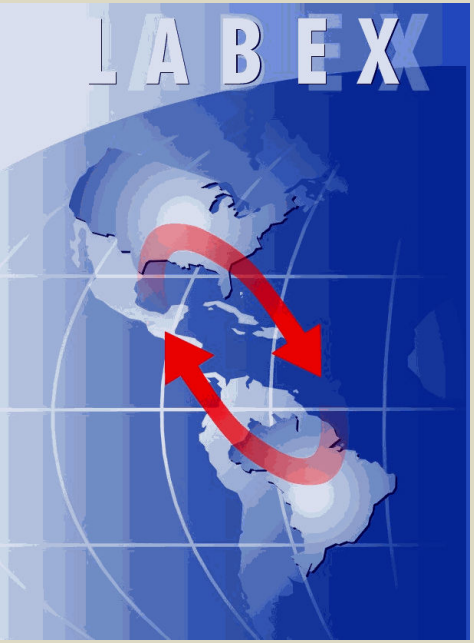
Brazil-US Labex program in plant biotechnology: experiences and insights

Alexandre Lima Nepomuceno
Brazilian Agricultural Research Corporation
Embrapa Labex USA

What is Labex ?



The Labex – Brazilian Agricultural Research Corporation (Embrapa)'s Virtual Laboratory Abroad is an instrument of international scientific cooperation in strategic areas and themes

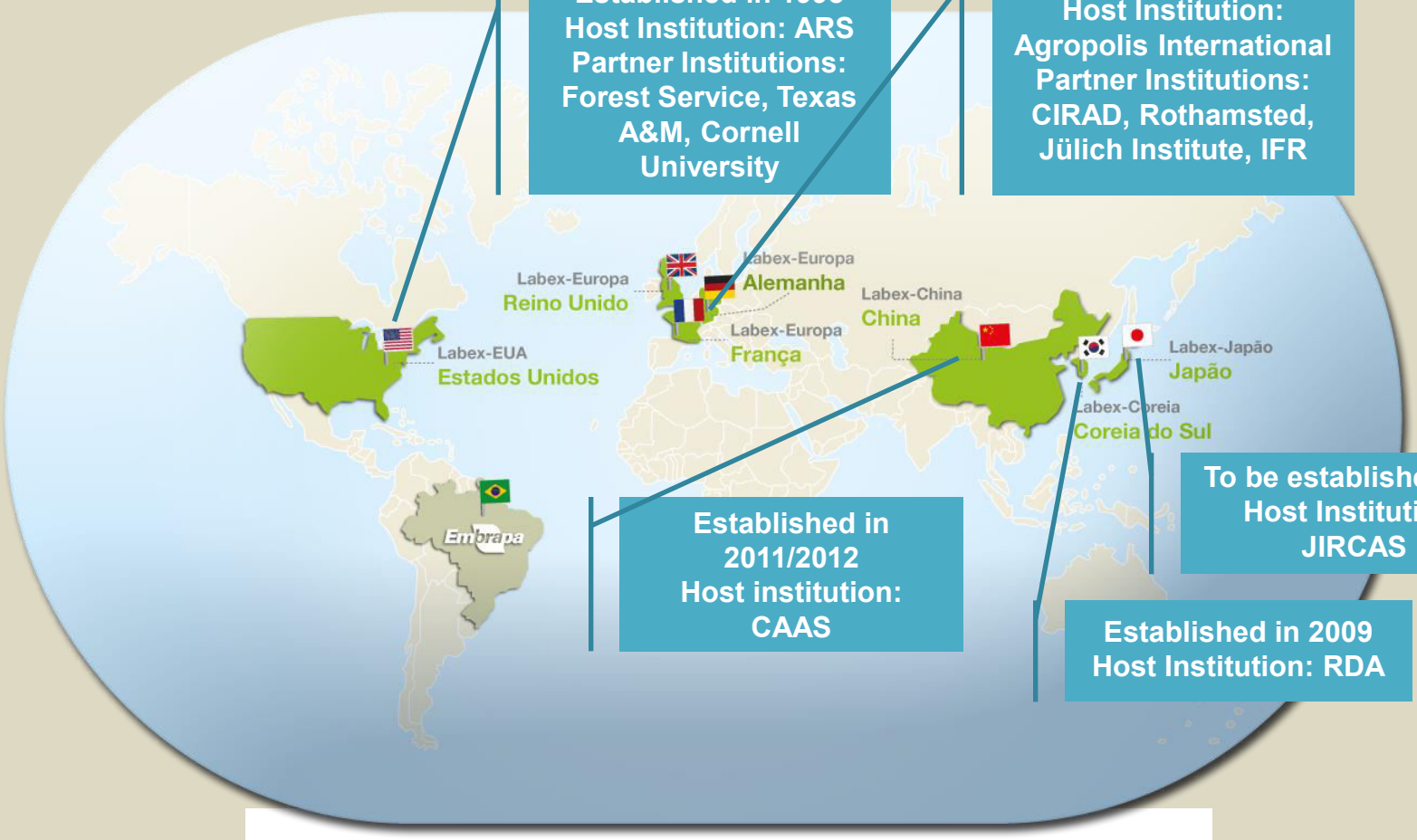
The Labex seeks to reduce time and cost in the development of research, through the exchange of senior researchers with institutions of excellence in different countries or regions

Knowledge Exchange

LABEX

Established in 1998
Host Institution: ARS
Partner Institutions:
Forest Service, Texas A&M, Cornell University

Established in 2002
Host Institution:
Agropolis International
Partner Institutions:
CIRAD, Rothamsted, Jülich Institute, IFR

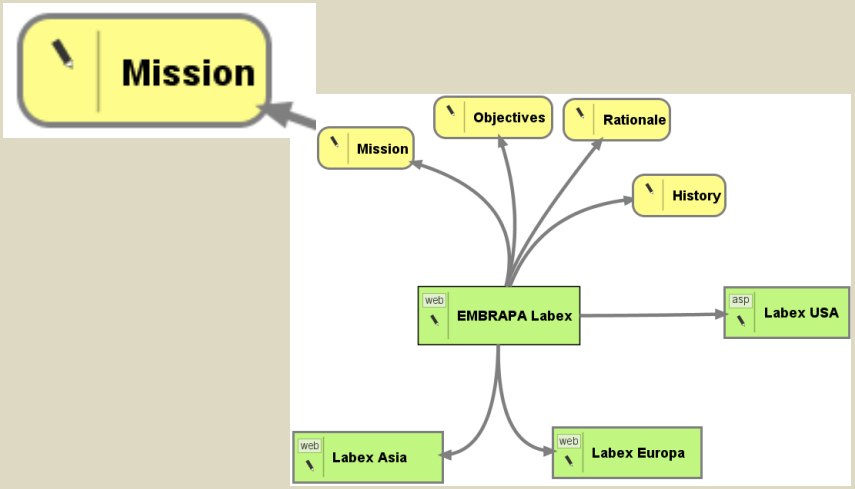


Established in 2011/2012
Host institution:
CAAS

To be established in a Host Institution:
JIRCAS

Established in 2009
Host Institution: RDA

Labex



To promote opportunities for international cooperation, in agricultural research of shared interest to the partner countries and organizations

Labe USA - Areas

- ✓ *Food safety*
- ✓ *Nanotechnology*
- ✓ *Plant & animal genetic resources*
- ✓ *Bioenergy*
- ✓ *Biotechnology (Genome Editing, RNAi topic, etc)*
- ✓ *Animal health*
- ✓ *Forestry management*
- ✓ *Integrated disease & pest management*
- ✓ *New products*
- ✓ *Precision farming*
- ✓ *Climate changes*
- ✓ *Natural resources*
- ✓ *Bioactive compounds*
- ✓ *Plant Biotechnology (Drought, GWS)*
- ✓ *Citrus Huanglongbing*

Priorities

LABEX US Plant Biotechnology

Location

Plant Gene Expression Center (PGEC)

East San Francisco Bay; Albany - CA

PLANT & MICROBIAL BIOLOGY
UNIVERSITY OF CALIFORNIA, BERKELEY

EMBRAPA LABEX
United States of America

PLANT GENE EXPRESSION CENTER

Agricultural Research Service
United States Department of Agriculture

Plant & Microbial Biology
University of California, Berkeley

Home | Labs | People | Seminars | Links | Directions

Arabidopsis images, Fletcher Lab

Welcome!

