

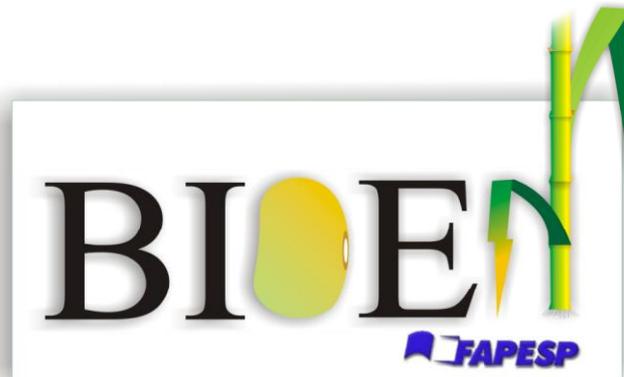
Gene regulation and biotechnology of drought tolerance in sugarcane

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Challenges and opportunities

- Sugarcane productivity: more energy per unit of area
- Energy distribution in sugarcane:

1/3 sugar (fermentation -> ethanol)

1/3 bagasse

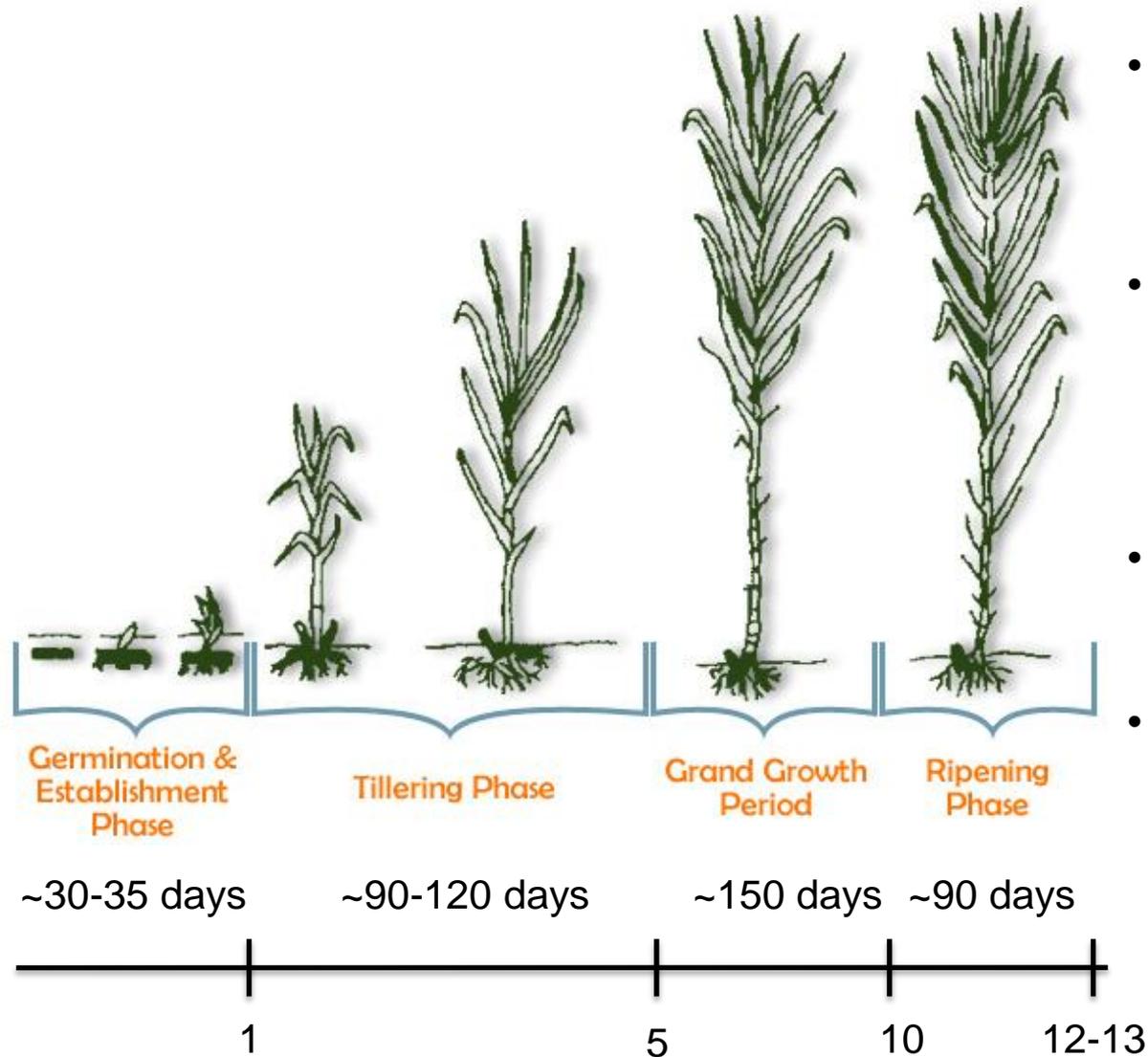
1/3 straw



new technologies to use this energy

- ~ 70% of ethanol costs rely on sugarcane production
- Technologies that reduce losses in the fields and the environmental impacts will have a key role for the bioenergy sector

Sugarcane development and drought



- 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

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

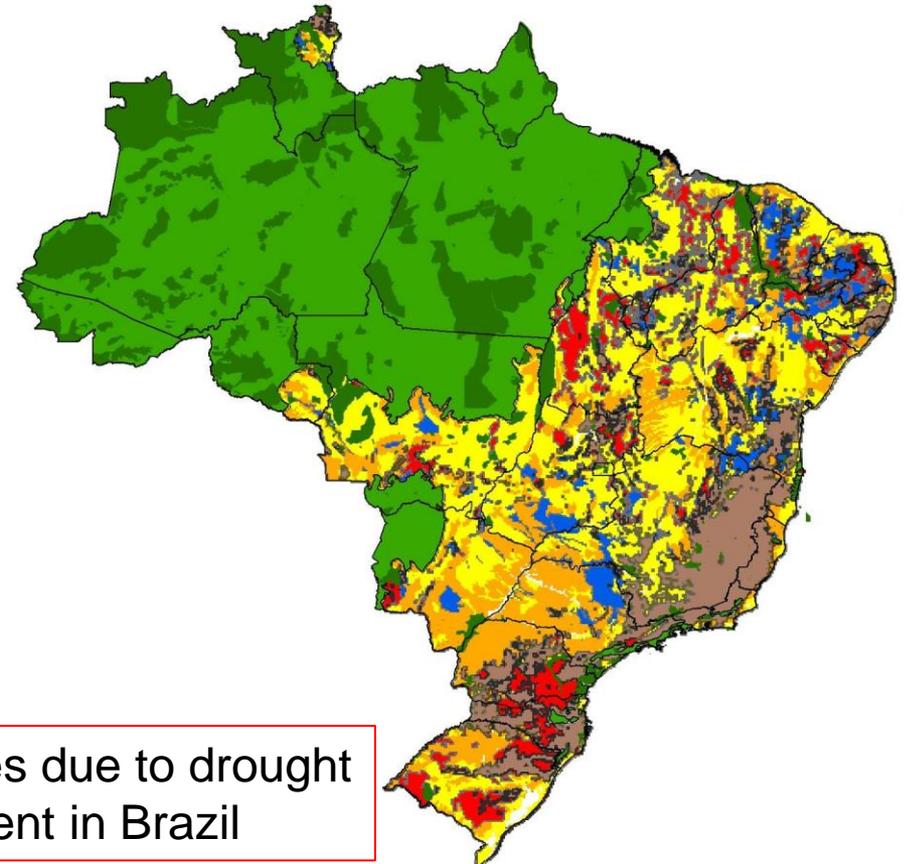
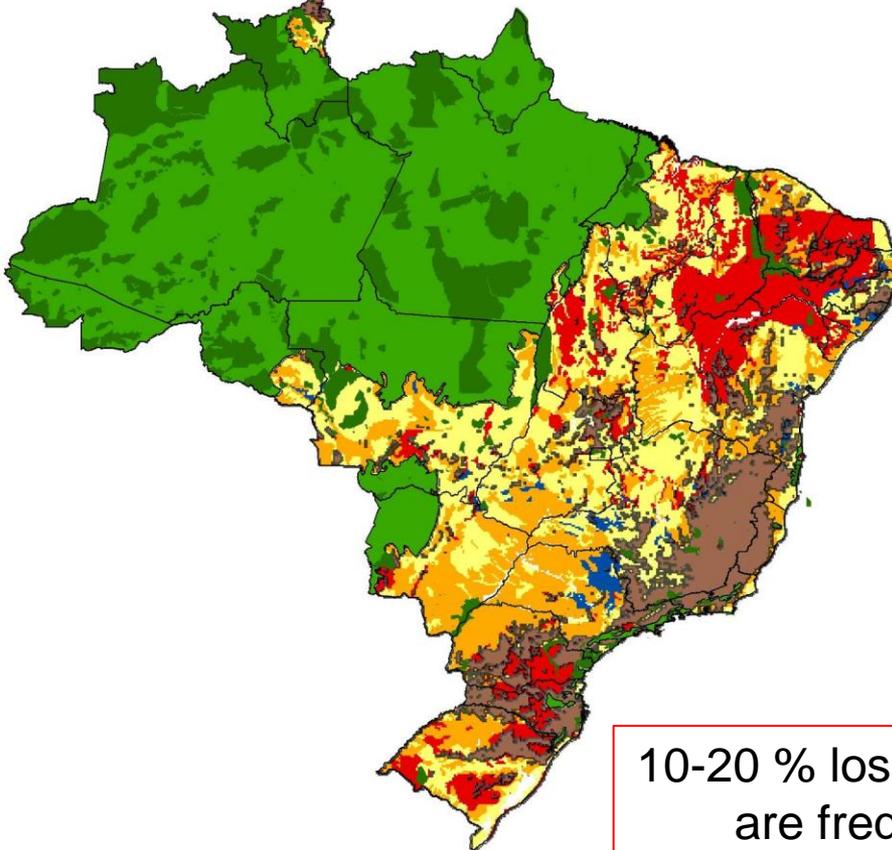
Source: Kuyper, 1952. <http://www.sugarcane crops.com/>

Effect of drought on sugarcane cultivation



No irrigation

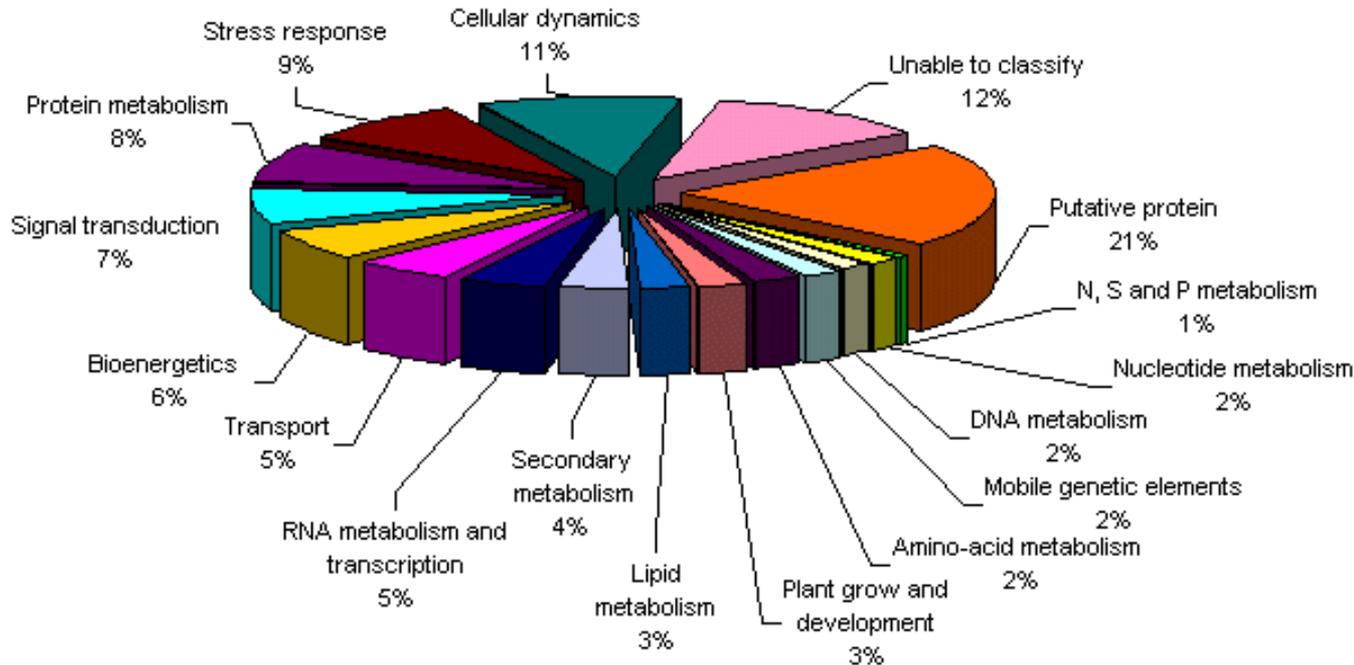
With irrigation



10-20 % losses due to drought are frequent in Brazil

The SUCEST project

43,143 genes in sugarcane



SUCEST
The Sugar Cane EST Project

Sugarcane responses to hormones and stress

Plants grown in a greenhouse

- Hormones: Methyl jasmonate (insects, etc), ABA (drought)
- Caterpillar infestation
- Phosphate deficiency
- Interaction with nitrogen-fixing bacteria
- Drought

http://www.biomedcentral.com/1471-2164/8/71

Table 1:

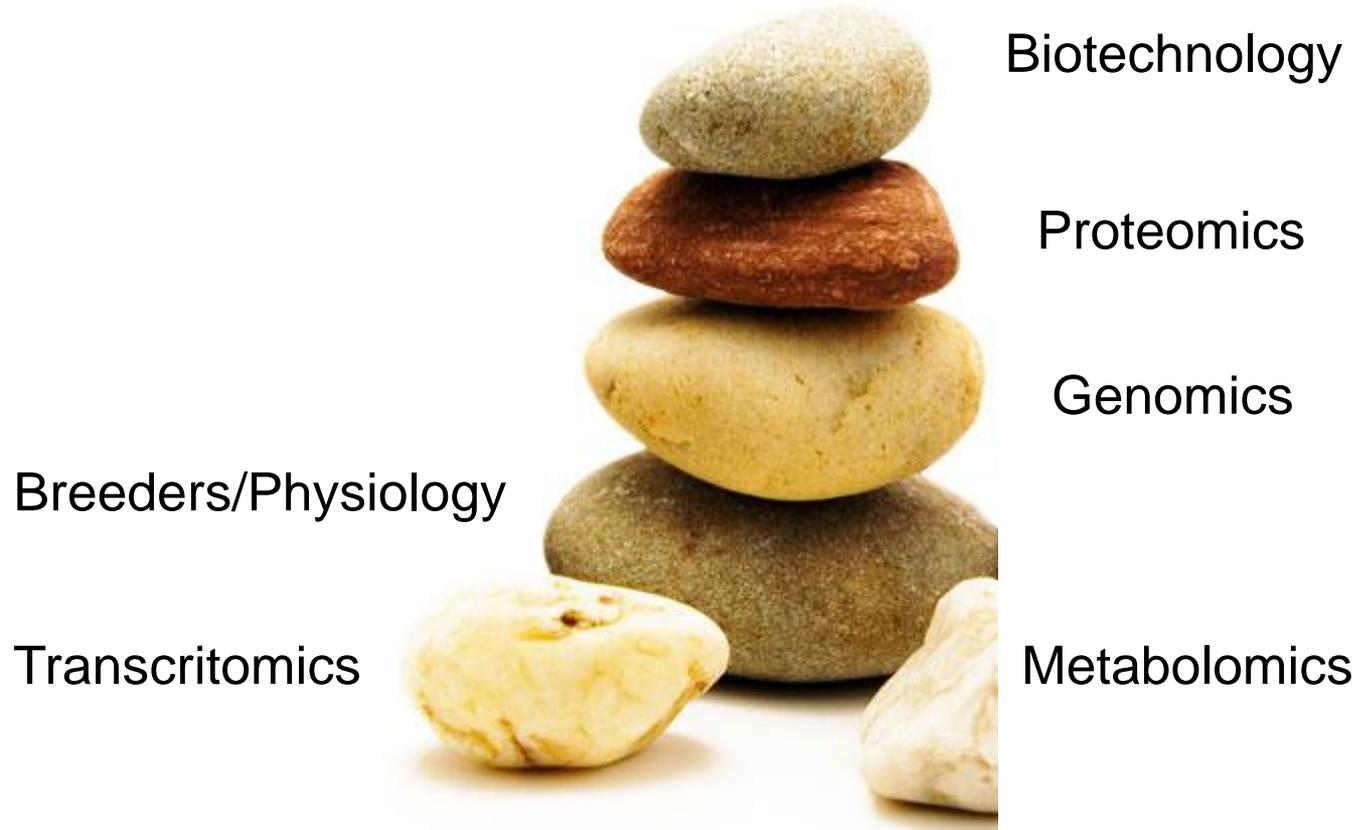
MeJA		ABA		Herbivory		Phosphate starvation		Gluconacetobacter		Herbaspirillum		Drought			
Cy3	Cy5	Cy3	Cy5	Cy3	Cy5	Cy3	Cy5	Cy3	Cy5	Cy3	Cy5	Cy3	Cy5		
0h (C1)	vs. 1h (E1)	0h (C1)	vs. 30min (E1)	30min (E1)	vs. 30min (C1)	6h (E1)	vs. 6h (C1)	E1	vs. C1	E1	vs. C1	24h (C1)	vs. 24h (E1)		
0h (C1)	vs. 6h (E1)	0h (C1)	vs. 1h (E1)	24h (E1)	vs. 24h (C1)	12h (E1)	vs. 12h (C1)	C2	vs. E2	C2	vs. E2	72h (C1)	vs. 72h (E1)		
0h (C1)	vs. 12h (E1)	0h (C1)	vs. 6h (E1)	30min (C2)	vs. 30min (E2)	24h (E1)	vs. 24h (C1)					120h (C1)	vs. 120h (E1)		
1h (E2)	vs. 0h (C2)	0h (C1)	vs. 12h (E1)	24h (C2)	vs. 24h (E2)	48h (E1)	vs. 48h (C1)					24h (E2)	vs. 24h (C2)		
6h (E2)	vs. 0h (C2)	30min (E2)	vs. 0h (C2)			6h (C2)	vs. 6h (E2)					72h (E2)	vs. 72h (C2)		
12h (E2)	vs. 0h (C2)	1h (E2)	vs. 0h (C2)			12h (C2)	vs. 12h (E2)					120h (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			

107, 8:71

Page 5 of 22
(page number not for citation purposes)

179 genes, from which 93 were modulated by drought

An integrated approach to study drought in sugarcane



The key objective is to identify the changes that take place in sugarcane plants during drought stress in order to produce new varieties with increased tolerance to drought

Consortium for physiology, omics and biotec of sugarcane responses to drought stress

Laurício Endres (UFAL)

Monalisa Sampaio Carneiro (UFSCAR)

Rejane J.M.C. Mansur (UFRPE)

Tercílio Calsa Jr. (UFPE)

Helaine Carrer (ESALq-USP)

Glaucia Mendes Souza (IQ-USP)

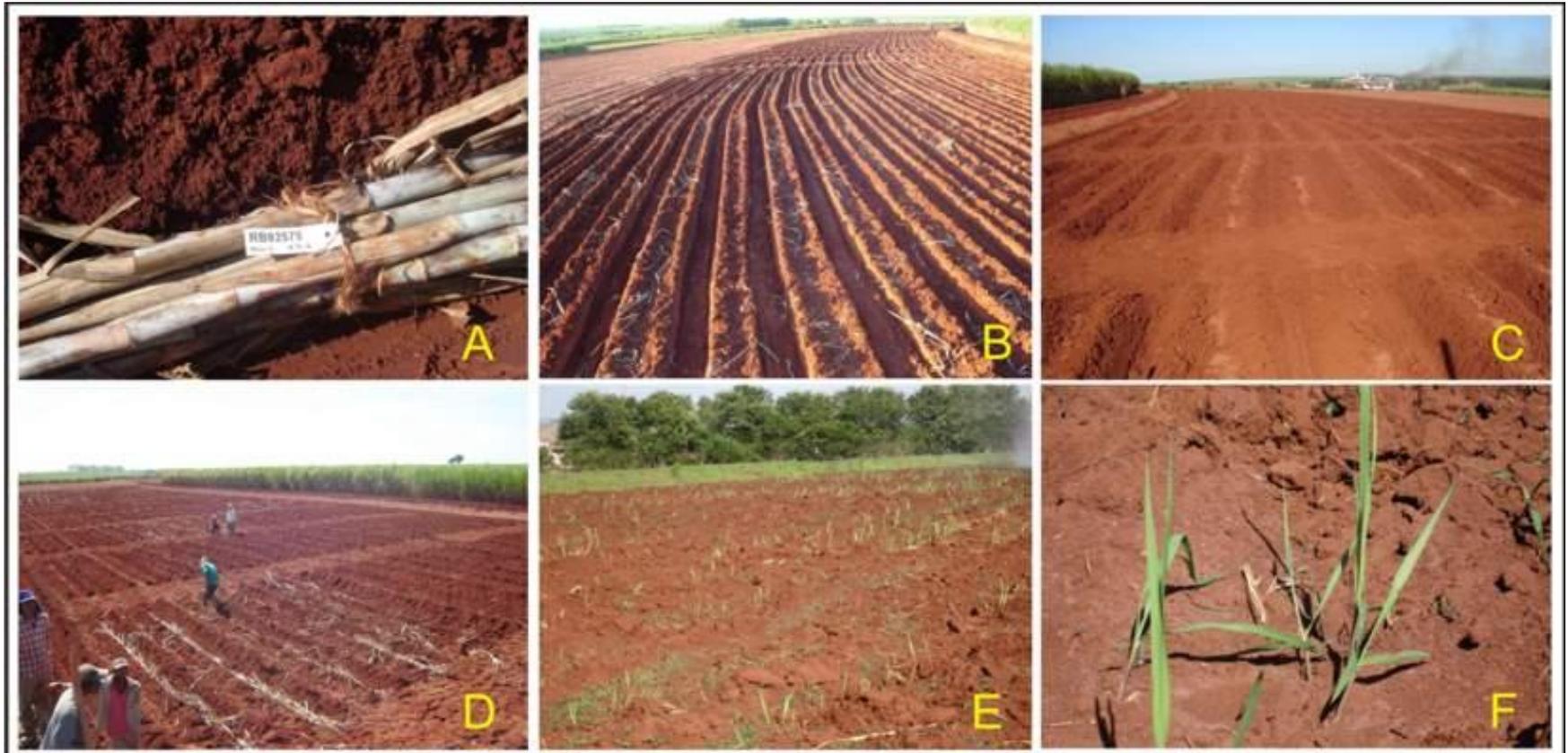
Marcelo Menossi (Coordinator, IB-UNICAMP)



Drought in sugarcane

Field experiments in AL, PE and two in SP.

Three time points: 3, 7 and 11 months after planting



Drought in sugarcane

Plants from six sugarcane varieties:

Lower tolerance: RB72454, RB855536 e RB855113

Higher tolerance: RB92579, RB867515 e SP79-1011

Plants were grown in the field under irrigation or without irrigation;

Leaf samples were taken after 3, 7 and 11 months;

Several parameters were evaluated during the growing season (gas exchange, proline content, leaf area, internode diameter, yield, etc)

Work led by Lauricio Endres (UFAL)



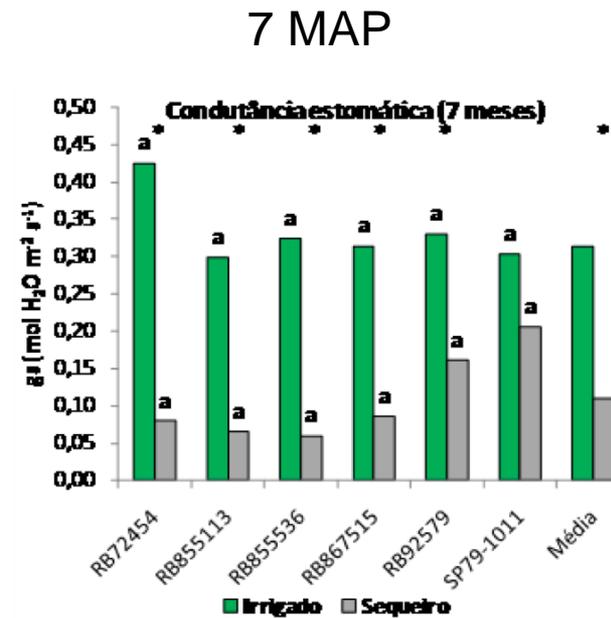
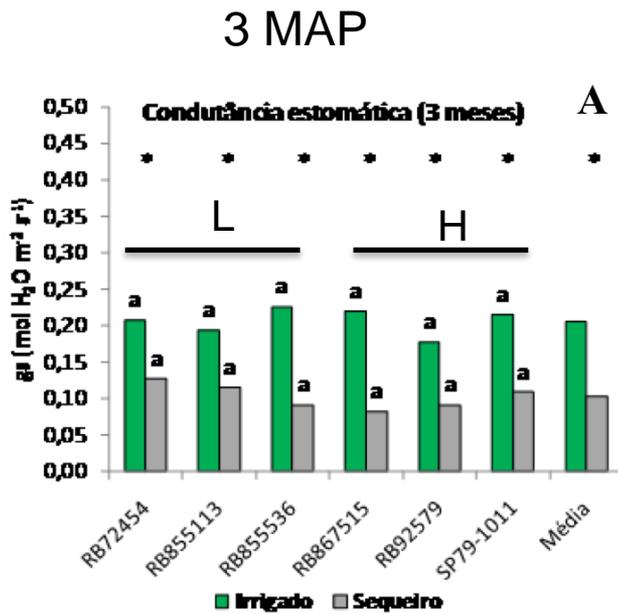
No irrigation



