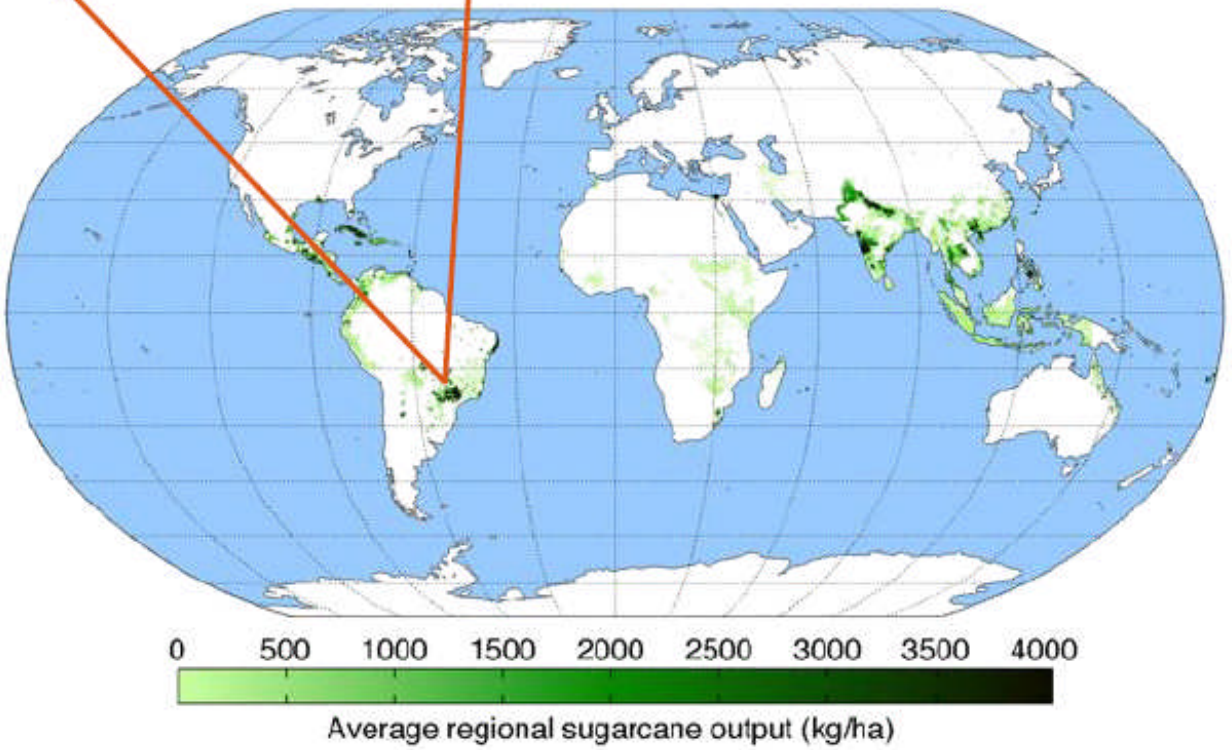
Molecular strategies developed by Embrapa to improve drought tolerance in sugarcane

Bárbara Andrade Dias B. da Cunha, MSc
Embrapa Agroenergy

- ✓ **Introduction**
- ✓ **MP3 - Water deficit-induced expression of *AREB* gene in sugarcane**
- ✓ **MP6 - Overexpression of *AtDREB2A CA* gene in sugarcane**
- ✓ **MP4 - Metabolite changes in transgenic sugarcane expressing drought tolerance-related gene**

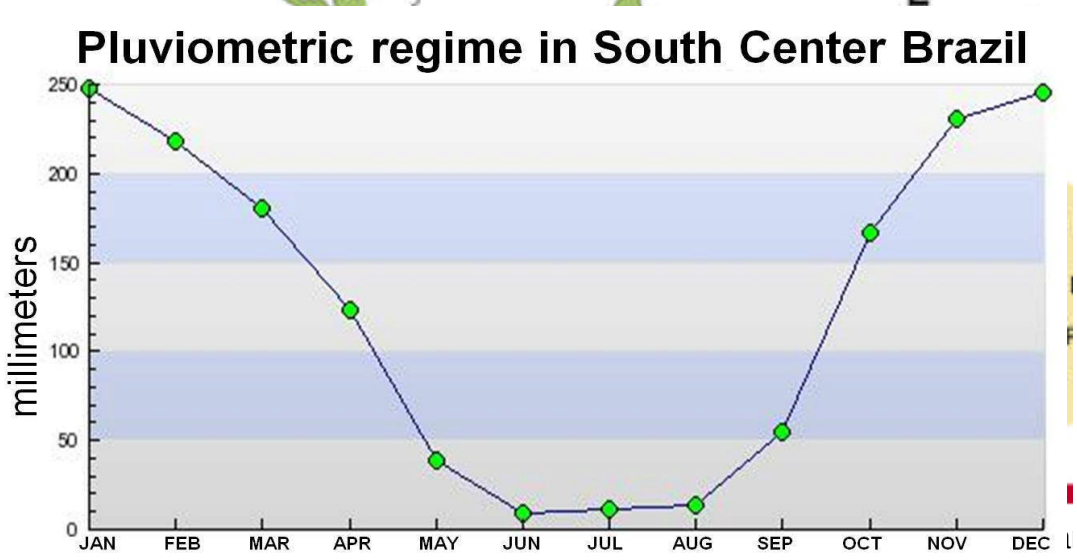
BRAZIL IS THE WORLD'S LARGEST SUGARCANE PRODUCER