The Plant Gene Expression Center co
in plant molecular biology. Researche
transduction pathways responsible for
environmental and cellular cues. We a
resistance, light perception, the circad
and reproduction. Essential genes an
they operate are elucidated using molecular, genetic and bio

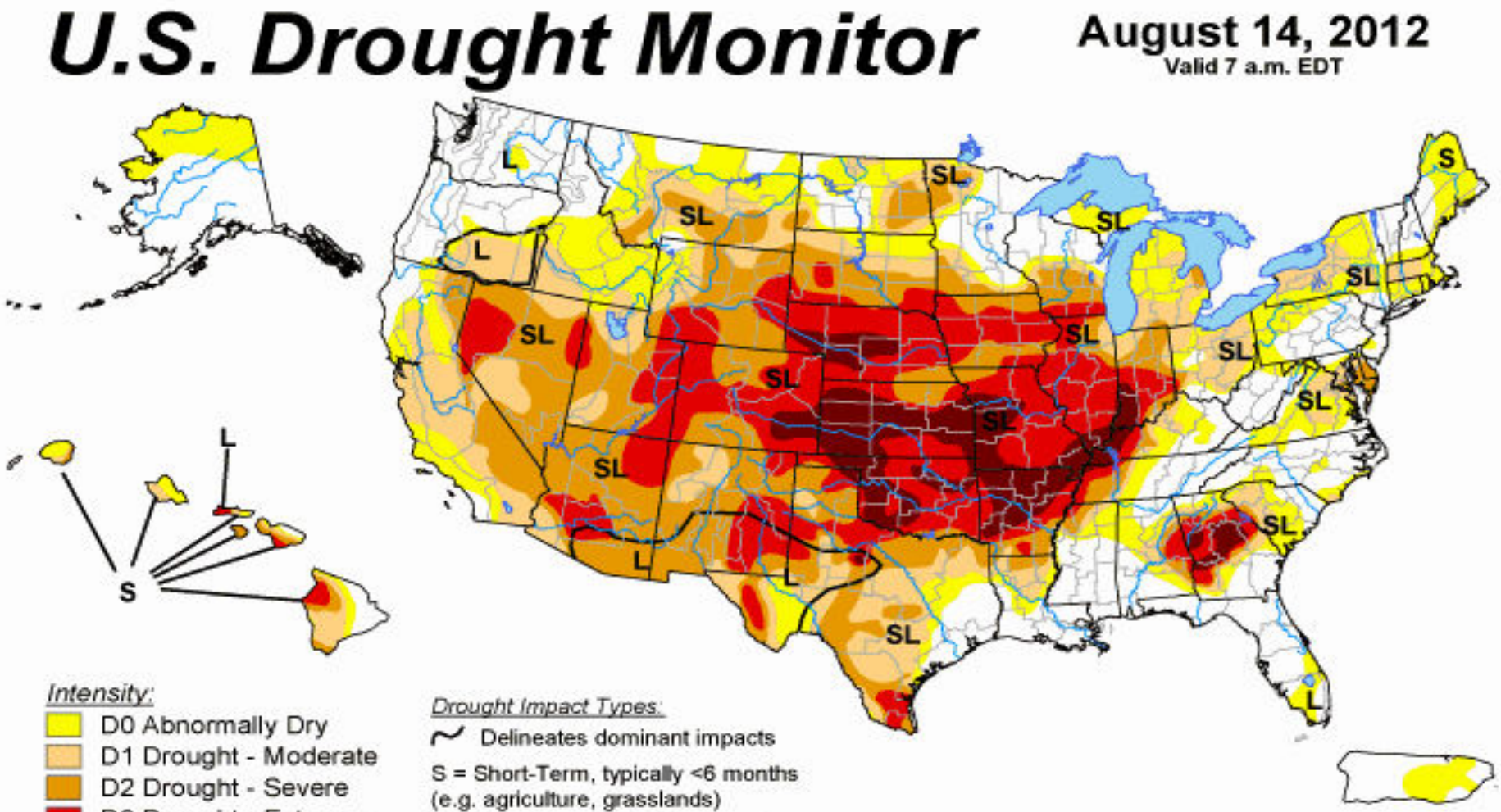
PGEC is a collaboration of the Agricultural Research Service
Agriculture and the Plant & Microbial Biology Department of t
Berkeley. The Center's principal investigators are faculty at U
opportunities are available in our laboratories for graduate ar

Sarah Hake Inducted into the Science Ha

Sarah Hake was recently inducted into the Agricultural Research Service

Sheila McCormick | Frank Harmon | **Sarah Hake, Center Director** | Jennifer Fletcher | Barbara Baker | **Peter Quail, Research Director**

We need to find a Main Focus: Plant Responses to Drought



Soybean Losses due Drought Events South of Brazil 1999/2000 to 2008/2009

	Production (10^3 ton)	Produtivity (%)	US$ Billion
PR	11.788	10,24	4,7
RS	28.552	28,6	11,4
PR/RS	40.340	38,84	16,1
Brasil	47.110	8,34	18,8

Source: Farias et al, 2009

Base Harvesting Season: 2002/2003

Circadian Clock x Abiotic Stress

the plant journal

SEB SOCIETY FOR EXPERIMENTAL BIOLOGY

The Plant Journal (2010) **63**, 715–727 doi: 10.1111/j.1365-313X.2010.04274.x

Time of day shapes Arabidopsis drought transcriptomes

Olivia Wilkins, Katharina Bräutigam and Malcolm M. Campbell*

Department of Cell and Systems Biology, University of Toronto, 25 Willcocks Street, Toronto, ON M5S 3B2, Canada, and Centre for the Analysis of Genome Evolution and Function, University of Toronto, 25 Willcocks Street, Toronto, ON M5S 3B2, Canada

The EMBO Journal (2009) 28, 3745–3757 | © 2009 European Molecular Biology Organization | All Rights Reserved 0261-4189/09
www.embojournal.org

THE EMBO JOURNAL

TOC1 functions as a molecular switch connecting the circadian clock with plant responses to drought

Tommaso Legnaioli[1], Juan Cuevas[1] and Paloma Mas*

Circadian Clock X Productivity

OPEN ACCESS Freely available online

PLoS one

Expression of the *Arabidopsis thaliana BBX32* Gene in Soybean Increases Grain Yield

Sasha B. Preuss[1]*, Robert Meister[1], Qingzhang Xu[1¤a], Carl P. Urwin[1], Federico A. Tripodi[1], Steven E. Screen[1], Veena S. Anil[2¤b], Shuquan Zhu[1], James A. Morrell[1], Grace Liu[1], Oliver J. Ratcliffe[3], T. Lynne Reuber[3], Rajnish Khanna[3], Barry S. Goldman[1], Erin Bell[1], Todd E. Ziegler[1], Amanda L. McClerren[1], Thomas G. Ruff[1], Marie E. Petracek[1]

1 Monsanto Company, St. Louis, Missouri, United States of America, **2** Monsanto Research Centre, Monsanto Company, Hebbal, Bangalore, India, **3** M Inc., Hayward, California, United States of America

AtBBX32 in soybean alters transcript levels of the soybean clock genes GmTOC1 and LHY-CCA1-like2 (GmLCL2). Modulation of the abundance of circadian clock genes during the transition from dark to light, the timing of critical phases of reproductive development are altered.

Table 1. *AtBBX32* transgenic soybean plants demonstrate improved grain yield over non-transgenic controls.

	Season 1 United States N = 10		Season 2 United States N = 19		Season 3 Argentina N = 14		Meta-analysis across seasons N = 43			
Line	Yield (kg/h)	% change vs control	Yield (kg/h)	% change vs control	Yield (kg/h)	% change vs control	Yield (kg/h)	% change vs control	ΔDOF	Δ MAT
1	4725	3.2	3968	8.5**	3766	7.7**	4068	6.9**	0	1.6**
2	4707	3.7	4040	7.2**	3661	3.1	4076	5.3**	−0.4	1.4**
3	4604	−1	3953	6.1**	3481	4.4	3966	4.1**	1.0**	1.8**
4	4277	−6.4**	3777	1.8	3287	−7.3**	3762	−2.3	0.4	0
5	4693	0.3	3972	7.1**	3655	6.4*	4040	5.6**	−0.2	1.3**
6	4814	0.1	3957	8.7**	3519	1.4	4014	4.8**	−0.2	0.9**
7	4491	−4.8*	3867	4.4*	3550	2.4	3917	1.9	−0.7**	0.2
8	4731	5.3*	3902	5.8**	3696	6.5*	4019	5.9**	−0.5	1.0**

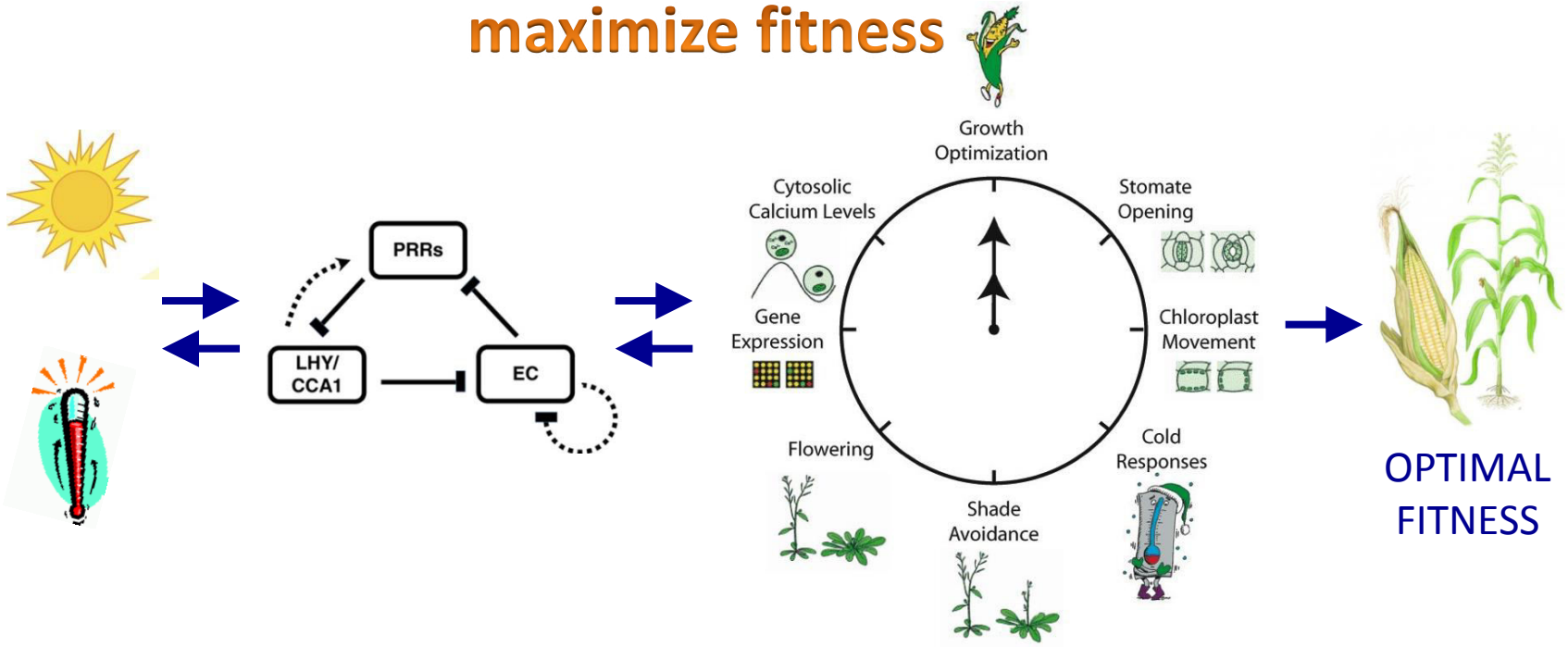
Mean yield values (kilograms per hectare) and percent improvement over controls for transgenic plots are shown for three growing seasons. The difference in the day of flowering (DOF) between the transgenic lines and control was calculated to determine delta DOF. The difference in day of final maturity (MAT) was examined in transgenic lines and compared to control to determine delta MAT (units = days). The low yielding event 4 produced no detectable transcript. N represents the number of environments tested. p-values were based on the difference between the transgenic lines and wildtype control.

