (IAN)
Nitrilase
Indole-3-acetic acid (IAA)



category	sub category 1	sub category 2	HB x LB	MI x II	Drought
hormone biosynthesis	auxin	nitrilase	1		2
hormone biosynthesis	auxin	nitrilase		2	2
hormone biosynthesis	auxin	nitrilase	1	1	2
transcription factor	Aux/IAA	.		4	2
transcription factor	Aux/IAA	.		2	
transcription factor	Aux/IAA	.		2	
hormone related	auxin	auxin repressed	1	2	



SNF-like Protein kinases



Sucrose biosynthesis

ABA

14-3-3 SnRK1 MAPK

Drought

ABA Osmotic Stress Calcium

SnRK2 CBL-SnRK3 CDPK MAPK

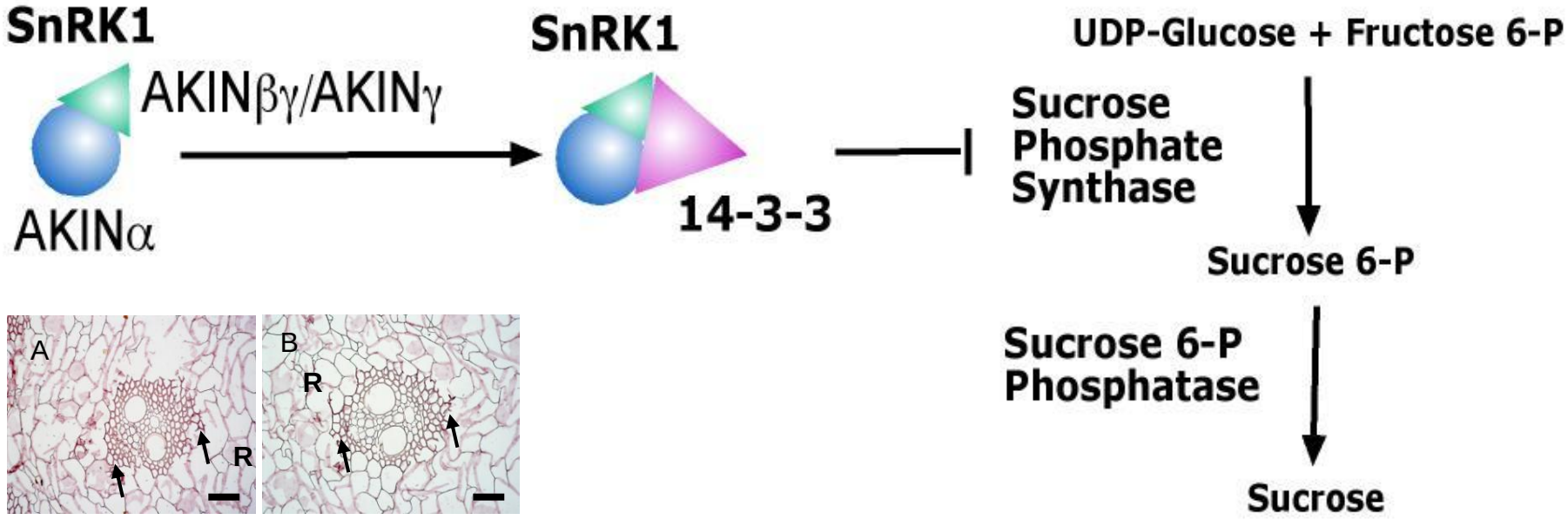
Rocha et al. (2007). BMC Genomics 8:71.

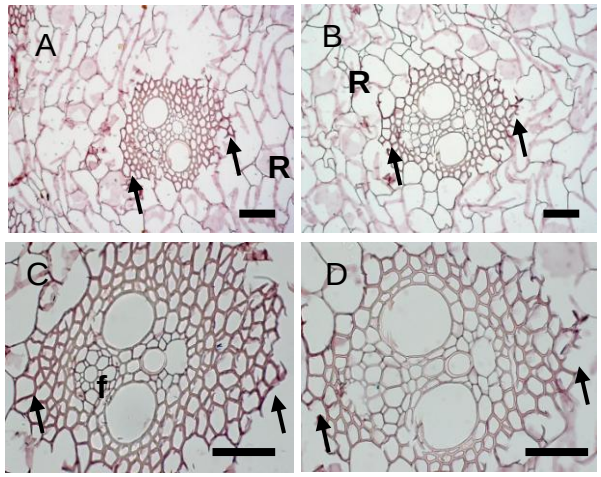
Boudsocq & Lauriere (2005)
Plant Physiol. 138,1185-1194

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	

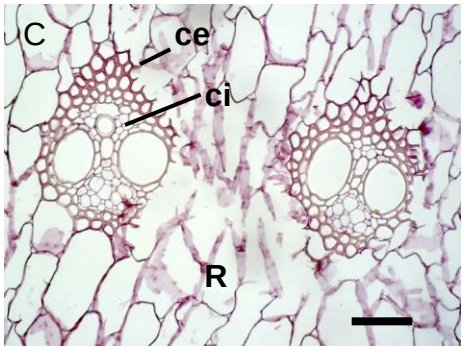


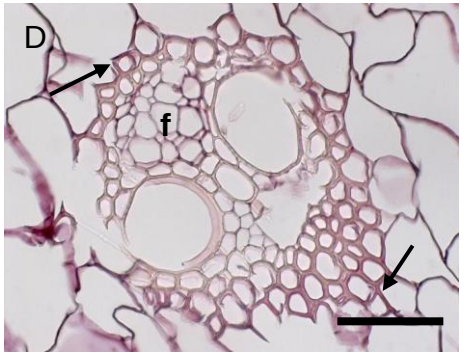


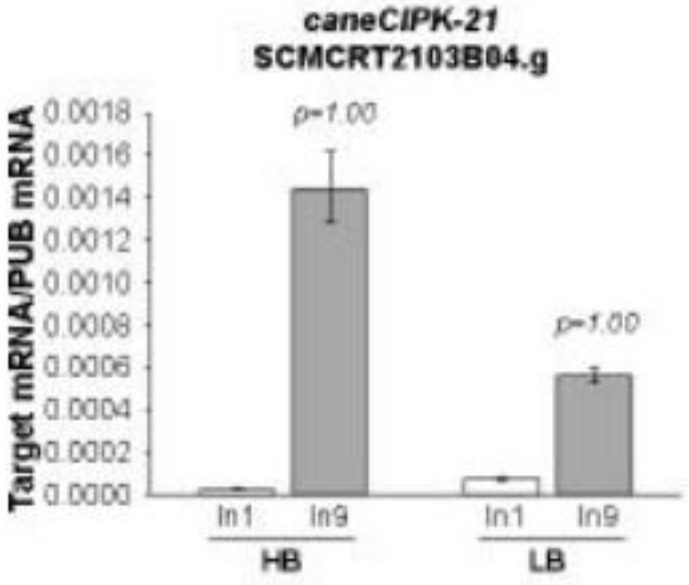
Storage parenchyma (R), Phloem (f), Fiber and bundle parenchymal cells (arrows), Bundle distal cells (ce), Bundle proximal cells (ci)

SnRK2 is up-regulated in plants with high sucrose

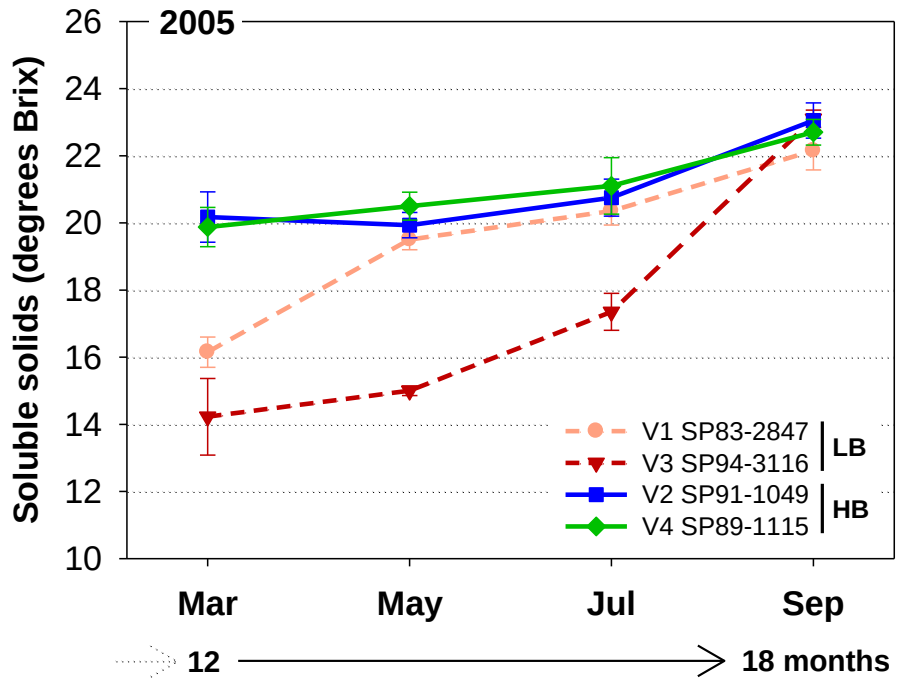
sub category 1	sub category 2	HB vs LB	MIn vs IIn	Drought	ABA	Sucrose	Glucose
MAPK/MAPKK/MAPKKK	caneMAPK-4	1	2		3		
SNF-like kinase	caneCIPK-14		1				
SNF-like kinase	caneCIPK-16	1				3	
SNF-like kinase	caneCIPK-21	2	1			3	
SNF-like kinase	caneCIPK-3	1				3	3
SNF-like kinase	caneCIPK-9		2				
SNF-like kinase	caneOSA PK-1		2			3	3
SNF-like kinase	caneOSA PK-7		1				
SNF-like kinase	canePKABA1-1	2				3	3
SNF-like kinase	canePKABA1-3	1				3	3