- 658 million ton harvested in 2013/14.
- Brazil's South-Central region account for 93,1% of all harvested sugarcane.
- Estimated production increase in 2014/15: up to 2%.

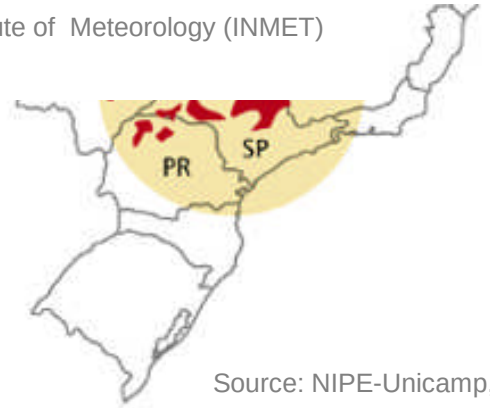


Source: University of Minnesota Institute on the Environment; Brazilian Sugarcane Industry Association (UNICA), Food and Agricultural Organization (FAO)

Economic impacts of drought in sugarcane



Source: National Institute of Meteorology (INMET)



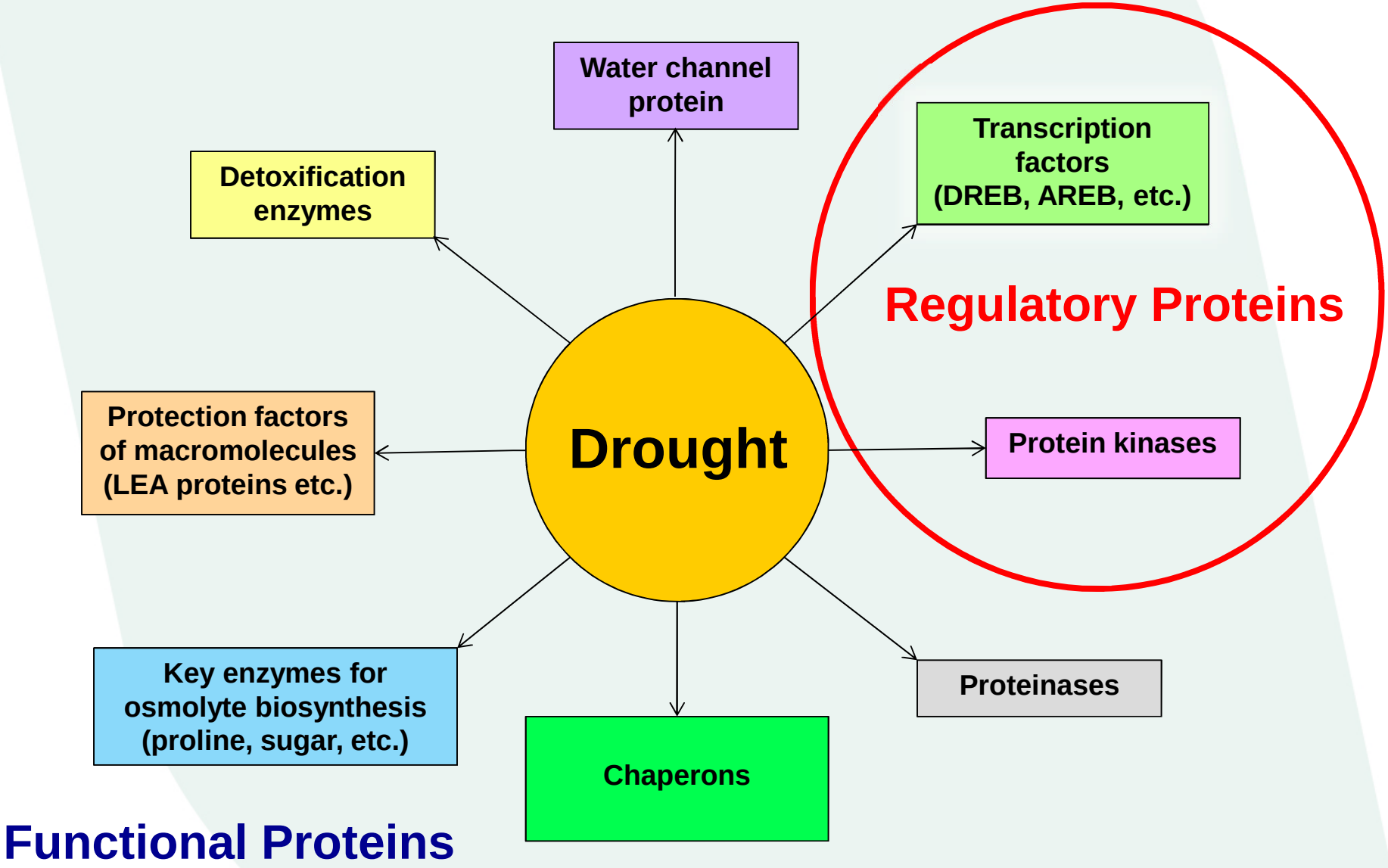
Source: NIPE-Unicamp, IBGE and CTC

Table 1 | Estimated losses in sugarcane fields due to drought stress.

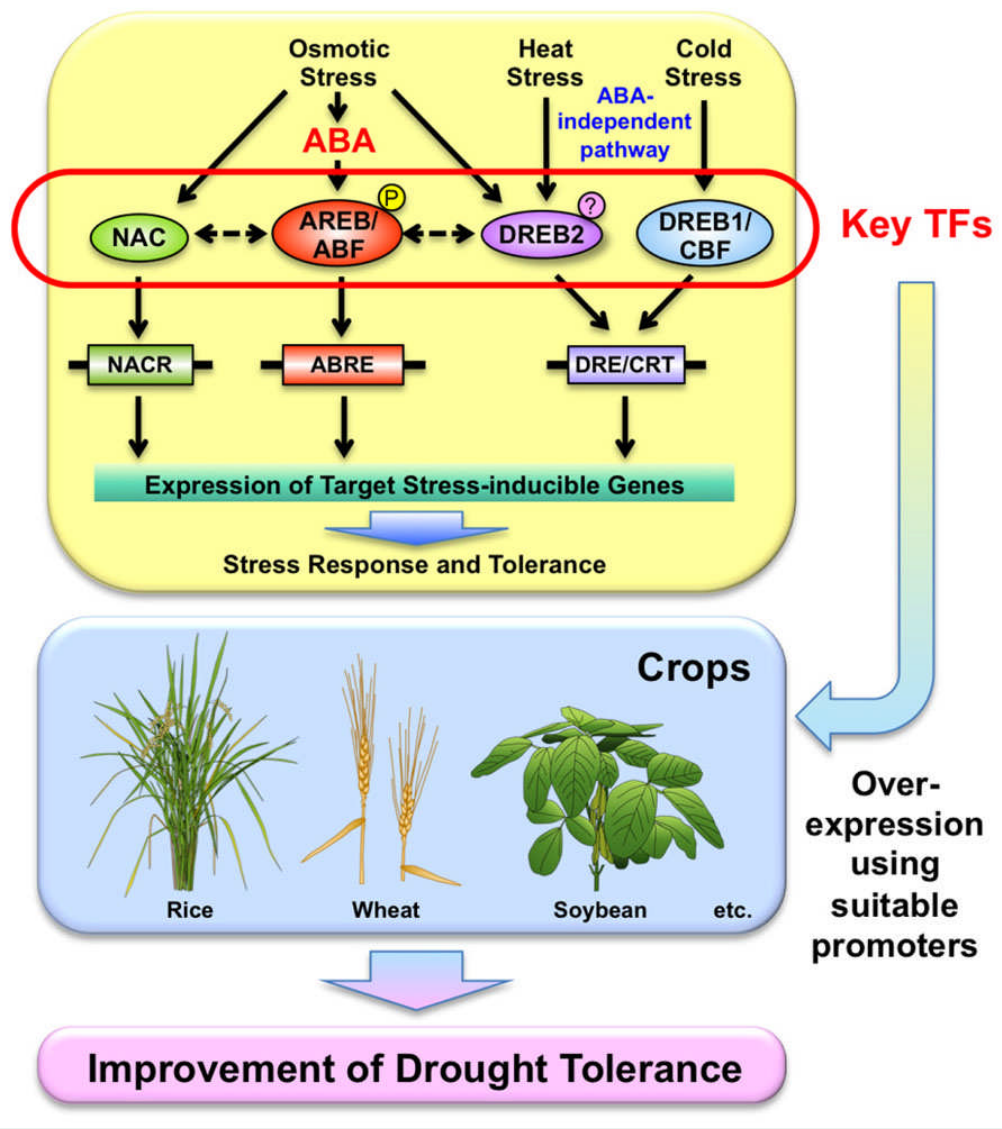
Year	Region	Losses	Rain (mm H_20)/ percentage of the expected rain
2008	São Paulo State	6.3% (Castro, 2008)	419.5/49%
2010	Zona da Mata (Pernambuco State)	40% (Cavalcanti, 2010)	300/50%
2012	Alagoas State	20% (Agência Globo, 2012; Sindaçucar, 2012)	774/48.6%
2012	Pernambuco State	35% (Associação dos fornecedores de cana de pernambuco, 2012; Camarotto, 2012)	629.4/50.7%
2013	Paraiba State	30% (G1 Agency, 2012; Silva, 2013)	n.a./up to 58.7%
2013	Zona da Mata (Pernambuco State)	25% (Brasilagro, 2013)	821/48.7%
2014	Ribeirão Preto (São Paulo State)	15% (Palhares, 2014)	480/51.6%

Gentile A, Dias LI, Mattos RS, Ferreira TH and Menossi M (2015) MicroRNAs and drought responses in sugarcane. Front. Plant Sci. 6:58.