*$p \leq 0.05$,

**$p \leq 0.01$.

doi:10.1371/journal.pone.0030717.t001

The Circadian clock acts by transcription/transduction of key genes activated by the Environmental cues to synchronize all plant physiological processes and maximize fitness



Environmental Cues

Molecular Clock

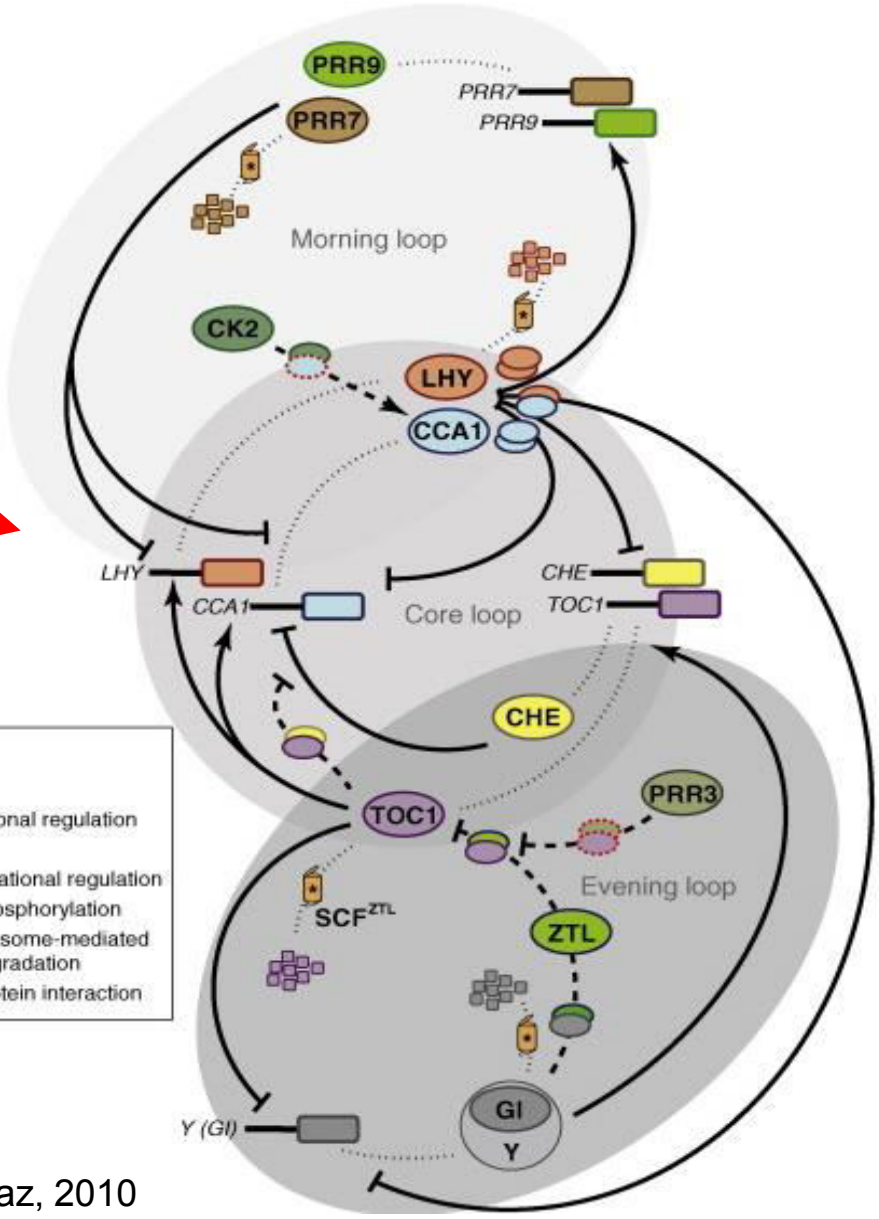
Circadian regulation of physiological processes

Synchrony among plant processes and with environmental conditions

Transcription/Translation Arabidopsis Circadian Clock Model

photoreceptors

Red
FR
Blue



Key:
- Gene
- Transcriptional regulation
- Protein
- Post-translational regulation
- Protein phosphorylation
- 26S proteasome-mediated protein degradation
- Protein-protein interaction

Feedback Loops

- At least three loops.
- Core: CCA1/LHY (-) and were recently, **TOC1** was confirmed as a transcriptional repressor that inhibits expression of transcription factors expressed in the morning.

Pruneda-Paz, 2010

Objectives

Investigate

- How the soybean transcriptome is affected along the day under drought conditions?
- How the soybean clock behaves: it resembles the *A. thaliana* clock model?
- How drought affects soybean circadian clock genes?
- How the time of day affects soybean drought responsive genes?
- Can we identify candidate genes to design genetic engineering strategies to improve abiotic stress tolerance?

Projects

Soybean Circadian Clock Genes responses during dehydration and how it affects defences against drought

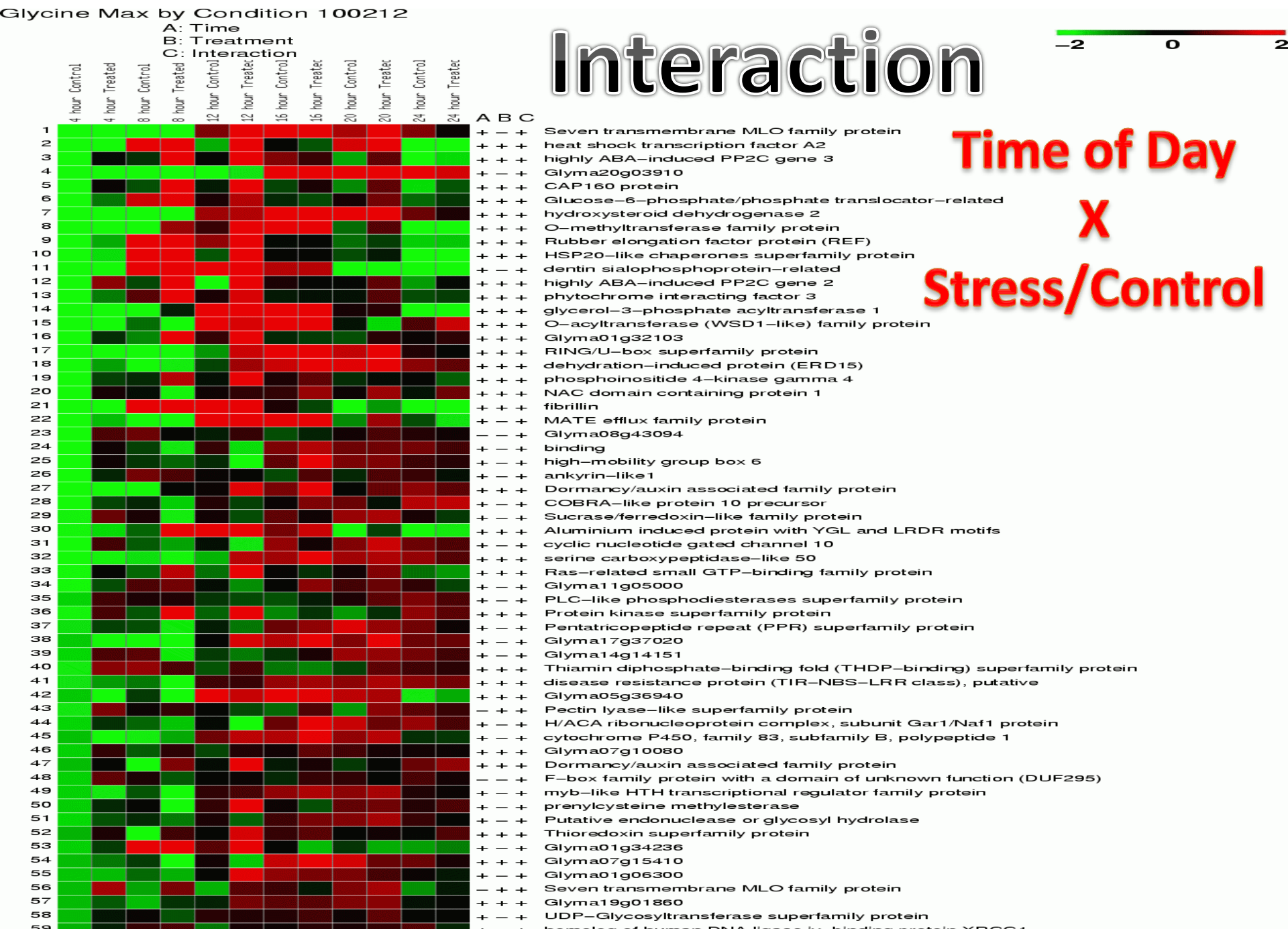
Project : Evaluation of soybean wide transcriptome responses during drought and how circadian clock genes are affected

Project : Quantitative gene expression evaluation of Soybean/A.thaliana clock gene orthologs in response to drought clock genes are affected

Project : Design of strategies to develop Geneticaly Modified Plants.

