ADVANCES IN GENOMICS OF SUGARCANE

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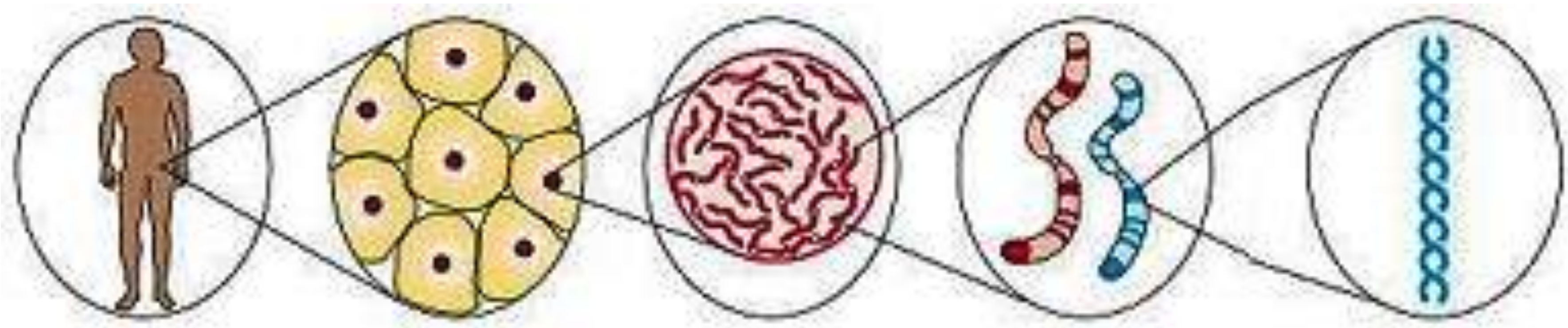
São Paulo Advanced School on the Present and Future of Bioenergy. Campinas, 10.11.2014

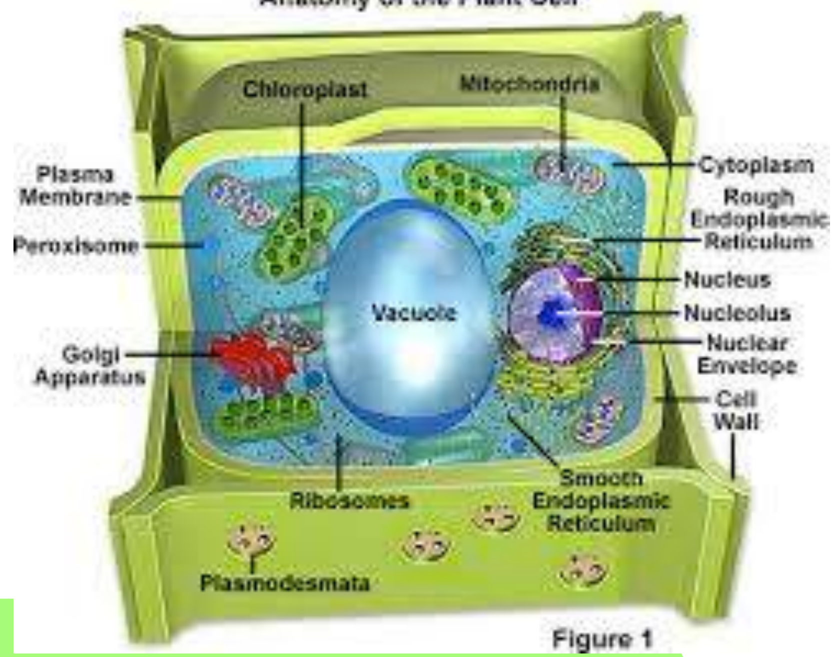
Talk overview

- Fundamentals in genes, genomes and biotechnology
- What is genomics
- Genomic resources for sugarcane
- Strategies to discover useful genes
- Biotechnology for biomass production

DNA and genomes

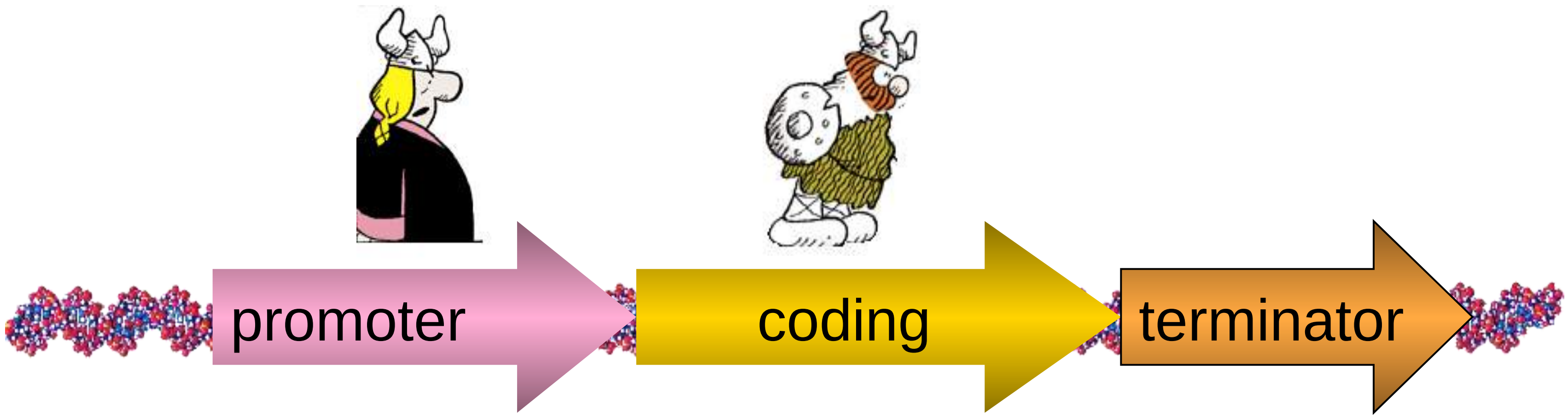


organism cells nucleus chromosomes DNA



Genome is the entire DNA content of an organism (nucleus, mitochondria and chloroplasts)

Molecular structure of a gene



...ATCGTGATATACTAGACGATGCATGACCACCCAACTGAACTGTAACTTTAAC...

Where:
- leaves
- roots

When:
- germination
- insect attack

How much:
- 1, 10, 1000.

What:
Bactericidal protein
Iron transporter
Sucrose synthase

End of the gene

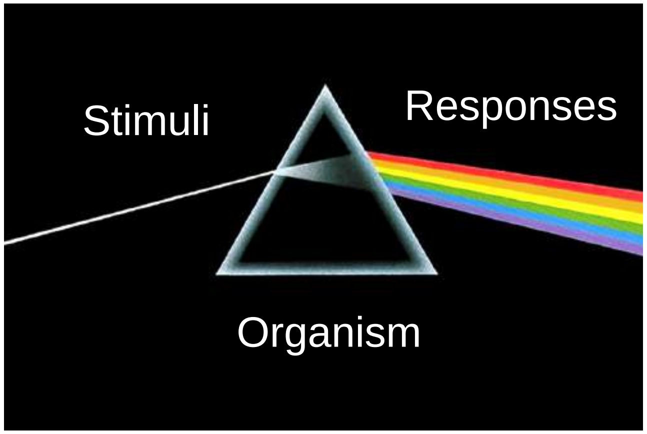
Selecting interesting genes

Goose bumps

High hear rate

Adrenaline increases

Pass out



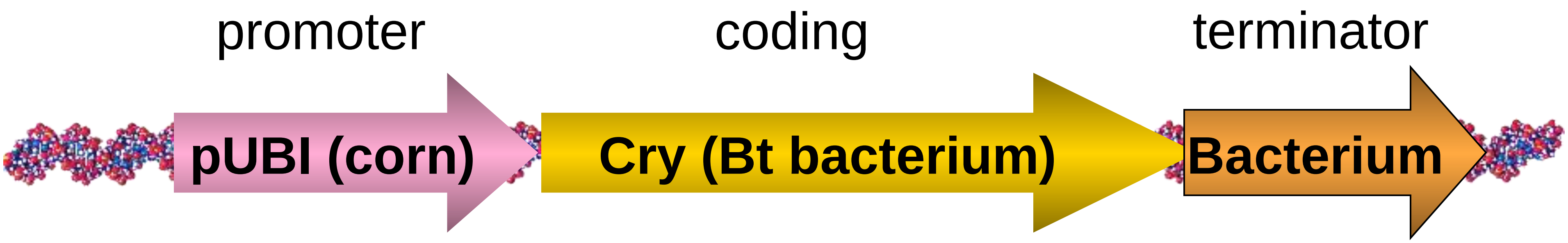
What are the defenses activated by a plant under stress?

Do individuals with distinct levels of tolerance have different responses?

Maximizing artificially a response might increase plant performance?

Transgenes & Recombinant DNA

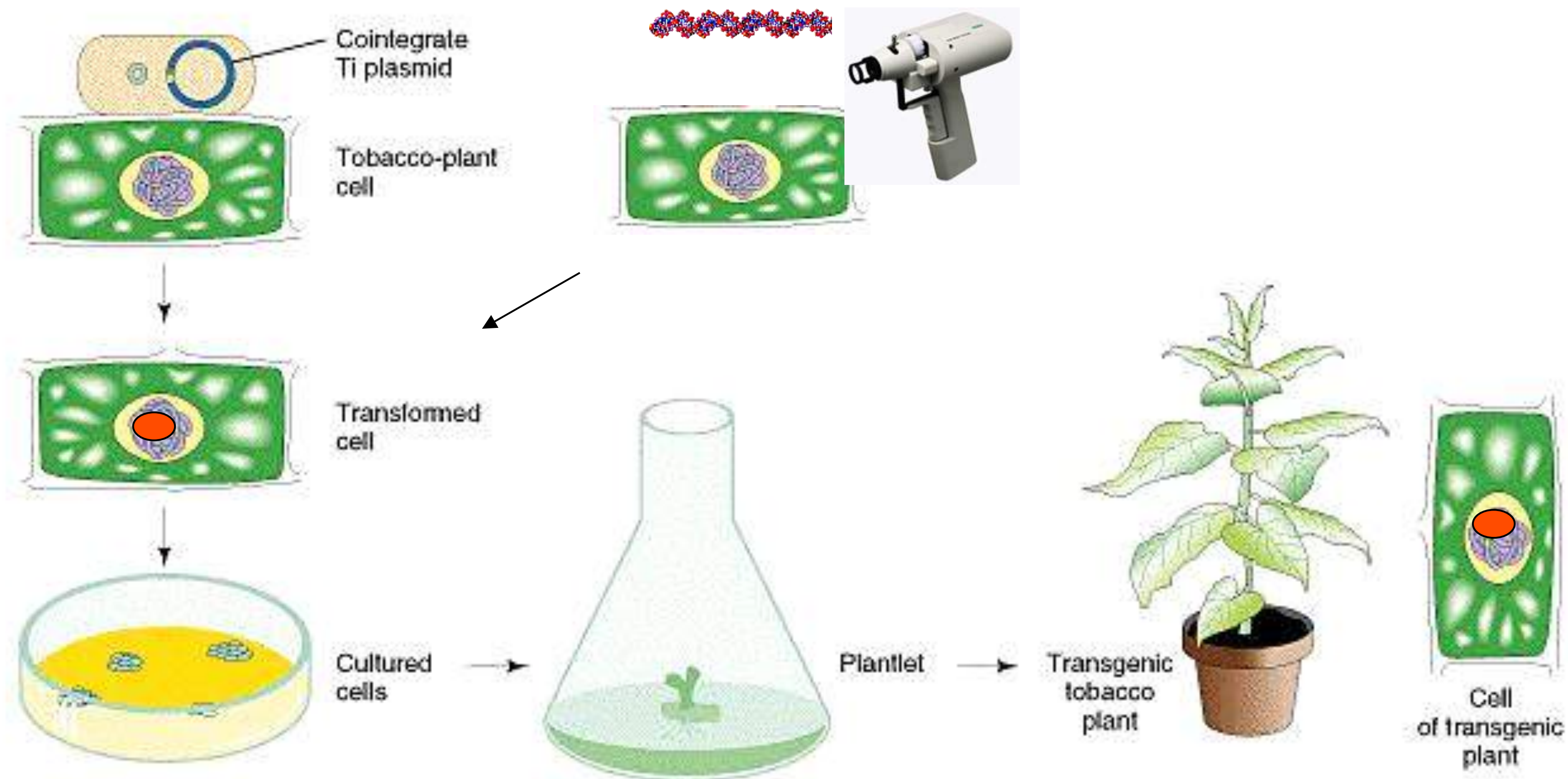
M14.2 Khimaira battles Pegasos & Bellerophon



Expression cassette: contains a promoter, a coding region and a terminator.
It is a synthetic gene

Genetically modified organism

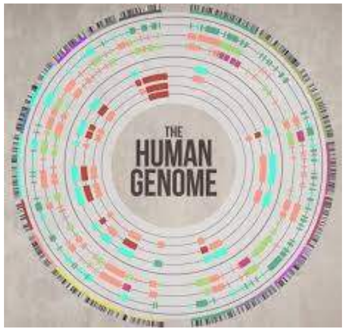
Agrobacterium Biolistics



- A GMO contains one or more synthetic genes integrated in its genome
- Transgenes can increase the production of a given protein (overexpression) or can reduce the levels of an endogenous protein (suppression).