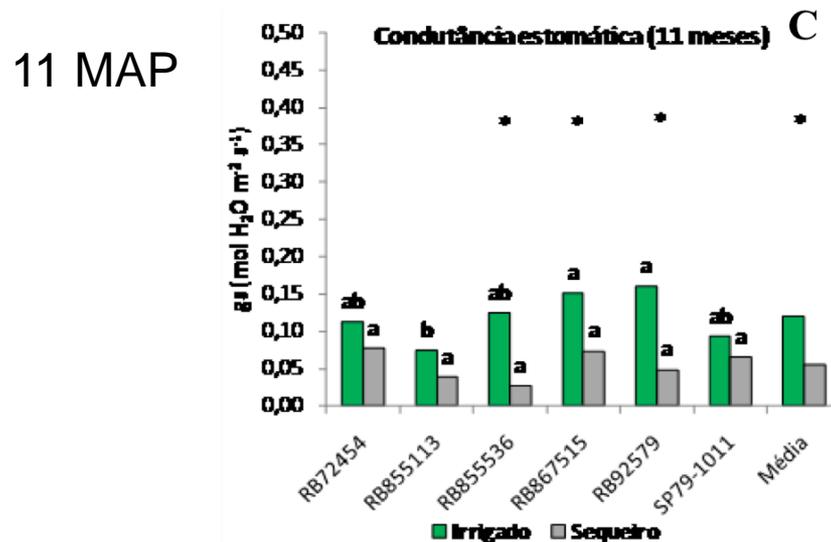
Irrigated

RB867515, 7 months after planting

Stomatal conductance

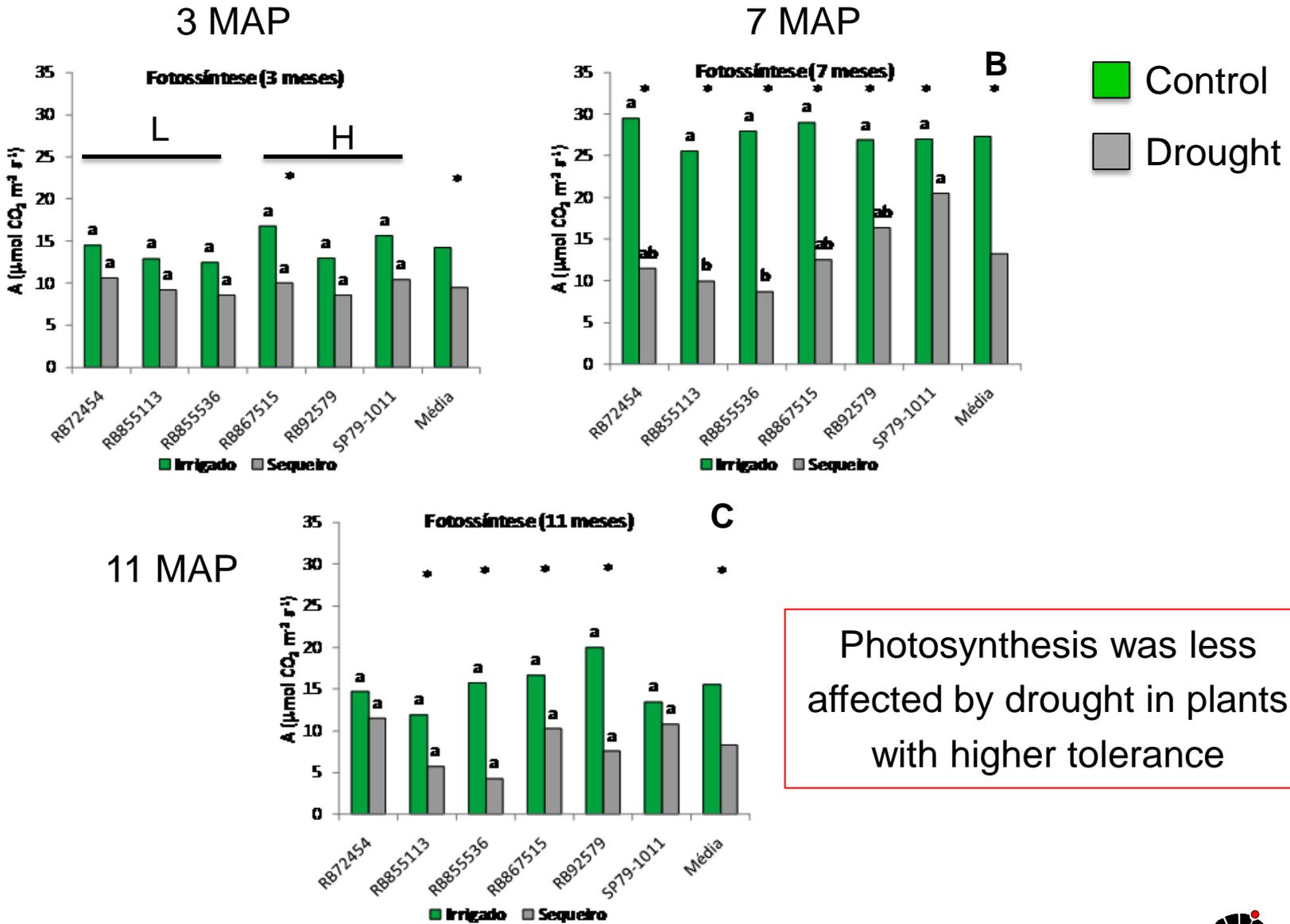


Control
Drought



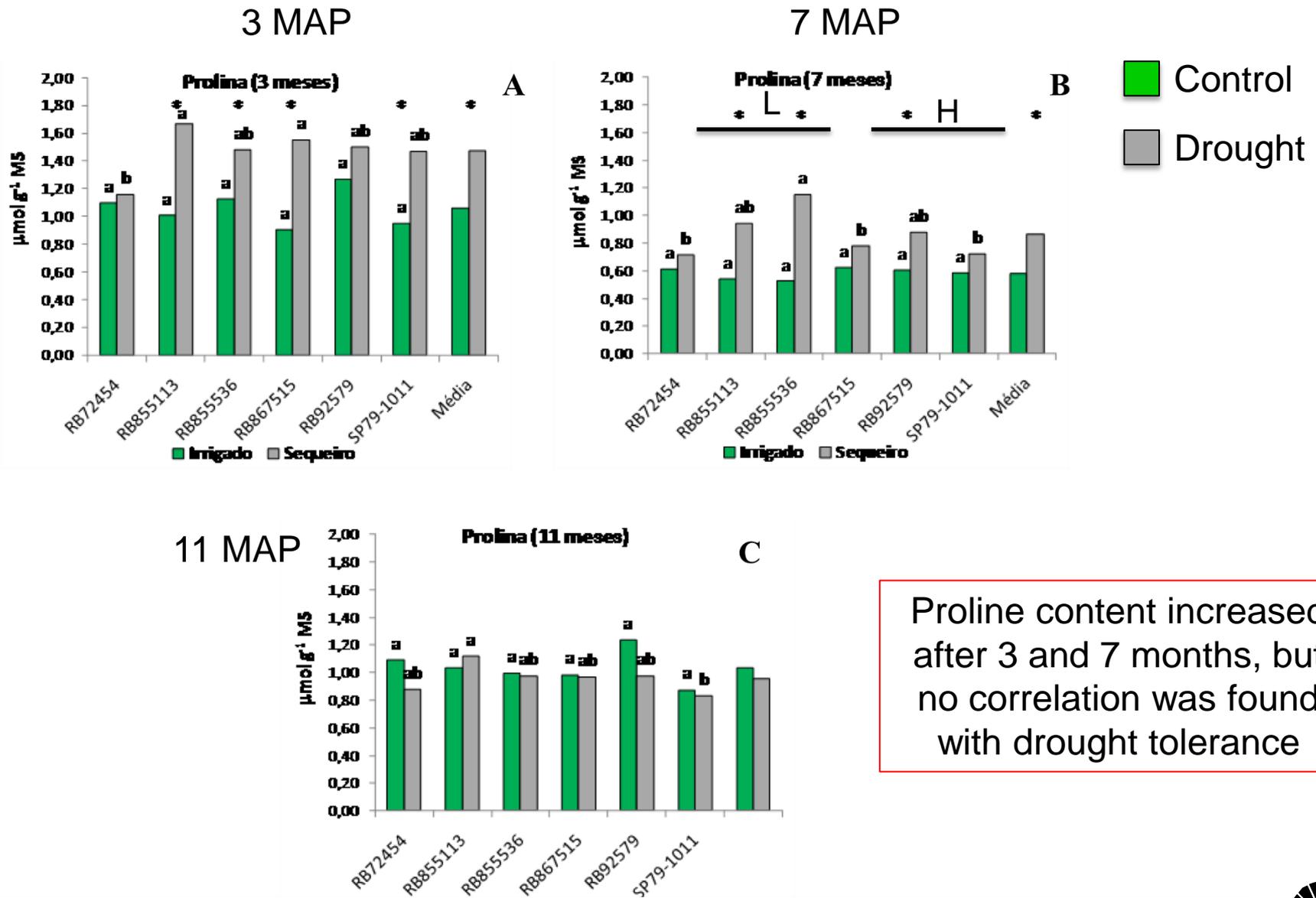
During drought stress there was a trend higher gs in plants with higher tolerance to drought

Photosynthesis



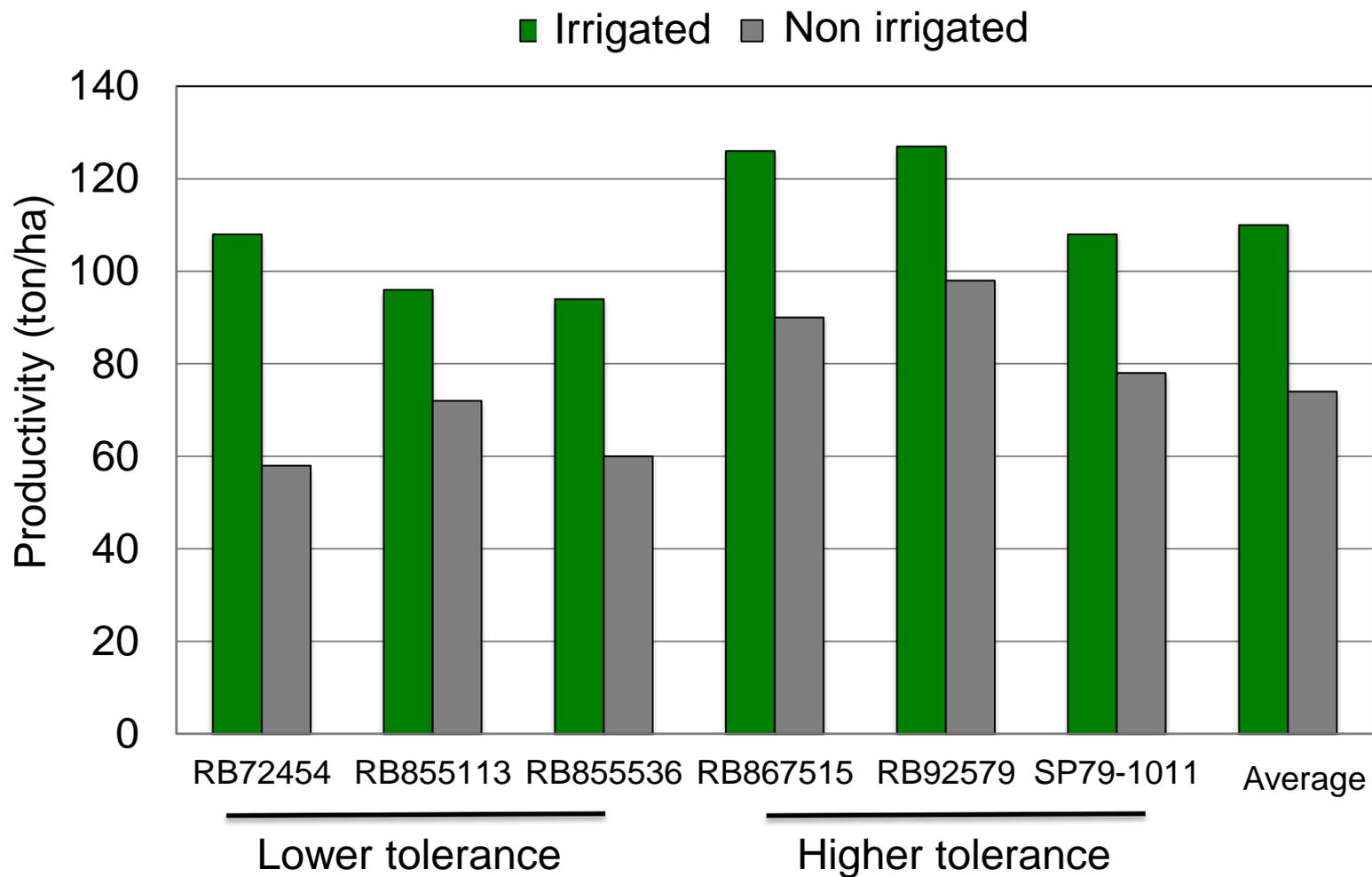
Photosynthesis was less affected by drought in plants with higher tolerance