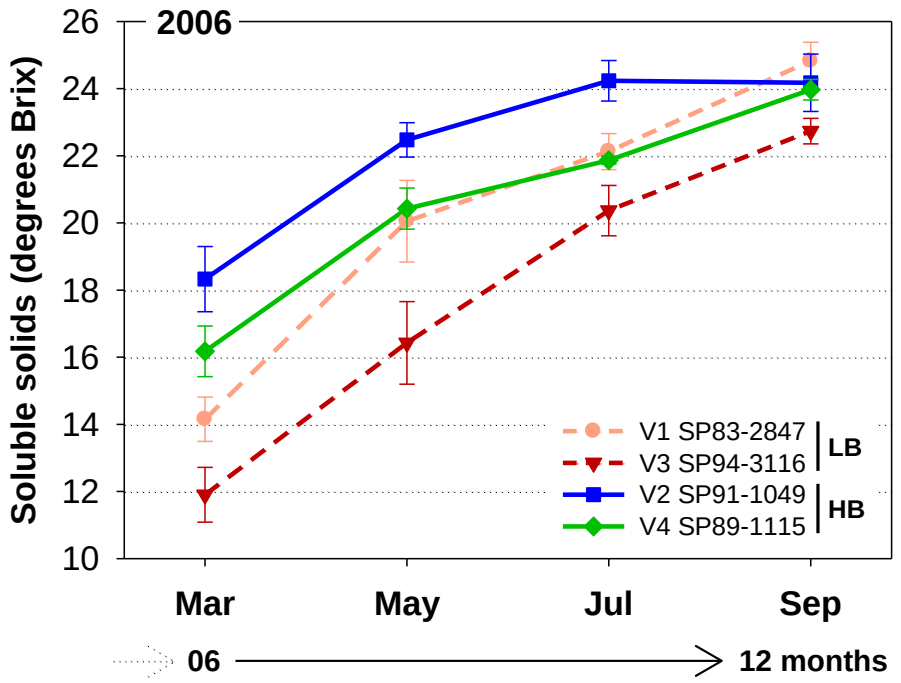




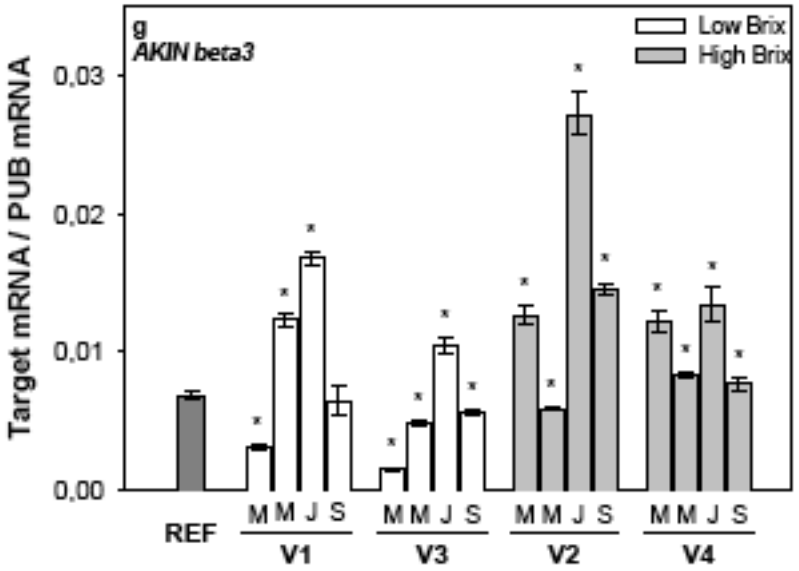


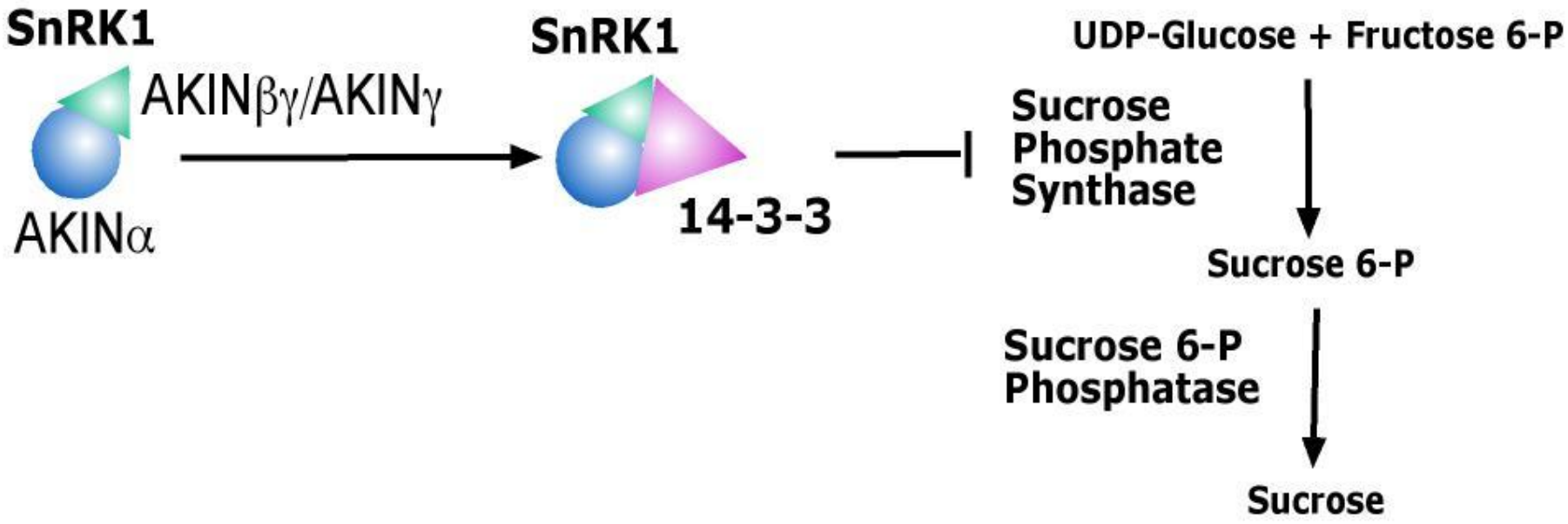
Sucrose accumulation dynamics



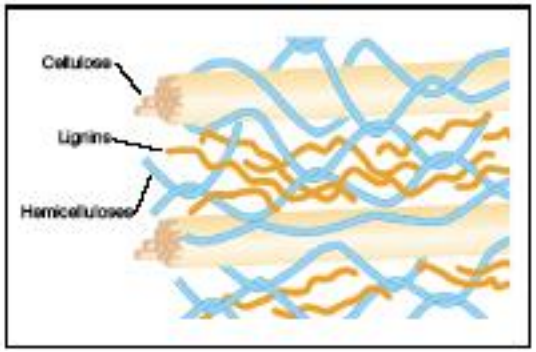


SnRK1 regulatory subunits

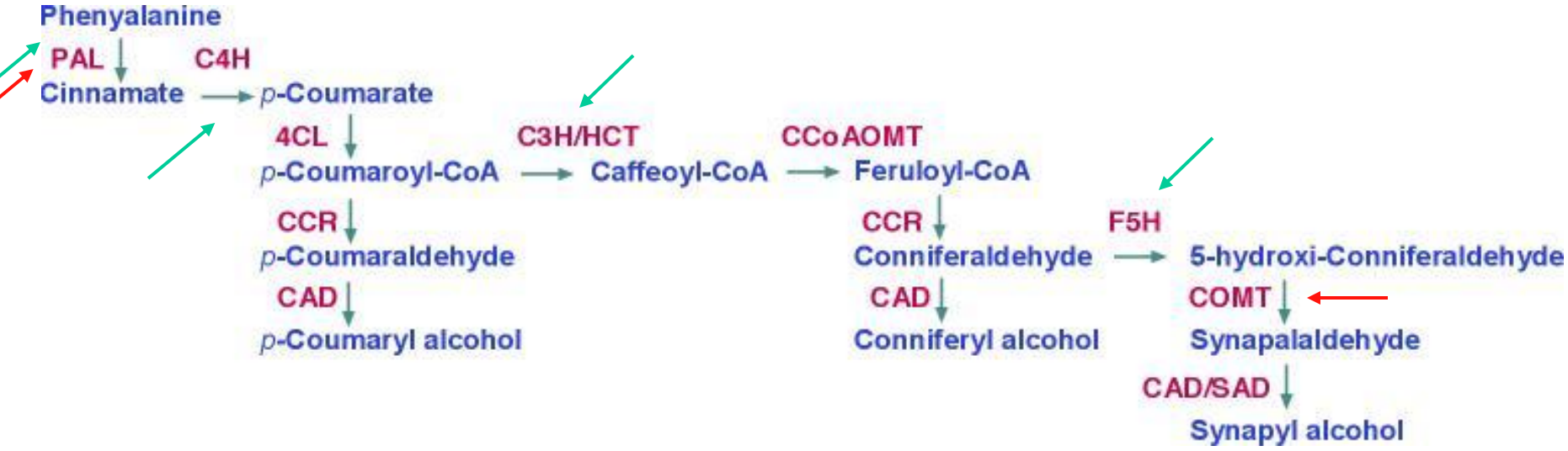




Lignin Biosynthesis is associated to sucrose content

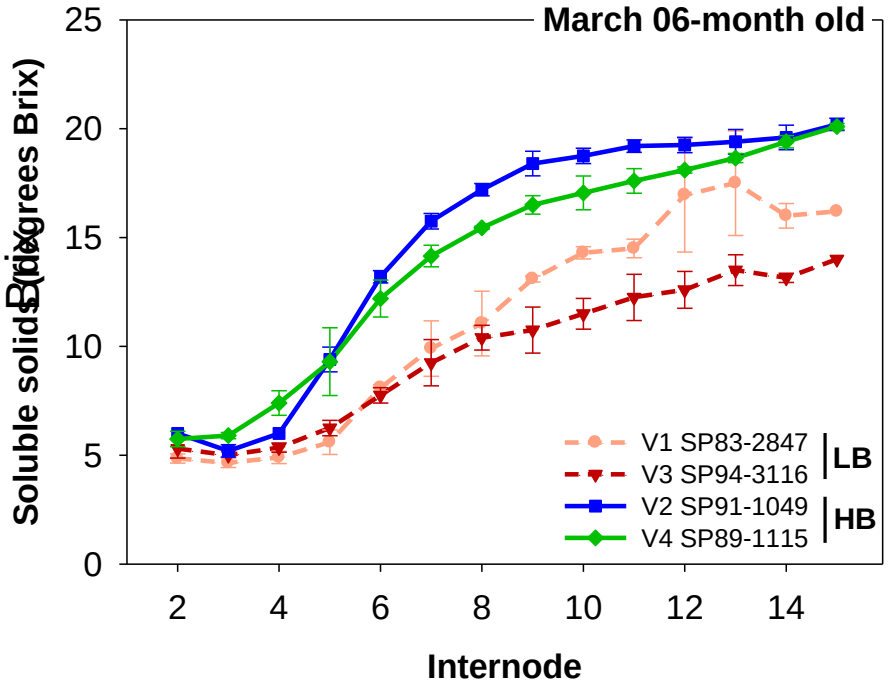


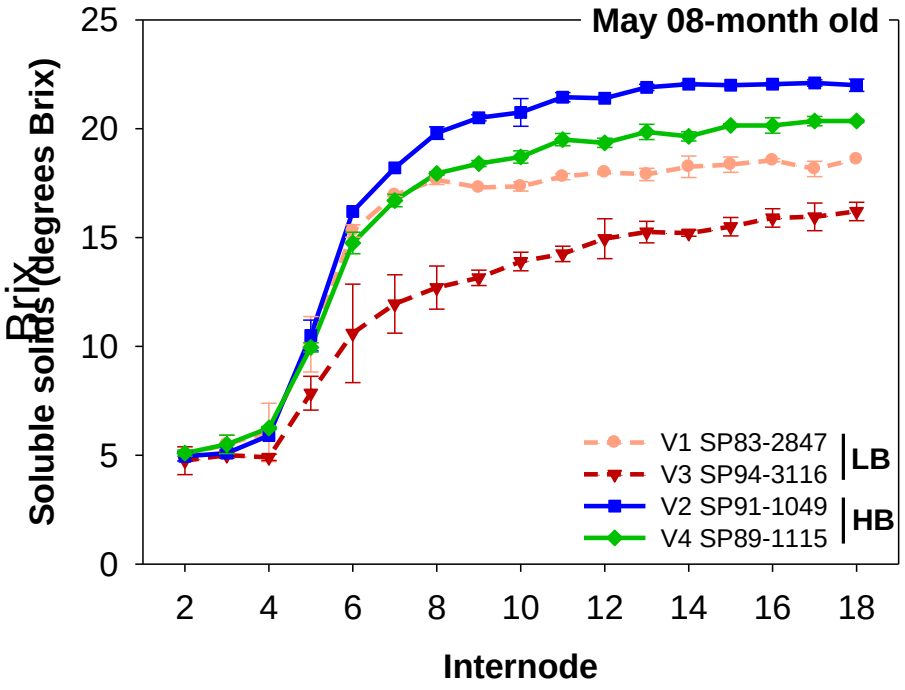
category	sub category 1	sub category 2	HB vs LB	MIn vs IIn	Drought
cell wall metabolism	expansin	EXPA11	1	2	
cell wall metabolism	expansin	OsEXPA23	1		
cell wall metabolism	cytochrome P450	P-coumaroyl shikimate 3'-hydroxylase	2		
cell wall metabolism	cytochrome P450	Ferulate-5-hydroxylase	1	2	
cell wall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



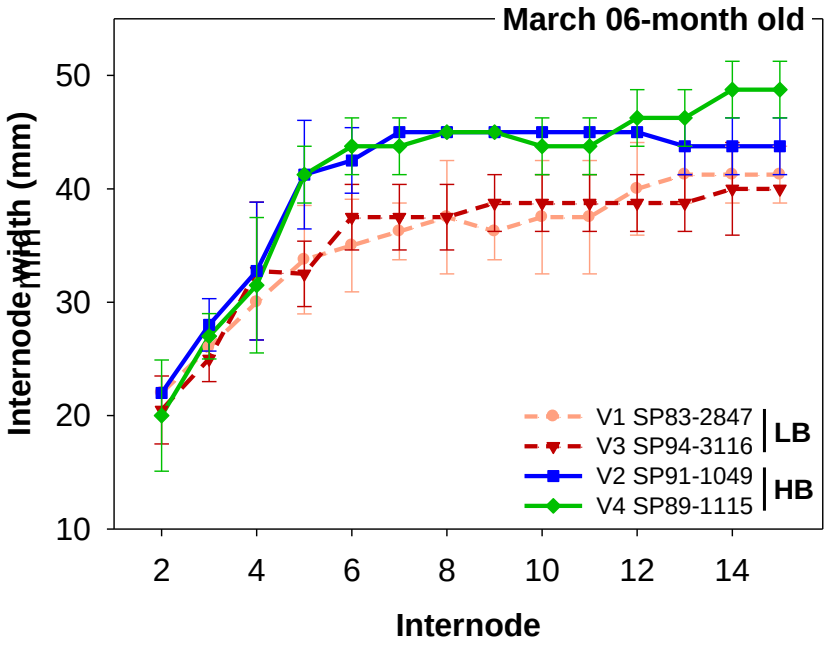
Boudet, A. M., Kajita, S., Grima-Pettenati, J., Goffner, D. (2003). Trends Plant Sci. 8, 576-581