LABEX Research Group at ARS/USDA - PGEC

PhD Students **Pos-Docs** **Embrapa Researchers**



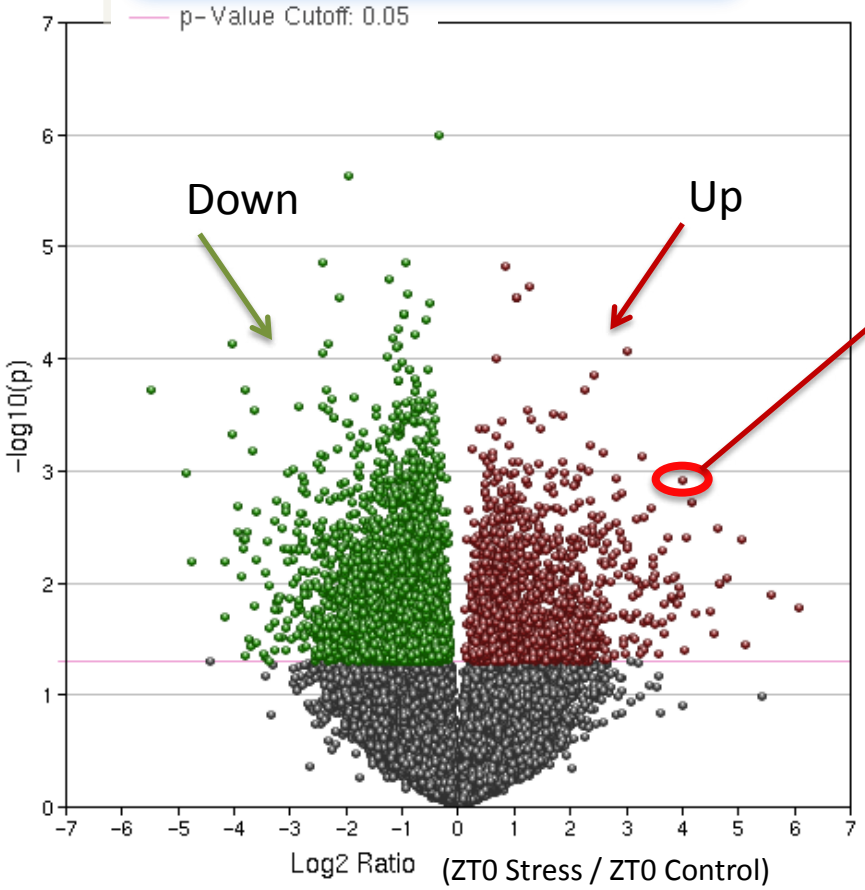
Interaction

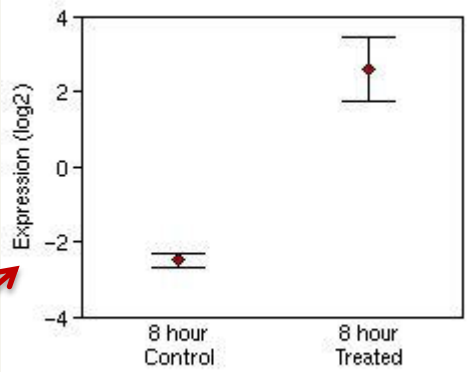
Time of Day X Stress/Control

Rodrigues, Harmon, Nepomuceno, *unpublished*

Project : Design of strategies to develop Geneticaly Modified Plants.

Soybean - RNA Seq



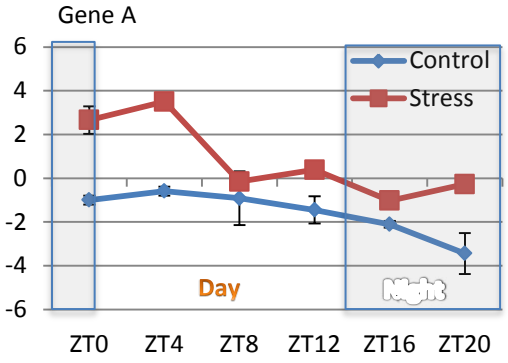


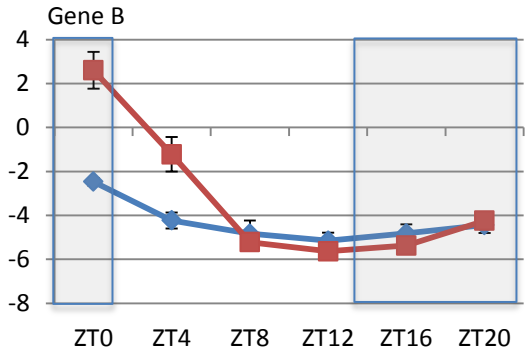
Glyma X Expressed at ZT0 (8am) under Water Deficit was up-regulated 33.6 fold when compared to Control.

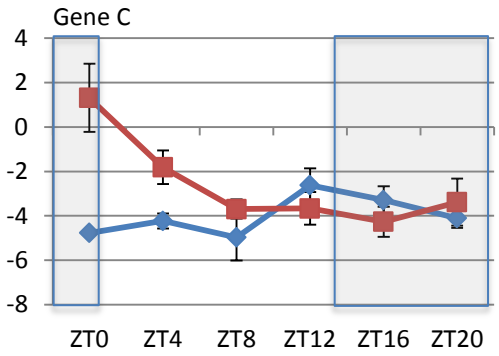
ZT0= when light are turned on

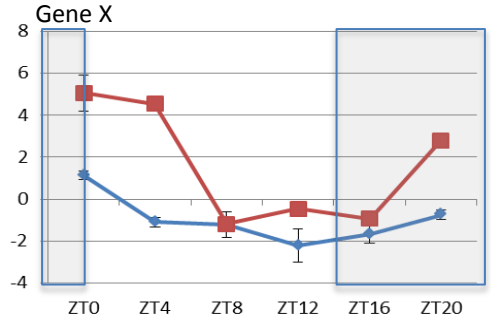
Source: Nepomuceno and Harmon, 2012 – ARS/USDA PGEC / Embrapa LABEX US

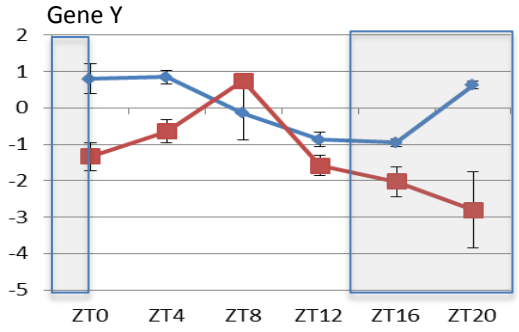
Candidate Genes for Transformation aiming improvement of Abiotic Stress Tolerance

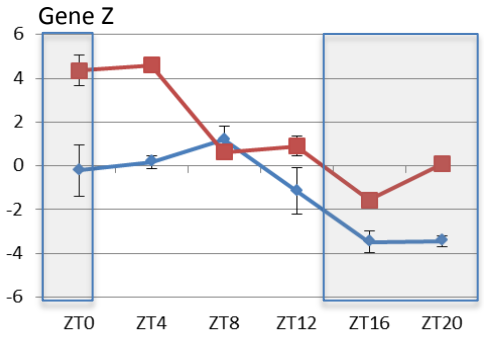


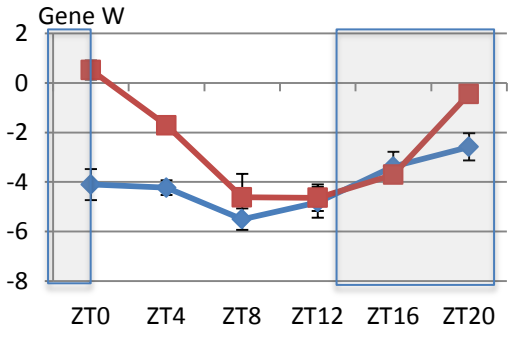


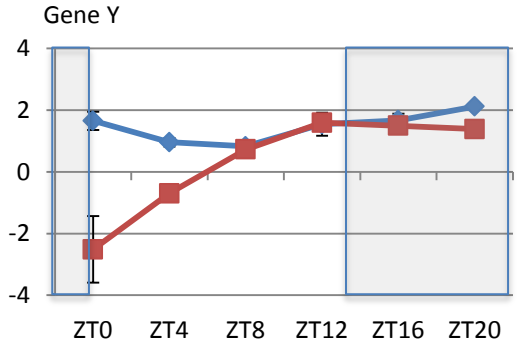


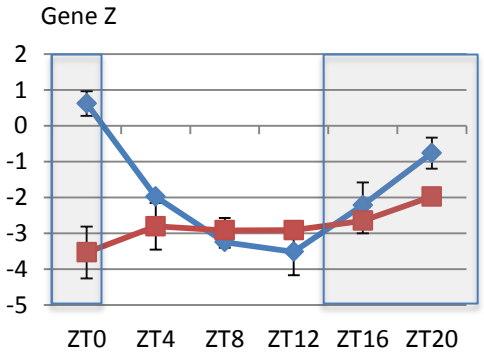












Source: Nepomuceno and Harmon, 2012 – ARS/USDA PGEC / Embrapa LABEX US

Drought tolerance: Soybean canditate Genes expressed and edited in A.thaliana

WT

F5-8

F3-6

EVENTS WITH ENHANCED DROUGHT TOLERANCE

DIÁRIO OFICIAL DA UNIÃO

Publicado em: 22/01/2018 | Edição: 15 | Seção: 1 | Página: 2-8
Órgão: Ministério da Ciência, Tecnologia, Inovações e Comunicações / Comissão Técnica Nacional de Biossegurança