Proline content



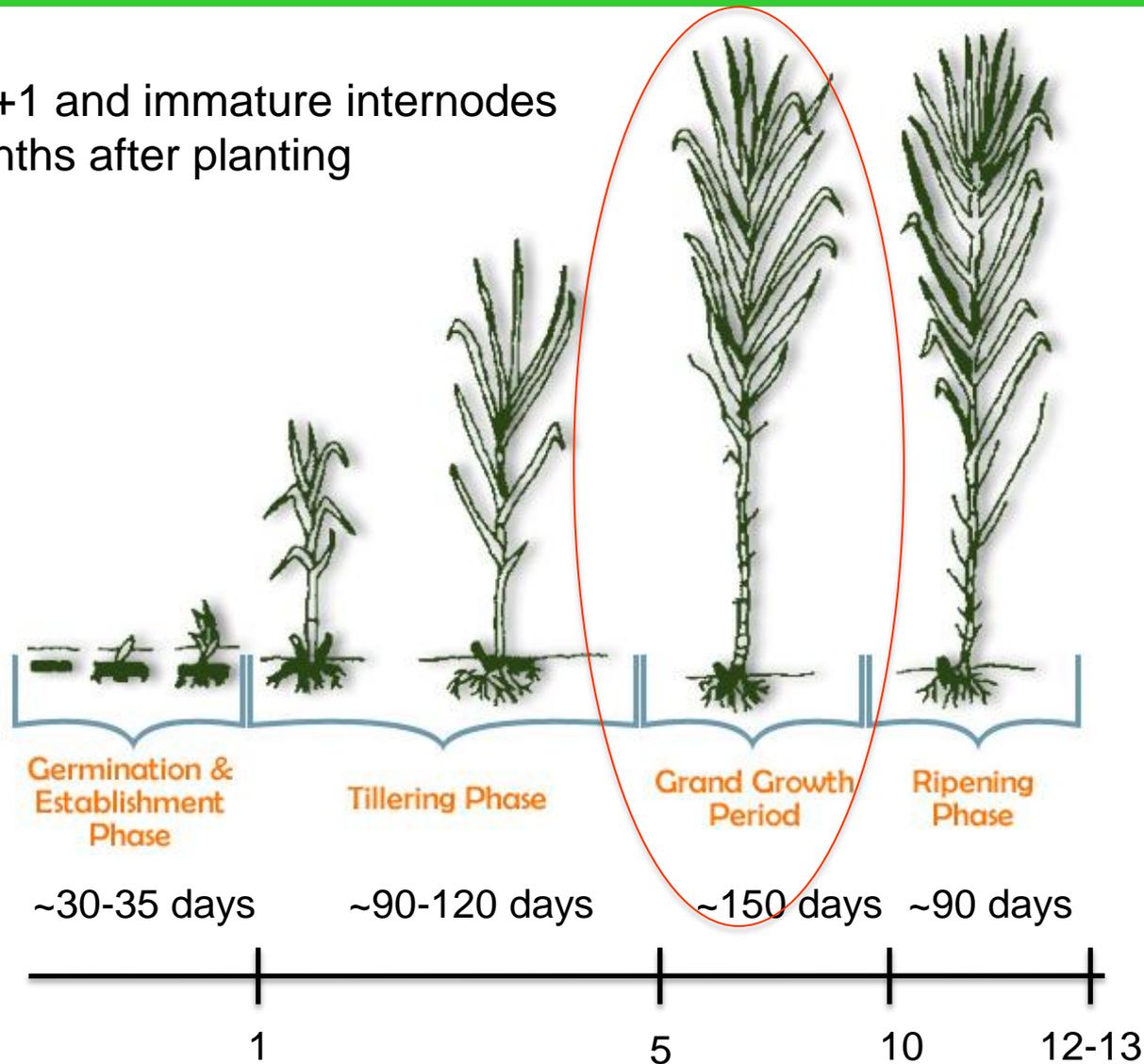
Proline content increased after 3 and 7 months, but no correlation was found with drought tolerance

Productivity of six sugarcane varieties (11 months)

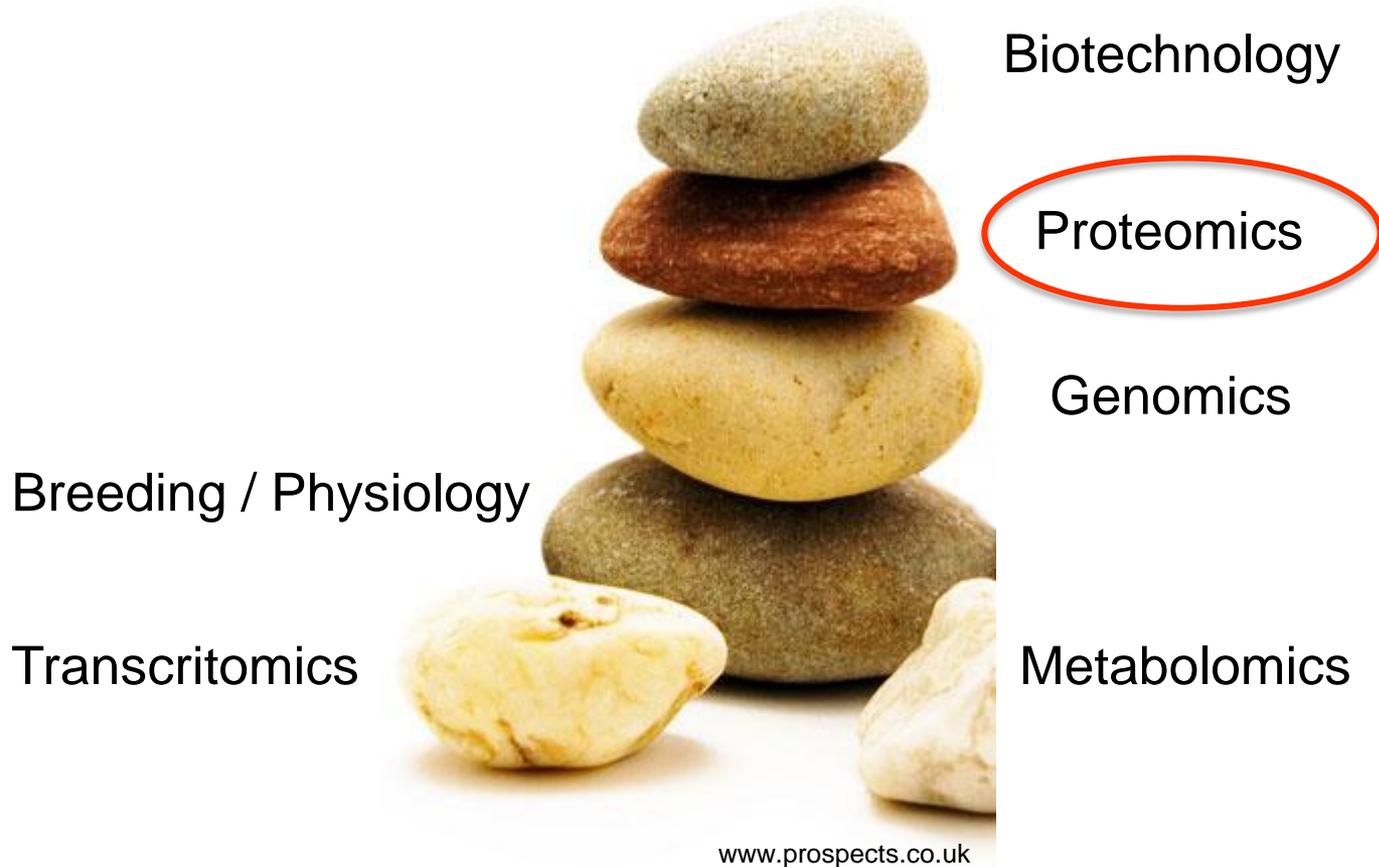


Sugarcane development and drought effects

Leaf +1 and immature internodes
7 months after planting



An integrated approach to study drought in sugarcane



Proteomics of sugarcane leaves

Work led by Tercilio Calsa Jr. (UFPE)

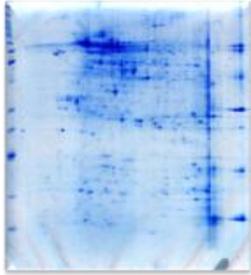
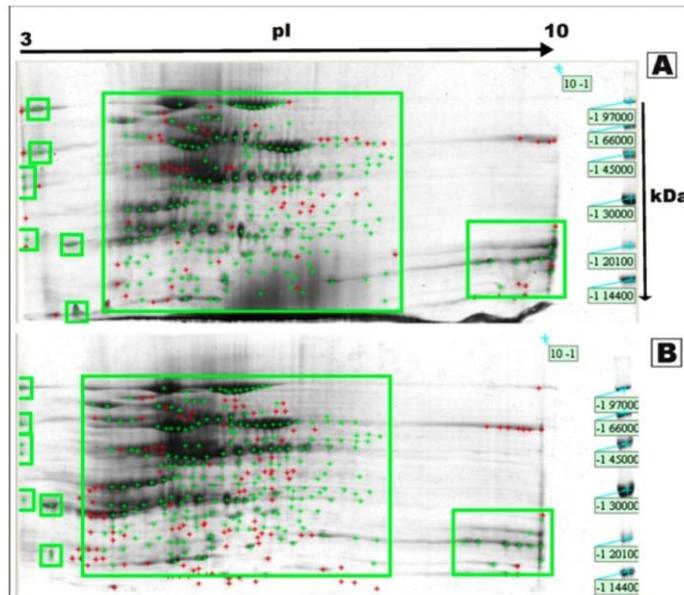
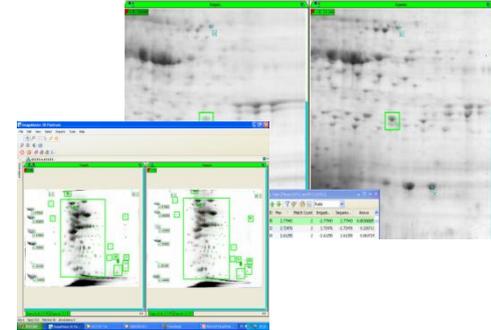
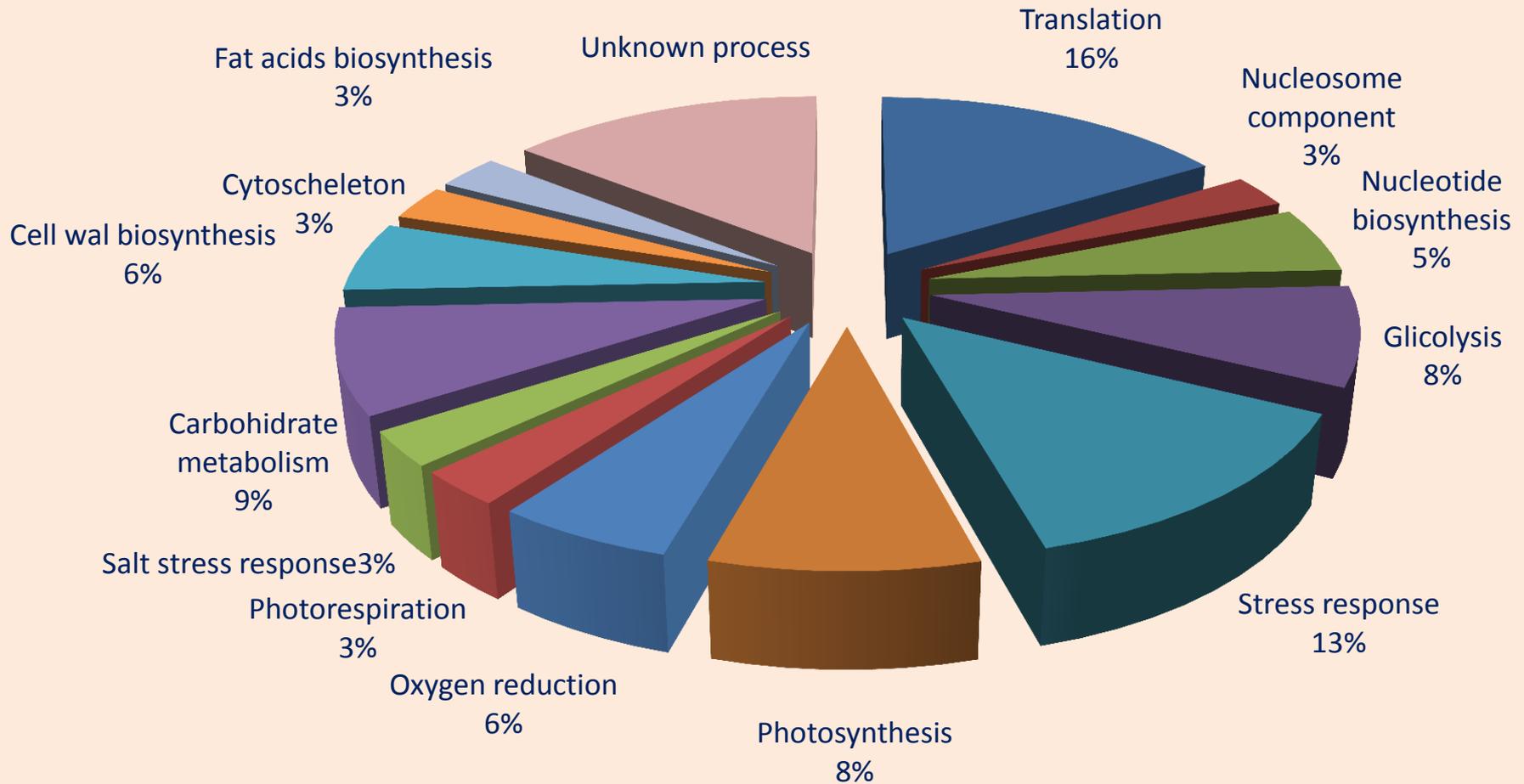


IMAGE SCANNER III (GE LIFE SCIENCES)