Genomics

Genomics aims the uncover the structure of genomes and how the different pieces of a genome work during the lifetime of an organism.



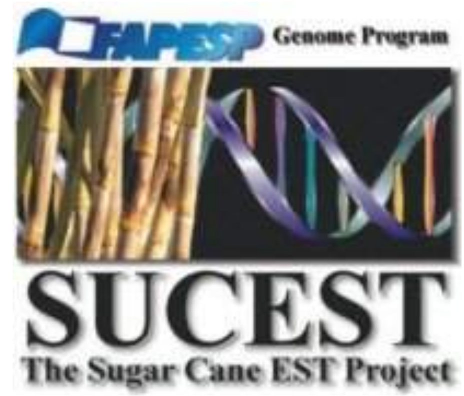
Genomics uses DNA sequencing methods and computing science (bioinformatics) not only to sequence but also to estimate how different genes are modulated during the development, in response to environmental clues, etc.

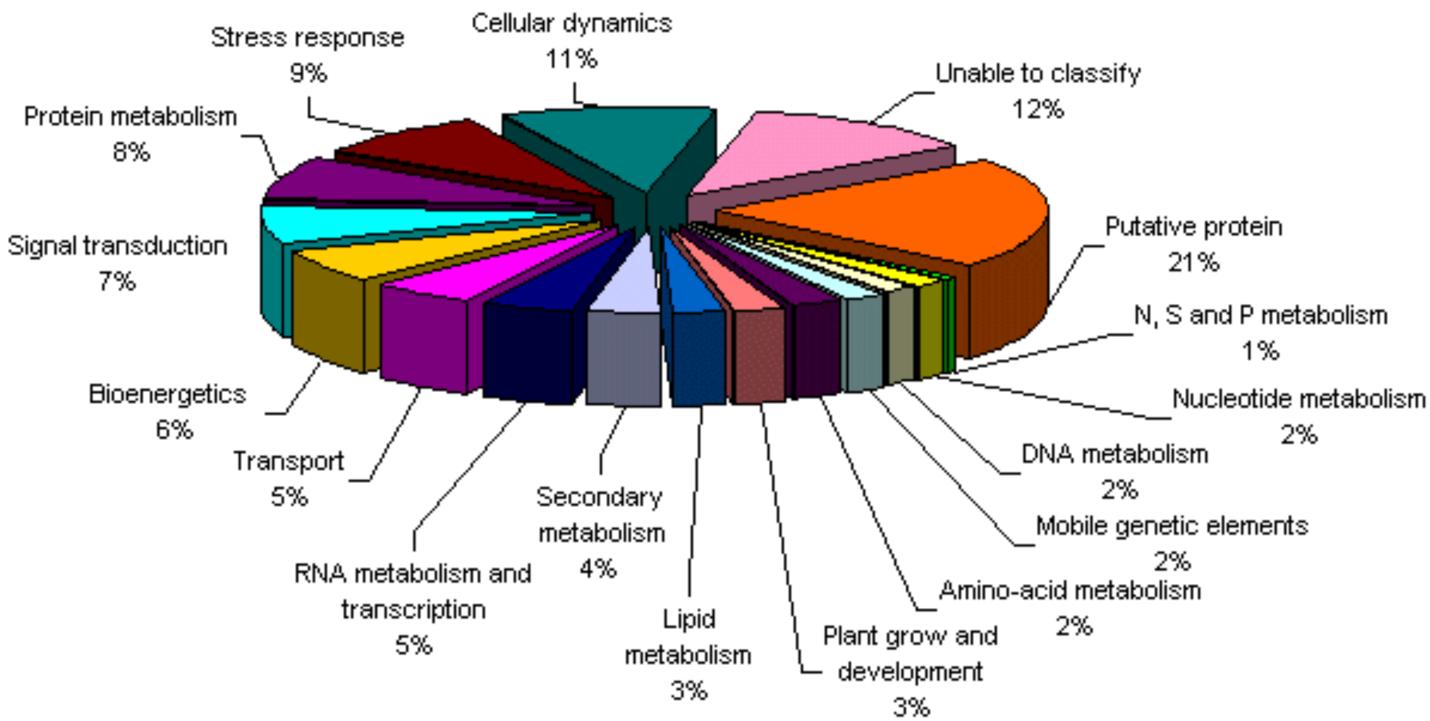
Recombinant DNA, genetic engineering and biotechnology are also used in genomics to understand genome structure and gene function

Genetic Engineering
Rob Frankle

Genomic resources: Sugarcane EST Project – SUCEST

Finished in 2003
Allowed the identification of 43,143 sugarcane genes





Genomic resources: SUCEST-FUN

SUGARCANE FUNCTIONAL GENOMICS DATABASE

http:://sucest-fun.org (Glaucia Souza´ lab)

Contains all sugarcane EST sequences

68,383 expression data points (DNA chips, 2012)

Proteomics, metabolomics and other data to be integrated in the future

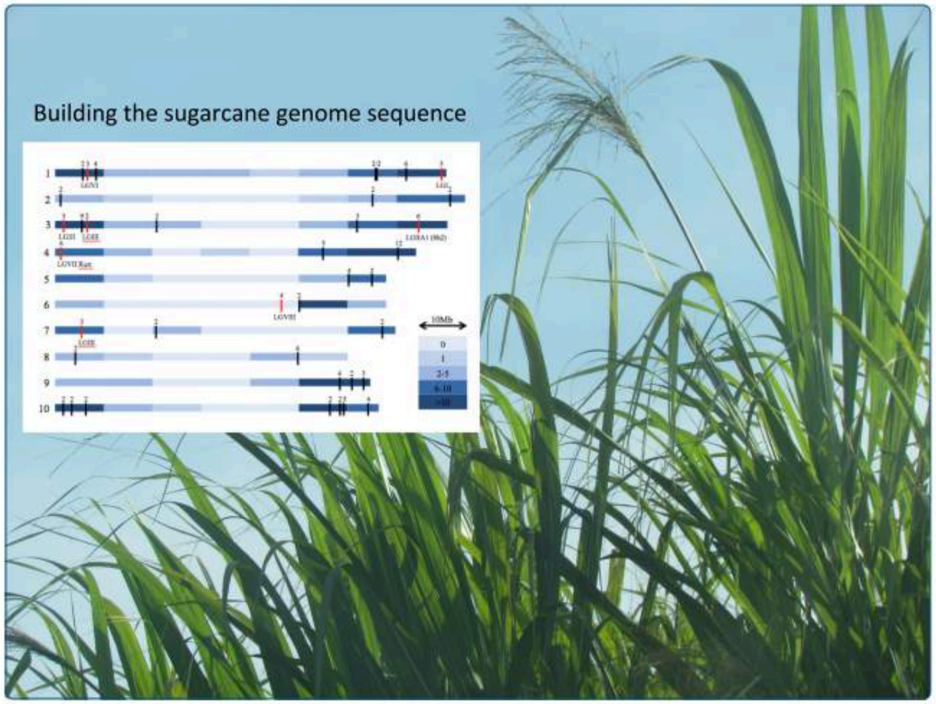
Genomic resources: Sugarcane genome

317 Bacterial Artificial Chromosomes (130 kb on average)

36.58 Mb sequenced (3.7% of the 1 Gb sugarcane genome)

Repetitive elements correspond to 50% (49.4% transposable elements and 0.43% satellite repeats)

1,400 gene sequences were found



Work leaded by
Marie-Anne van Sluys´group

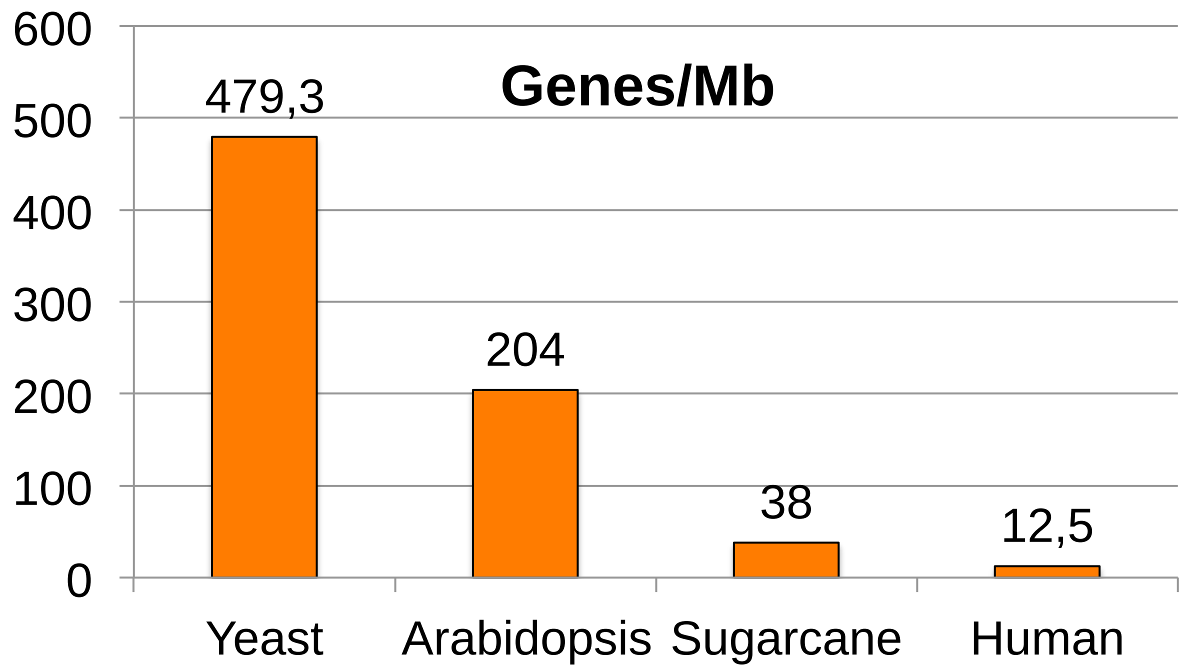
Building the sugarcane genome for biotechnology and identifying evolutionary trends