RESOLUÇÃO NORMATIVA Nº 16,.DE 15 DE JANEIRO DE 2018

ANEXO I

Estabelece os requisitos técnicos para apresentação de consulta à CTNBio sobre as Técnicas Inovadoras de Melhoramento de Precisão

A COMISSÃO TÉCNICA NACIONAL DE BIOSSEGURANÇA - CTNBio, no uso de suas atribuições legais e regulamentares e em observância às disposições contidas nos incisos XV e XVI do art. 14 da Lei nº 11.105, de 24 de março de 2005;

CONSIDERANDO a necessidade de avaliar as Técnicas Inovadoras de Melhoramento de Precisão (TIMP), do inglês Precision Breeding Innovation (PBI) e que também englobam as denominadas Novas Tecnologias de Melhoramento, do inglês New Breeding Technologies -NBTs, à luz dos preceitos previstos na Lei nº 11.105, de 24 de março de 2005;

Considerando que a Lei nº 11.105, de 2005, define moléculas de ADN/ARN recombinante, engenharia genética e organismo geneticamente modificado - OGM nos incisos III, IV e V de seu art. 3º, respectivamente;

Considerando que as TIMP abrangem um conjunto de novas metodologias e abordagens que diferem da estratégia de engenharia genética por transgenia, por resultar na ausência de ADN/ARN

OPEN ACCESS Freely available online

PLOS ONE

Diurnal Oscillations of Soybean Circadian Clock and Drought Responsive Genes

January 2014 | Volume 9 | Issue 1 | e86402

Juliana Marcolino-Gomes[1,2], Fabiana Aparecida Rodrigues[1], Renata Fuganti-Pagliarini[1], Claire Bendix[3], Thiago Jonas Nakayama[4], Brandon Celaya[3], Hugo Bruno Correa Molinari[5], Maria Cristina Neves de Oliveira[1], Frank G. Harmon[3], Alexandre Nepomuceno[1,5]*

1 Embrapa Soybean, Brazilian Agricultural Research Corporation, Londrina, Paraná, Brazil, **2** Department of Biology, State University of Londrina, Londrina, Paraná, Brazil, **3** Plant Gene Expression Center, ARS/USDA, Albany, California, USA and Department of Plant and Microbial Biology, University of California-Berkeley, Berkeley, California, USA, **4** Department of Crop Science, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil, **5** Embrapa LABEX US Plant Biotechnology, Plant Gene Expression Center-ARS/USDA, Albany, California, United States of America

Rodrigues *et al. BMC Genomics* (2015) 16:505
DOI 10.1186/s12864-015-1731-x

BMC Genomics

RESEARCH ARTICLE Open Access

Daytime soybean transcriptome fluctuations during water deficit stress

Fabiana Aparecida Rodrigues[1], Renata Fuganti-Pagliarini[1], Juliana Marcolino-Gomes[1,2], Thiago Jonas Nakayama[1,3], Hugo Bruno Correa Molinari[4,8], Francisco Pereira Lobo[5], Frank G Harmon[6,7] and Alexandre Lima Nepomuceno[1,8*]

frontiers in Plant Science

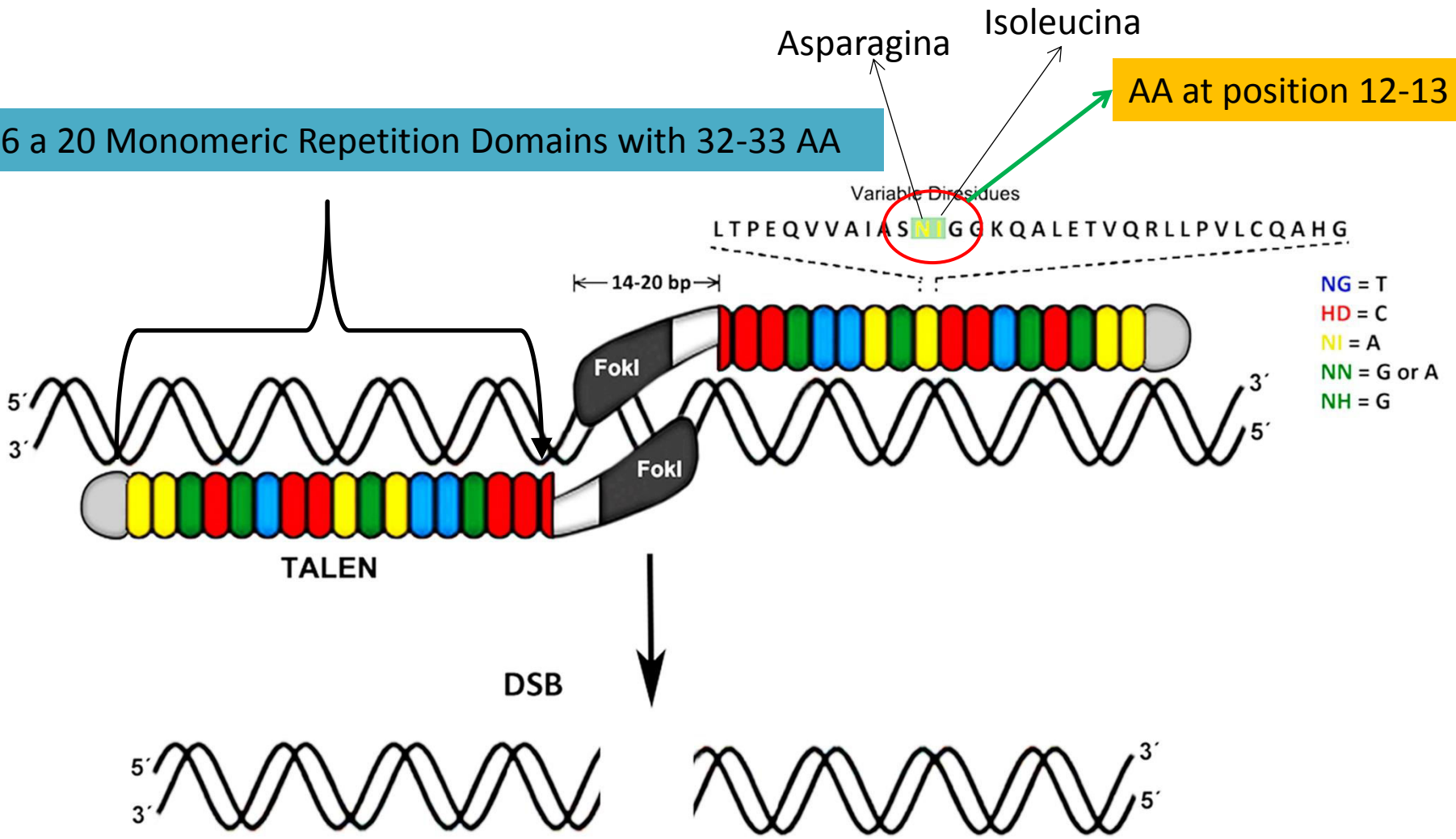
ORIGINAL RESEARCH
published: 20 April 2017
doi: 10.3389/fpls.2017.00618

Functional Characterization of a Putative *Glycine max ELF4* in Transgenic Arabidopsis and Its Role during Flowering Control

*Juliana Marcolino-Gomes[1], Thiago J. Nakayama[2], Hugo B. C. Molinari[2], Marcos F. Basso[2], Liliane M. M. Henning[1], Renata Fuganti-Pagliarini[1], Frank G. Harmon[3,4] and Alexandre L. Nepomuceno[1]**

TALENs = Transcription Activator-Like Effector Nucleases.

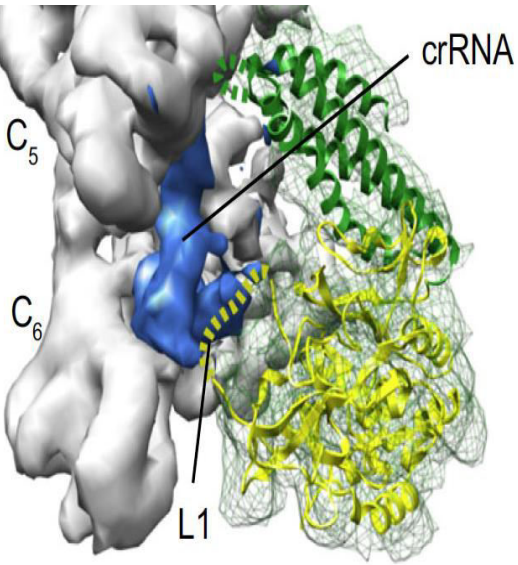
16 a 20 Monomeric Repetition Domains with 32-33 AA