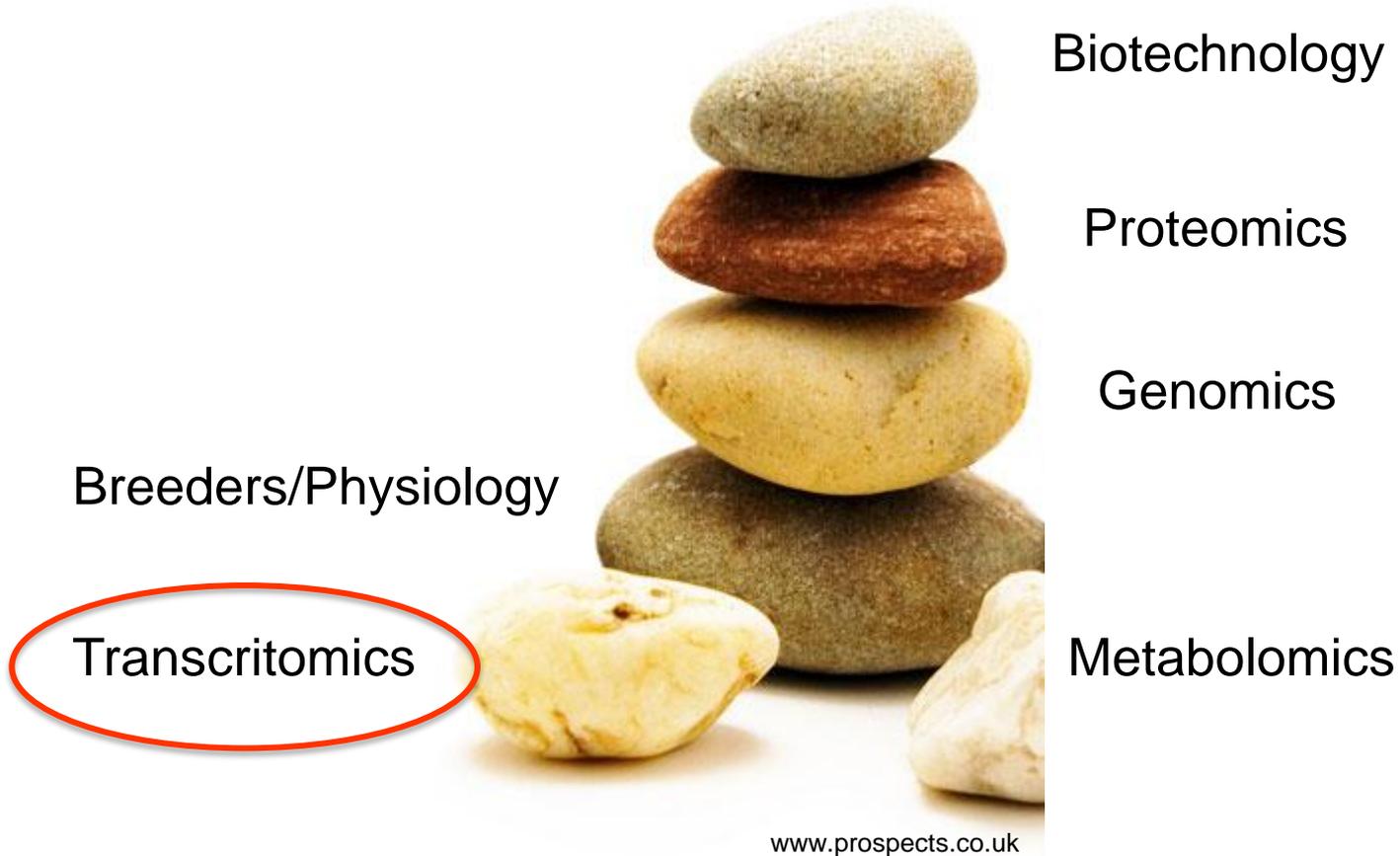


Proteomics of sugarcane leaves

Biological process

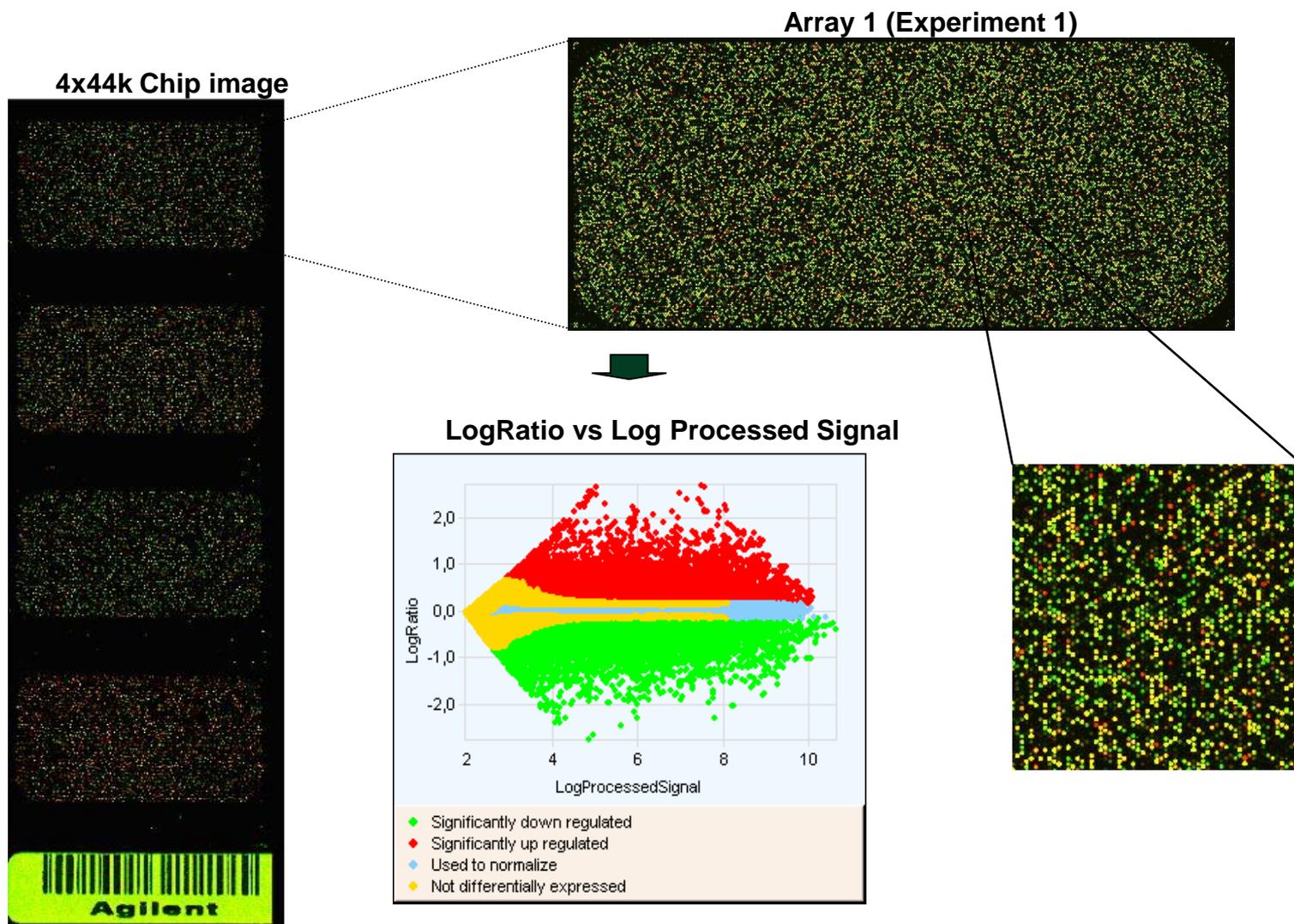


An integrated approach to study drought in sugarcane

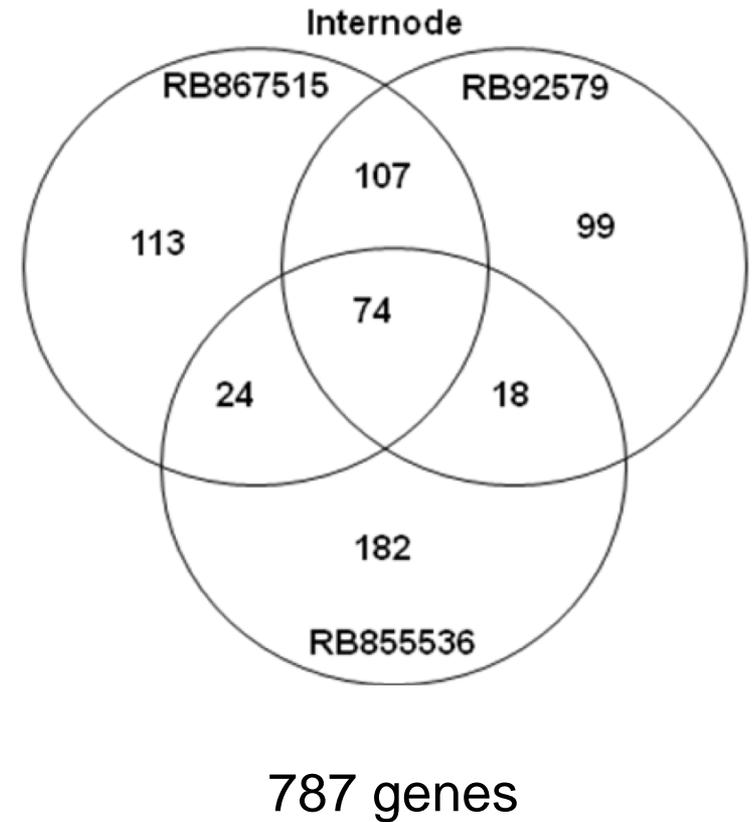
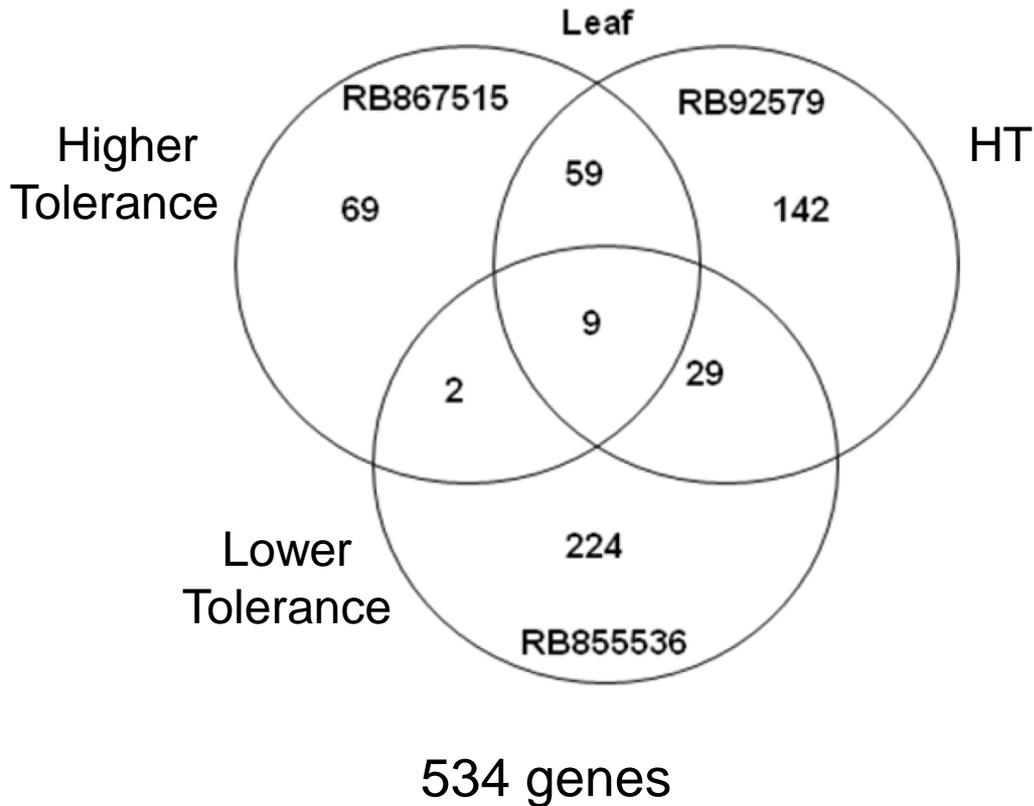


Transcriptomics of drought responses in sugarcane

Work led by Glaucia Souza (USP)