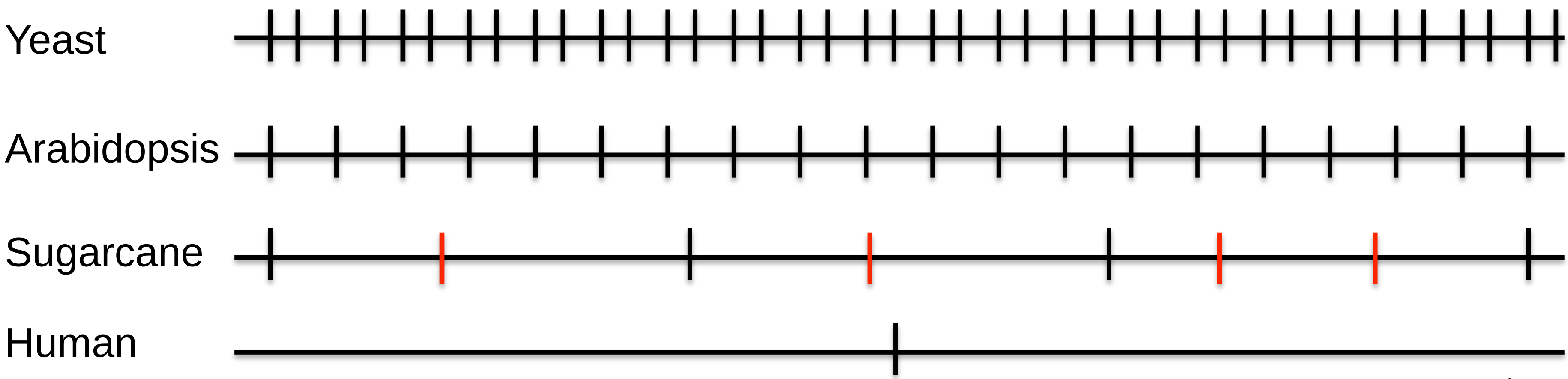
de Setta *et al.*

BioMed Central

de Setta *et al. BMC Genomics* 2014, **15**:540
http://www.biomedcentral.com/1471-2164/15/540

Gene density in different organisms



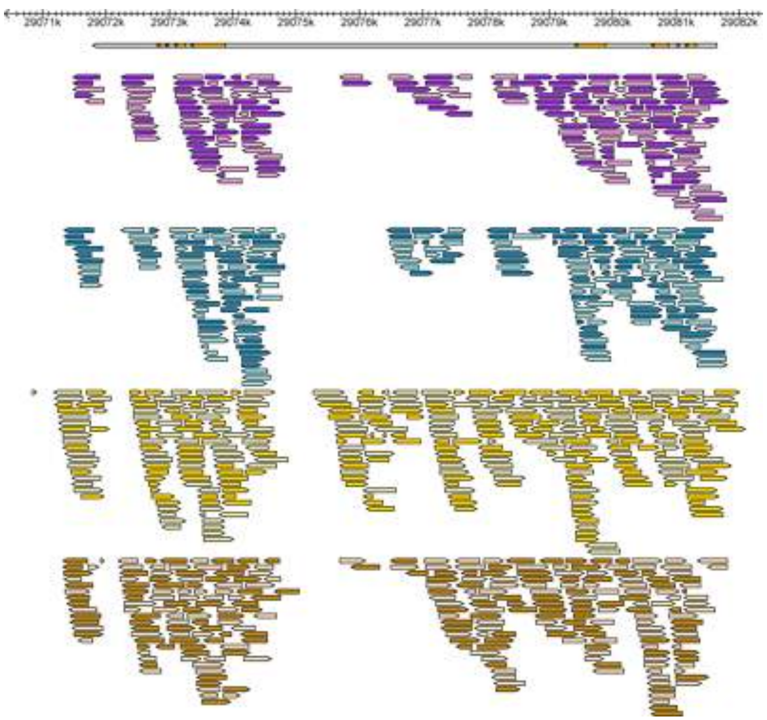


Genomic resources - the missing link: RNAseq database

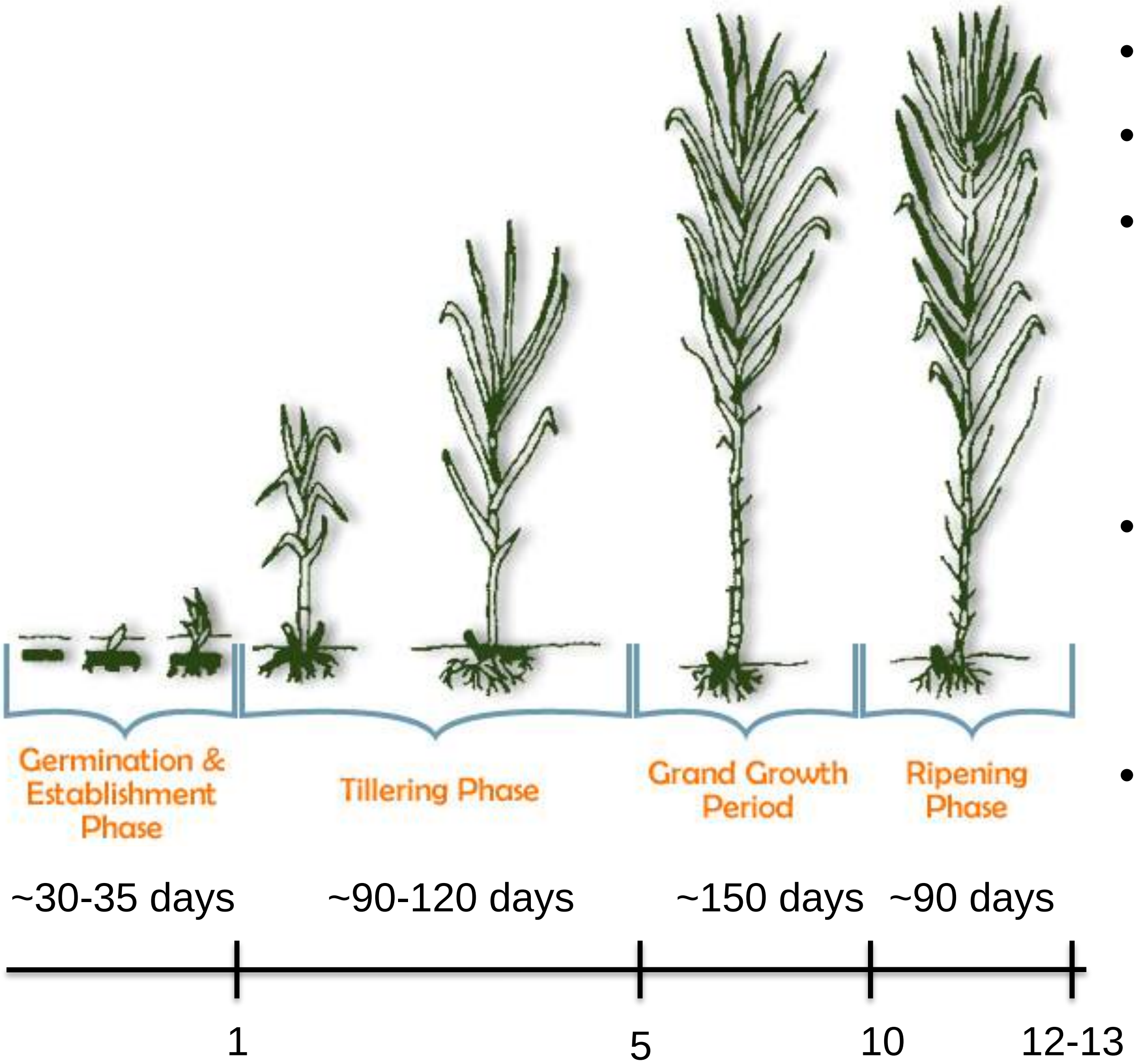
RNAseq is a method that sequences the transcribed RNA in a sample

Nowadays, it is the most powerful method to identify genes that are expressed and also to quantify their expression levels

There are hundreds of sugarcane samples that have been sequenced by RNAseq, but there is no database with this information organized in a user-friendly way.



Biomass production



- 84 t/ha/year fresh weight;
- 21 t/ha/year of sugar;
- 39 t/ha of dry stalks and trash per year, higher than Miscanthus (29.6), switchgrass (10.4) and maize (17.6);
- Ethanol production 7,500 L/ha, and can be increased to 13,000 L/ha with cellulosic ethanol
- Theoretical maximum 381 t/ha

Source: Kuyper, 1952. http://www.sugarcanecrops.com/

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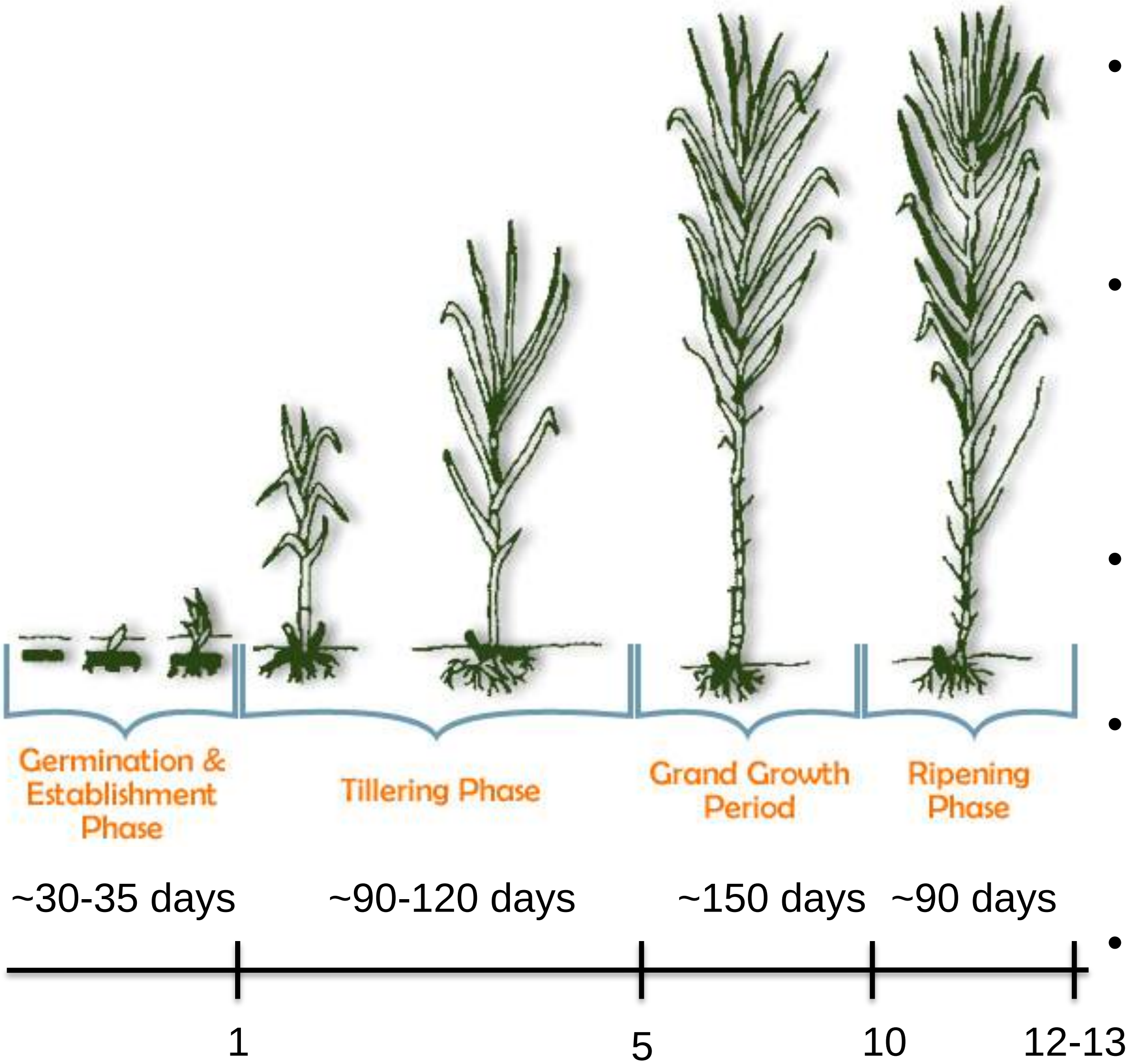
Sugarcane development and drought



Inman-Bamber and Smith. Field Crops Research 92 (2005) 185–202

- Drought reduces germination, tillering and expansion from leaves and stalks
- During early expansive growth, drought has little effect on sucrose yield at the harvest
- Water supply during stalk elongation phase is critical
- During the ripening phase, water deficit contributes to increased sucrose levels
- Losses of ~5-10% in sugar yield are usual every year in regions scattered around Brazil

Source: Kuyper, 1952. http://www.sugarcanecrops.com/

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Yield penalties due to drought in Brazil

Year	Region	Losses
2008	São Paulo State	6.3% (Castro, 2008)
2010	Zona da Mata (Pernambuco State)	40% (Cavalcanti, 2010)
2011	Mata Norte (Pernambuco State)	30% (Associação dos Fornecedores de Cana de Pernambuco, 2010)
2012	Colônia Leopoldina (Alagoas State)	20% (Agência Glogo, 2012)
2012	Northeast Region	20% (Fraga, 2012)
2012	Pernambuco State	35% (Camarotto, 2012)
2013	Paraiba State	30% (Silva, 2013)
2013	Alagoas State	15% (Sindaçucar, 2013)
2013	Zona da Mata (Pernambuco State)	25% (Brasilagro, 2013)

Main questions

- What is the genetic and physiological basis underlying the different levels of biomass accumulation under drought observed in sugarcane cultivars?