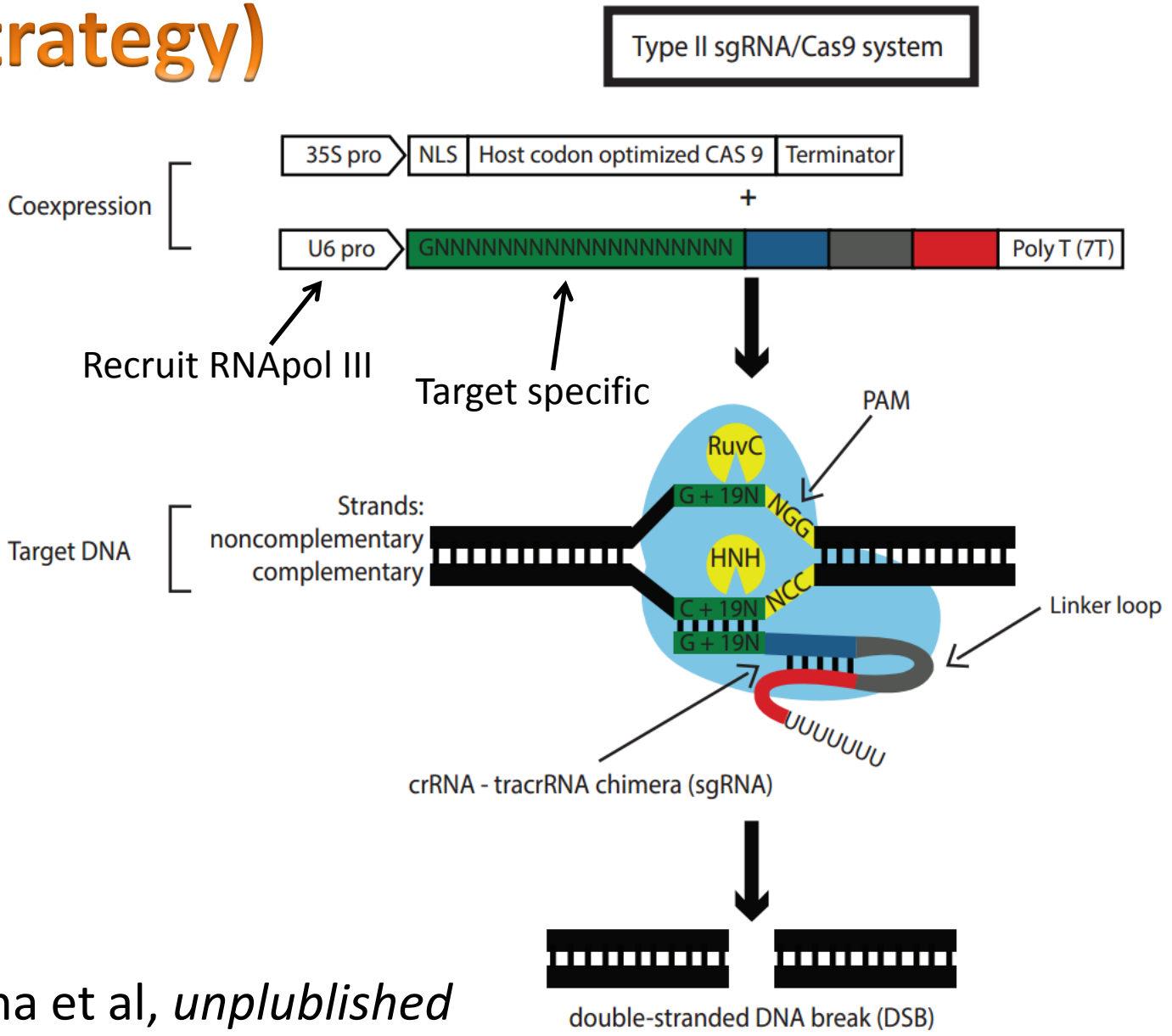


Source: Curtin et al, 2013

CRISPRs

(design strategy)





Source: Nakayama et al, *unplublished*

Project : Evaluation of Maize Wide Transcriptome Responses During Drought and How Circadian Clock Genes Are Affected

Research Team:
Renata Fuganti-Pagliarini - Embrapa
Newton Carneiro – Embrapa M&S
Francisco Lobo – Embrapa IA
Frank Harmon – PGEC ARS/USDA
Sarah Hake - PGEC ARS/USDA

Project : Phenotypic and Genomic Selection in Forage Breeding: A Comparison of Accuracy and Genetic Gains by Applying Different Methods of Selection

Research Team:
Rosangela M. Simeão - Embrapa
Michael D. Casler – ARS/USDA
Marcos Deon V. de Resende – Embrapa/UFV

USDA ARS

Embrapa Beef Cattle

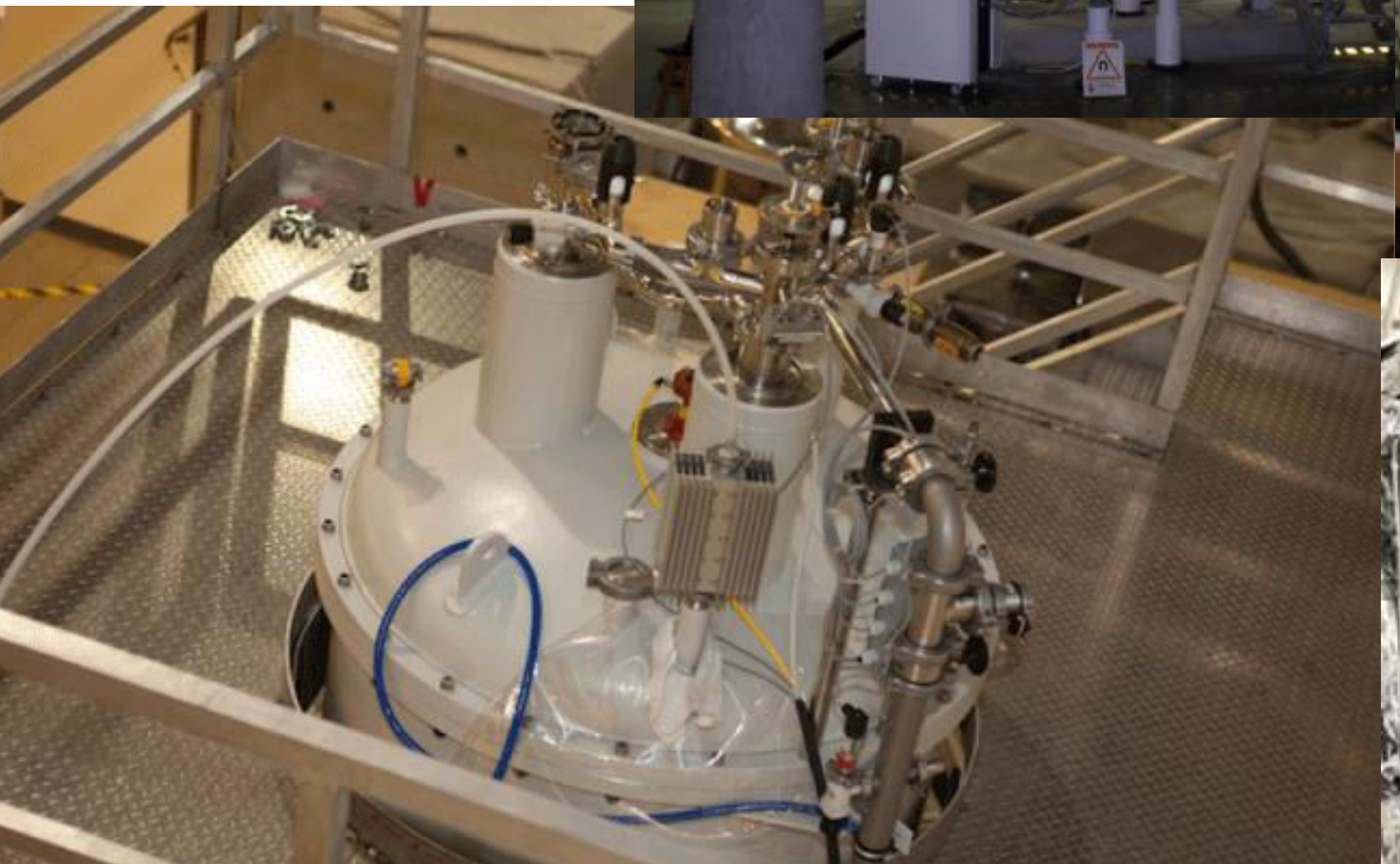
Project : Study Cell Wall Composition of GM Plants by Nuclear Magnetic Resonance (NMR)

Wet chemistry or/and instrumental (chromatographic/spectrophotometric) methods for lignocellulosic biomass characterization have been used for providing quantitative information about the main components of the cell wall. The use of NMR to characterize cell wall has the advantage of providing detailed structural information that is not obtained with other methods and can be performed without a previous fractionation to isolate a cell wall component, which could change the structure of the polymer under study.

Dr. Kevin Holtman

Dr. Patricia Abrao de Oliveira

UC Berkeley NMR SPECTROSCOPY FACILITY



2D HSQC NMR spectra of GM sugarcane cell walls
Aliphatic region

Dr Anthe George

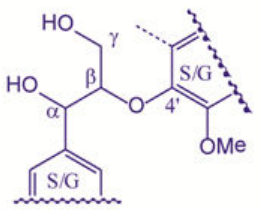
DOE-JBEI

Joint BioEnergy Institute

Proposal APPROVED

CNPq Visiting Researchers special call

2014-2016 at Embrapa Agroenergy



A
β-aryl ether (β-O-4)

cell wall has the advantage of providing detailed structural information that is not obtained with other methods and can be performed without a previous fractionation to isolate a cell wall component, which could change the structure of the polymer under study.