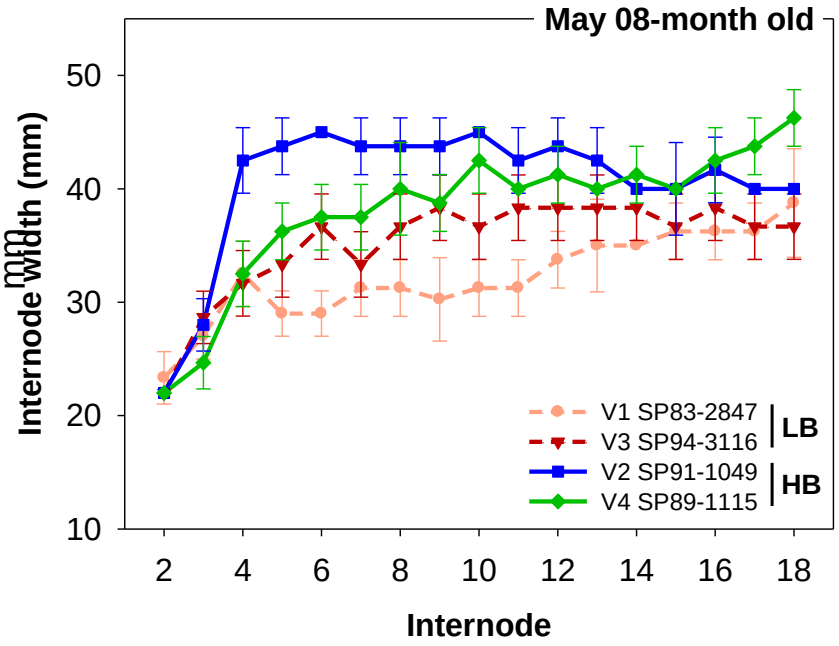
Internode Brix

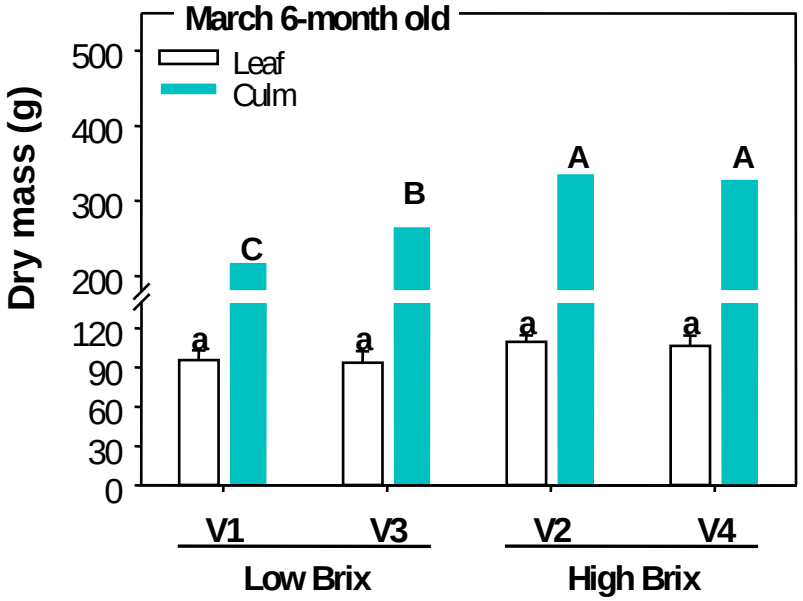


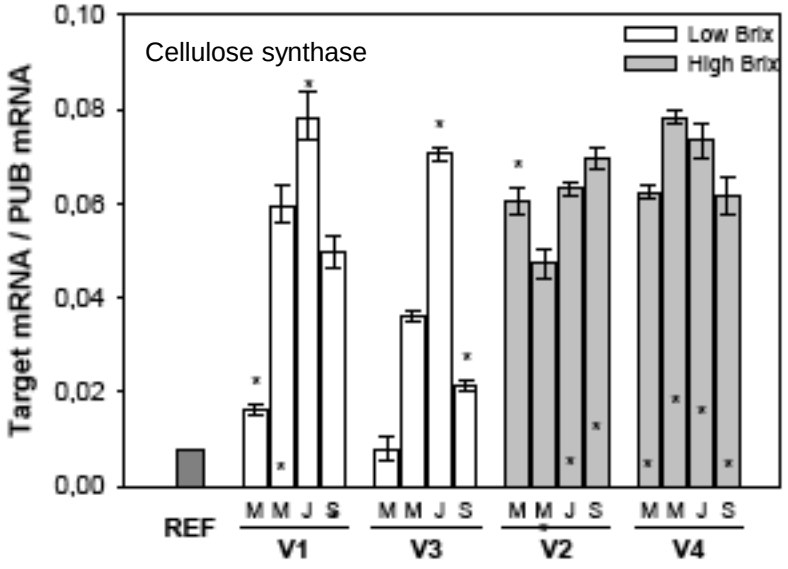


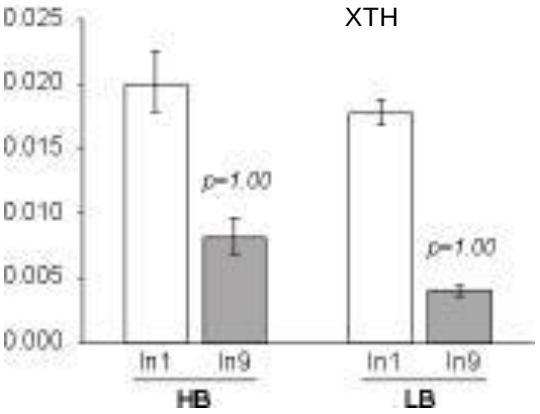
Internode thickness

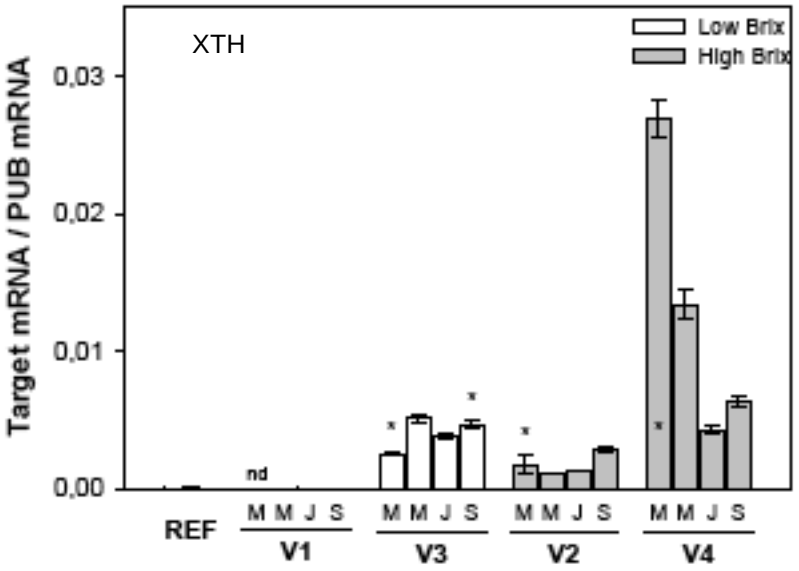





March 6-month old
Leaf
Culm
Dry mass (g)
500
400
300
200
120
90
60
30
0
C
B
A
A
a
a
a
a
V1
V3
V2
V4
Low Brix
High Brix


Cellulose synthase
Low Brix
High Brix
Target mRNA / PUB mRNA
0,10
0,08
0,06
0,04
0,02
0,00
REF
M M J S
V1
V3
V2
V4


XTH
0.025
0.020
0.015
0.010
0.005
0.000
p=1.00
p=1.00
In1
In9
In1
In9
HB
LB

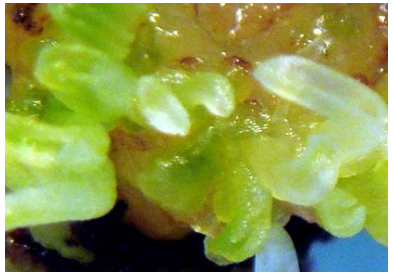

XTH
Low Brix
High Brix
Target mRNA / PUB mRNA
0,03
0,02
0,01
0,00
nd
REF
M M J S
V1
V3
V2
V4

Cell wall metabolism associated to biomass

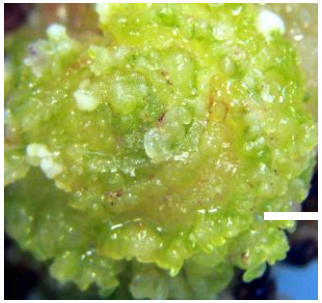
Cell Wall Metabolism	category	sub category 1	sub category 2
cell wall modification	Development	maturation	ripening regulated protein
cell wall protein	development	expansin	**OsEXPA**
cell wall protein	development	expansin	**OsEXPA**
cell wall protein	development	expansin	**OsEXPA**
cellulose synthesis	Carbohydrate metabolism	cellulose synthase (UDP-forming)	**Cellulose synthase (UDP-forming)**
cellulose synthesis	Carbohydrate metabolism	cellulose synthase (UDP-forming)	**Cellulose synthase (UDP-forming)**
cellulose synthesis	Carbohydrate metabolism	Sucrose synthase	.
cellulose synthesis	Carbohydrate metabolism	Sucrose synthase	.
disease response	cell wall hydroxylation methylation	infected library	S-adenosylmethionine synthetase
disease response	flavonoid	wound-induced	Chalcone synthase
disease response	flavonoid	wound-induced	Chalcone synthase
disease response	Pathogenicity	R-genes transduction	PR
disease response	Pathogenicity	R-genes transduction	NPR1
lignin synthesis	Hormone biosynthesis	Salicylic acid	**Phenylalanine ammonia-lyase (PAL)**
lignin synthesis	hormone biosynthesis	salicylic acid	**Phenylalanine ammonia-lyase (PAL)**
lignin synthesis	hormone biosynthesis	salicylic acid	**Phenylalanine ammonia-lyase (PAL)**
lignin synthesis	hormone biosynthesis	salicylic acid	**Phenylalanine ammonia-lyase (PAL)**
lignin synthesis	secondary metabolism	Caffeic acid 3-O-methyltransferase (COMT)	**Caffeic acid 3-O-methyltransferase (COMT)**
lignin synthesis	secondary metabolism	cinnamoyl CoA reductase (CCR)	**cinnamoyl-CoA reductase [Saccharum officinarum] (CCR**
lignin synthesis	secondary metabolism	cinnamoyl CoA reductase (CCR)	**cinnamoyl-CoA reductase [Saccharum officinarum] (CCR**
lignin synthesis	secondary metabolism	CYP98A (P-coumaroyl shikimate 3'-hydroxylase)	**CYP98A (P-coumaroyl shikimate 3'-hydroxylase) (C3H/H**
lignin synthesis	secondary metabolism	cytochrome P450	**CYP73A5 (Cinnamic acid 4-hydroxylase) (C4H)**
lignin synthesis	secondary metabolism	cytochrome P450	**CYP84A1 (ferulate-5-hydroxylase) (F5H)**
lignin synthesis	secondary metabolism	cytochrome P450	**CYP98A (P-coumaroyl shikimate 3'-hydroxylase) (C3H/H**
polysaccharide modifying enzyme	Carbohydrate metabolism	Xylanase precursor	**Endo-Beta-1,4 Xylanase**
polysaccharide modifying enzyme	Carbohydrate metabolism	pathogen-induced	Beta-1,3-glucanase-like protein
polysaccharide modifying enzyme	Carbohydrate metabolism	Beta-glucosidase	**Beta-glucosidase**
polysaccharide modifying enzyme	Carbohydrate metabolism	Beta-glucosidase, putative	**Beta-glucosidase**
polysaccharide modifying enzyme	Carbohydrate metabolism	Pentose and Glucuronate interconversions	Pectinesterase
polysaccharide modifying enzyme	carbohydrate metabolism	polysaccharide metabolism/secretory pathway	**Xyloglucan endotransglycosylase (XTH)**
polysaccharide modifying enzyme	carbohydrate metabolism	polysaccharide metabolism/secretory pathway	**Xyloglucan endotransglycosylase (XTH)**
polysaccharide modifying enzyme	carbohydrate metabolism	polysaccharide metabolism/secretory pathway	**Xyloglucan endotransglycosylase (XTH)**
polysaccharide modifying enzyme	carbohydrate metabolism	polysaccharide metabolism/secretory pathway	**Xyloglucan endotransglycosylase (XTH)**
polysaccharide modifying enzyme	Carbohydrate metabolism	Starch and sucrose metabolism	**beta-Glucosidase, Gentiobiase, Cellobiase**
polysaccharide modifying enzyme	Secondary metabolism	arabinosidase	**arabinosidase**
signaling	Carbohydrate metabolism	Similar to AKINbetagamma	interacts with the SNF1 complex
signaling	Development	transcriptional adaptor	auxin-independent growth promoter
signaling	hormone biosynthesis	auxin	Nitrilase
signaling	hormone biosynthesis	auxin	Nitrilase
signaling	hormone biosynthesis	ethylene	ACC oxidase
signaling	hormone biosynthesis	ethylene	ACC oxidase
signaling	Hormone biosynthesis	Giberellin	oxidases
signaling	protein kinase	SNF-like kinase	caneCIPK-21
signaling	protein phosphatase	serine/threonine PPM family	PP2C-like
signaling	Receptors	RSTK	Wall associated
signaling	Small GTPases	Sar1	.
signaling	Stress response	response to stress	Abscisic acid-and stress-inducible protein
transcription	Nucleic acid metabolism	nucleosome assembly	Argonaute protein
transcription	transcription factor	CCAAT	Hap
transcription	transcription factor	Homeobox	.
transcription	transcription factor	hormone-related/auxin/Aux/IAA	Auxin-responsive protein IAA16
transcription	transcription factor	LIM (protein-protein interaction)	pollen specific LIM domain protein
transcription	transcription factor	MYB	Transcription factor (myb)
transcription	transcription factor	Transcription factor MADS37	SHP1