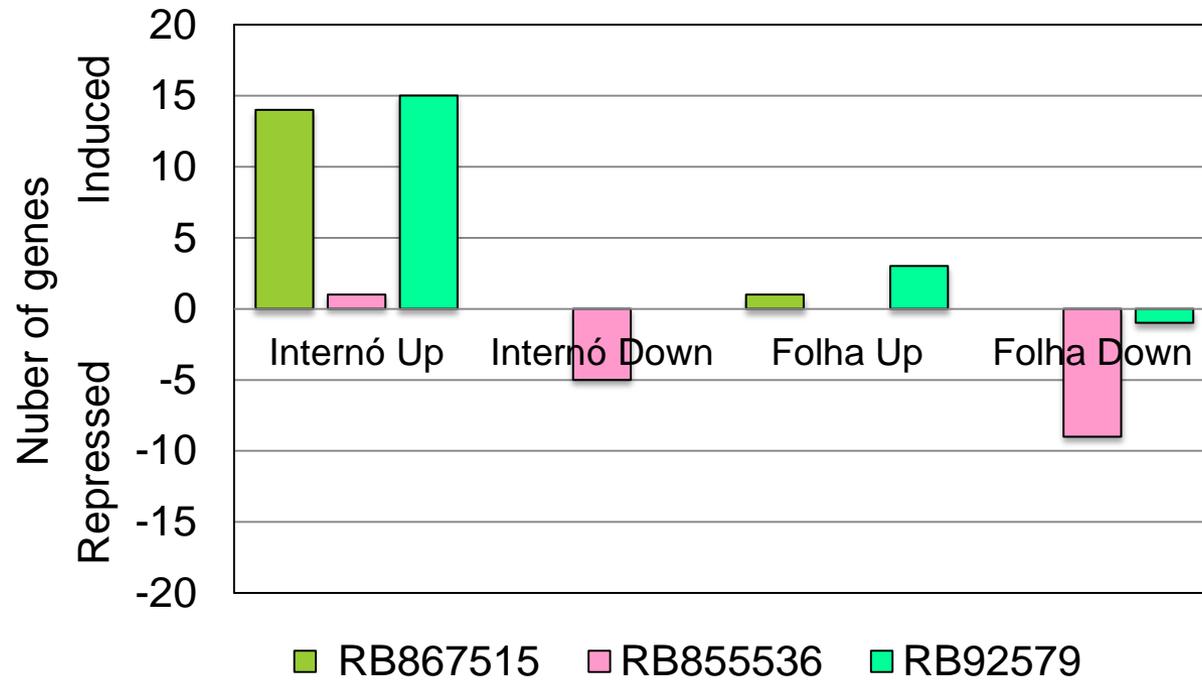
Gene expression in response to drought stress



Gene expression in response to drought stress

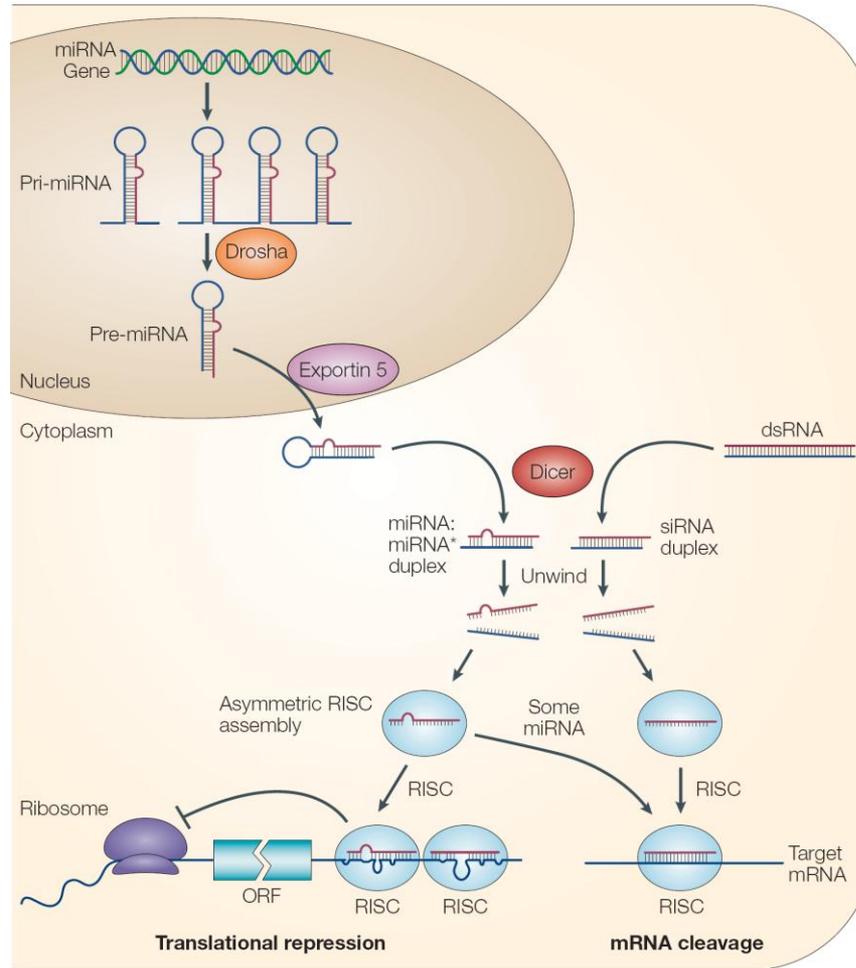
	Higher tolerance		Lower tolerance		Higher tolerance	
	RB867515	RB867515	RB855536	RB855536	RB92579	RB92579
category	Internode	Leaf	Internode	Leaf	Internode	Leaf
Osmolyte accumulation	UP	UP	DOWN	up/DOWN	-	up/DOWN
Oxidative stress	UP/down	UP/down	up/DOWN	up/down	UP/down	up
Photosynthesis	UP/down	UP/down	up/DOWN	-/DOWN	UP	UP/down
Water transport	-/DOWN	UP/-	-/DOWN	up/DOWN	-/DOWN	UP/-
Protein folding	UP/-	UP/-	up/DOWN	down	UP/-	UP/down
Hormone biosynthesis						
Ethylene	- / -	-/DOWN	-/DOWN	-/-	-/DOWN	up/DOWN
Jasmonate	- / -	- / -	-/DOWN	up/down	- / -	-/DOWN
Gibberelin	-/DOWN	-/-	/DOWN	/DOWN	/DOWN	/DOWN
Auxin	-/DOWN	up/DOWN	-/DOWN	/DOWN	-/-	-/DOWN
ABA	UP/-	UP/-	-/-	up/DOWN	UP/-	UP/-
Cell cycle	-/DOWN	-/DOWN	-/DOWN	-/DOWN	-/DOWN	-/DOWN

Chaperones



miRNA from sugarcane

Work led by Marcelo Menossi (UNICAMP).



miRNA expression by RNAseq

7 months-old plants: RB867515 (T) and RB855536 (S) in the field, irrigated (I) or not (S).

	TI	TS	SI	SS
Total reads	12 900 755	8 346 703	10 978 514	11 397 847
Known miRNAs	334	229	269	265
Unknown miRNAs	758	422	441	415
siRNAs	103 305	35 464	60 533	47 797
Non annotated (%)	56,03	47,92	49,74	54,71



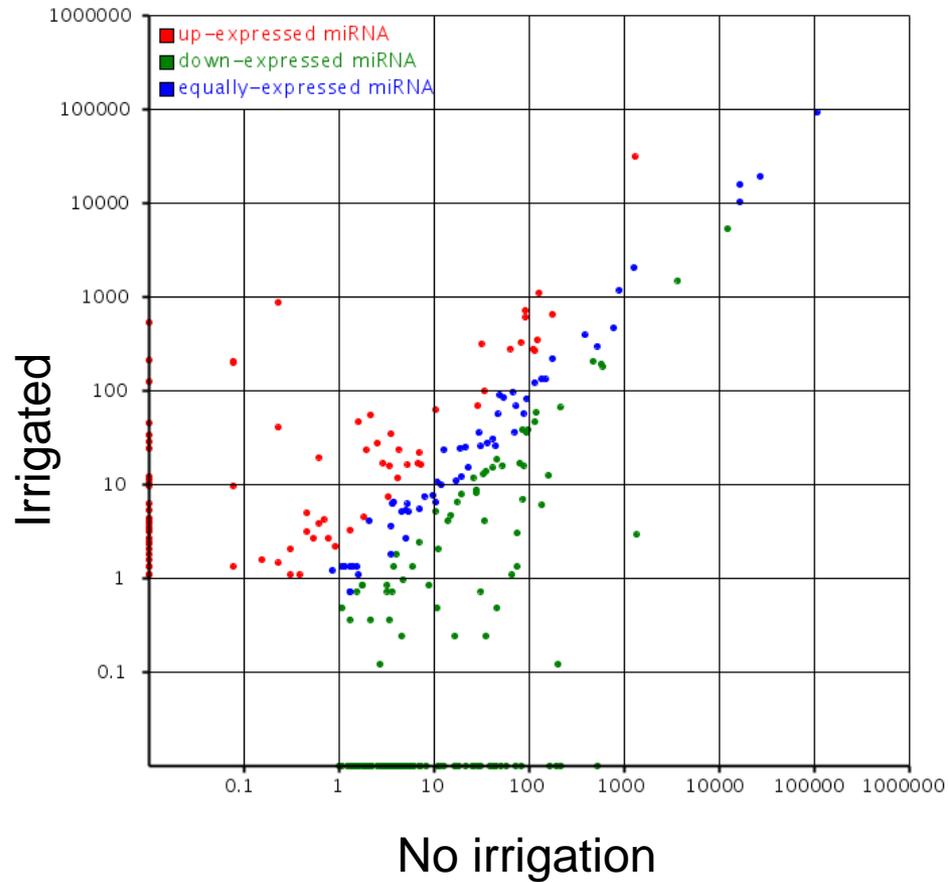
<http://www.genomics.cn>



Illumina HiSeq2000

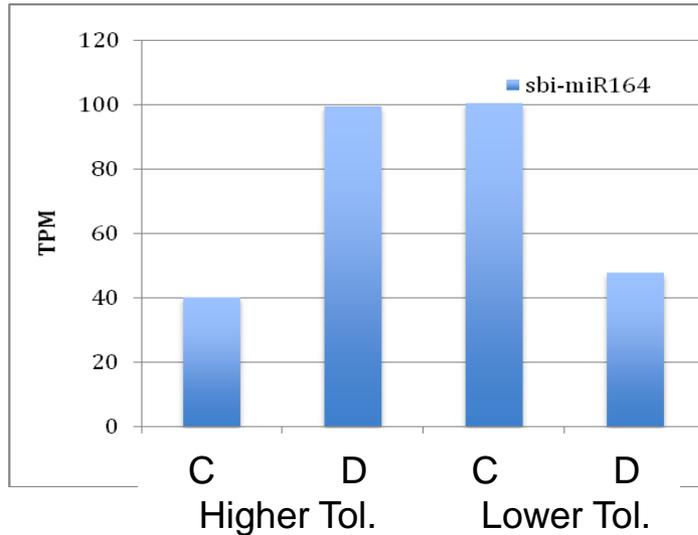
miRNA expression in 7 MAP plants in the field

RB867515 (T)

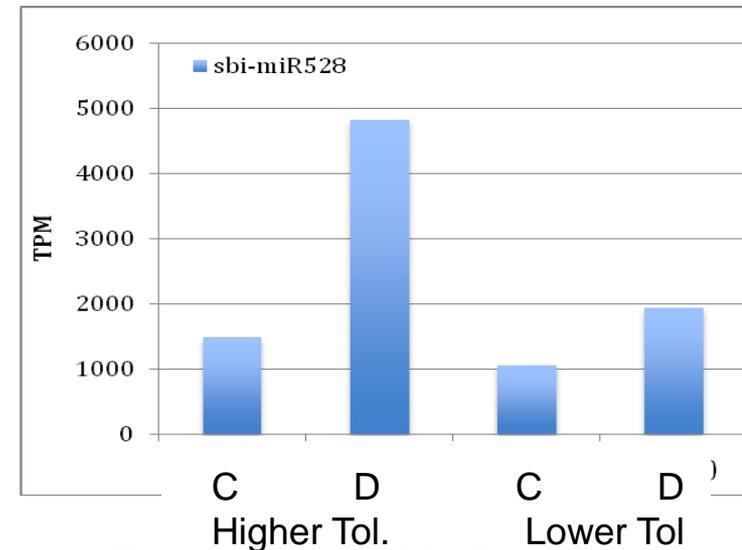
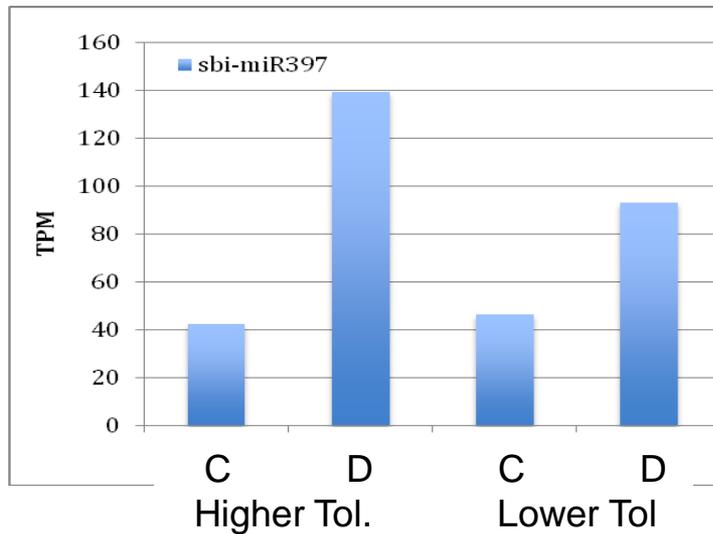
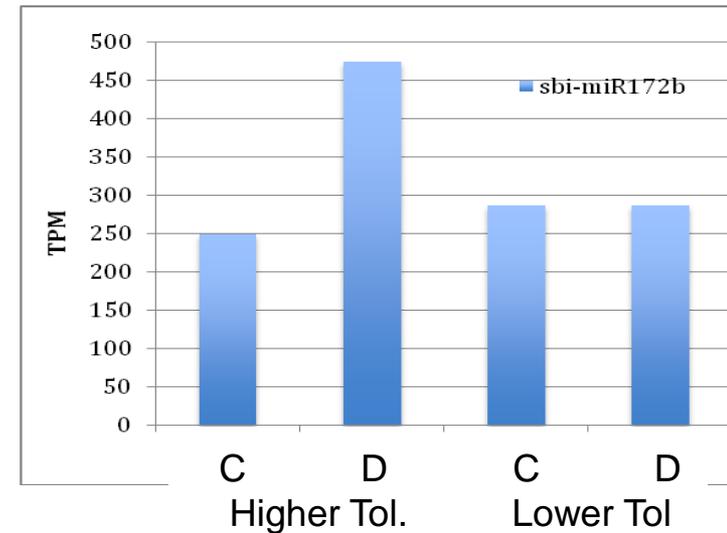