Project : Differential Proteomics Analysis of Escherichia Coli O145 Pst1 and Dam Methylase Mutants

Research Team:

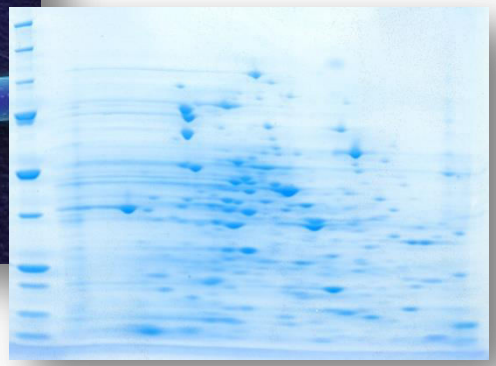
Douglas Gomes (Embrapa/UEL)

Michelle Carter (WRRC-USDA)

Leslie Harden (WRRC-USDA)

Mariangela Hungria (Embrapa)

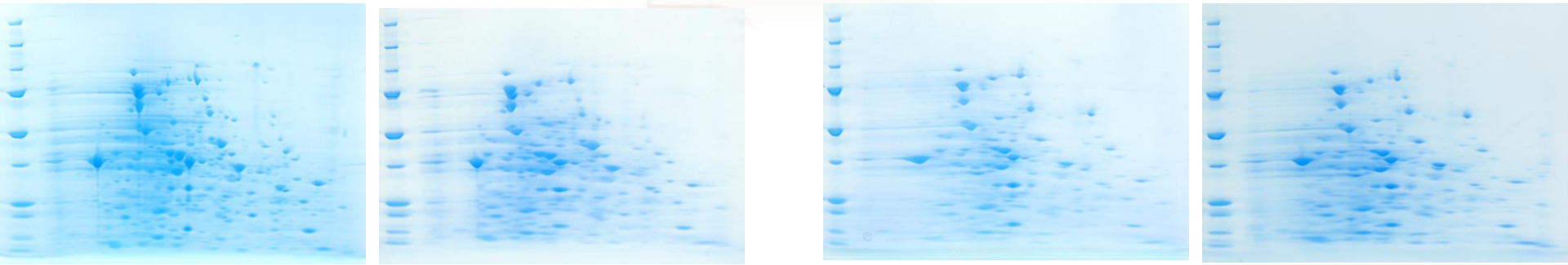
Douglas F. Gomes



Differential Proteomics Analysis

***E. coli* (*WILD TYPE*)** X ***E. coli* (PST1 AND Dam MUTANT)**

The methodology will be used in Epigenetic studies in Bradyrhizobium sp



RESULTS: The *pst1* and *dam* methylase genes knockout resulted in the differential expression of 68 and 80 proteins, respectively. → **HELP ELUCIDATE THIS BACTERIAL PATHOGENICITY MECHANISM**

NOTA TÉCNICA Nº 06

A Nova Era Genômica e a Biodiversidade Brasileira

Alexandre Lima Nepomuceno, LABEX US Biotecnologia
Ricardo Elesbão Alves, LABEX US Biodiversidade
Isabel Rodrigues Gerhardt, Embrapa Florestas
Ricardo Augusto Dante, Embrapa Agropecuária Oeste
Carlos Eduardo Lazarini da Fonseca, LABEX US Coordenação

Milhões de anos de evolução da biodiversidade possibilitaram o surgimento de mecanismos moleculares, bioquímicos e fisiológicos que, em combinação, foram responsáveis pela adaptação das espécies aos seus ecossistemas. O Brasil detém de 15 a 25% dessa biodiversidade, fonte potencial para a geração de novos materiais genéticos, capazes de atender a demanda crescente da sociedade moderna por novos produtos na agricultura, medicina e indústria. Até recentemente, as ferramentas disponíveis para explorar essa riqueza eram caras e ineficientes. No entanto, uma nova geração de equipamentos e técnicas genômicas tem permitido conhecer não só a sequência de DNA dos organismos, como também detalhes de expressão gênica, possibilitando entender como uma miríade de genes é regulada de forma coordenada, produzindo proteínas e metabólitos que, no final, são responsáveis pelas características das espécies que são de interesse do homem.

LABEX US Technical Note

LABEX NOTA TÉCNICA Nº 5/2012

Seleção Genômica no Melhoramento de Plantas e Animais

Alexandre Nepomuceno, LABEX USA Biotecnologia Vegetal
Rosangela Simeão, Embrapa Gado de Corte
Magda Benavides, LABEX USA Sanidade Animal
Carlos Eduardo Lazarini da Fonseca, LABEX USA Coordenação

Desenvolver genótipos superiores e selecioná-los com precisão é a base de qualquer programa de melhoramento genético eficiente, seja vegetal ou animal. A seleção genética tem sido praticada com base em dados fenotípicos obtidos em indivíduos, progênies, clones e populações avaliados experimentalmente em campo e em ambientes mais controlados. Na tentativa de aumentar a eficiência desse processo foi introduzida na década de 1990 a seleção auxiliada por marcadores moleculares (*Marker Assisted Selection* - MAS) que utiliza tanto dados fenotípicos quanto dados de marcadores moleculares que estejam em ligação gênica próxima a locos controladores de características quantitativas (QTL) de interesse agronômico. Entretanto, a MAS tem se mostrado útil na análise de grandes progênies e para características de alta herdabilidade. Entre as estratégias de uso de MAS pelos programas de melhoramento esta primeiro a identificação de QTLs (*Quantitative Trait Loci*) e depois a estimativa de seus efeitos. Entretanto, se os QTLs forem identificados de populações obtidas dos cruzamentos entre parentais que não representam ou não tem o mesmo nível de diversidade alélica do programa de melhoramento como um todo a identificação de QTLs entre médias estimadas e efeitos estimados fica tendenciosa. É importante destacar que o custo de gerar populações representativas é muito alto, levando a geração de populações subestimadas do ponto de

LABEX Fort Pierce, FL, US - RNAi

RNA interference (RNAi) Topic Uses

- Natural mechanism in eukaryotic cells (gene regulation and antiviral defense);
- Enables silencing genes in a specific way (surgically) using dsRNAs.
- Broad application: Agriculture, human heath, etc..

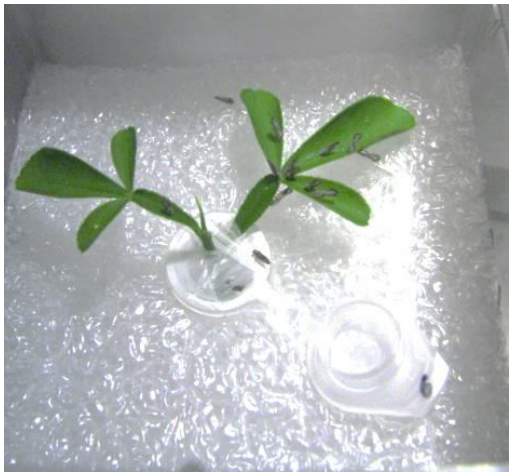
Dr. Eduardo Chumbinho de Andrade

RNAi research at EMBRAPA:

- Goals:
  - Pest control: insects, virus and fungi;
  - Plant trait modification.
- Strategies:
  - Transgenic (genetic traits: target genes and regulatory elements);
  - Non-transgenic (target genes and delivery approaches).

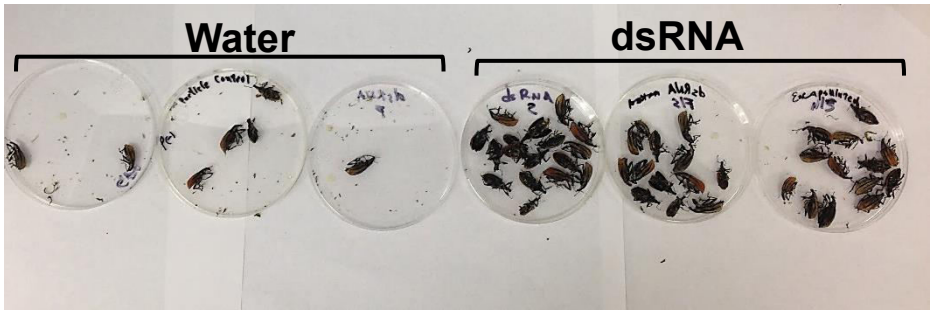
Root delivery (dsRNA solution) – sucking insects

Small scale:
dsRNA screening



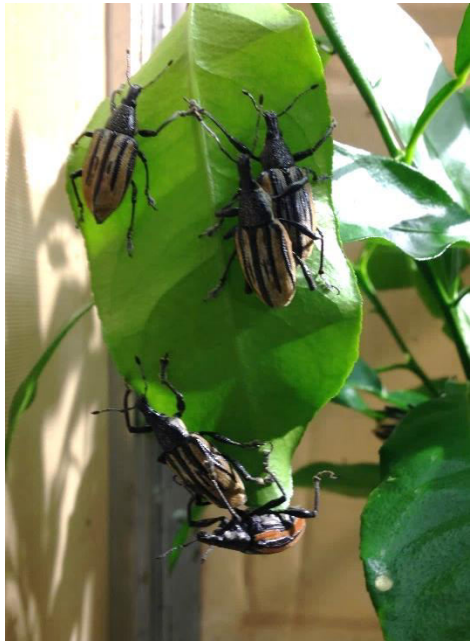
“Natural system”: insect development on treated plant

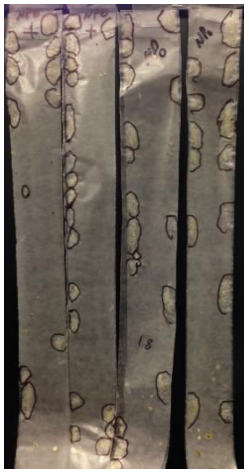
Foliar application (dsRNA solution) – chewing insects