Sugarcane Transgenics

Explants:
Immature Leaves

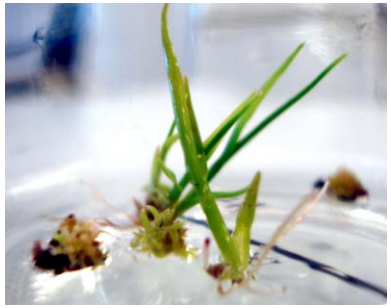


Direct
Embryogeneis



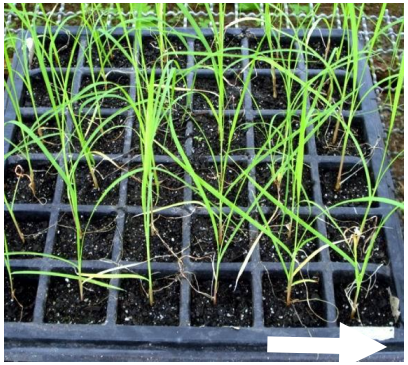
Callus
Induction

Regeneration
Selective
Medium

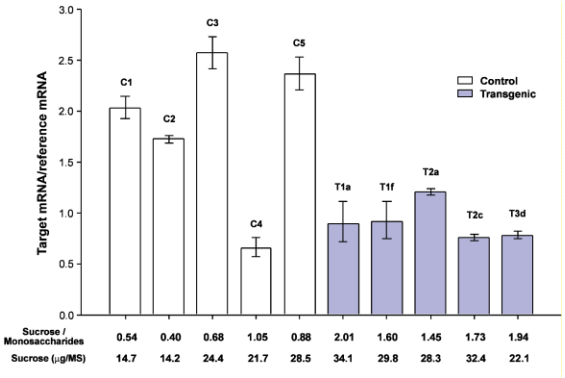


Rooting

Shoot Growth



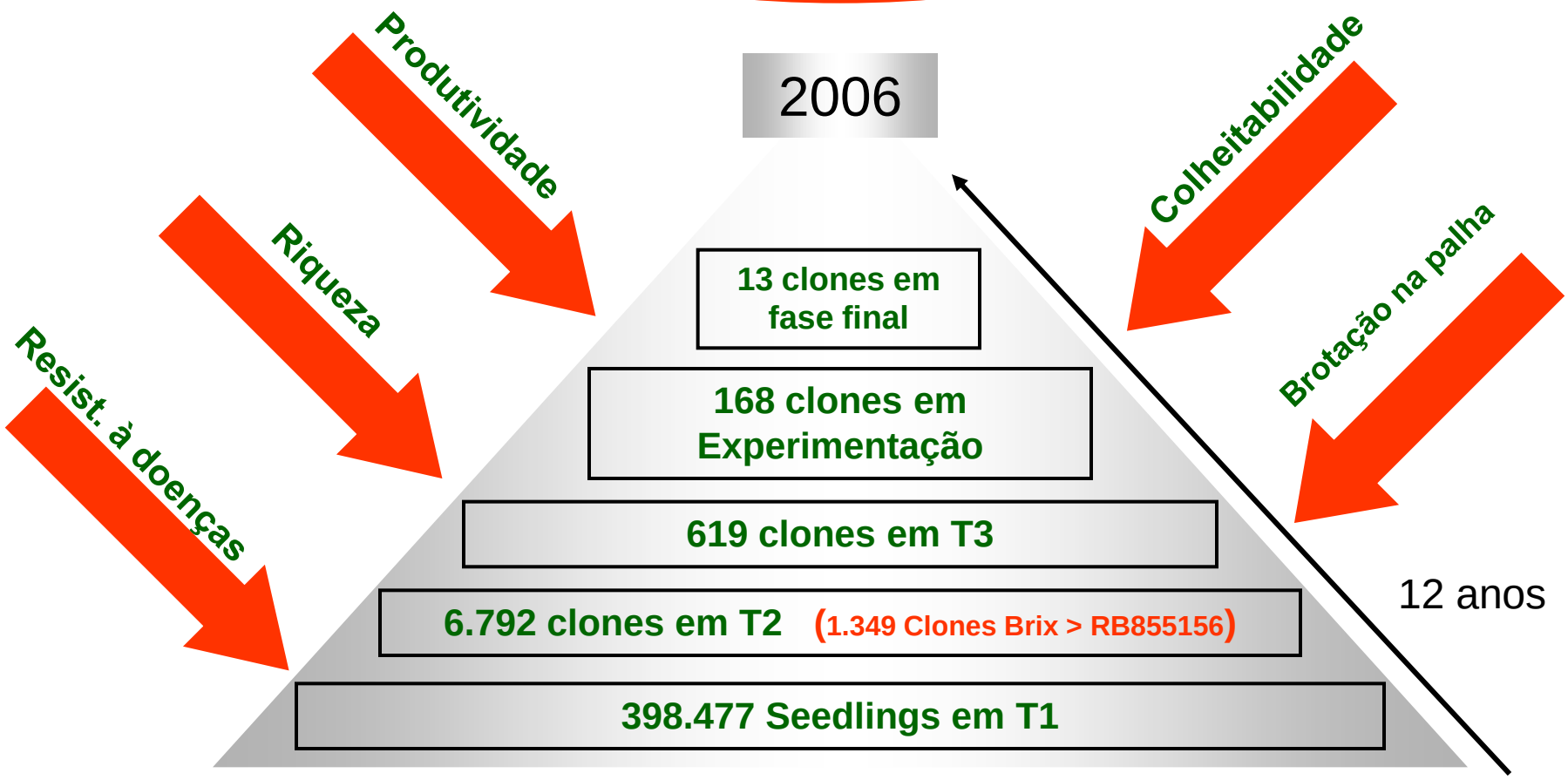
Greenhouse



Phenotyping

PMGCA

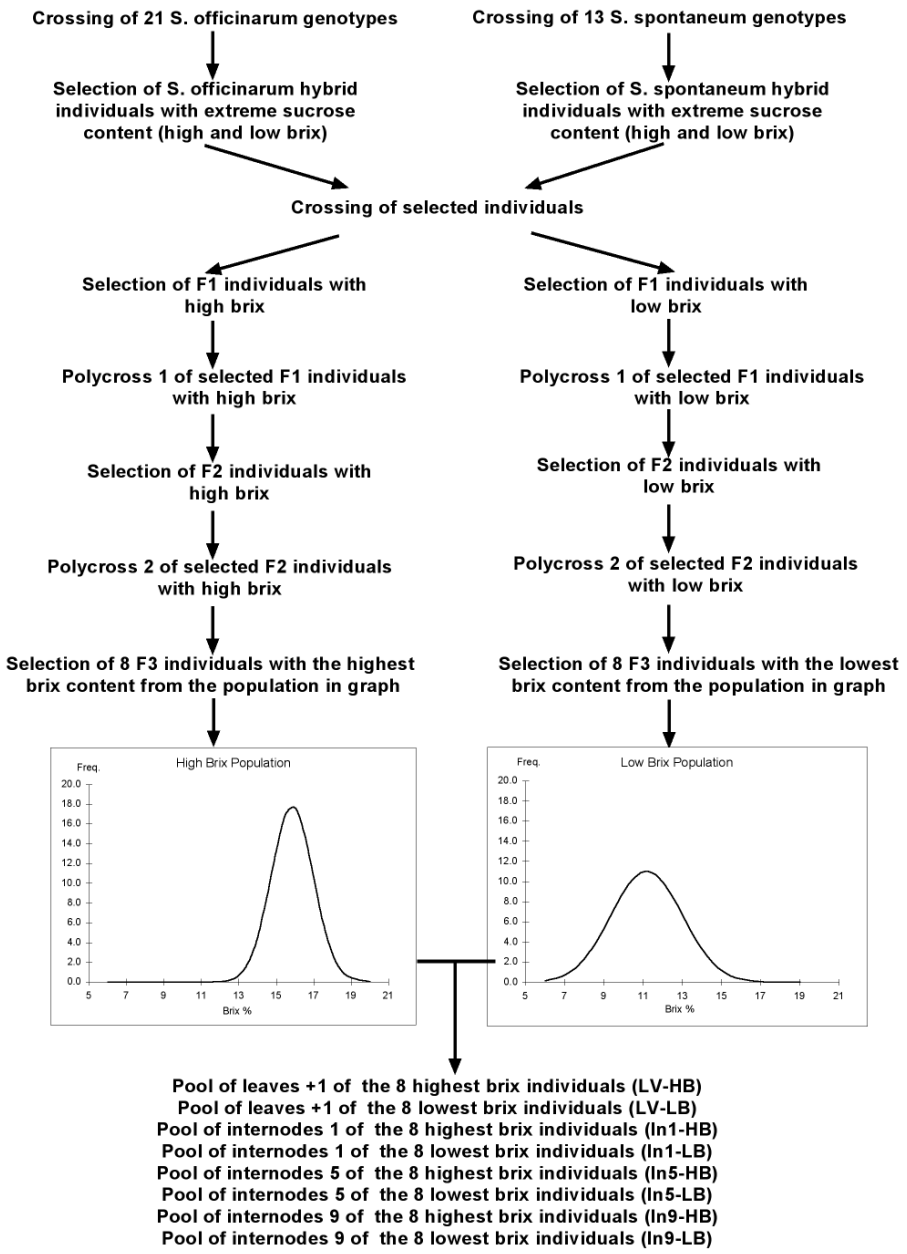
Novas Variedades



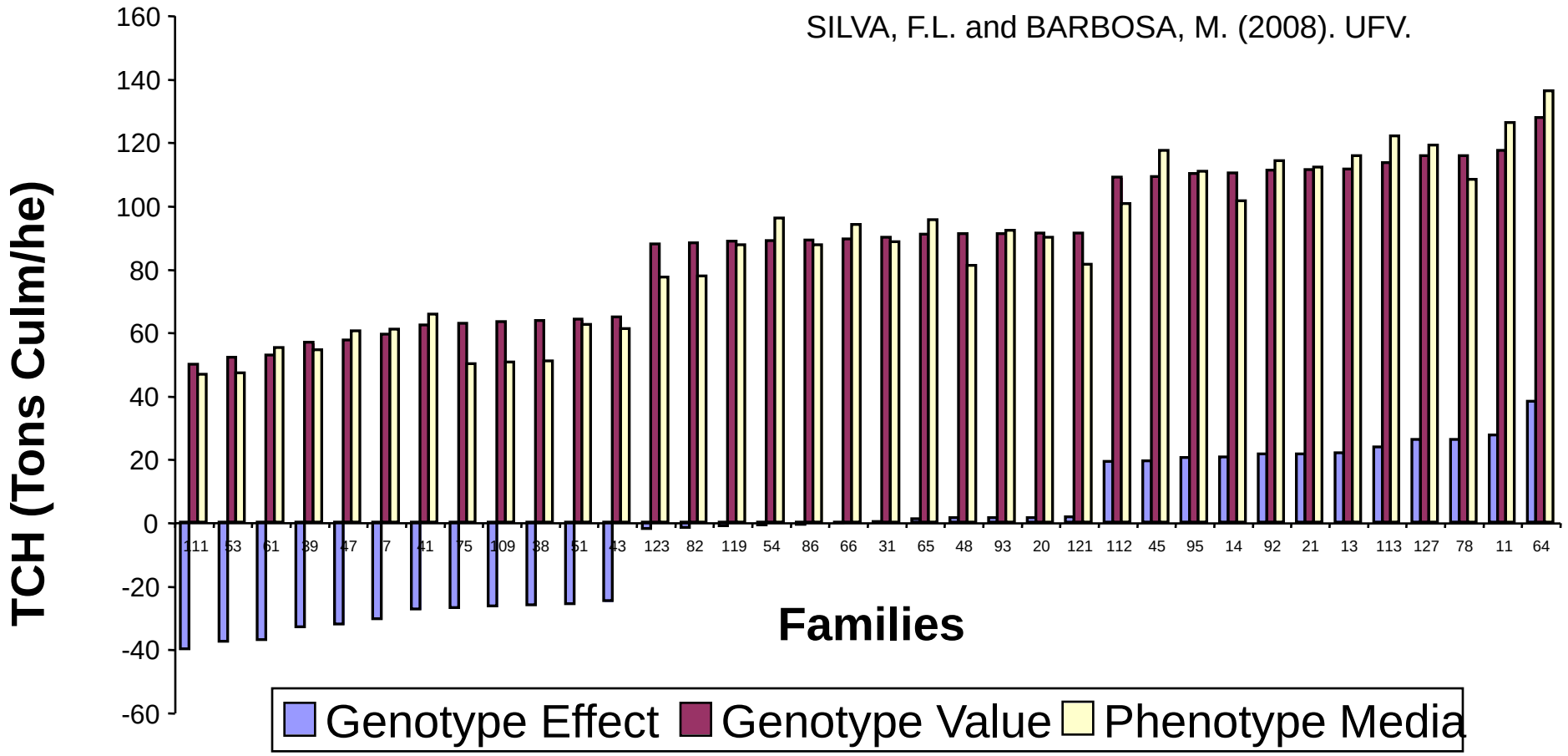
Total: 406.069 genótipos

Genetical Genomics of Traits of interest

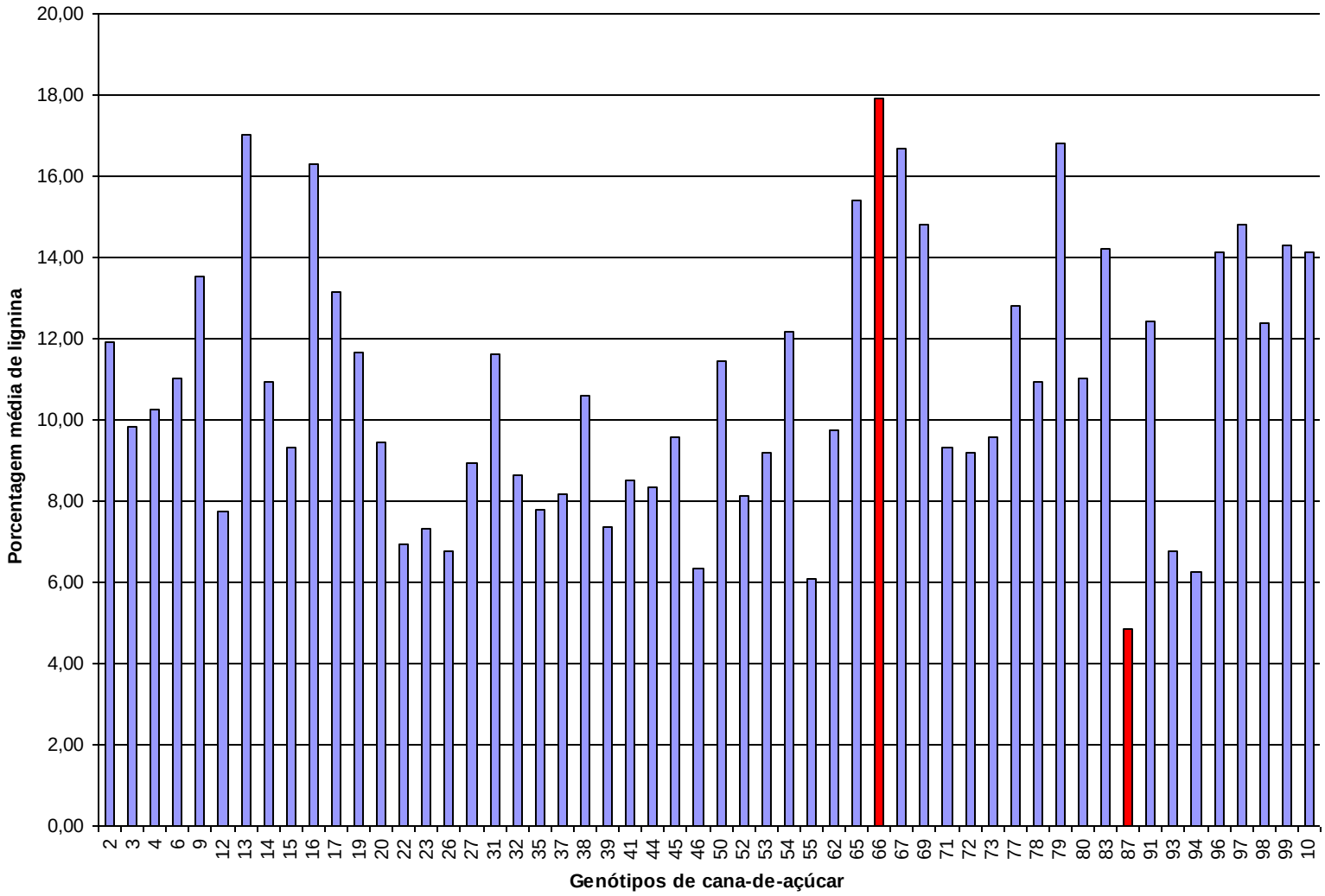
Progeny 1 Genotypes	Brix	Sucrose % m/m	Glucose % m/m	Fructose % m/m	Progeny 2 Genotypes	Brix
CTC98-241	18.00	7.311	1.322	0.988	C158	18.3
CTC98-242	18.60	9.183	1.430	1.014	C121	18.8
CTC98-243	19.20	10.956	0.649	0.602	C171	16.8
CTC98-244	14.60	11.161	0.633	0.646	C496	17
CTC98-246	18.80	10.974	0.709	0.545	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		