- What are the genes/proteins that will help us to enhance biomass accumulation under drought in sugarcane?

We use genomics approaches to address these questions

Recent data from other groups showing how genomics can change the composition of sugarcane biomass

Gene expression profiling in greenhouse plants

DNA chip with 1545 sugarcane genes

- Hormones: methyl jasmonate and ABA
- Insect attack
- Phosphate starvation
- Interaction with nitrogen-fixing bacteria
- Drought

Table 1:

MeJA			ABA			Herbivory			Phosphate starvation			*Gluconacetobacter*			*Herbaspirillum*			Drought		
Cy3		Cy5	Cy3		Cy5	Cy3		Cy5	Cy3		Cy5	Cy3		Cy5	Cy3		Cy5	Cy3		Cy5
0h (C1)	vs.	1h (E1)	0h (C1)	vs.	30min (E1)	30min (E1)	vs.	30min (C1)	6h (E1)	vs.	6h (C1)	E1	vs.	C1	E1	vs.	C1	24h (C1)	vs.	24 h (E1)
0h (C1)	vs.	6h (E1)	0h (C1)	vs.	1h (E1)	24h (E1)	vs.	24h (C1)	12h (E1)	vs.	12h (C1)	C2	vs.	E2	C2	vs.	E2	72h (C1)	vs.	72 h (E1)
0h (C1)	vs.	12h (E1)	0h (C1)	vs.	6h (E1)	30min (C2)	vs.	30min (E2)	24h (E1)	vs.	24h (C1)							120h (C1)	vs.	120 h (E1)
1h (E2)	vs.	0h (C2)	0h (C1)	vs.	12h (E1)	24h (C2)	vs.	24h (E2)	48h (E1)	vs.	48h (C1)							24 h (E2)	vs.	24h (C2)
6h (E2)	vs.	0h (C2)	30min (E2)	vs.	0h (C2)				6h (C2)	vs.	6h (E2)							72 h (E2)	vs.	72h (C2)
12h (E2)	vs.	0h (C2)	1h (E2)	vs.	0h (C2)				12h (C2)	vs.	12h (E2)							120 h (E2)	vs.	120h (C2)
			6h (E2)	vs.	0h (C2)				24h (C2)	vs.	24h (E2)									
			12h (E2)	vs.	0h (C2)				48h (C2)	vs.	48h (E2)									
SP80-3280												SP70-1143						SP90-1638		

179 genes, 93 modulated by drought stress (13 unknown proteins)

Integrating different approaches

Biotechnology

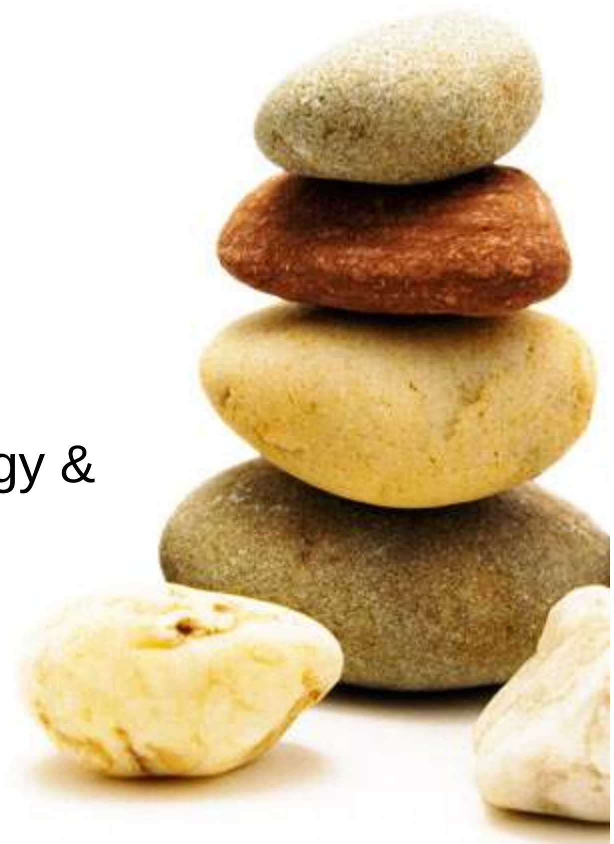
Proteomics

Transcriptomics &
Microtranscriptomics

Physiology &
Breeding

Metabolomics

Genomics



www.prospects.co.uk

Consortium for physiology, genomics, proteomics and biotechnology associated to drought stress in sugarcane

Laurício Endres (UFAL)

Monalisa S. Carneiro (UFSCAR)

Rejane J.M.C. Mansur (UFRPE)

Tercílio Calsa Jr. (UFPE)

Helaine Carrer (ESALQ/USP)

Glaucia M. Souza (IQ-USP)

Marcelo Menossi (Coordinator, IB-UNICAMP)

UFAL UFRPE UFPE UNICAMP

Field experiment

Lower tolerance: RB72454, RB855536 e RB855113
Higher tolerance: RB92579, RB867515 e SP79-1011

Plants cultivated in the field in AL, PE and SP,
Irrigated or rainfed (sequeiro)

Leaves and internodes collected after 3, 7 and 11 months;

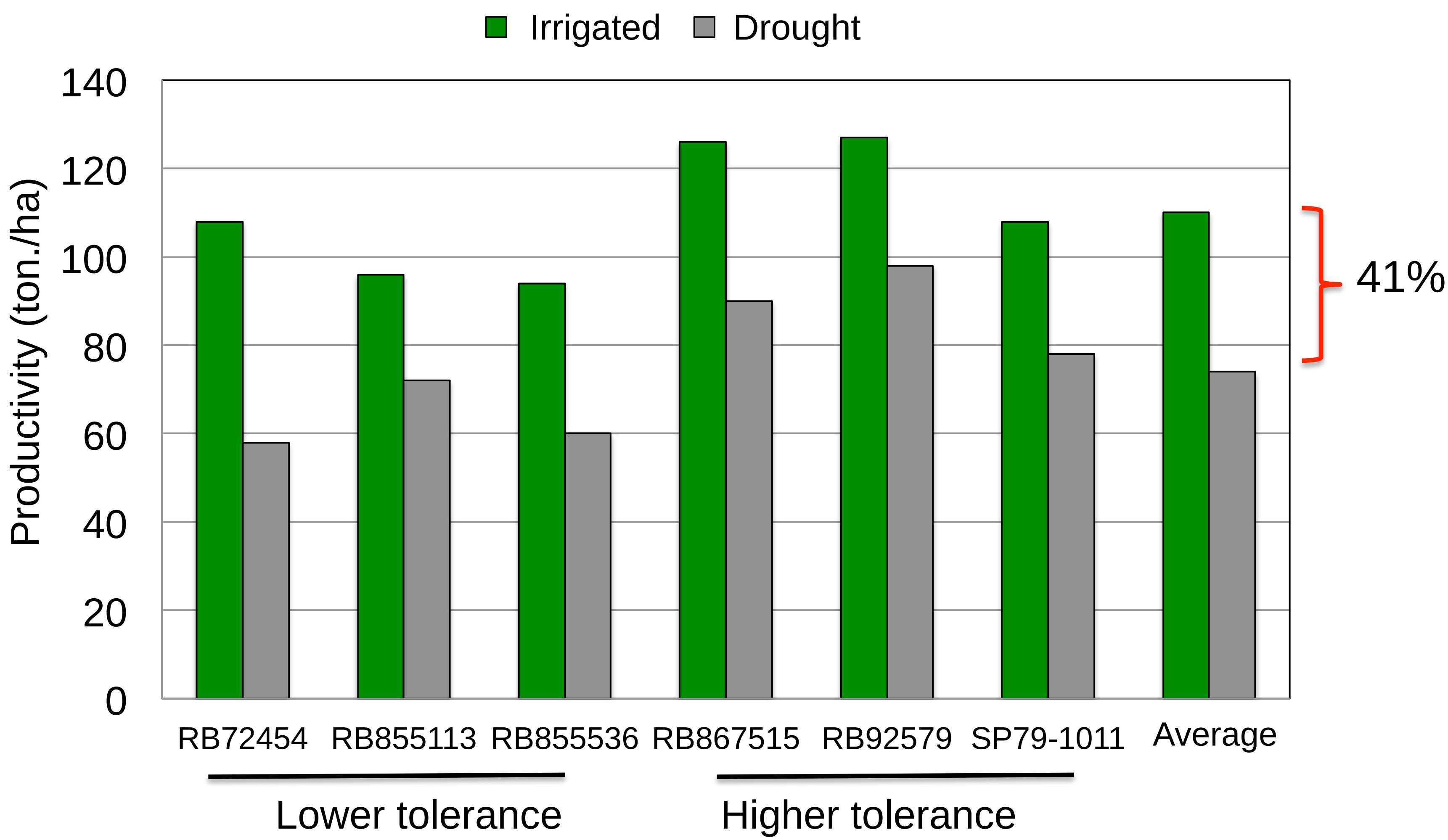
Several parameters were evaluated: gas exchange, proline content, leaf area, internode diameter, productivity, etc)

Drought (sequeiro)

Irrigated

RB867515, 7 months after planting

Drought and biomass accumulation in sugarcane

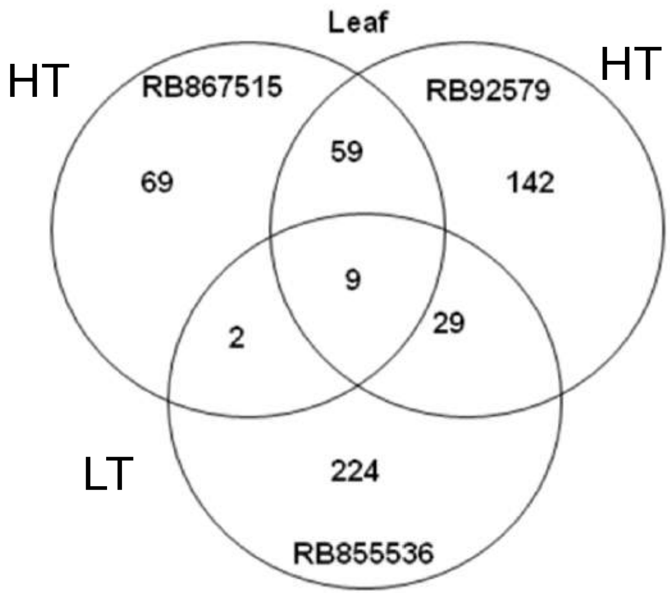


Sugarcane transcriptome under drought stress (7 MAP)

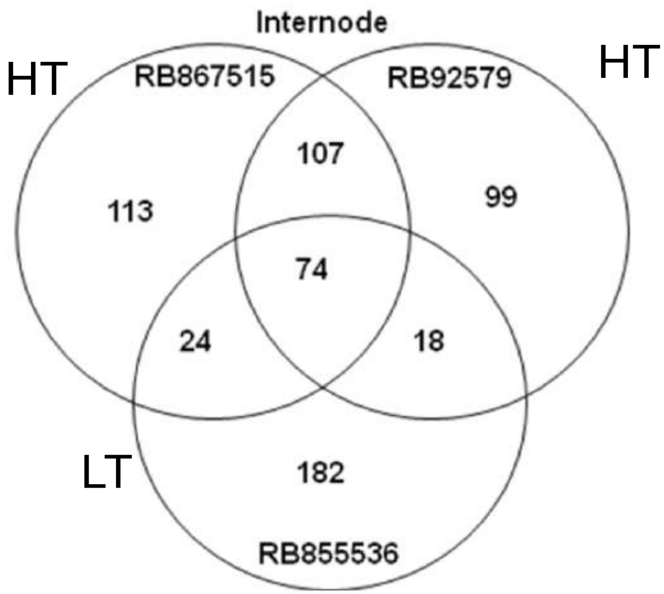
Agilent chips with 14,522 sugarcane genes
Leaves and first internode

Glaucia Souza

Maximiller Dal-Bianco



534 genes



787 genes

Functional analysis of selected genes

Cesar B. Souza

Andrea Hoshino

Kevin Begcy

Hypothesis

Genes that are induced by drought, when overexpressed in transgenic plants, enhance drought tolerance.