Selected miRNA with differential expression

Target: Nac domain TF



Target: APETALA2-like TF

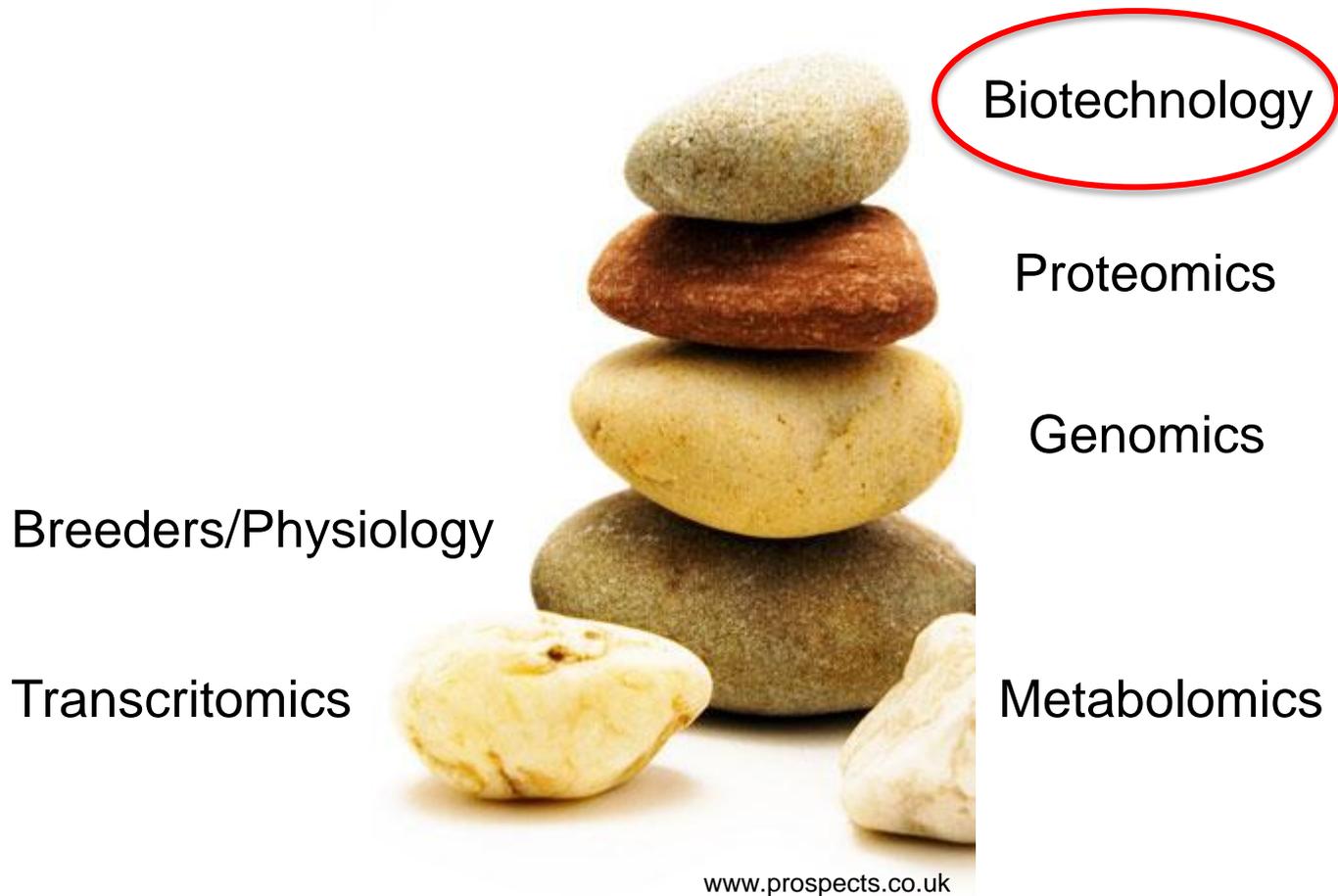


Target: Laccases, beta-6 tubulin,

Target: copper-binding proteins,

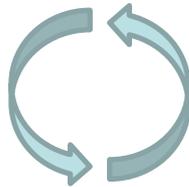


An integrated approach to study drought in sugarcane

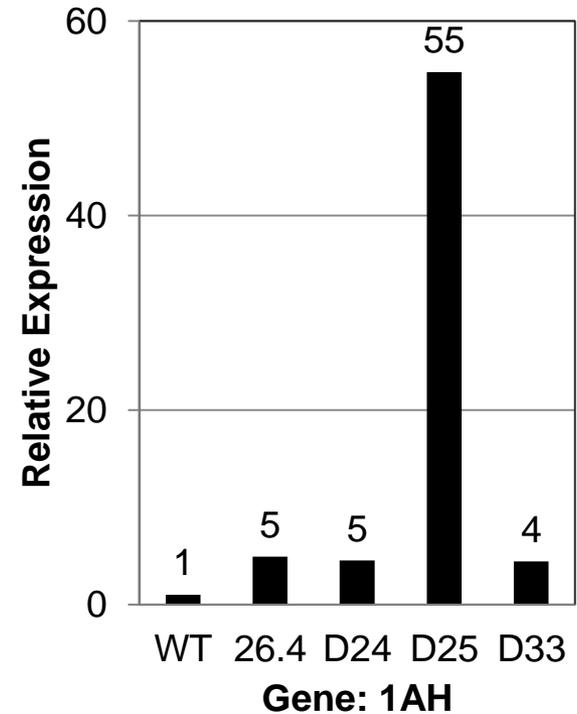
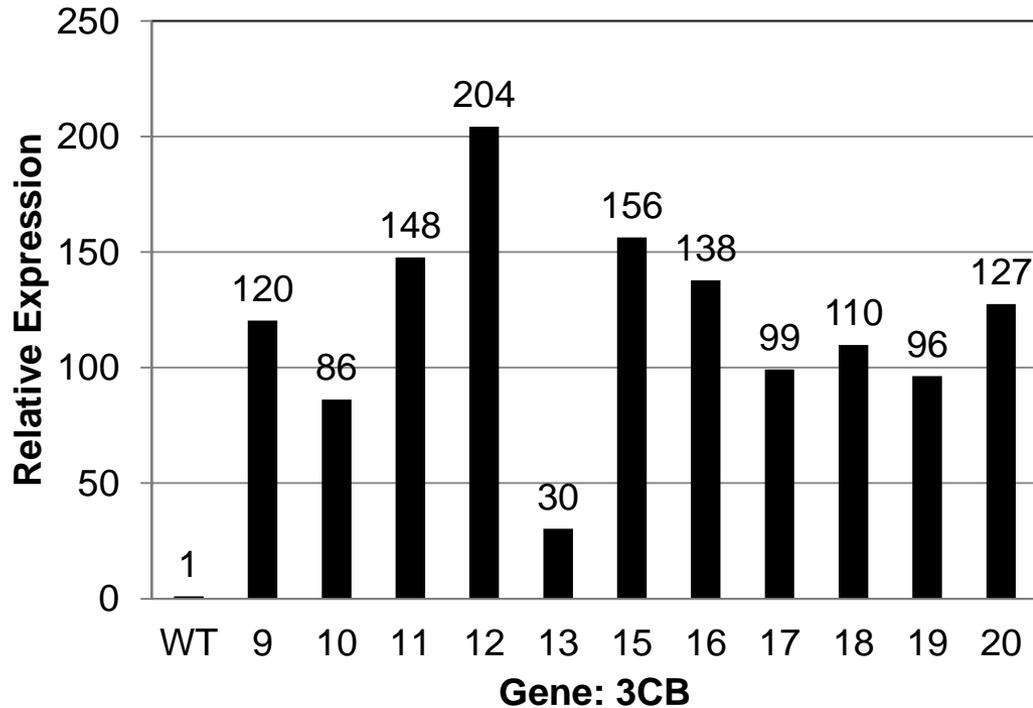


Production of transgenic sugarcane plants

Led by Marcelo Menossi (UNICAMP) in cooperation with Helaine Carrer (Esalq/USP)



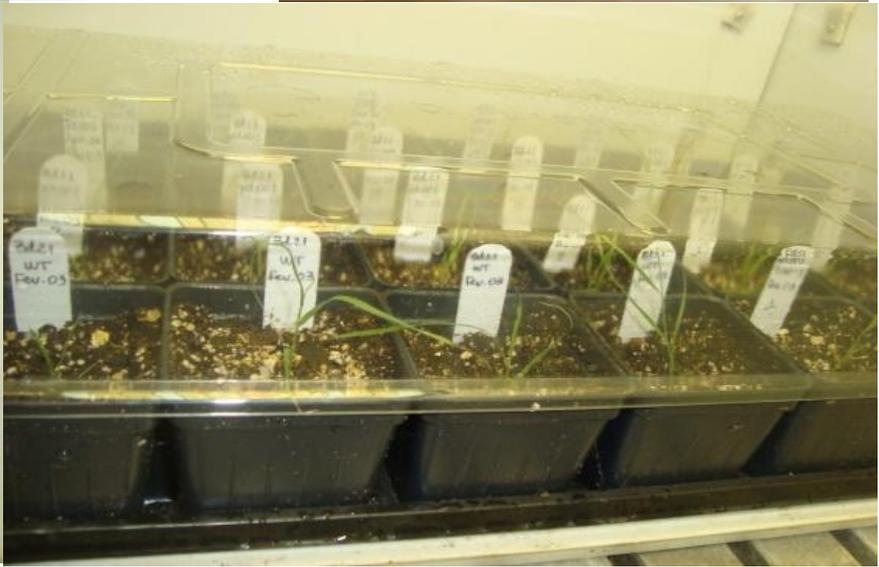
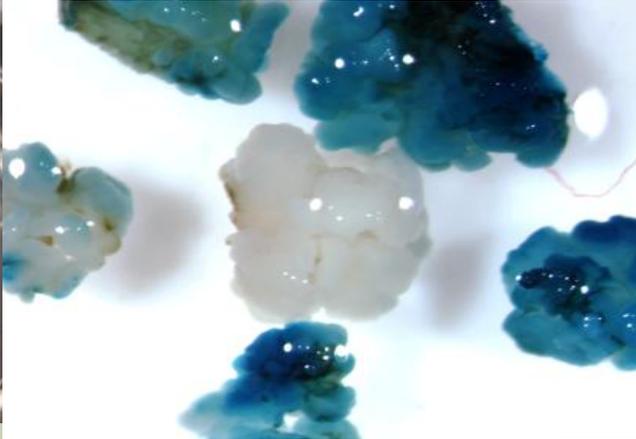
Production of transgenic sugarcane plants



Plants in the greenhouse will be tested soon

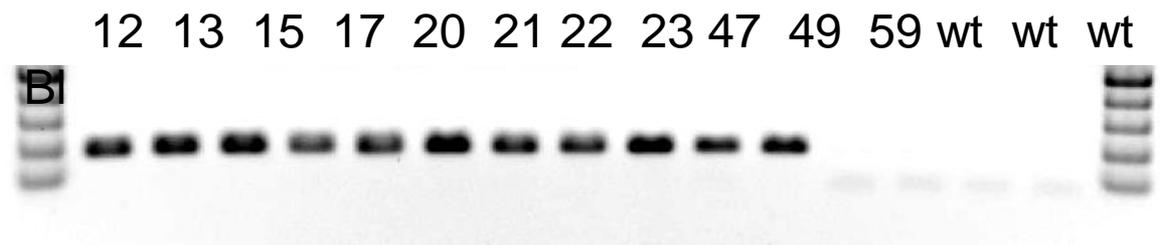
Production of transgenic Brachypodium plants