Family Yield



Cellulosic Ethanol: Energy Cane



Stress genes associated to sucrose content

Mature X Immature Internodes

High Brix X Low Brix

Stressed X Control

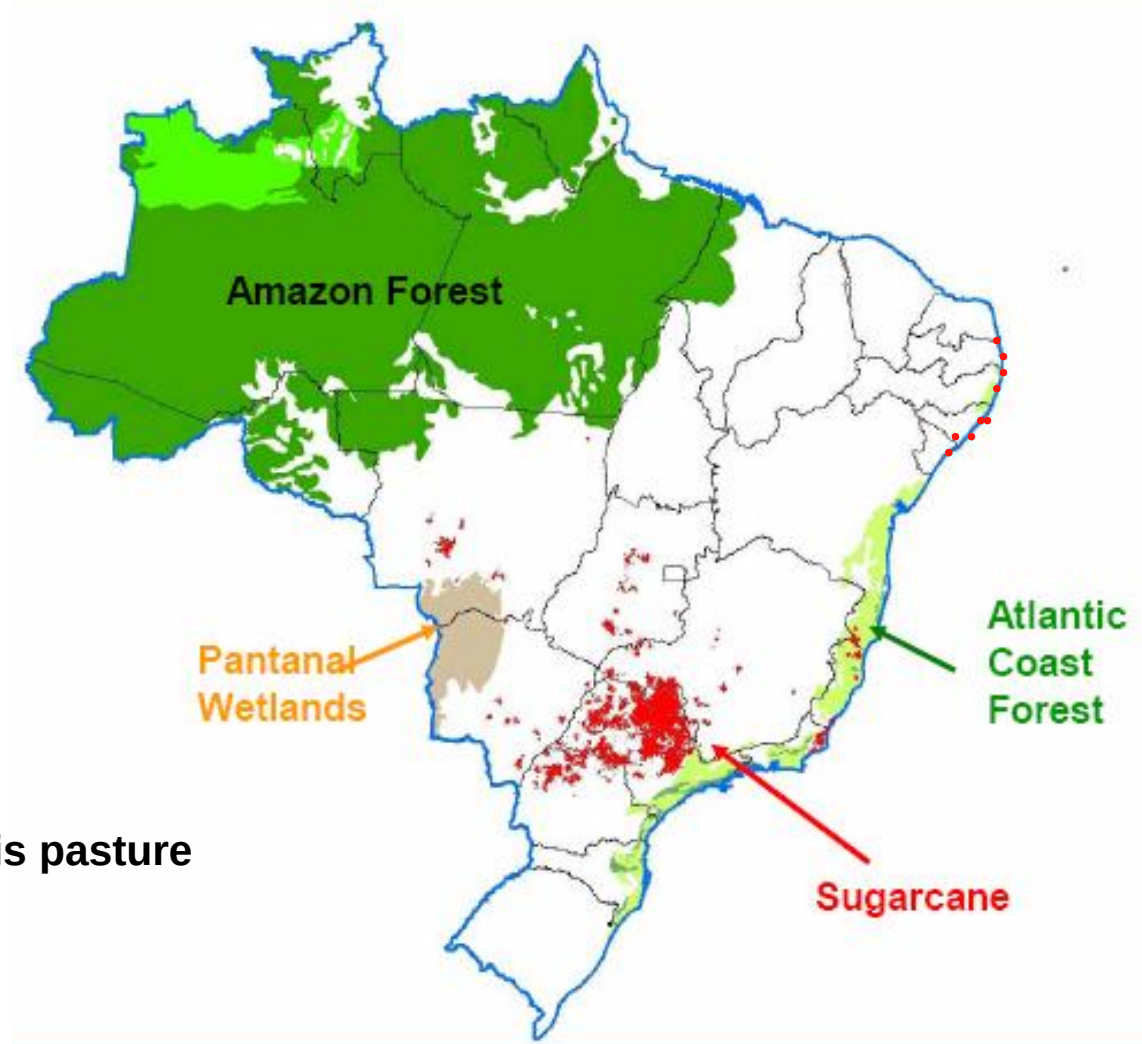
sub category 1	sub category 2	HB x LB	MI x II	Drough
oxidative stress	catalase		3	2
oxidative stress	glutathione S-transferase	2	3	2
oxidative stress	glutathione S-transferase	1	3	2
oxidative stress	metalothionein		2	2
oxidative stress	superoxide dismutase Cu/Zn		4	
oxidative stress	superoxide dismutase Cu/Zn		4	2
drought and cold response	Enolase		2	2
drought and cold response	Low temperature induced (LTI)	1		4
drought and cold response	Low temperature induced (LTI)		1	4
drought and cold response	Low temperature induced (LTI)	1	1	4
drought and cold response	putative aquaporin (tonoplast intrinsic protein)	2	1	
drought and cold response	putative aquaporin (tonoplast intrinsic protein)	1	2	
drought and cold response	putative aquaporin (tonoplast intrinsic protein)	1	1	
drought and cold response	putative aquaporin (tonoplast intrinsic protein)		2	
drought and cold response	putative aquaporin (tonoplast intrinsic protein)		2	
drought and cold response	reversibly glycosylated polypeptide		3	
drought-induced	.	1	3	4
wound-induced	.	3	1	
wound-induced	Chalcone synthase	1	3	
wound-induced	Ribonuclease	1		4