AT CELL LEVEL: DROUGHT-INDUCIBLE GENE PRODUCTS



Transcription factor network involved in response to abiotic stresses



Nakashima et al., 2014 *Front Plant Sci*.16:1-7.

MP3 - Water deficit-induced expression of *AREB* gene in sugarcane

Water deficit-induced expression of *AREB* gene in sugarcane

MP3



Explant donor plant – variety RB855156 with 6-10 month old



Surface sterilization
1' ETOH 70%
15' NaOCl 2,5%



2-3 mm transversal segments 30-45 days culture in de dark MS + 3 mg.L^{-1} 2,4 D



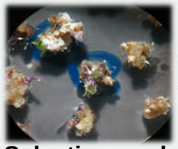
Osmotic treatment



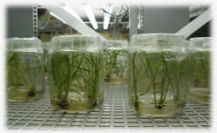
Shot with gene gun



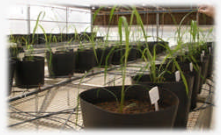
Osmotic treatment



Selection and regeneration plants



Selection of transformants in ammonium glufosinate 3 mg.L^{-1}



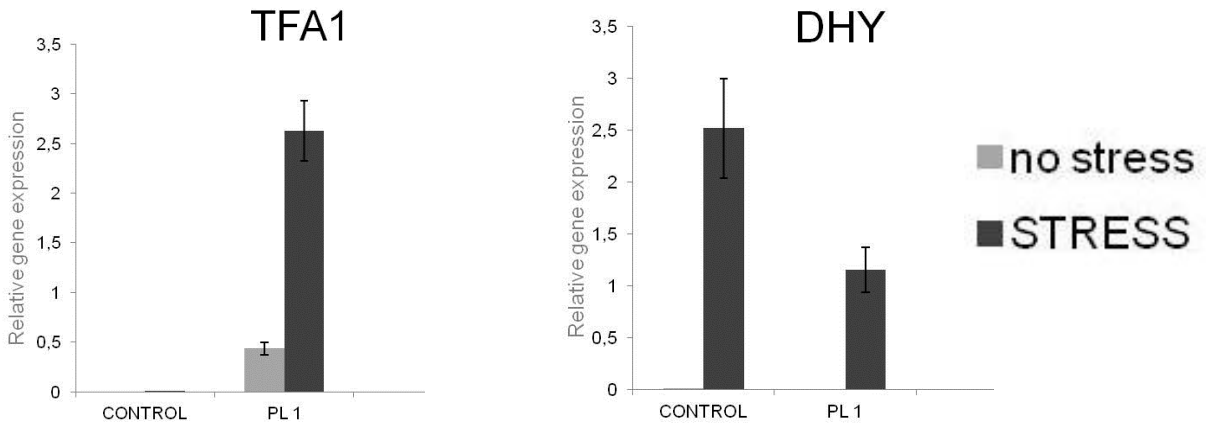
Acclimatization

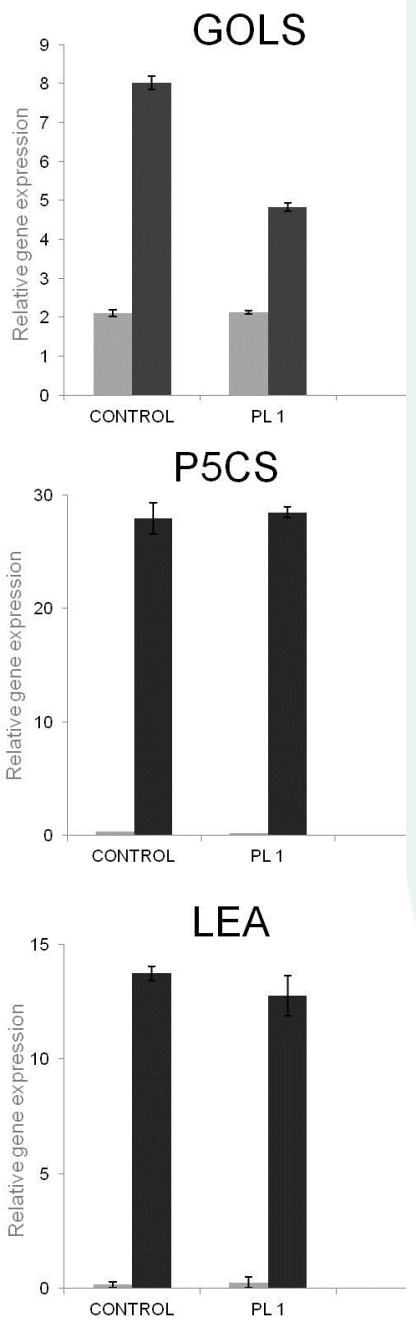
(Molinari et al., 2007)