In cooperation with Prof. David Garvin, University of Minnesota



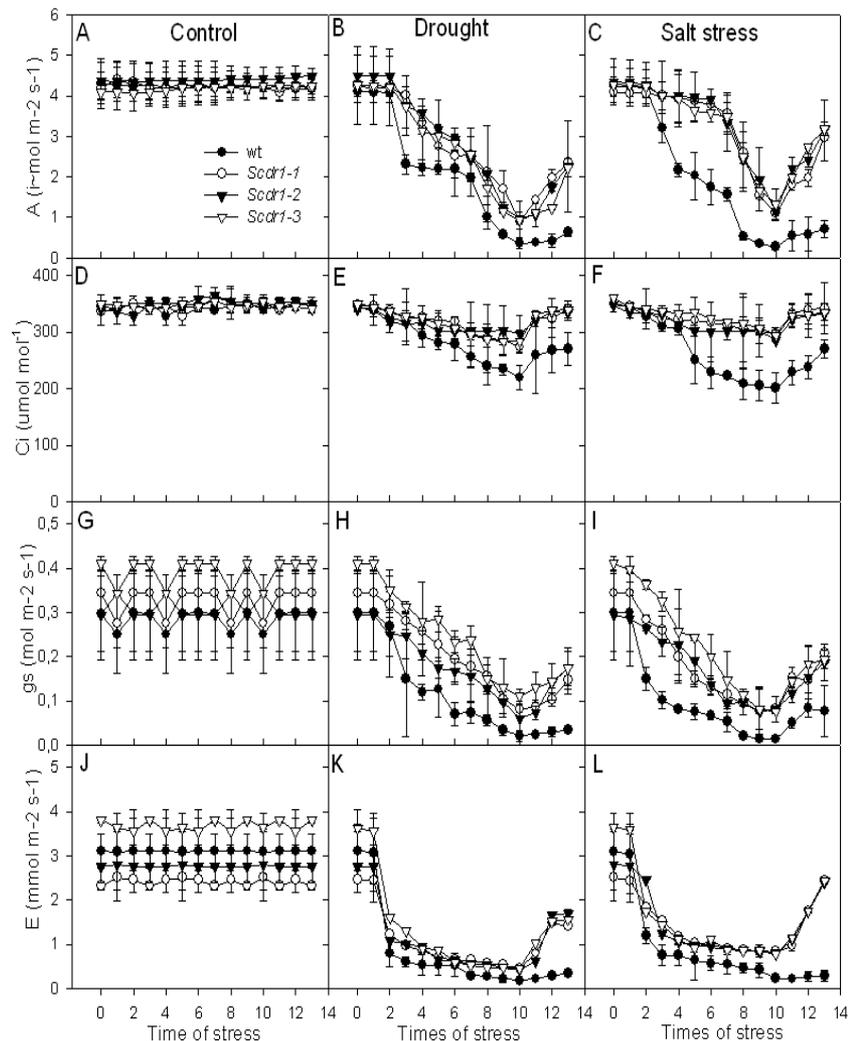
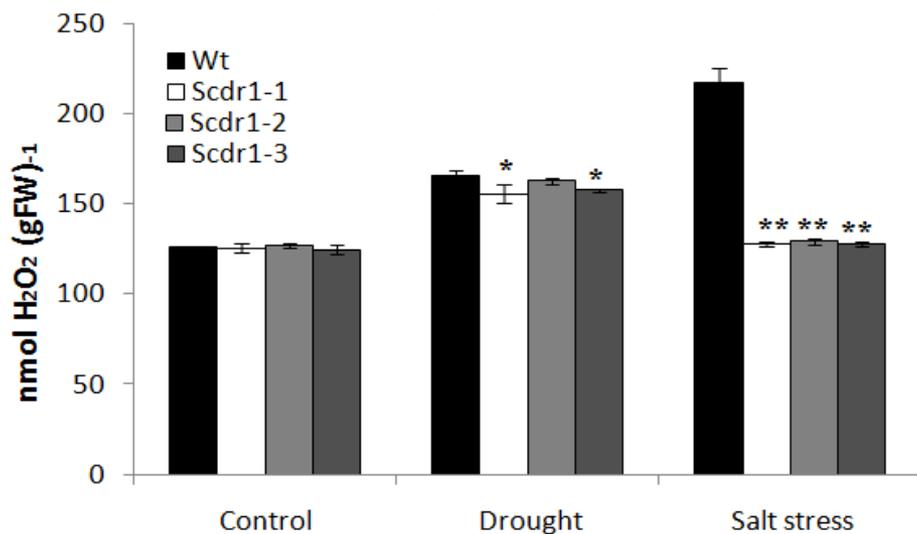
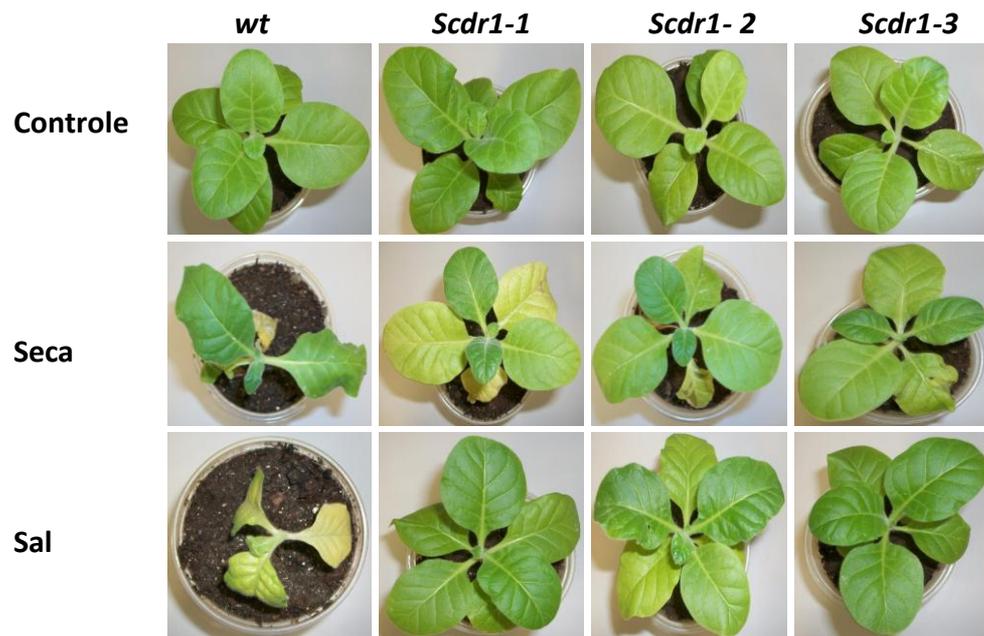
Production of transgenic *Brachypodium* plants

<i>Gene</i>	<i>Number of events</i>	<i>PCR +</i>	<i>RT-PCR +</i>
Bioenergy	26	10	9
Drought response	42	8	8
Photosynthesis	38	11	11



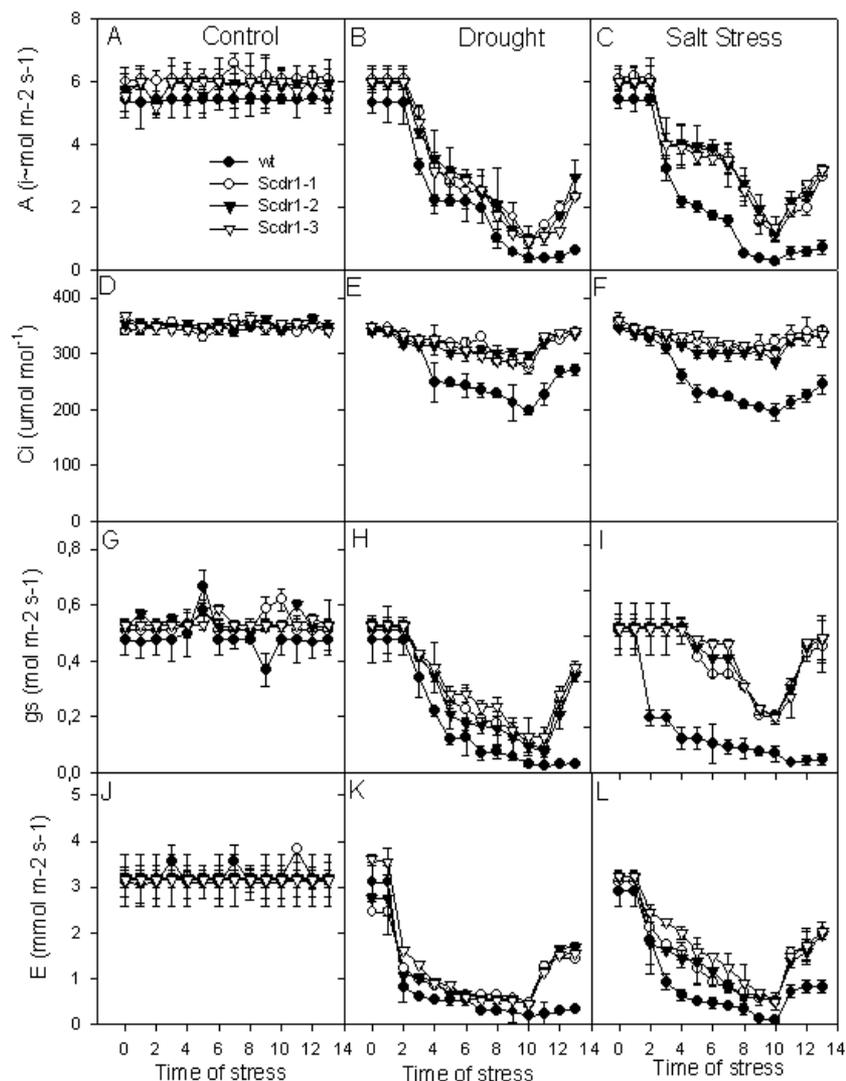
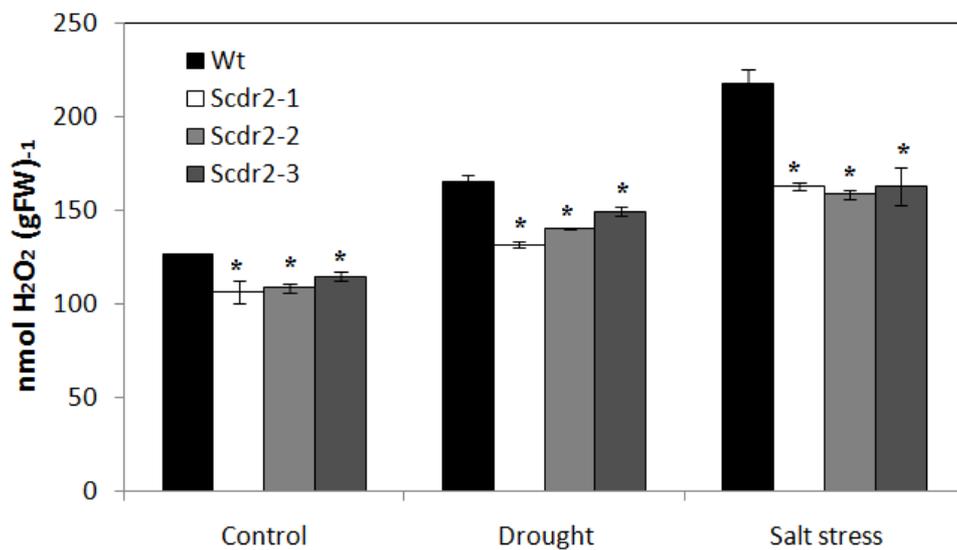
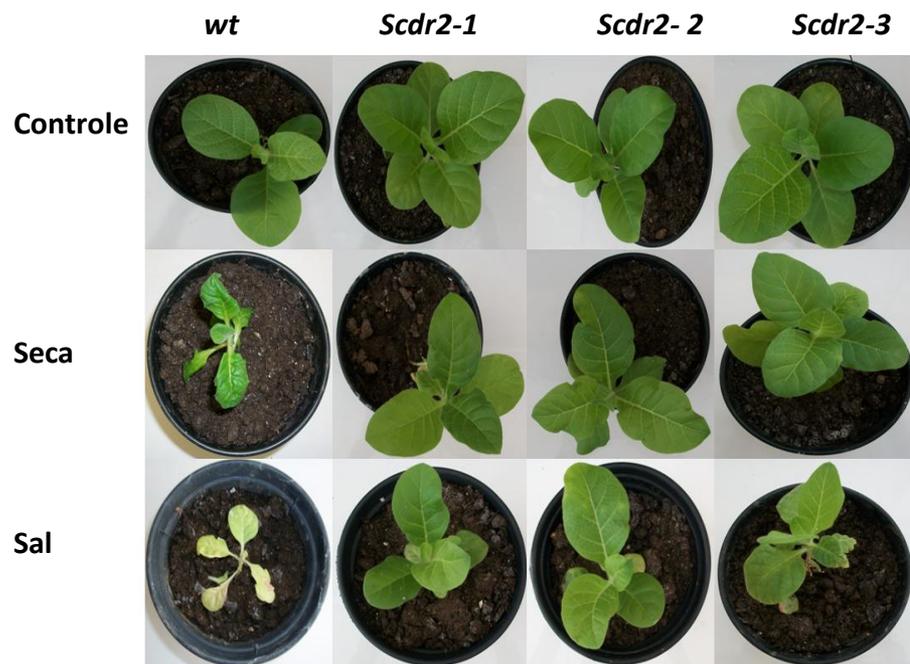
Plants waiting for import clearance (may take 9-12 months)

Gene *Scdr1* confers tolerance to drought in tobacco



Fonte: Begcy et al. (Pat. req.)

Gene *Scdr2* confers tolerance to drought in tobacco



Fonte: Begcy et al. (Pat. req.)

Concluding remarks and challenges

- Genomics and proteomics data highlighted differences between sugarcane varieties exposed to drought stress
 - Several new genes modulated by drought have been discovered, as those involved in protein folding, ABA synthesis and cell cycle
 - Selected genes from sugarcane can improve plant tolerance to drought stress
 - This is, to our knowledge, the first integrated study on sugarcane drought responses under field conditions
-
- The integration of data from physiology, proteomics, transcriptomics, microtranscriptomics and biotechnology will demand a large effort
 - A large scale facility to transform sugarcane is needed

Team

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Tadeu Patelo Barbosa
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Weverton G. Duarte
Wilson Libanio



UNICAMP

Thank you !

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