Cultivated land expansion vs. yield

Sucrose Content

High Biomass

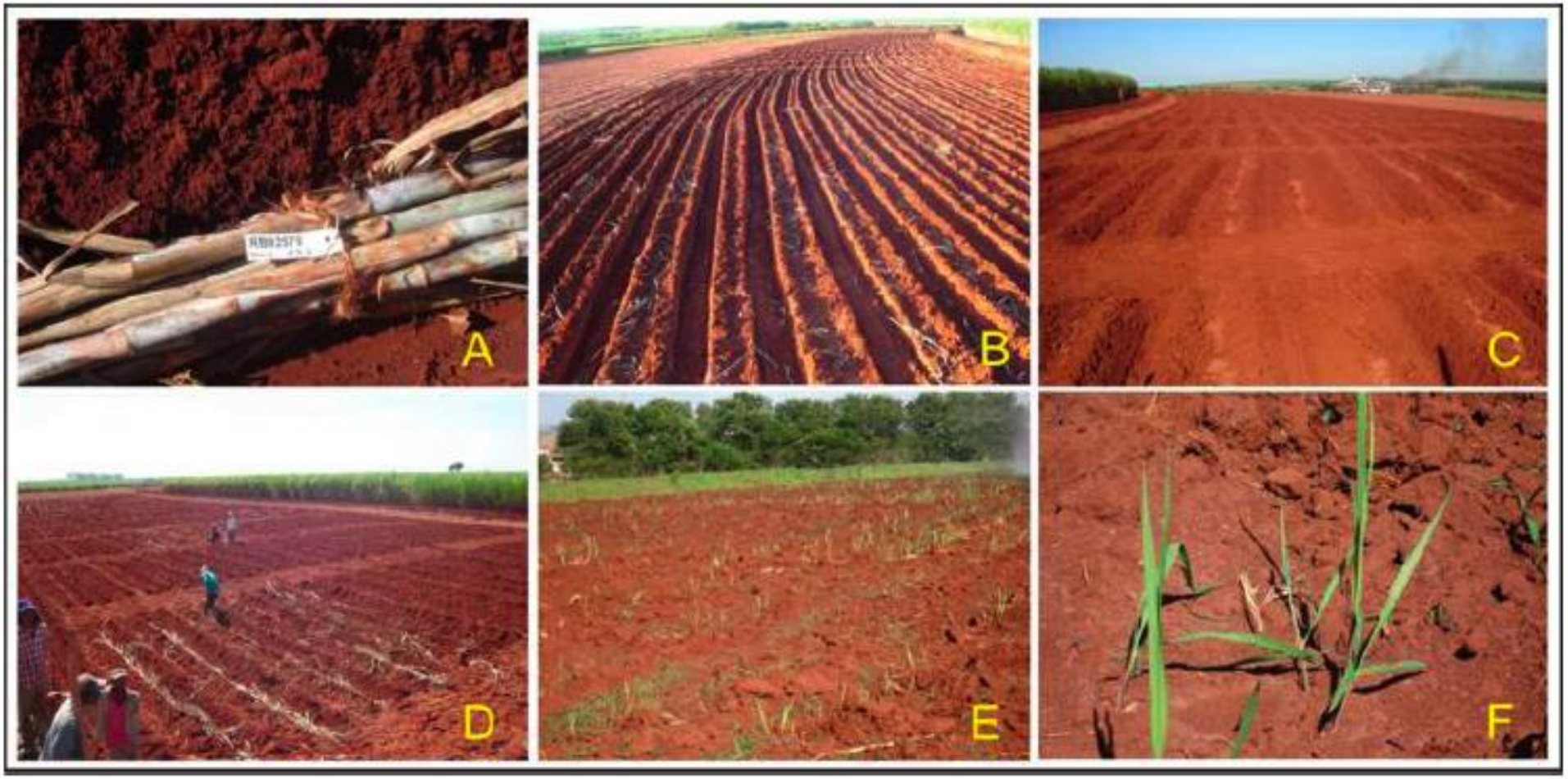
Drought Tolerance



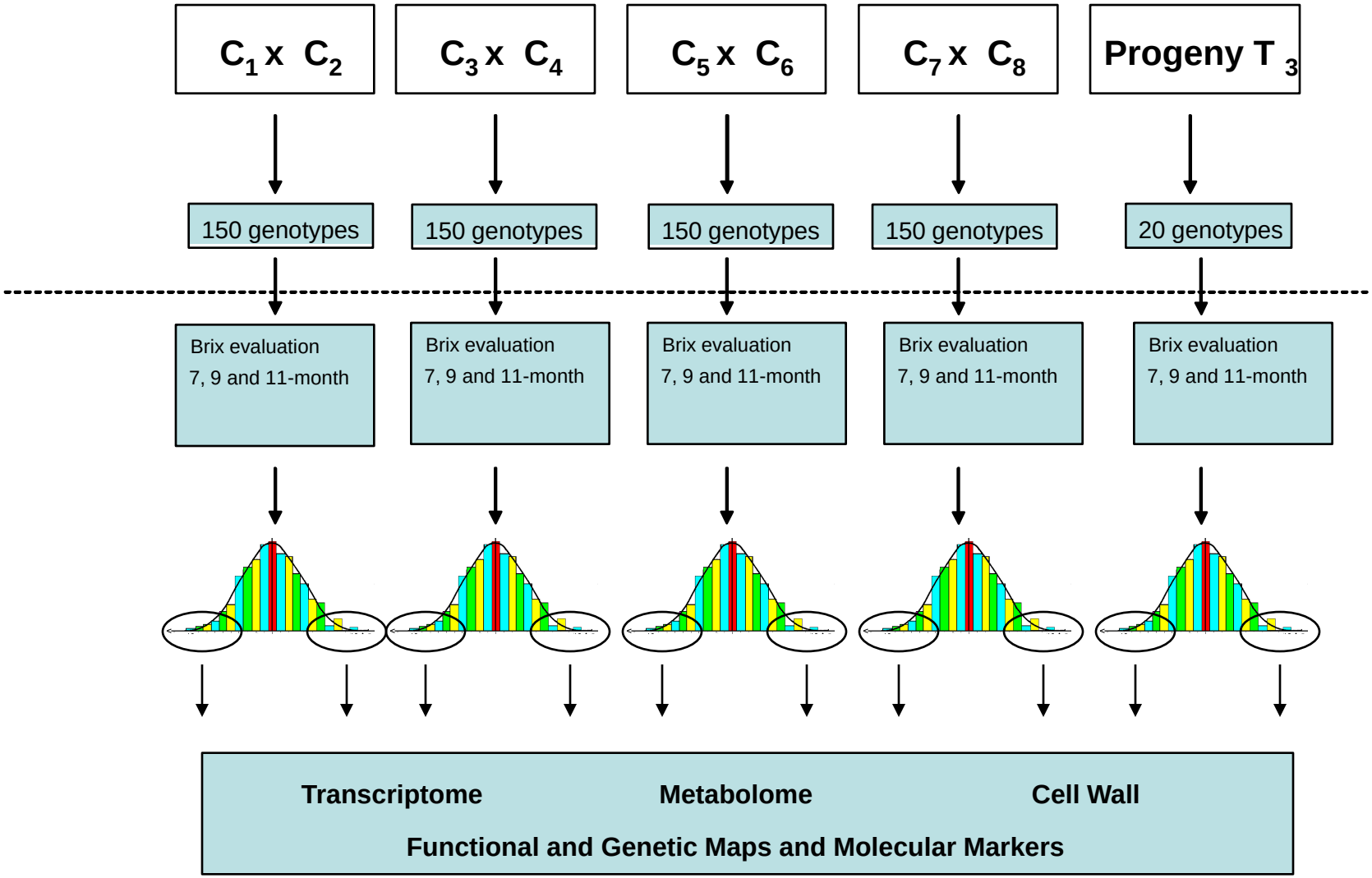
65% of expansion land is pasture

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

Drought Field Experiments: 4 locations



Sucrose Project



Cell wall metabolism associated to biomass

Cell Wall Metabolism	category	sub category 1	sub category 2
cell wall modification	Development	maturation	ripening regulated protein
cell wall protein	development	expansin	**OsEXPA**
cell wall protein	development	expansin	**OsEXPA**
cell wall protein	development	expansin	**OsEXPA**
cellulose synthesis	Carbohydrate metabolism	cellulose synthase (UDP-forming)	**Cellulose synthase (UDP-forming)**
cellulose synthesis	Carbohydrate metabolism	cellulose synthase (UDP-forming)	**Cellulose synthase (UDP-forming)**
cellulose synthesis	Carbohydrate metabolism	Sucrose synthase	.
cellulose synthesis	Carbohydrate metabolism	Sucrose synthase	.
disease response	cell wall hydroxylation methylation	infected library	S-adenosylmethionine synthetase
disease response	flavonoid	wound-induced	Chalcone synthase
disease response	flavonoid	wound-induced	Chalcone synthase
disease response	Pathogenicity	R-genes transduction	PR
disease response	Pathogenicity	R-genes transduction	NPR1
lignin synthesis	Hormone biosynthesis	Salicylic acid	**Phenylalanine ammonia-lyase (PAL)**
lignin synthesis	hormone biosynthesis	salicylic acid	**Phenylalanine ammonia-lyase (PAL)**
lignin synthesis	hormone biosynthesis	salicylic acid	**Phenylalanine ammonia-lyase (PAL)**
lignin synthesis	hormone biosynthesis	salicylic acid	**Phenylalanine ammonia-lyase (PAL)**
lignin synthesis	secondary metabolism	Caffeic acid 3-O-methyltransferase (COMT)	**Caffeic acid 3-O-methyltransferase (COMT)**
lignin synthesis	secondary metabolism	cinnamoyl CoA reductase (CCR)	**cinnamoyl-CoA reductase [Saccharum officinarum] (CCR**
lignin synthesis	secondary metabolism	cinnamoyl CoA reductase (CCR)	**cinnamoyl-CoA reductase [Saccharum officinarum] (CCR**
lignin synthesis	secondary metabolism	CYP98A (P-coumaroyl shikimate 3'-hydroxylase)	**CYP98A (P-coumaroyl shikimate 3'-hydroxylase) (C3H/H**
lignin synthesis	secondary metabolism	cytochrome P450	**CYP73A5 (Cinnamic acid 4-hydroxylase) (C4H)**
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polysaccharide modifying enzyme	carbohydrate metabolism	polysaccharide metabolism/secretory pathway	**Xyloglucan endotransglycosylase (XTH)**
polysaccharide modifying enzyme	carbohydrate metabolism	polysaccharide metabolism/secretory pathway	**Xyloglucan endotransglycosylase (XTH)**
polysaccharide modifying enzyme	carbohydrate metabolism	polysaccharide metabolism/secretory pathway	**Xyloglucan endotransglycosylase (XTH)**
polysaccharide modifying enzyme	carbohydrate metabolism	polysaccharide metabolism/secretory pathway	**Xyloglucan endotransglycosylase (XTH)**
polysaccharide modifying enzyme	Carbohydrate metabolism	Starch and sucrose metabolism	**beta-Glucosidase, Gentiobiase, Cellobiase**
polysaccharide modifying enzyme	Secondary metabolism	arabinosidase	**arabinosidase**
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signaling	hormone biosynthesis	auxin	Nitrilase
signaling	hormone biosynthesis	auxin	Nitrilase
signaling	hormone biosynthesis	ethylene	ACC oxidase
signaling	hormone biosynthesis	ethylene	ACC oxidase
signaling	Hormone biosynthesis	Giberellin	oxidases
signaling	protein kinase	SNF-like kinase	caneCIPK-21
signaling	protein phosphatase	serine/threonine PPM family	PP2C-like
signaling	Receptors	RSTK	Wall associated
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transcription	transcription factor	CCAAT	Hap
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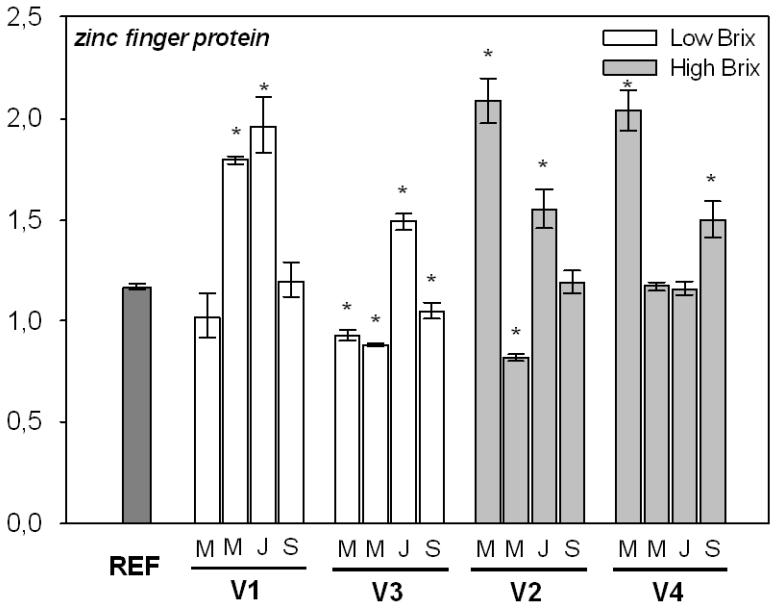
transcription	transcription factor	MYB	Transcription factor (myb)
transcription	transcription factor	Transcription factor MADS37	SHP1

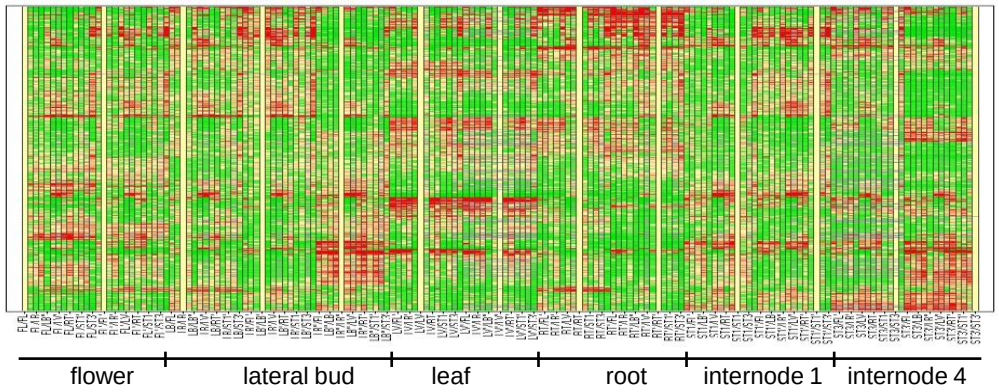
Tissue Profiling for Promoter Identification and Tool Development

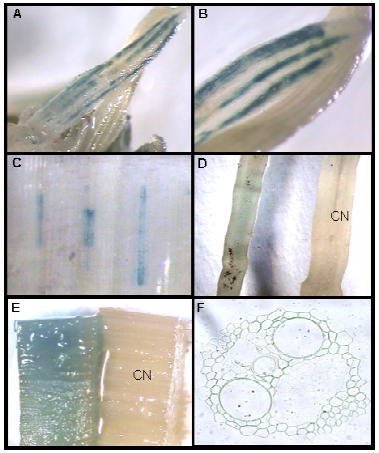
DNA Research 12, 27–38 (2005)

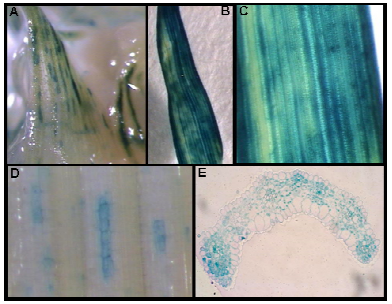
Transcription Profiling of Signal Transduction-Related Genes in Sugarcane Tissues

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,*]









Grassius
Grass Regulatory Information Server

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- Sugarcane
- Sorghum

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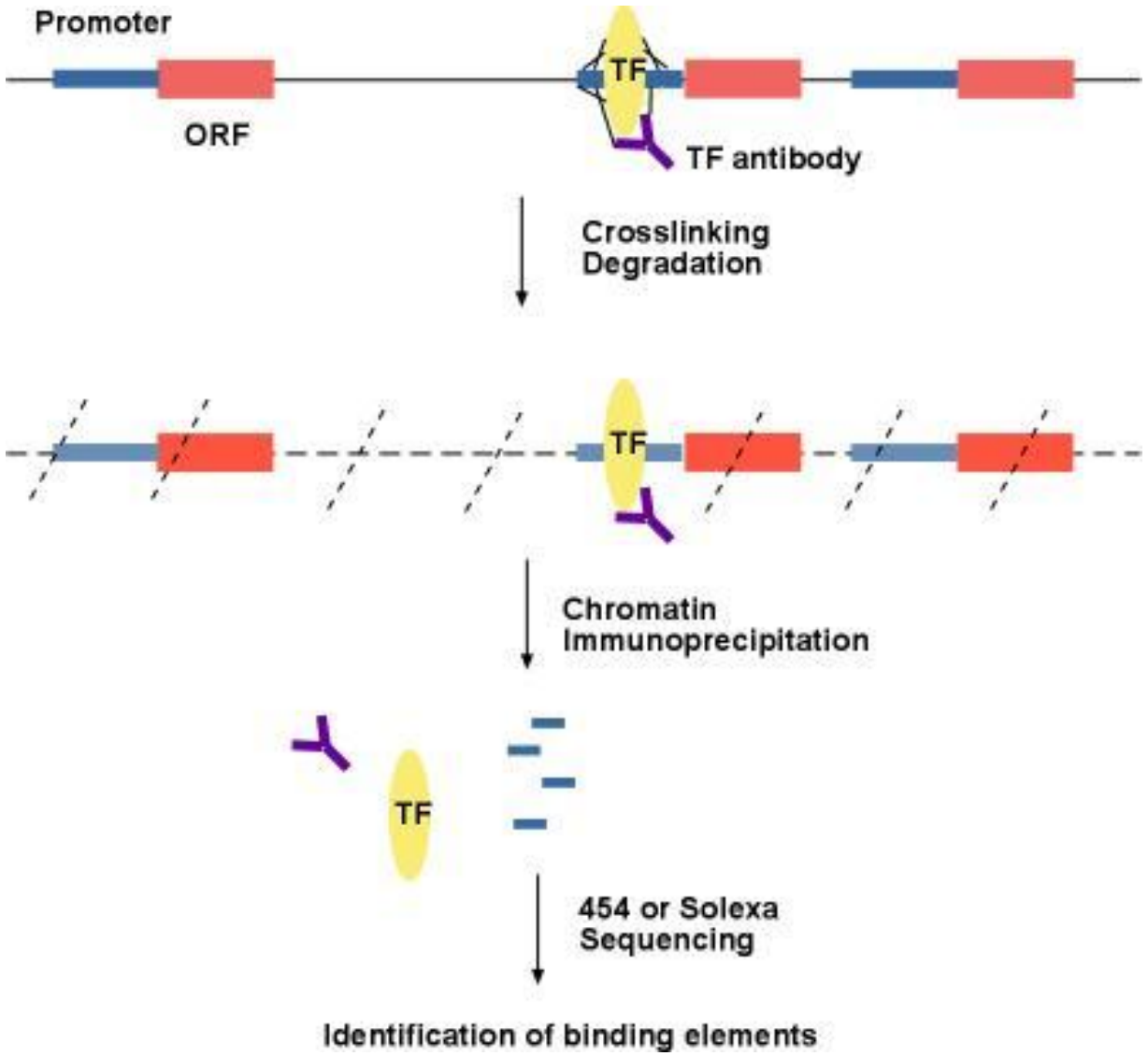
TFome Collection
Downloads
Community Contribution
NSF Plant Genome Project
People
Outreach
Links
Help

Saccharum officinarum

Sugarcane Transcription Factors

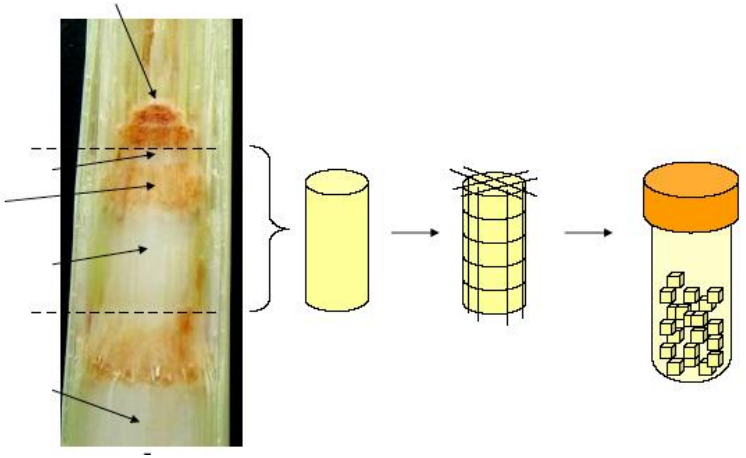
ABI3VP1 (33)	Alfin-like (25)	AP2-EREBP (86)
ARF (33)	ARID (3)	ARR-B
AS2 (1)	AtRKD	AUX/IAA (88)
BBR/BPC (2)	bHLH (42)	bZIP (59)
BZR (4)	C2C2-CO-like (14)	C2C2-Dof (6)
C2C2-GATA (2)	C2C2-YABBY (11)	C2H2 (26)
C3H (76)	CAMTA (5)	CCAAT
CCAAT-DR1 (5)	CCAAT-HAP2 (2)	CCAAT-HAP3 (5)
CCAAT-HAP5 (19)	CPP (9)	CSD
DBP	DDT	E2F-DP (5)
EIL (8)	FHA (12)	G2-like (38)
GeBP	GIF (11)	GRAS (32)

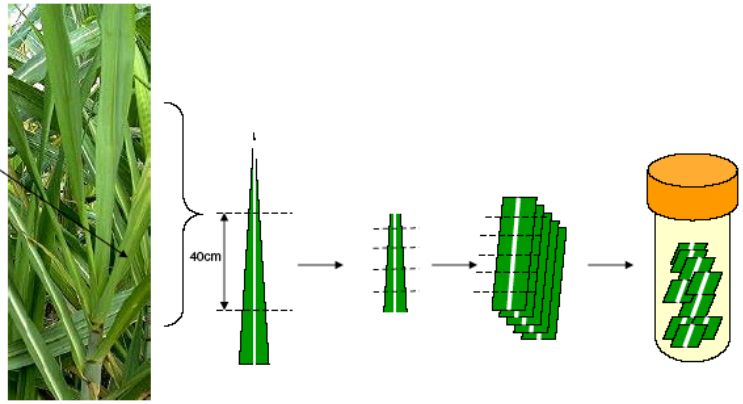
Species	Total Number of genes/proteins	Number of proteins from GRASSTFDB	Number of New TFs	Number of Total TFs
Saccharum oficinarum	43.141	1349	2889	4238
Zea mays	191.255	2897	4500	7397
Oryza sativa japonica	68.388	3139	4039	7178
Oryza sativa indica	38.861	2025	793	2818
Sorghum bicolor	36.338	1755	1753	3408

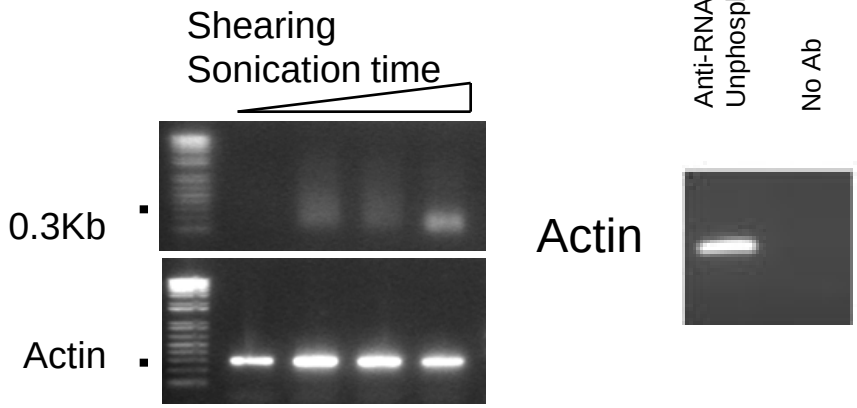

Promoter
ORF
TF
TF antibody
Crosslinking
Degradation
TF
Chromatin
Immunoprecipitation
TF
454 or Solexa
Sequencing
Identification of binding elements