TRANSFORMATION EFFICIENCY				
Vector	Nº explants	Selectable marker	N° of bar positive plant	Efficiency (%)
MISP::TFA1	921	*bar*	3	0,43

Real Time qPCR assays



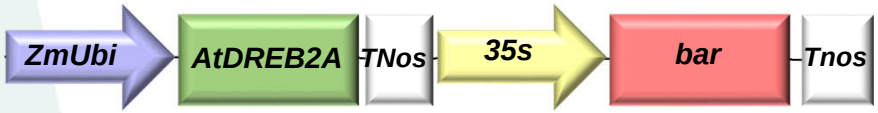


Net photosynthetic rate (µmol $m^{-2}s^{-1}$)		
events	No stress	stress
Control	14.15	8.67
Plant 1	24.96	5.41
Soil water potential (MPa)		
Control	-0.24	-1.93
Plant 1	-0.09	-4.04

MP6- Overexpression of *AtDREB2A CA* gene in sugarcane

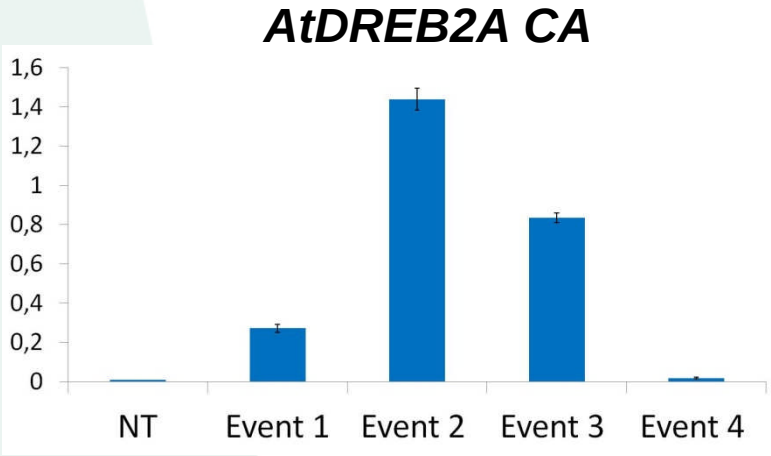
Overexpression of *AtDREB2A CA* gene in sugarcane

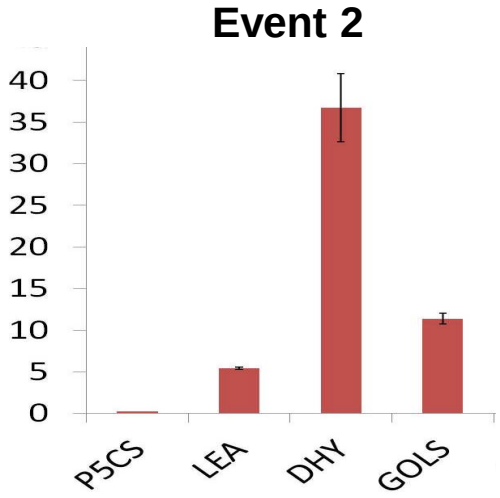
MP6

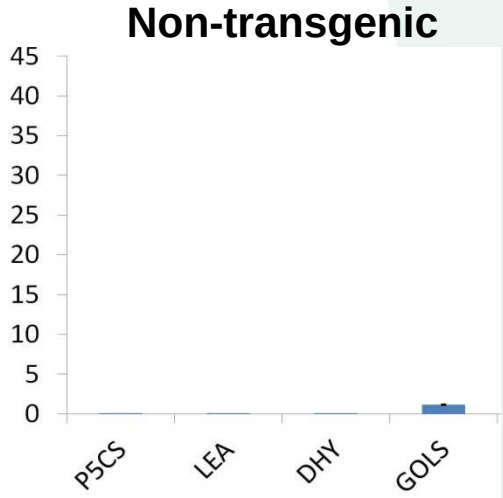


TRANSFORMATION EFFICIENCY				
Vector	Nº explants	Selectable marker	N° of bar positive plant	Efficiency (%)
Ubi:DREB	800	*bar*	4	0,5