- genes induced by drought
- not covered by patents

Biotechnology

Proteomics

Genomics

Breeding /Physiology

Metabolomics

Transcritomics

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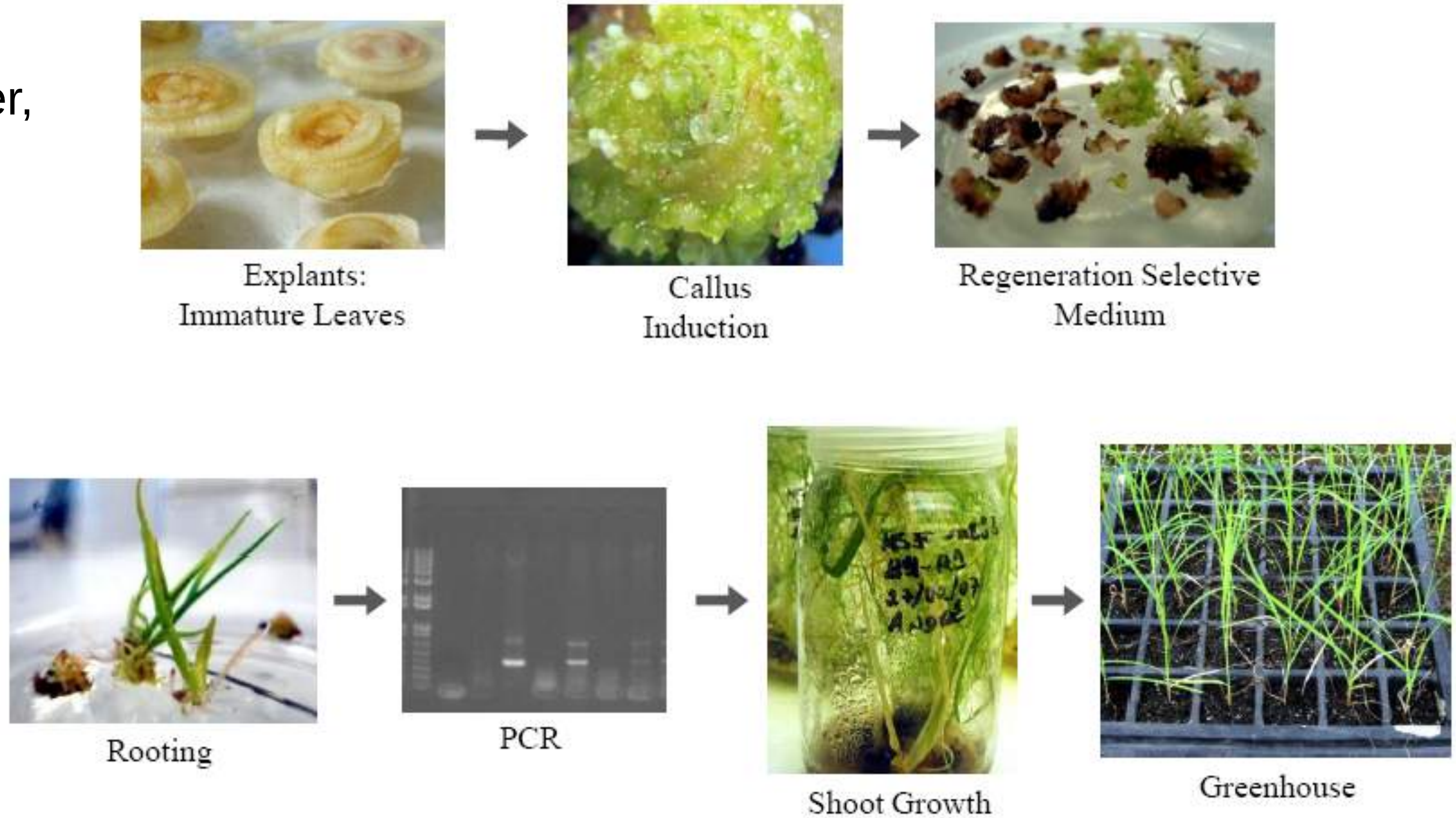
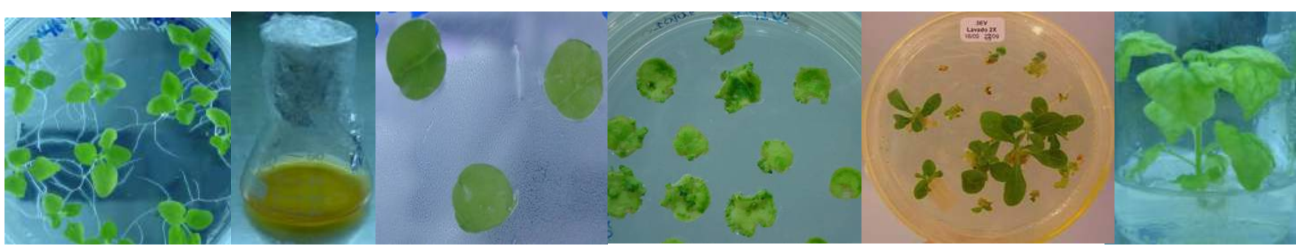
Assay in transgenic plants

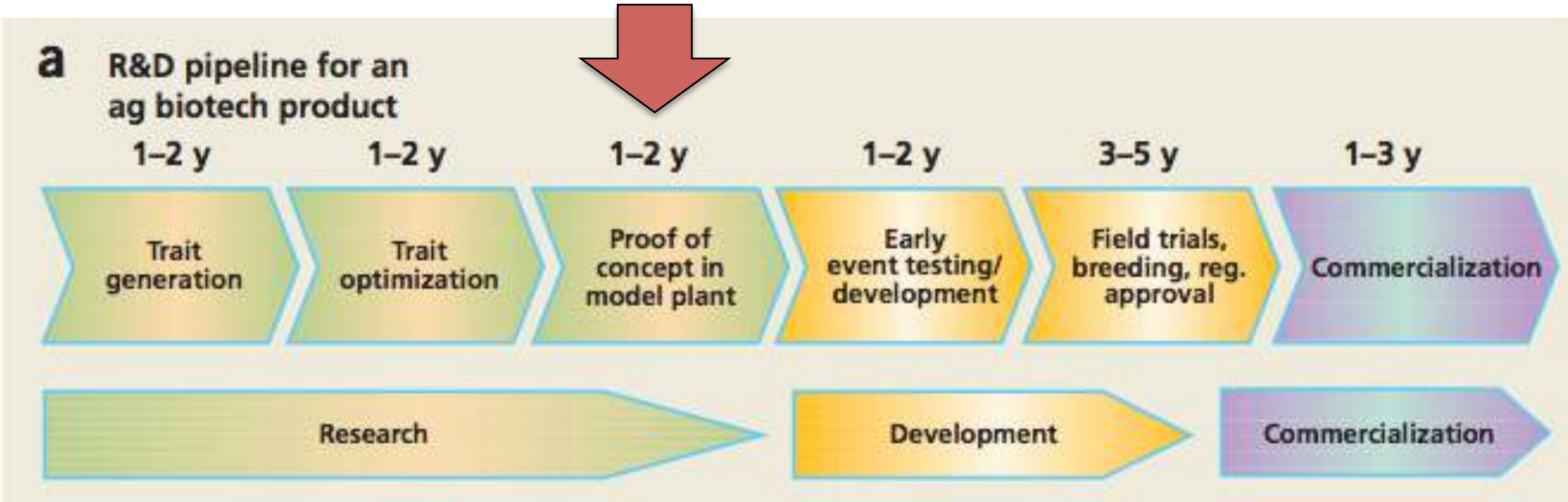
Sugarcane
(Helaine Carrer,
Esalq/USP)



Tobacco

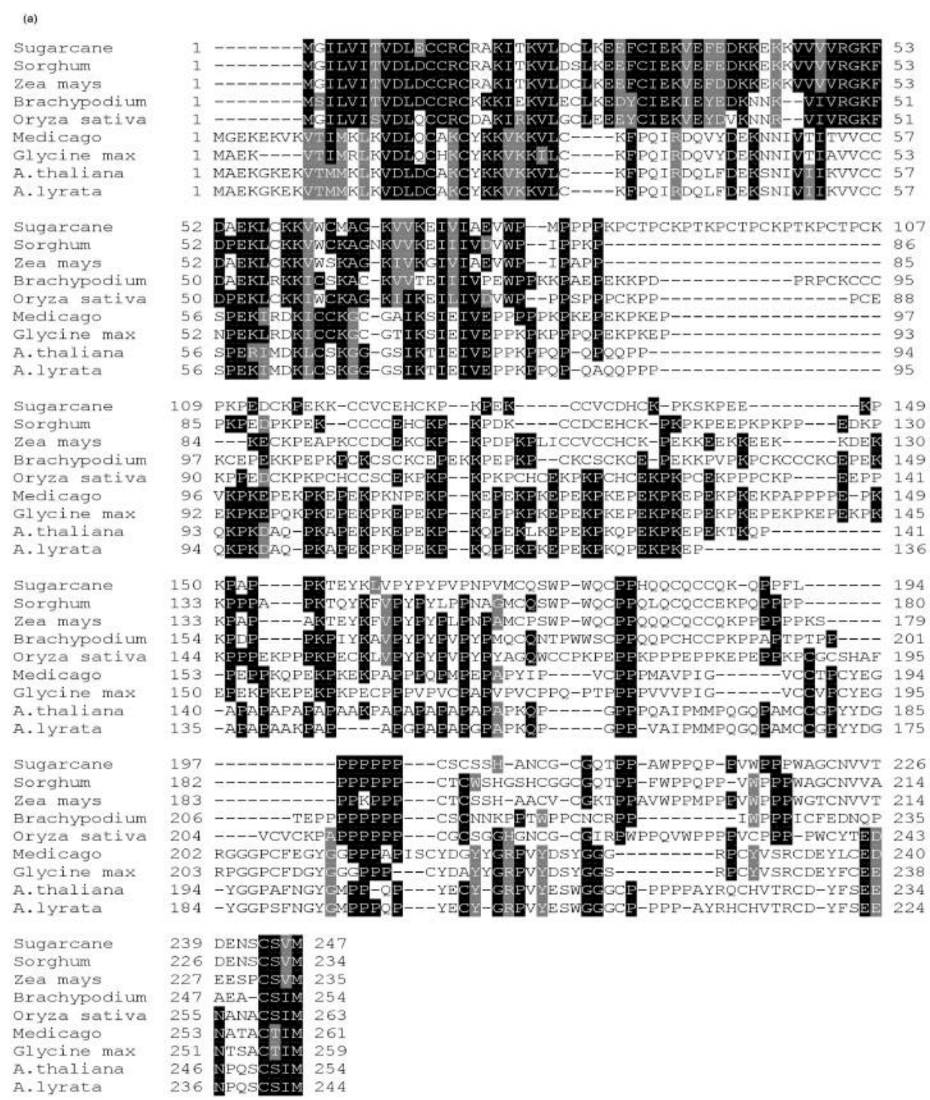


Assays in transgenic plants



McElroy (2004) Valuing the product development cycle in agricultural biotechnology—what's in a name? Nature Biotechnology 22:817- 822.