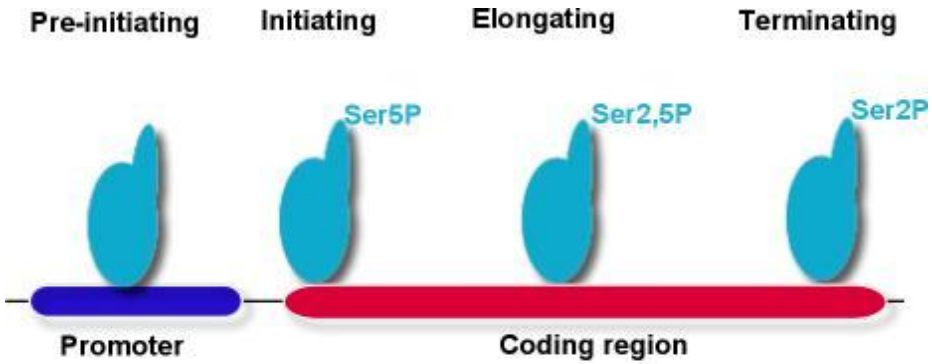
ChIP-Seq with RNA polymerase II repeat YSPTSPS antibody

To establish ChIP in sugarcane and promoter and active gene database









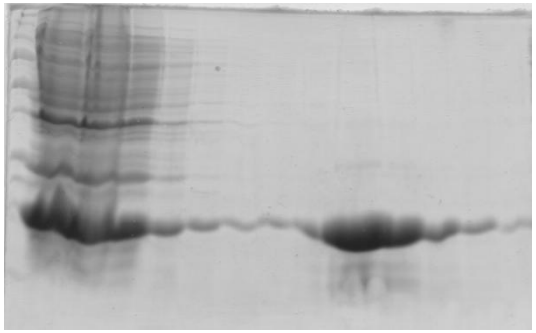
ChIP-Seq

Selected genes (10)

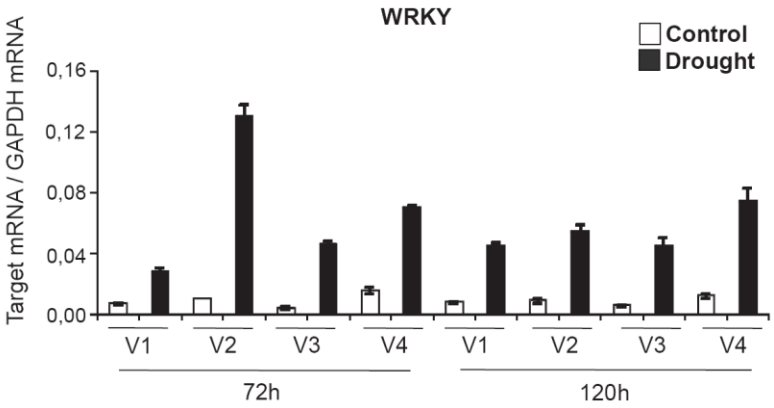
SAS	TF	Brix	Develop	Drought
SASGMS00068	MYB			
SASGMS00064	MYB			
SASGMS00071	NAM			
SASGMS00002	HLH			
SASGMS27191	AP1/MADS			
SASGMS00003	homeobox			
SASGMS00001	HLH			
SASGMS00062	WRKY			
SASGMS34742	NAM			
SASGMS00067	AP2/EREBP			

Cloned (8)
Expressed (7)

NAM- HIS

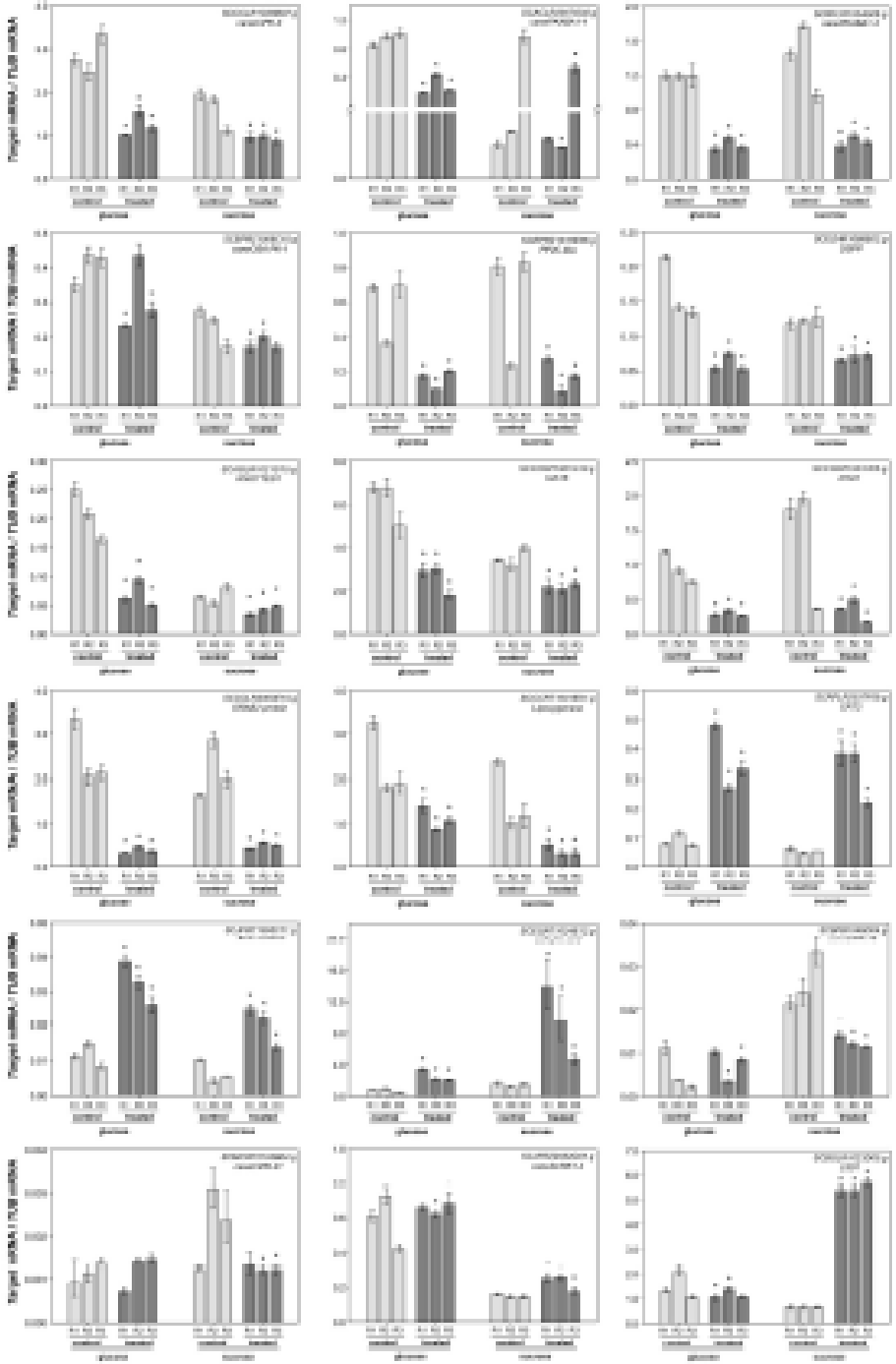


qRT-PCR (10)



Status

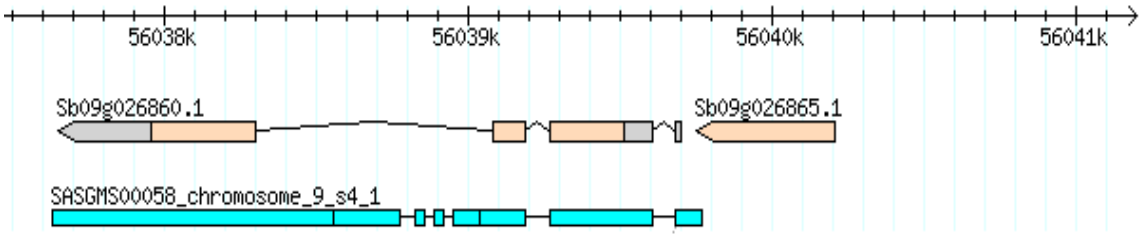
SAS	Antibody	RACE	cDNA	OGM
SASGMS00068	x			
SASGMS00064	x		x	x*
SASGMS00071			x	x
SASGMS00002	x		x	
SASGMS27191	x		x	
SASGMS00003	x		x	
SASGMS00001		x		
SASGMS00062	x	x		x*
SASGMS34742	x	x		x
SASGMS00067				



SAS total number: 43,131
SAS with homologues sequences in Sorgo: 18.216 (at least 95% of identity)

5Kb of Sorghum

BLAST genome on http://www.phytozome.net/



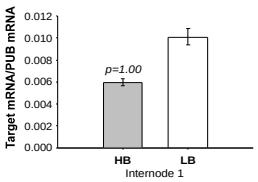
PLACE

Database of Plant Cis-acting Regulatory DNA Elements

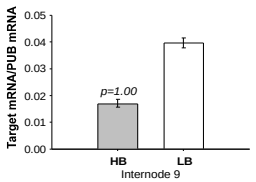
841 sites identified

135 different motifs

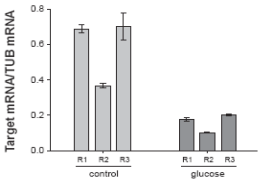
18 related to dehydration, ABA signalling or sucrose regulation