Real Time qPCR assays

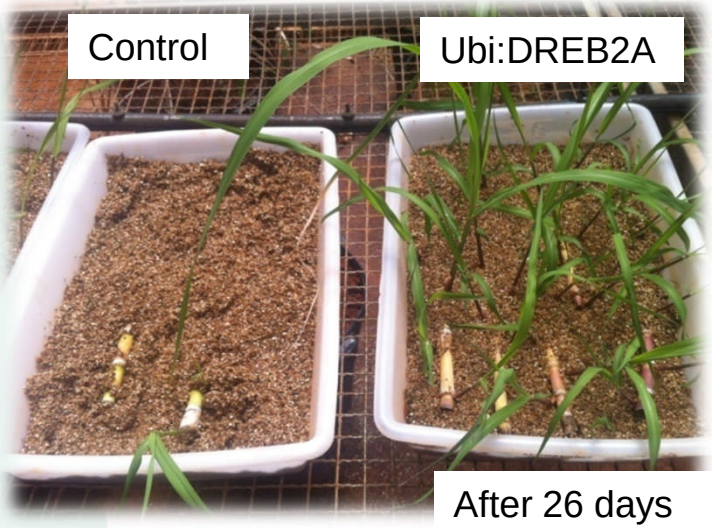






PHENOTYPIC ANALYSIS

Sugarcane sprouting in vermiculite

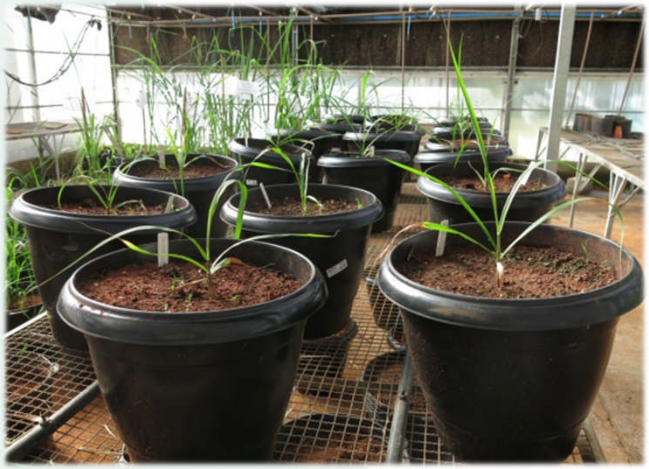


After 26 days



Three-month-old plants

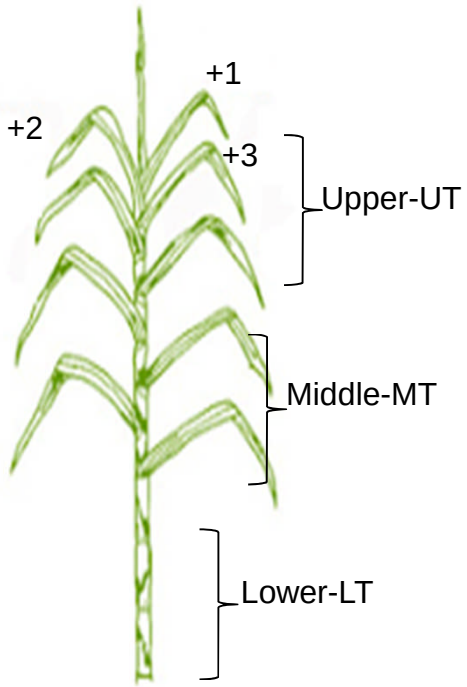
Plant multiplication for physiological analysis



MP4 - Metabolite changes in transgenic sugarcane expressing drought tolerance-related gene

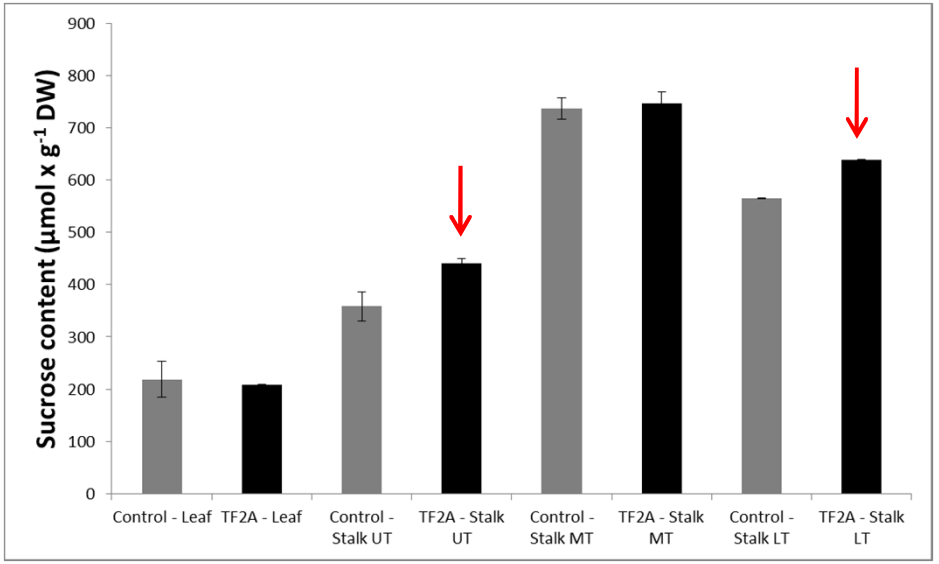
Metabolite changes in transgenic sugarcane expressing drought tolerance-related gene MP4