Scdr1: sugarcane drought-related 1



Menossi et al., Patent pending;
Begcy et al. (2012). A Novel Stress-Induced Sugarcane Gene Confers Tolerance to Drought, Salt and Oxidative Stress in Transgenic Tobacco Plants. PLoSONE 7(9): e44697

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Gene *Scdr1* confers tolerance to drought in tobacco

wt ***Scdr1-1*** ***Scdr1- 2*** ***Scdr1-3***

Control

Drought

Salt

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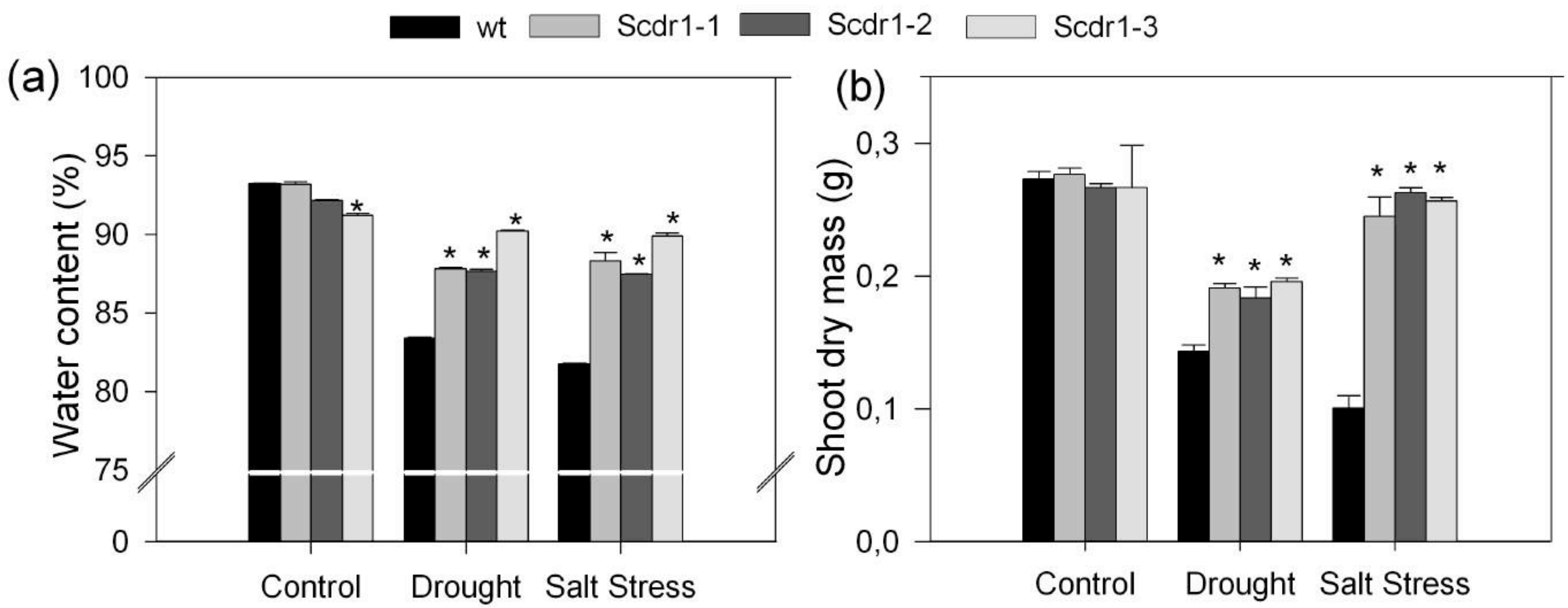
Water content and shoot biomass in *Scdr1* plants



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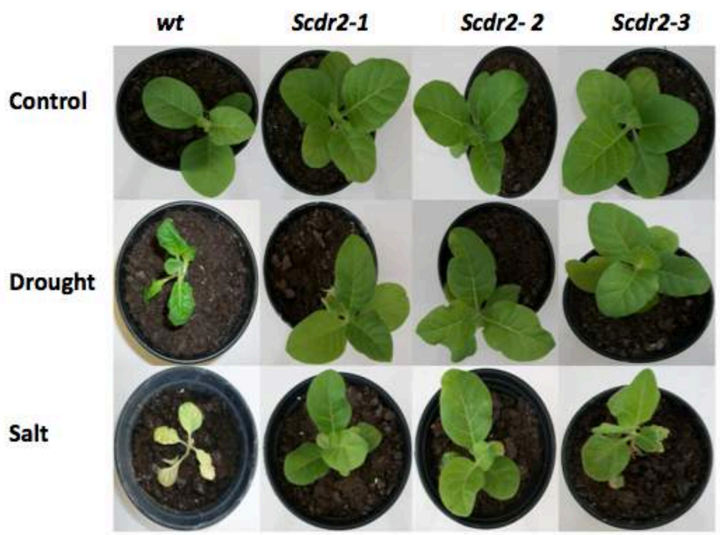
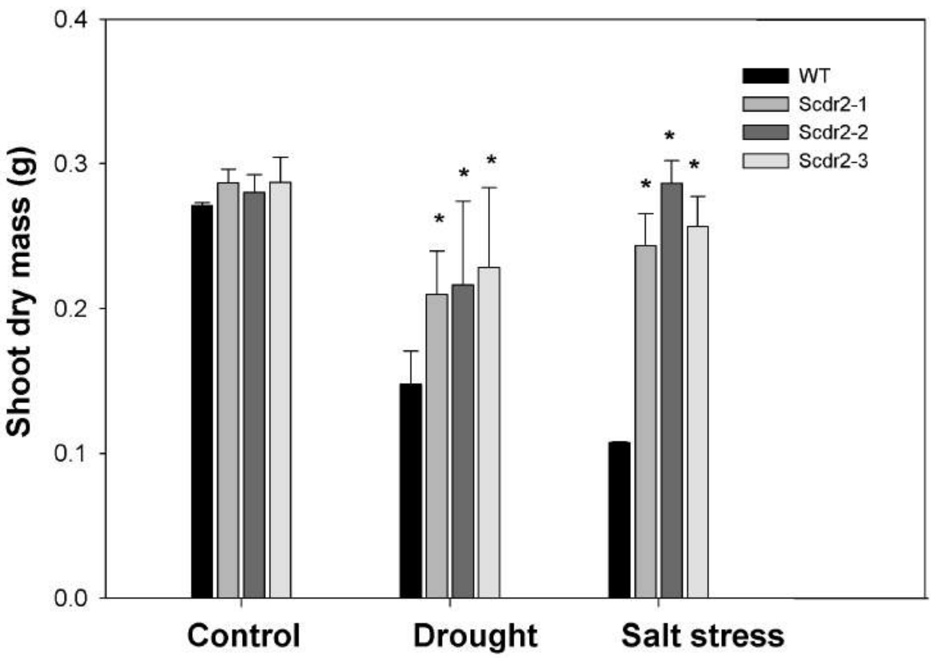
Scdr2 enhances biomass accumulation under stress



Kevin Begcy

Victoria Castro



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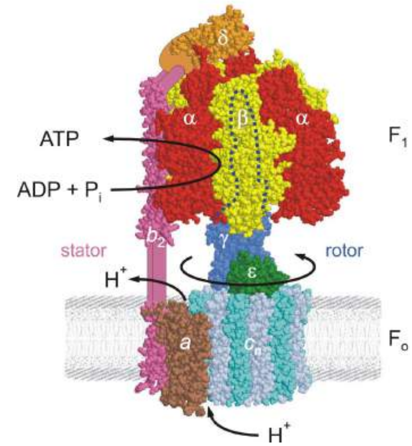
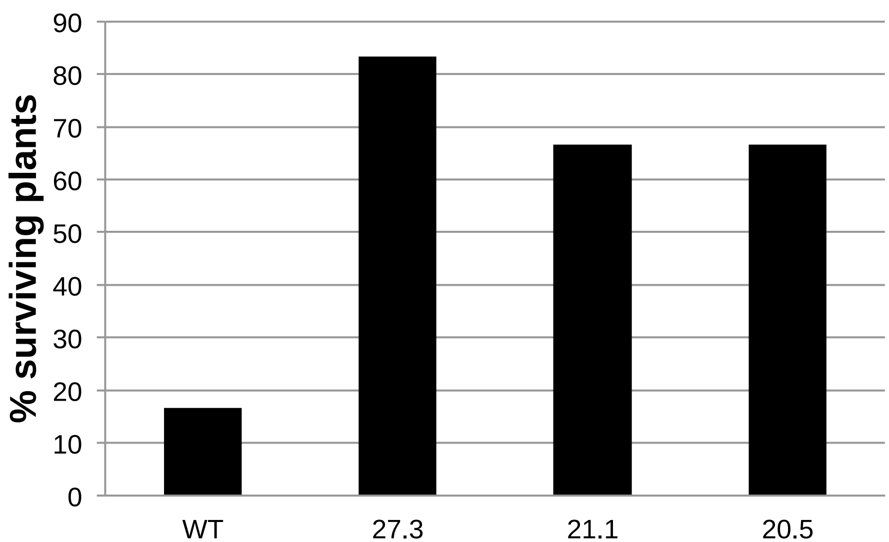
ScATPB - subunity of the ATP synthase complex

ATP synthase produces ATP from ADP in the presence of a proton gradient across the membrane



Two months-old plants, 15 days without watering and 3 days of rewatering



Marcelo Menossi

Menossi et al., Patent pending

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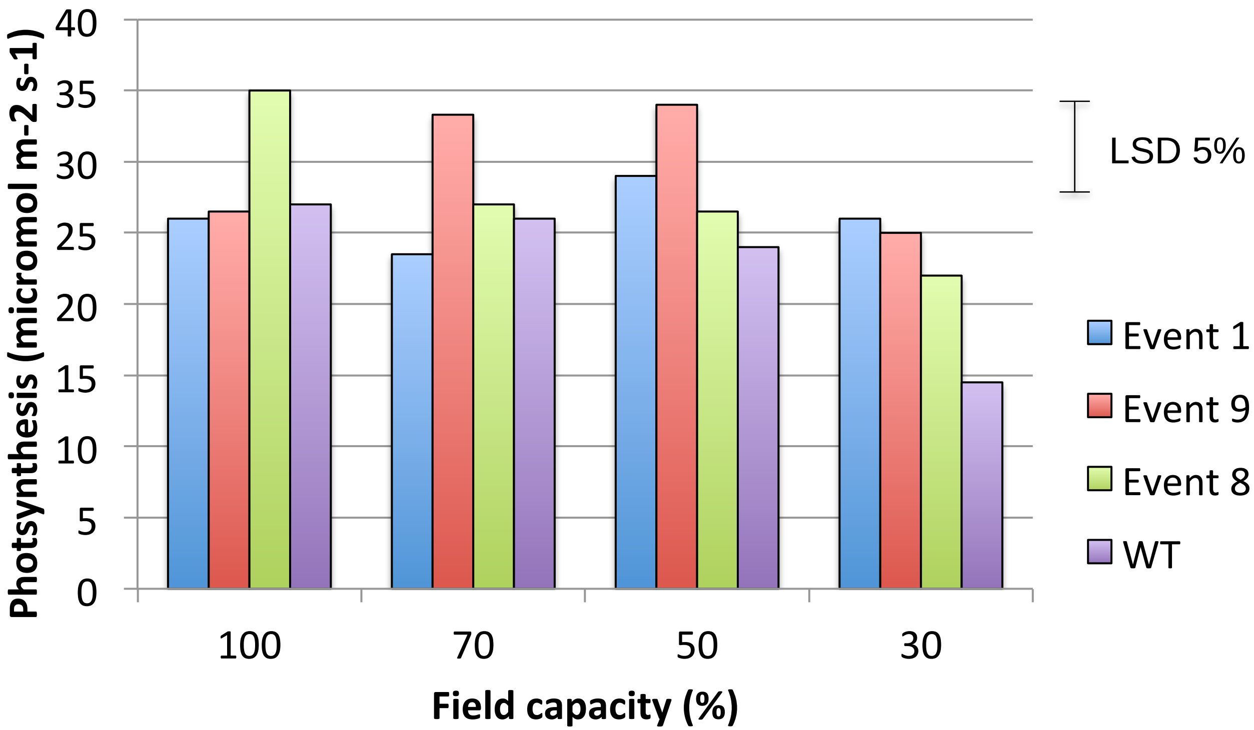
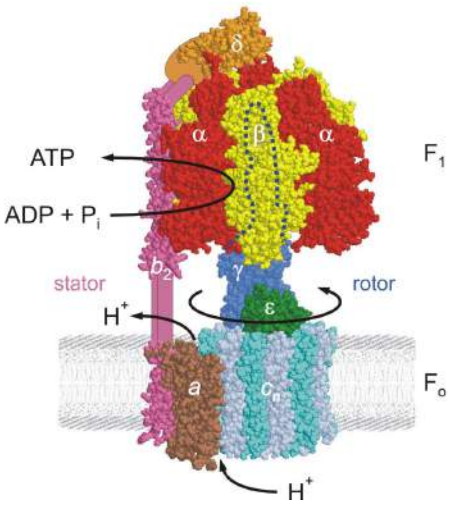
ScATPB overexpression in sugarcane