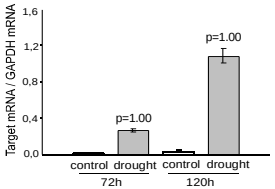
SP80-180 x SP80-4966

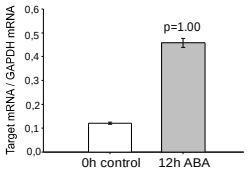


SP80-180 x SP80-4966



SURE2STPAT21 - Sucrose responsive element



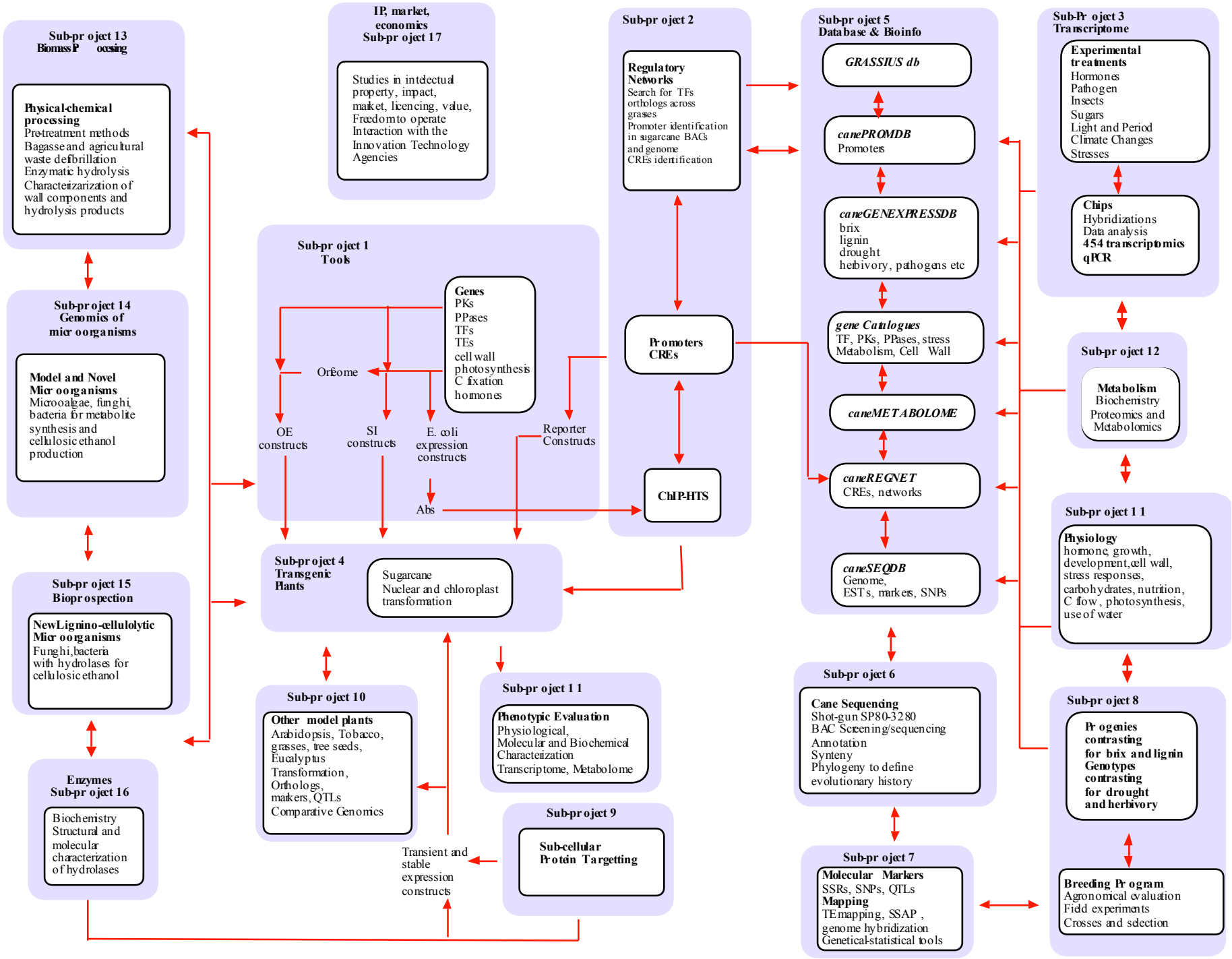


DRECRTCOREAT - core motif of DRE/CRT

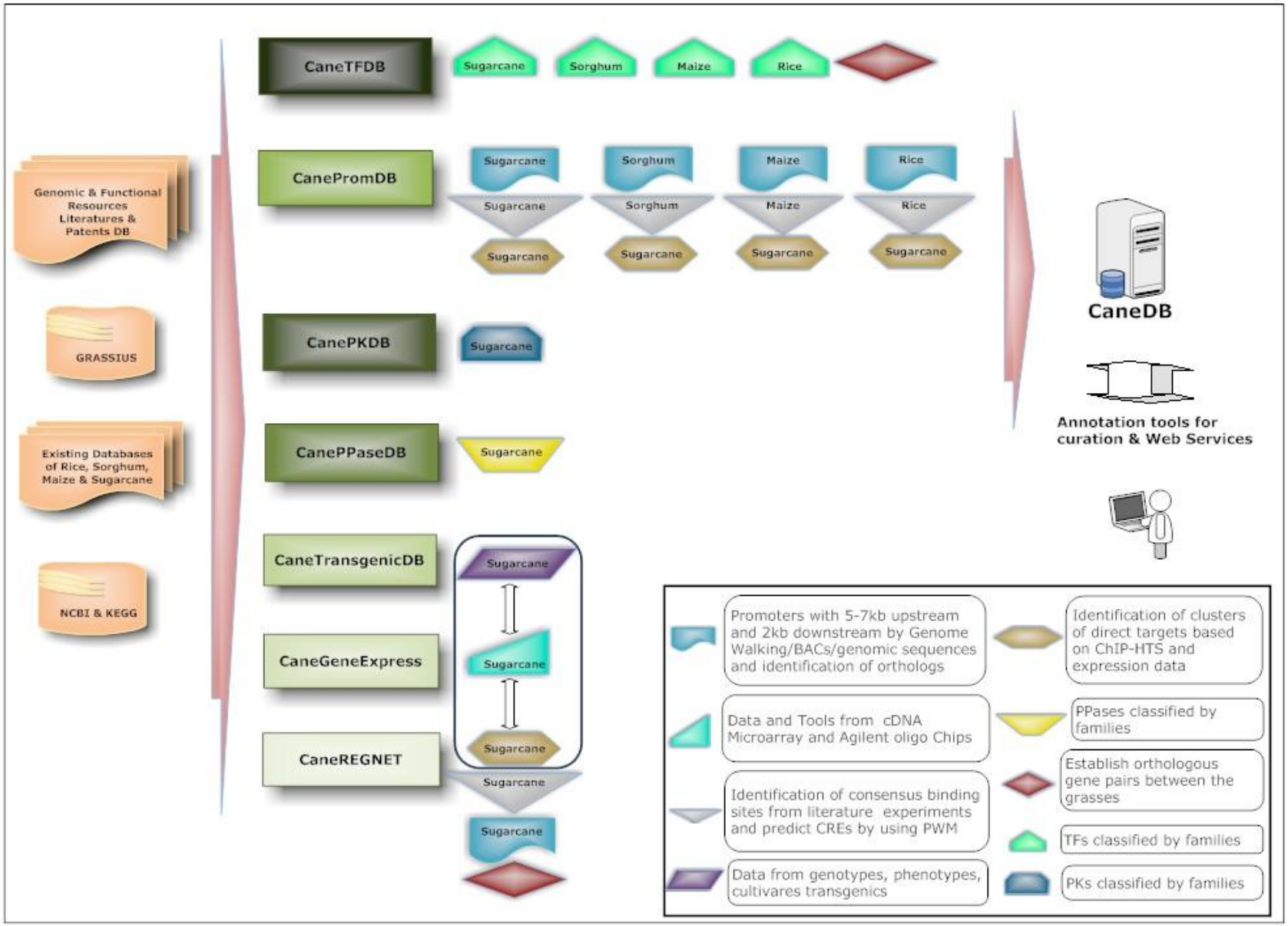
ABRELATERD1 - Responsive to dehydration

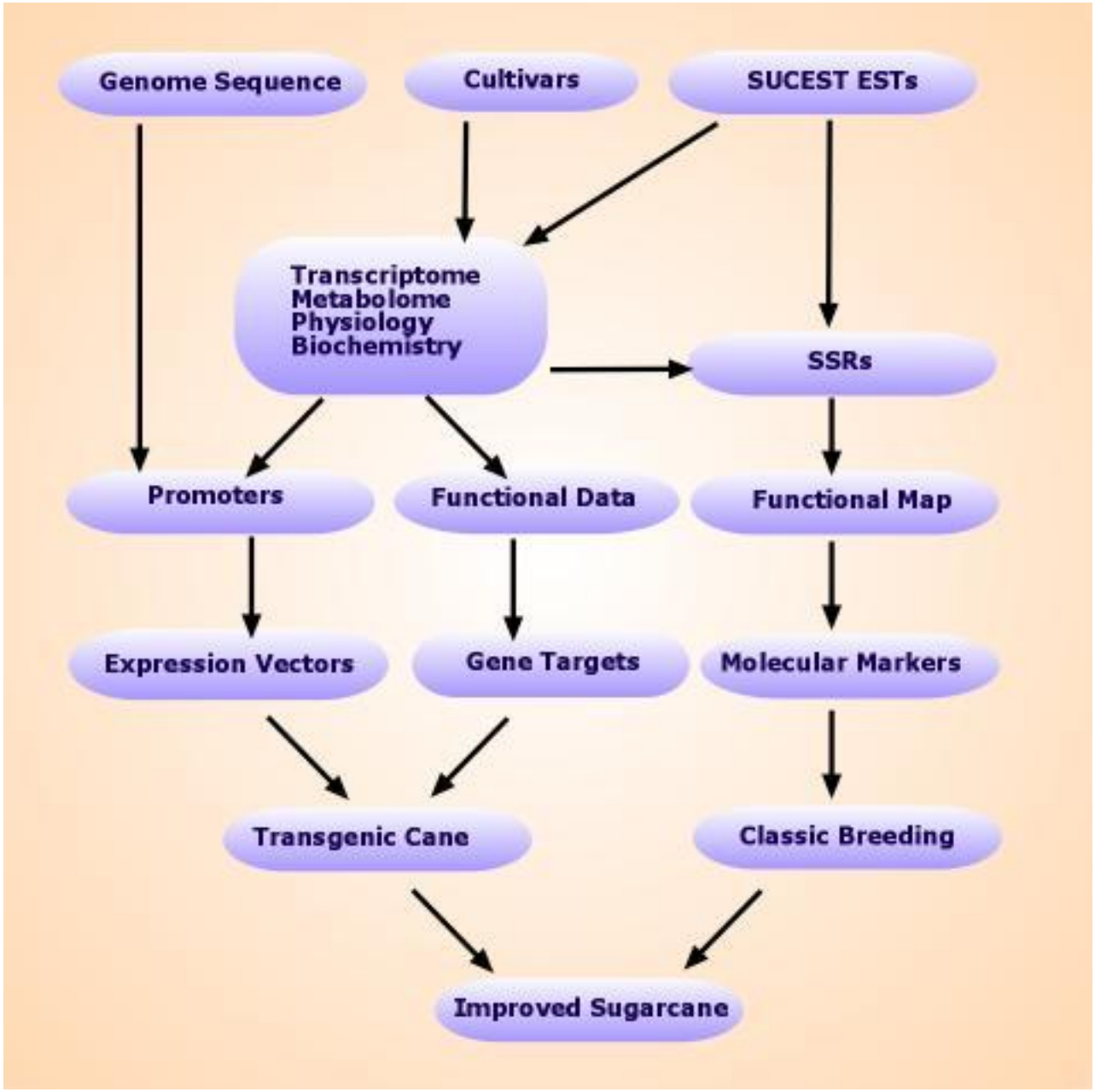
ABRE3HVA1 - ABA responsive element

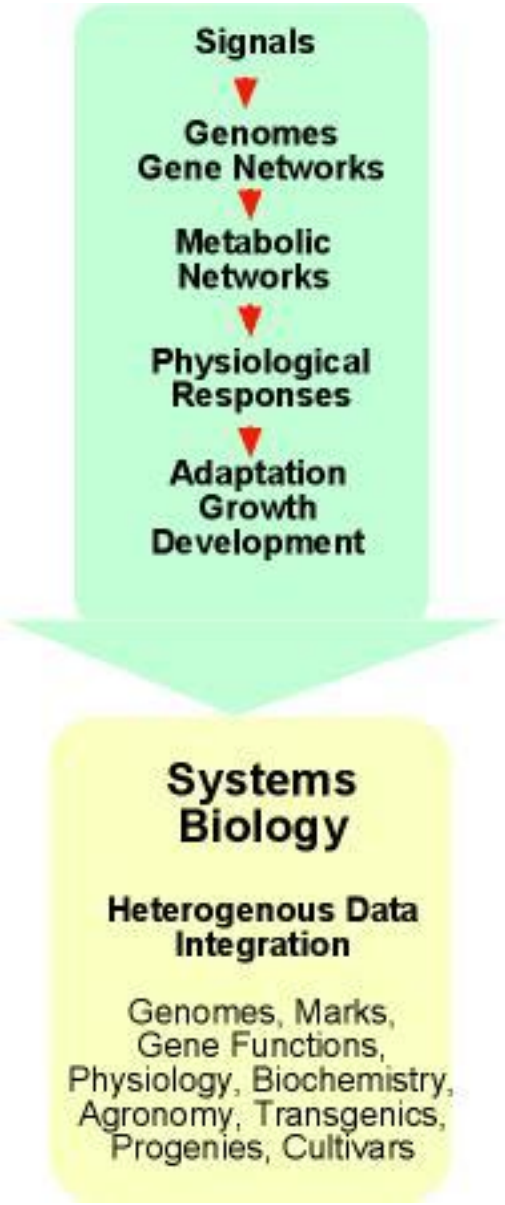
- Genome sequencing
- Comparative, structural and evolutionary genomics of grasses
- Development of new sugar cane cultivars
- Biochemical, physiological and agronomic evaluation of cultivars
- Discovery of genes associated with agronomic characteristics of interest
- Analysis of transcriptome, proteome and metabolome
- Signaling, regulation of gene expression and regulatory networks
- Genetic transformation of sugar cane and other grasses
- Molecular markers
- Physical, genetic and molecular mapping of genomes
- Metabolic networks of the production of carbohydrates and sucrose
- Cell wall structure and function, cellulolytic fungi
- Bioinformatics
- Impact of climate changes, sustainability and productivity
- Environmental impact of GM sugar cane and biosafety
- Intellectual property and technology transfer


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
Microoalgae, funghi, bacteria for metabolite synthesis and cellulosic ethanol production
Sub-project 15
Bioprospection
NewLignino-cellulolytic Microorganisms
Funghi, bacteria with hydrolases for cellulosic ethanol
Enzymes
Sub-project 16
Biochemistry
Structural and molecular characterization of hydrolases
IP, market, economics
Sub-project 17
Studies in intelectual property, impact, market, licencing, value, Freedom to operate
Interaction with the Innovation Technology Agencies
Sub-project 1
Tools
Genes
PKs
PPases
TFs
TEs
cell wall
photosynthesis
C fixation
hormones
Orfeome
OE constructs
SI constructs
E. coli expression constructs
Abs
Reporter 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 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 11
Phenotypic Evaluation
Physiological, Molecular and Biochemical Characterization
Transcriptome, Metabolome
Transient and stable expression constructs
Sub-project 9
Sub-cellular Protein Targeting
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
Sub-project 11
Physiology
hormone, growth, development, cell wall, stress responses, carbohydrates, nutrition, C flow, photosynthesis, use of water
Sub-project 8
Progenies contrasting for brix and lignin
Genotypes contrasting for drought and herbivory
Breeding Program
Agronomical evaluation
Field experiments
Crosses and selection

CaneDB


Genomic & Functional Resources Literatures & Patents DB
GRASSIUS
Existing Databases of Rice, Sorghum, Maize & Sugarcane
NCBI & KEGG
CaneTFDB
Sugarcane
Sorghum
Maize
Rice
CanePromDB
Sugarcane
Sorghum
Maize
Rice
CanePKDB
Sugarcane
CanePPaseDB
Sugarcane
CaneTransgenicDB
Sugarcane
CaneGeneExpress
Sugarcane
CaneREGNET
Sugarcane
Sugarcane
Sugarcane
CaneDB
Annotation tools for curation & Web Services
Promoters with 5-7kb upstream and 2kb downstream by Genome Walking/BACs/genomic sequences and identification of orthologs
Identification of clusters of direct targets based on ChIP-HTS and expression data
Data and Tools from cDNA Microarray and Agilent oligo Chips
PPases classified by families
Establish orthologous gene pairs between the grasses
Identification of consensus binding sites from literature experiments and predict CREs by using PWM
TFs classified by families
Data from genotypes, phenotypes, cultivares transgenics
PKs classified by families


Genome Sequence
Cultivars
SUCEST ESTs
Transcriptome
Metabolome
Physiology
Biochemistry
SSRs
Promoters
Functional Data
Functional Map
Expression Vectors
Gene Targets
Molecular Markers
Transgenic Cane
Classic Breeding
Improved Sugarcane



•Basic and biotechnological research on sugar cane and other biofuel plants

•Broad and interdisciplinary

•Systemic approaches on sugar cane biology

•Relation among genome, metabolism, physiological and adaptive responses to the environment

•Effective impact on the improvement of cultivars of interest

•Paths to genetically manipulate the energy metabolism of cultivated plants

•New alternatives of biofuel

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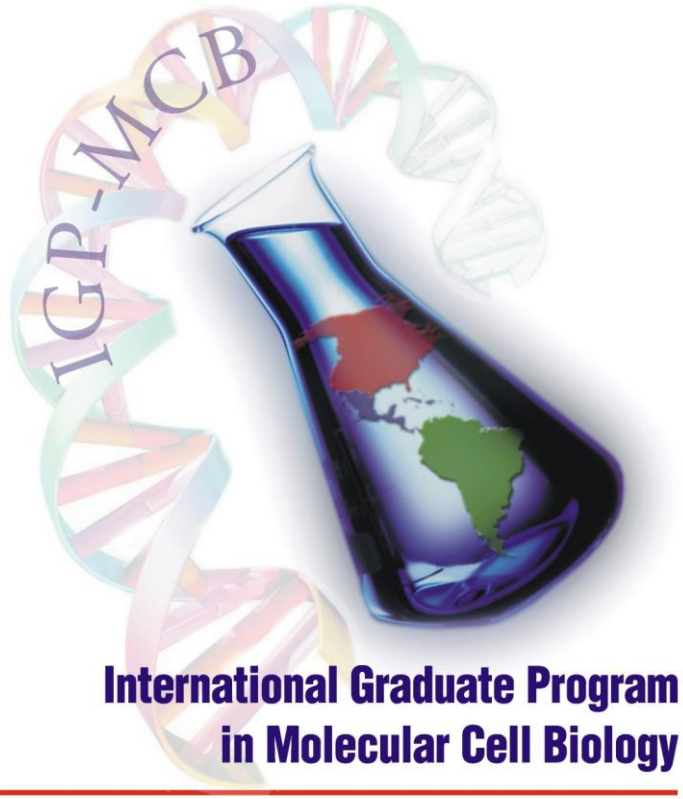

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"Plant Sciences"

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