Insect mortality

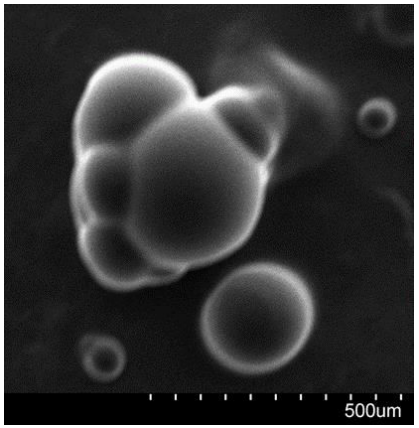
Leaf damage





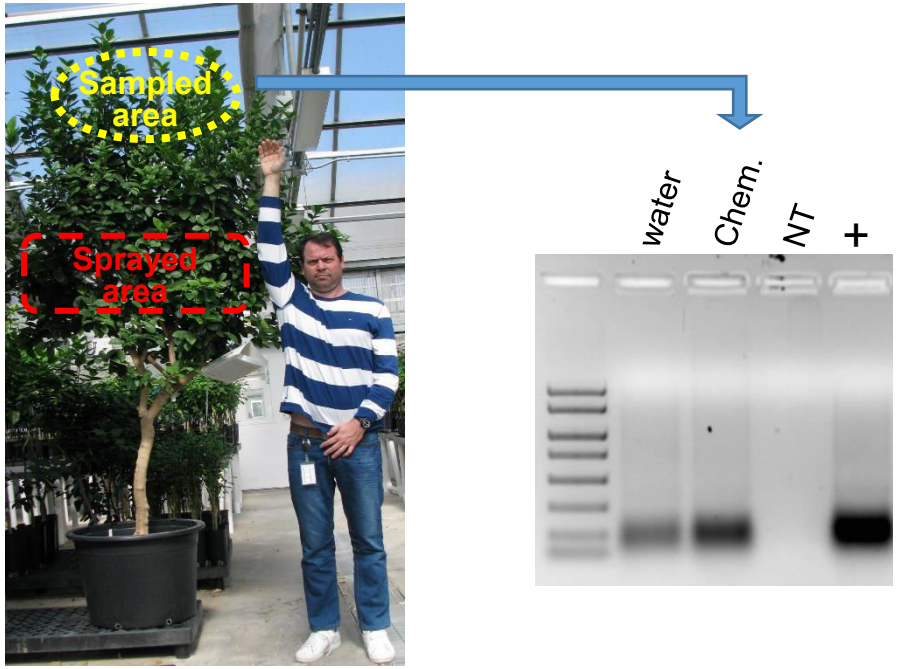
Reduced oviposition

Delivery approaches



- dsRNA nanoencapsulation:
  - Using natural, biodegradable materials;
  - Improve efficacy and longevity of dsRNA;

- Chemical for dsRNA delivery:
  - Topical and systemic delivery of dsRNA (targeting plant and pest);
  - Improve efficacy of dsRNA;

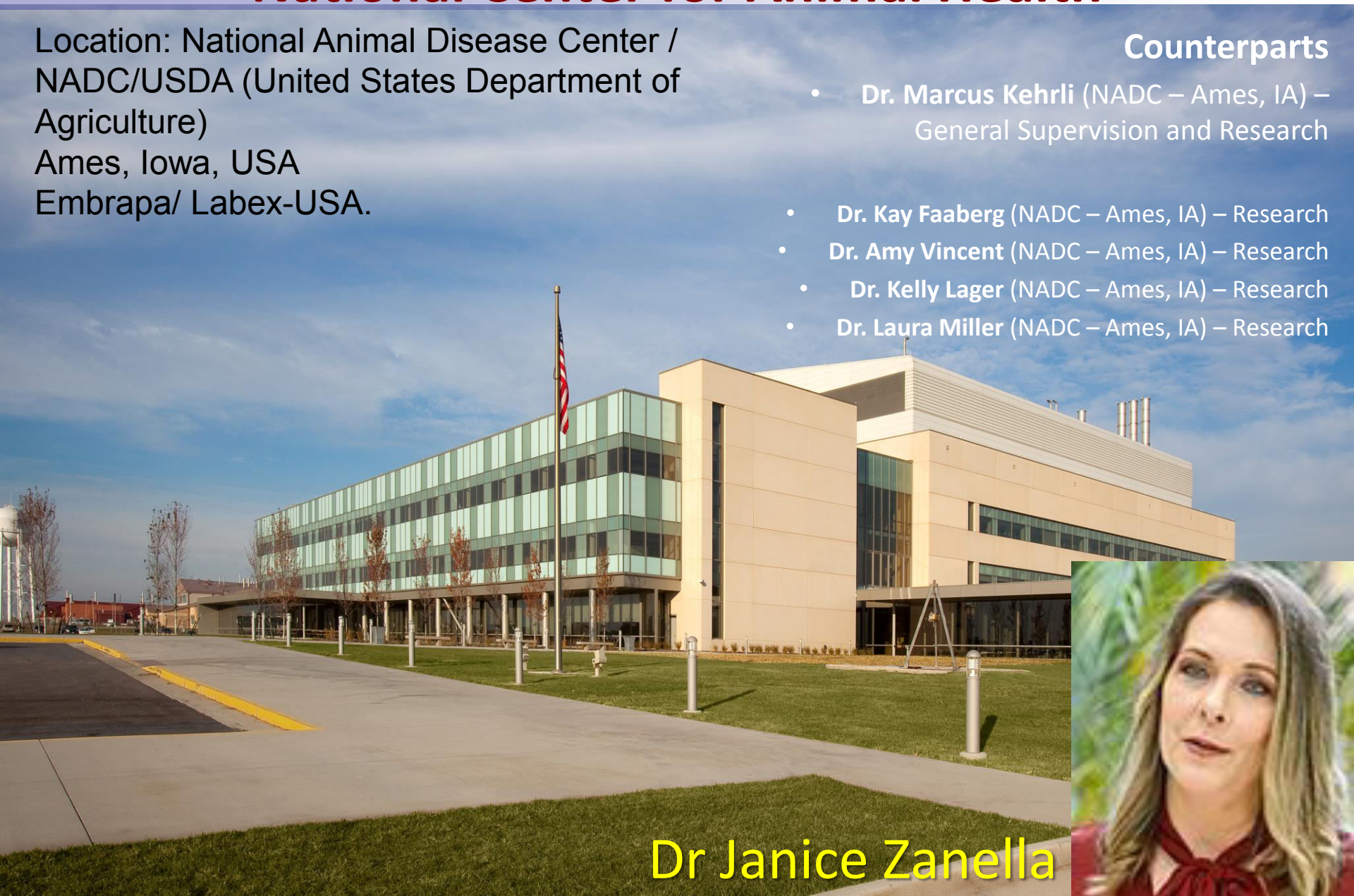


LABEX at
National Center for Animal Health

Location: National Animal Disease Center / NADC/USDA (United States Department of Agriculture)
Ames, Iowa, USA
Embrapa/ Labex-USA.

Counterparts

- **Dr. Marcus Kehrli** (NADC – Ames, IA) – General Supervision and Research
- **Dr. Kay Faaberg** (NADC – Ames, IA) – Research
- **Dr. Amy Vincent** (NADC – Ames, IA) – Research
- **Dr. Kelly Lager** (NADC – Ames, IA) – Research
- **Dr. Laura Miller** (NADC – Ames, IA) – Research



Dr Janice Zanella

Janice Ciacci-Zanella, DVM, M.Sc., PhD
Labex-ARS, NADC Visiting Scientist from 2008 - 2010

Labex-USA Animal Health - (2008-2010)

- Visit of leading researchers Dr. Marcus Kehrli Jr. and Dr. Steven Olsen and counterpart researchers Amy Vincent and Kelly Lager of the NADC to Embrapa Research Units in Brazil.
- Labex Animal Health Workshop

- As a Labex / USA researcher, I continued to guide students and approved projects in Brazil.
- Dozen of Graduate students CNPq/Labex and visiting scientists from Embrapa at NADC
- Animal Health Research Portfolio that I was president from 2012 to 2015 was based on Labex.

LABEX US– Plant Biotechnology in SFO

ARS USDA - Dairy Forage Research Center

ARS USDA – Plant, Soil, Nutrition Center

ARS USDA – Western Regional Research Center

ARS USDA - National Center For Genetic Resources Preservation

Biotech Private Companies

Amyris

Dow

Keygene

BGI

Eurofins

LABEX at ARS/USDA PGEC

US Universities

UC Berkley (Dr Hake/Dr Harmon)

UC Davis (Dr Blumward)

UC Riverside (Dr Bailey-Serres)

UC S.Diego (Dr Maarten Chrispeels)

Virginia Tec (Dr Fukao)

Cornell University (Dr. Jean Luc)

Colorado State University (Dr John McKay/Dr Antunes)

Biotechnology Public Institutes

DOE-JGI

DOE-JBEI

Technical Notes,
Book Chapters,
Journal Articles,
Workshops, Courses,
Unit Visits,
Email, Face, Phone
Joint Projects

Embrapa Units

Soybean, M&S, R&B, Cenargen, Beef Cattle, Temperate Climate, Acre, Agroenergy, etc

Brazilian Universities

UFPE, UFES, UFRGS, UEL, UEM, UFV

CNPq

CAPES

Final Thoughts – International Partnerships

- physical presence matters...
- eye to eye conversation matters...
- drinking a beer with your counter part makes the difference...

- It builds up Confidence, Trust, and Friendship.

- It helps new ideas to pop up, new projects to be designed, etc

- Sinergy, joint growth and mutual gain appears.

Embrapa
MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO
BRASIL GOVERNO FEDERAL

謝謝

Alexandre.Nepomuceno@embrapa.br