- 3 months-old plants were subjected to three different levels of water stress
- Preliminary data indicate the events with higher expression of the transgene Ubi::ScATPB have higher photosynthetic capacity under severe stress

Thaís H. Ferreira

Prakash Lakshmanan





Marcelo Menossi

Menossi et al., Patent pending

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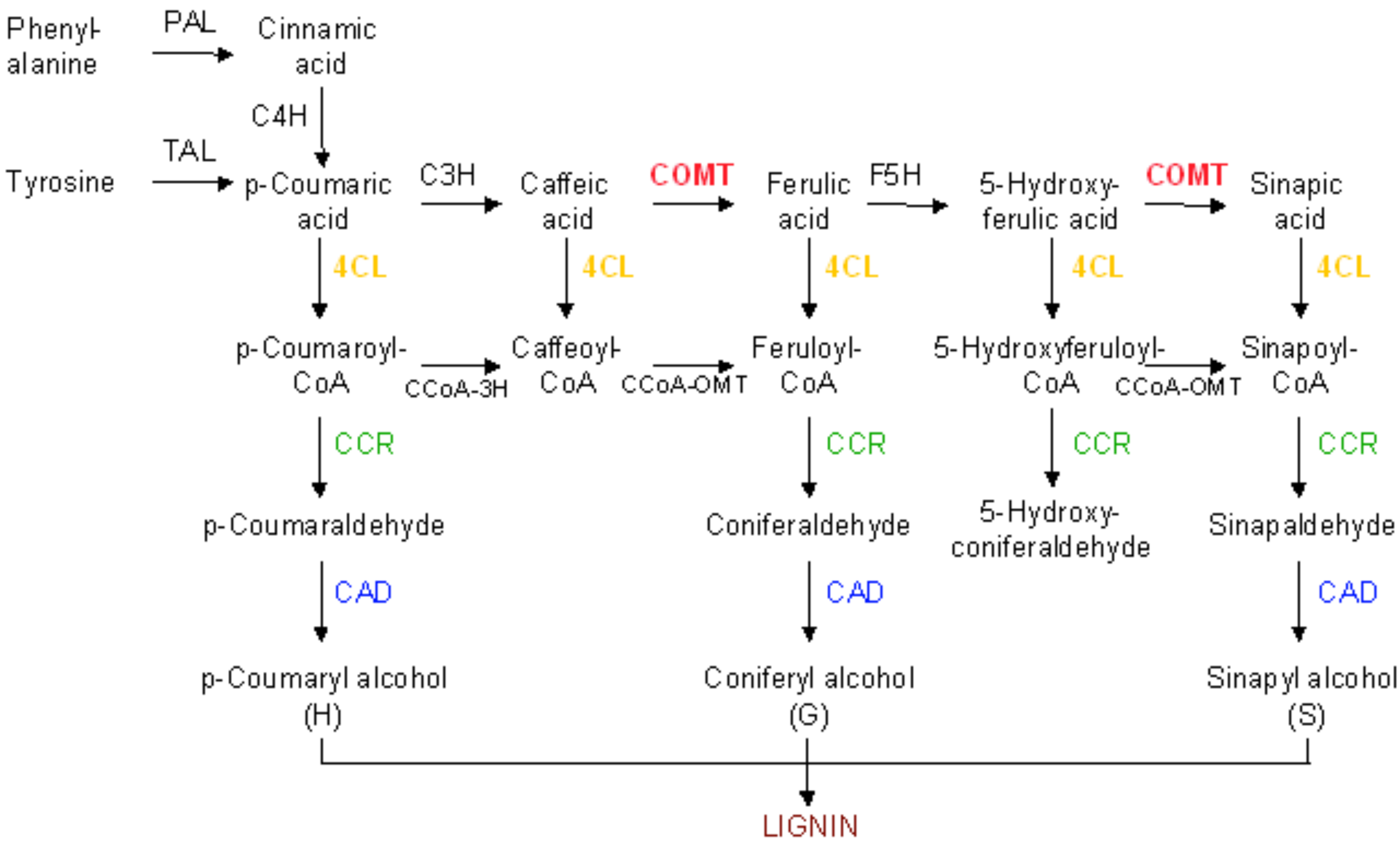
Suppresion of lignin biosynthesis in sugarcane

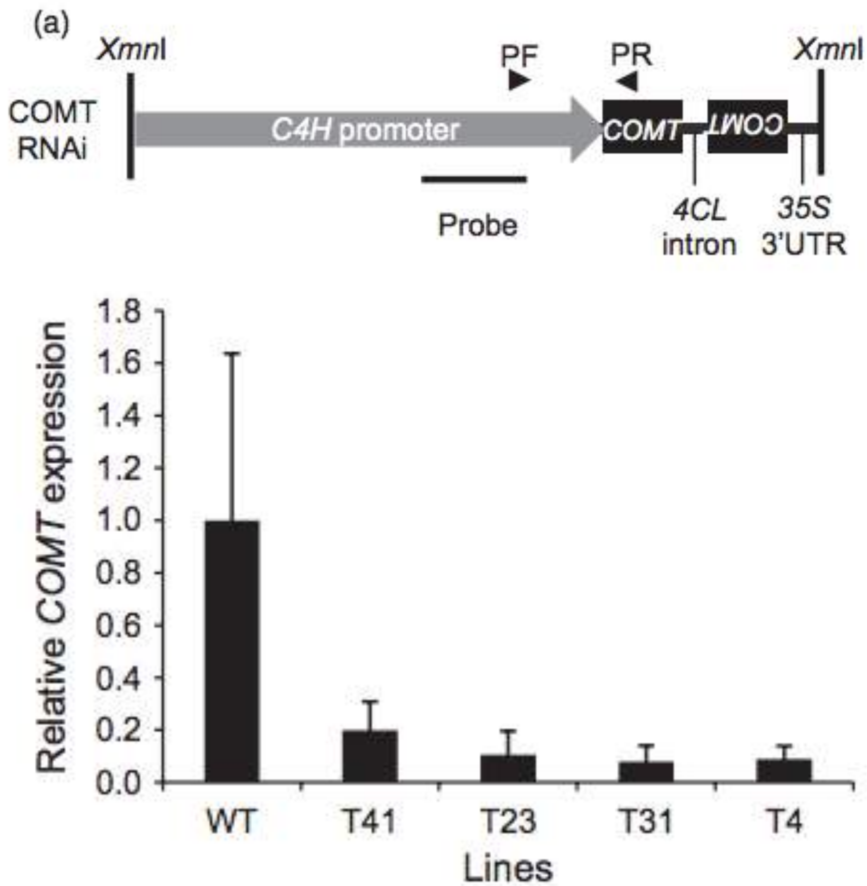


p-hydroxyphenil guaicyl syringil

Suppresion of lignin biosynthesis in sugarcane

Jung et al. used RNA interference to suppress COMT expression

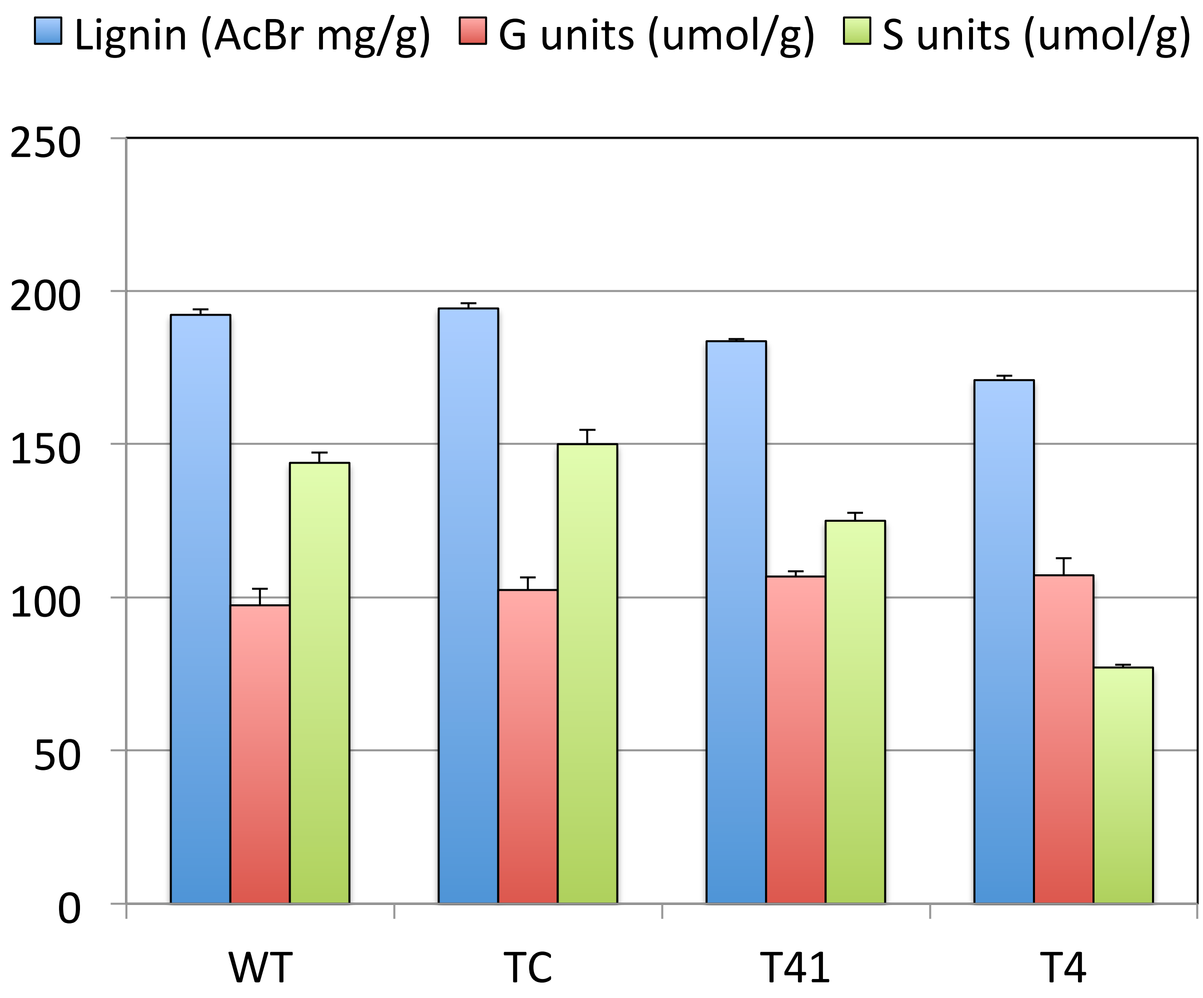
Reducing the availability of the building blocks would decrease lignin content



Jung et al. (2012). Plant Biotechnol. J. 10:1067–1076.
Jung et al. (2013). Plant Biotechnol. J. 11:709–716

Suppresion of lignin biosynthesis in sugarcane

Transgenic plants had similar lignin levels, but lower amounts of syringyl



Jung et al. (2012). Plant Biotechnol. J. 10:1067–1076.
Jung et al. (2013). Plant Biotechnol. J. 11:709–716