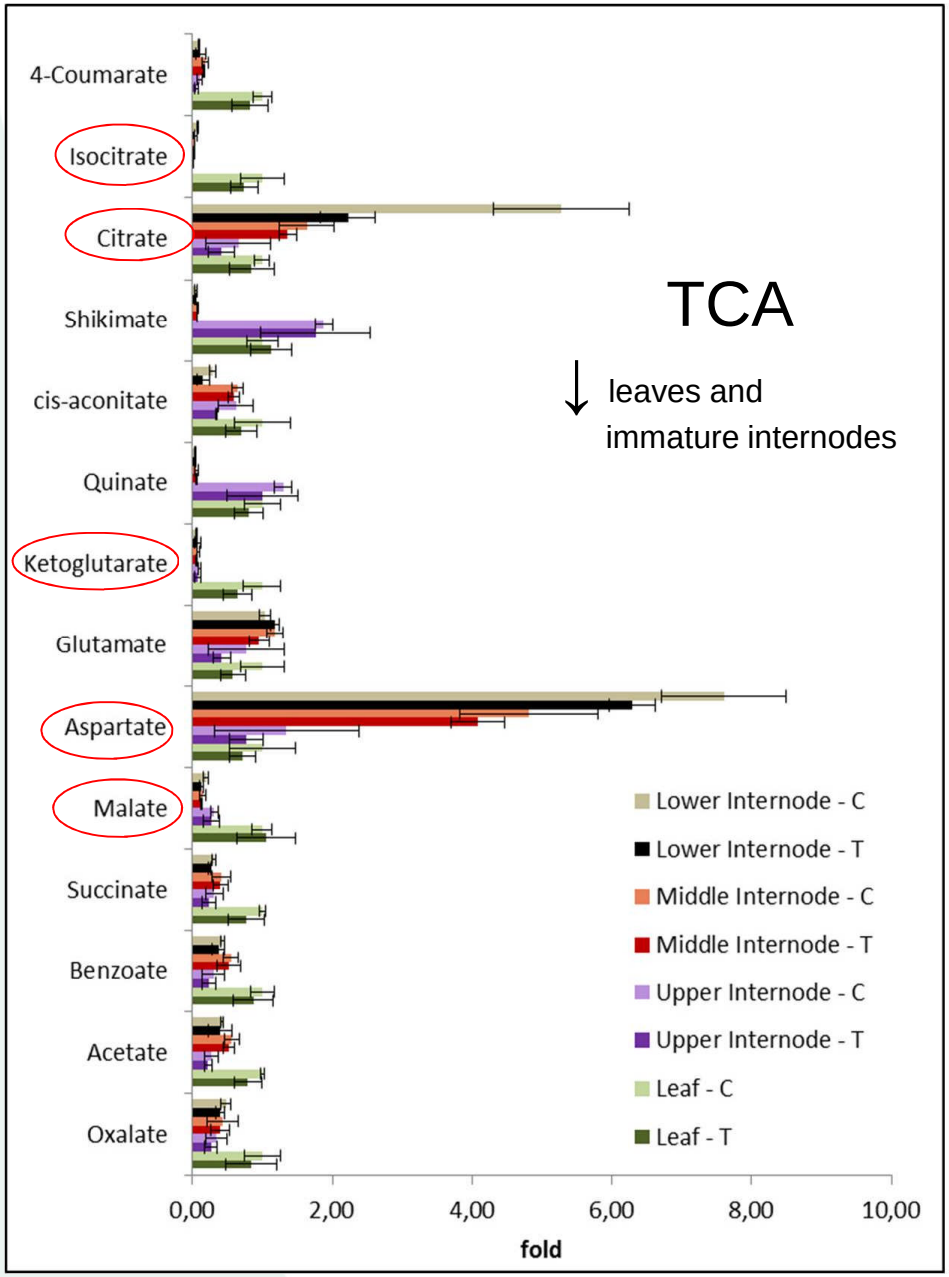


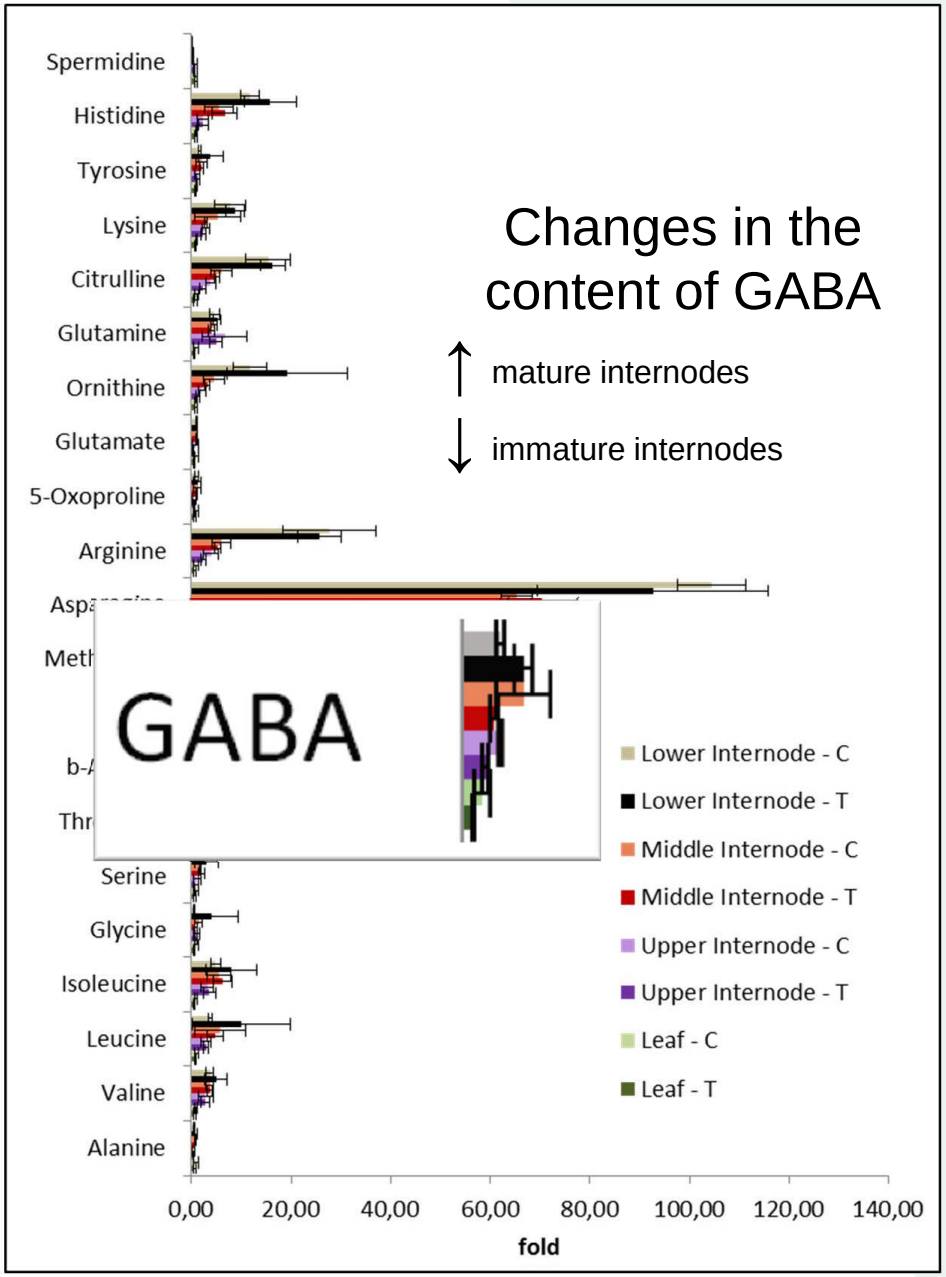
Eight-month-old transgenic and non-transgenic plants

➢ GC-MS result:



- ✓ Could not show a clear pattern of changes in sugar between the transgenic and control plants.
- ✓ However, sucrose level increased in immature and mature internodes.



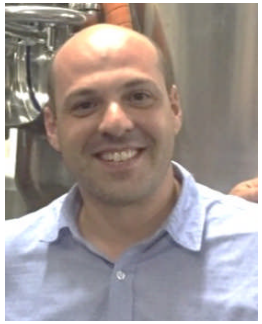


✓ Together, these results indicate a slight decrease on the metabolism of immature internodes of the transgenic, resulting in accumulation of carbon as sucrose in those tissues.





Sugarcane/Setaria Team



Hugo Bruno C. Molinari, PhD



Adilson Kenji Kobayashi, PhD



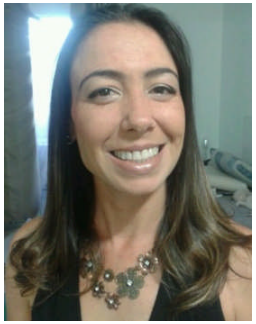
Carlos A. F.de Souza, PhD



Letícia Jungmann Cançado, PhD



Bárbara A. D. B. da Cunha, MSc



Polyana Kelly Martins, PhD



Ana Paula Ribeiro, PhD student

Thank you

barbara.dias@embrapa.br

Embrapa

Ministry of Agriculture, Livestock and Food Supply

BRAZILIAN GOVERNMENT BRASIL