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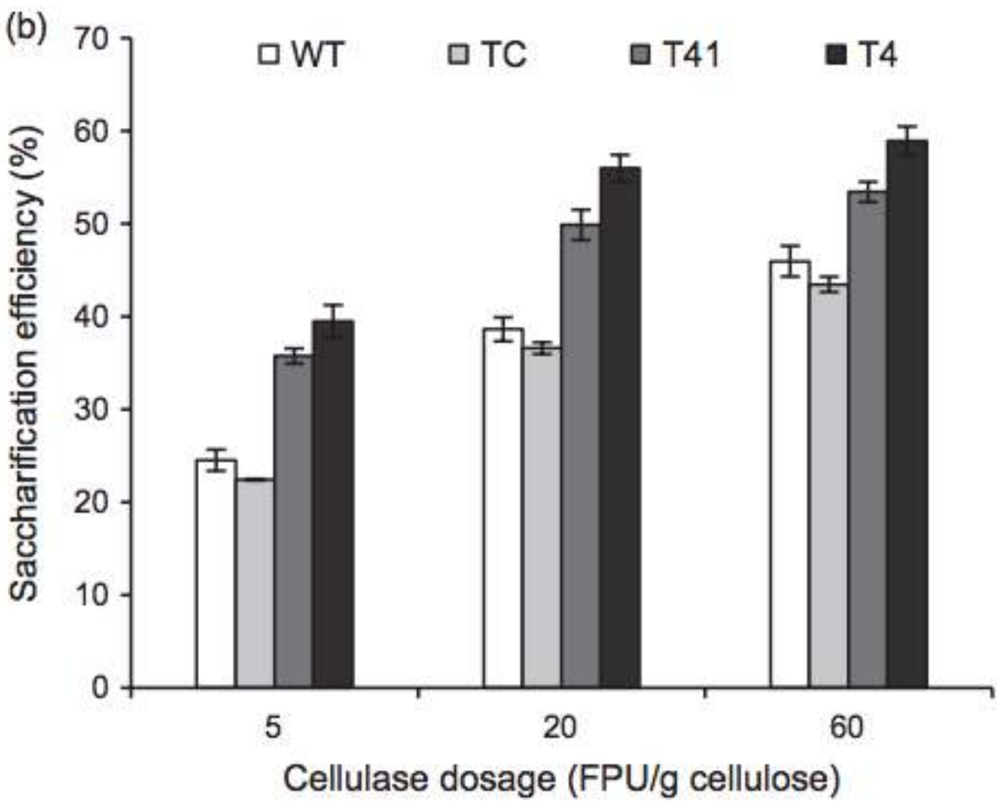
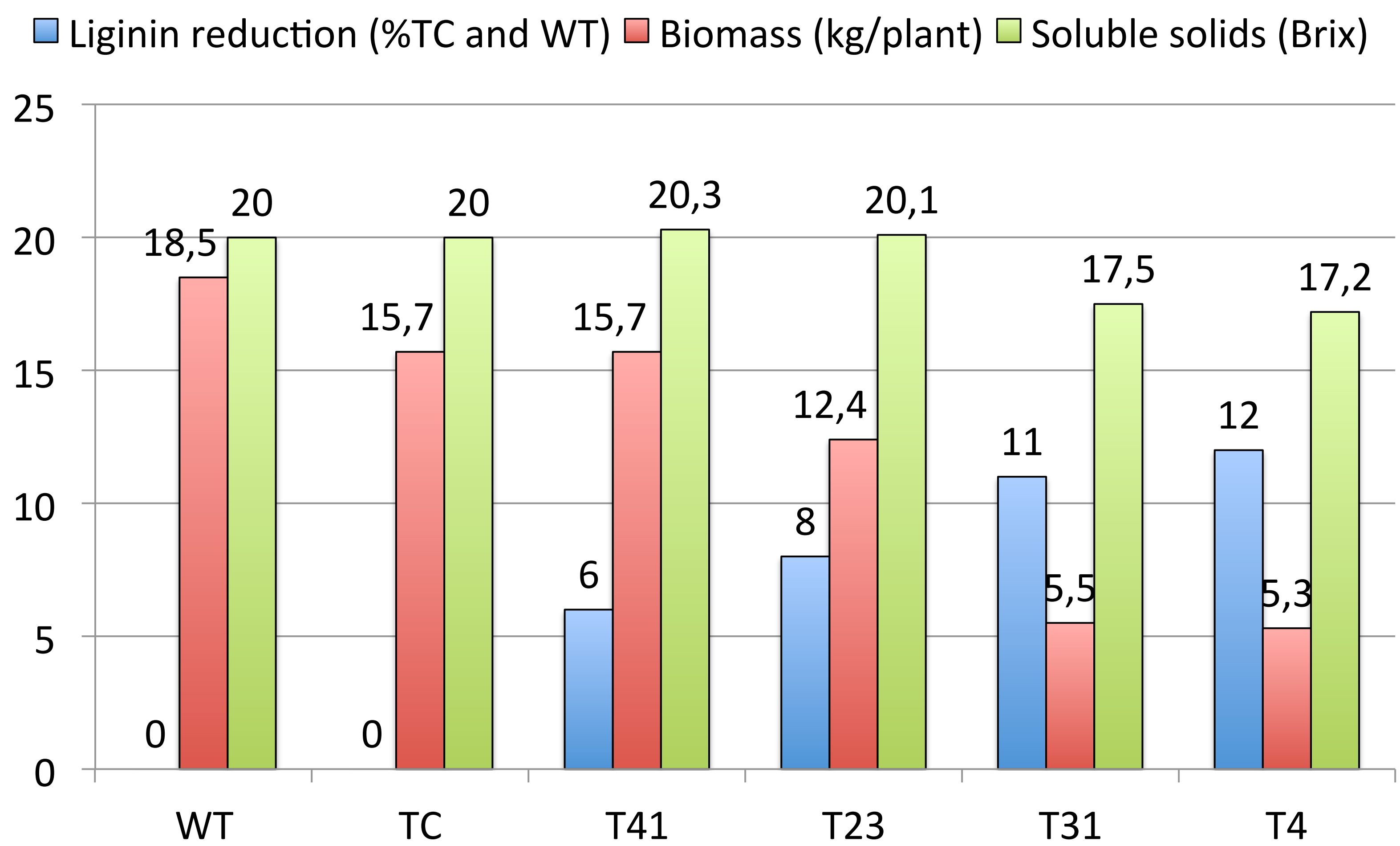
Saccharification efficiency is higher in suppressed lines



Jung et al. (2013). Plant Biotechnol. J. 11:709–716

COMT suppression has side effects



Jung et al. (2013). Plant Biotechnol. J. 11:709–716

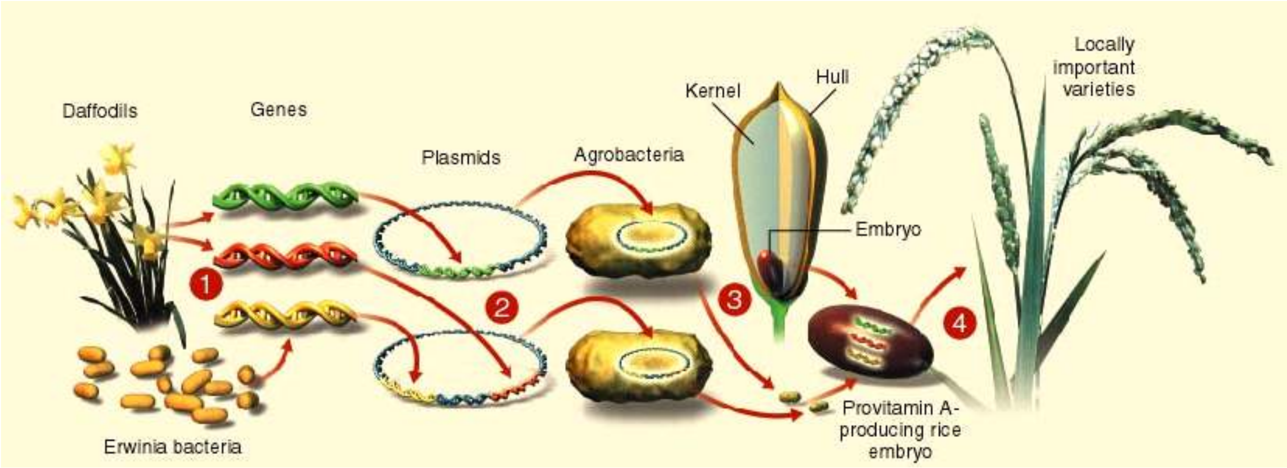
Side effects are genotype-specific

COMT expression, total lignin and sugar content of *COMT-AS* lines SP80-3280 and RB 835486

Helaine Carrer, work in progress: similar levels of COMT silencing had no deleterious effects in some sugarcane germoplasms

Challenges: Intellectual property issues

Case: golden rice



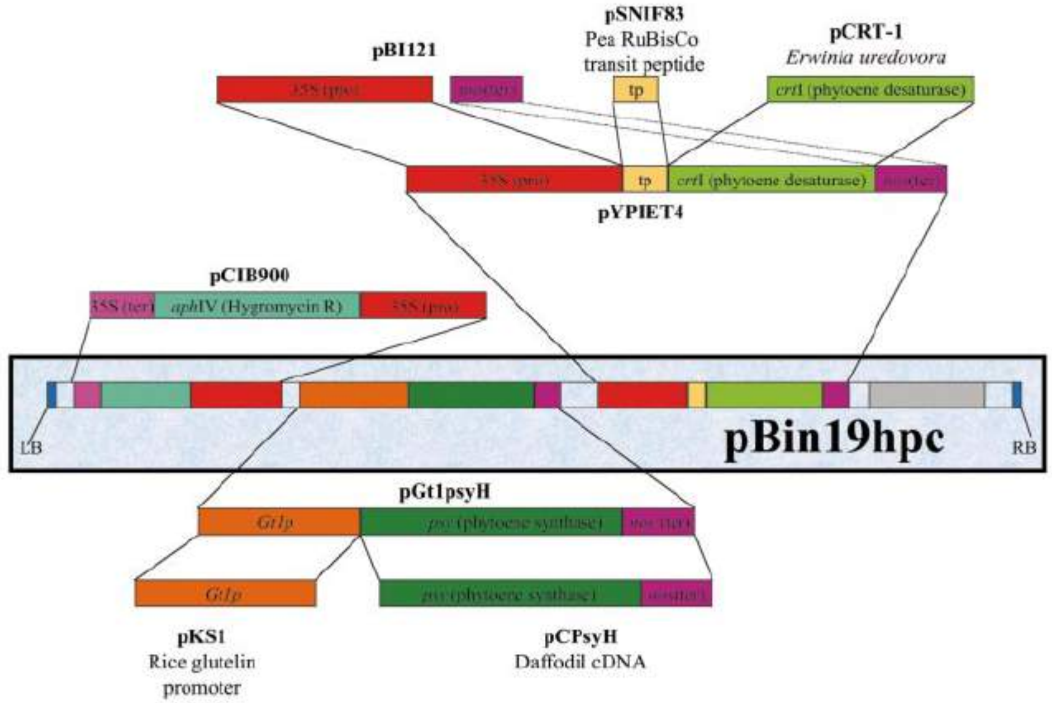
Non-GM rice

Golden rice

- Three genes were transferred to rice and a higher level of pro-vitamin A was produced
- Patent was granted, but do the inventors have the conditions for using the technology?

Case: golden rice

The use of the three genes is novel, but the use of each gene is protected



Golden rice: 72 patents, 40 organizations (Kryder et al., 2000).

Conclusions and perspectives

• Biomass accumulation under stress differ widely between sugarcane cultivars

• Sugarcane cultivars have very different responses to drought at the molecular level

• Gene expression profiling have allowed the discovery of genes that can enhance biomass production under drought stress in transgenic plants

• Analysis in transgenic sugarcane plants and field assays are a challenge

• Transgenic approaches such as COM-T suppression need a fine tuning to achieve the desired trait without compromising plants´ overall agronomic performance

• Since this field has great chances to reach the market, intellectual property issues should be monitored at all stages of the research

Different approaches, such as high-throughput functional genomics, integration with QTL mapping, are needed to increase our capacity to screen sugarcane genes related to biomass accumulation

Thank you !

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