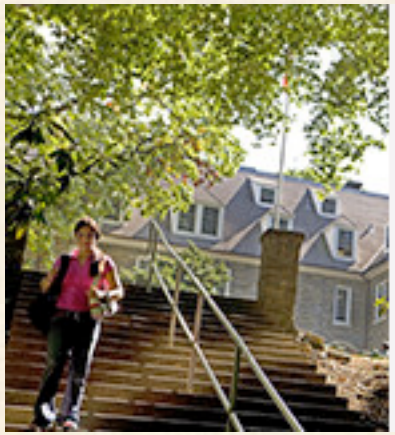


Genomics of Cacao Disease Resistance

Mark Guiltinan, Drew Fister and Siela Maximova

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Understanding the Plant Immune System

How quick and strong plant reacts to pathogen determines the outcome.

Immunity

Disease

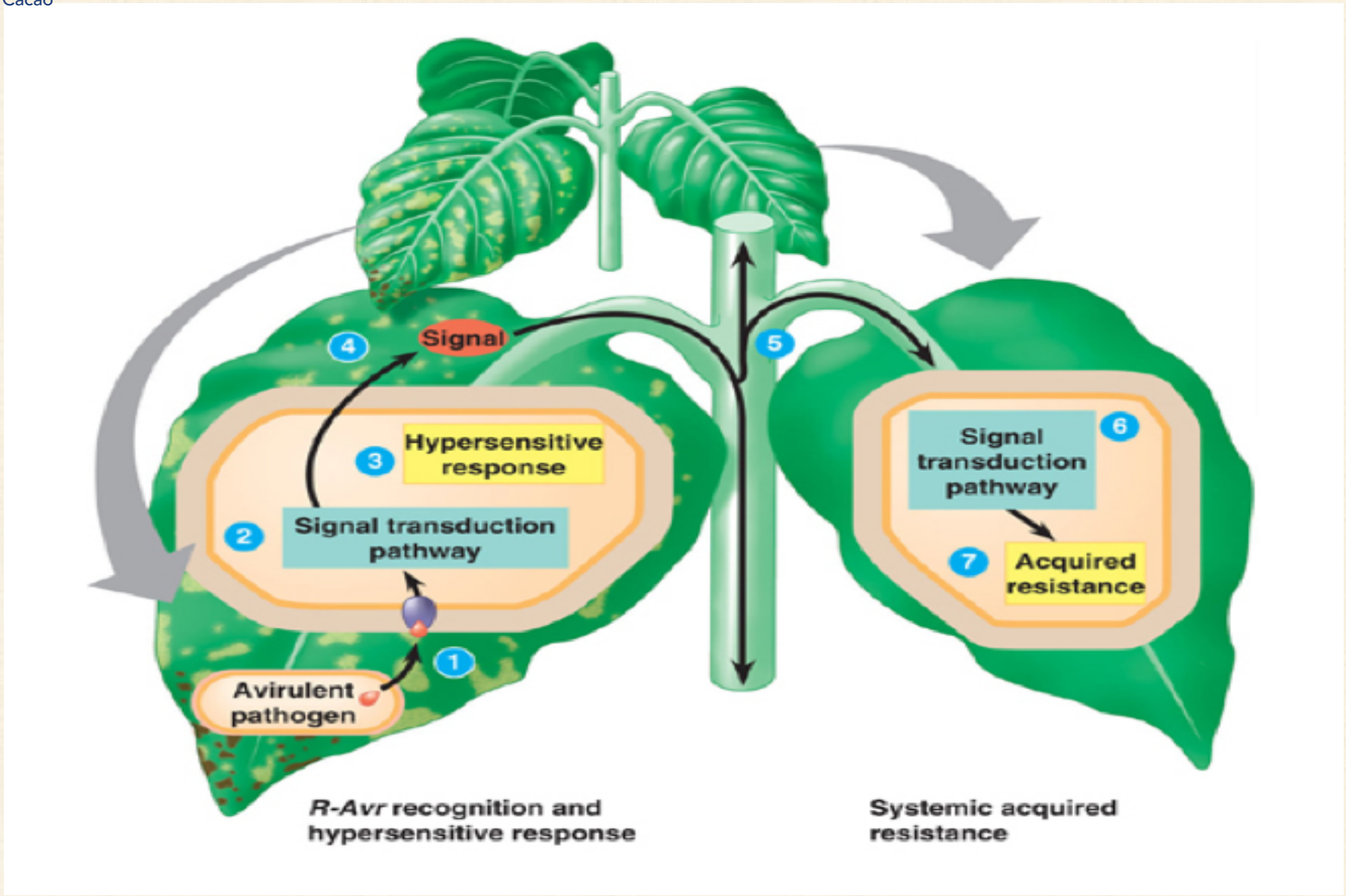
Key Steps in Plant Pathogen Interactions

1) Recognition of Pathogen Presence

2) Signal Transduction and Amplification

3) Complex Mechanisms of Response

Overview of the Plant Immune System



http://www.bio.miami.edu/dana/226/226F08_21.html

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Understanding the Plant Immune System

Three Main Categories of Genes

1. Receptor Proteins
2. Signal Transduction Proteins
3. Pathogenesis Related Response Proteins

Main Questions

What are the most important mechanisms of resistance for cacao?

What are the genes controlling these mechanisms?

Can we discover useful variants of these genes?

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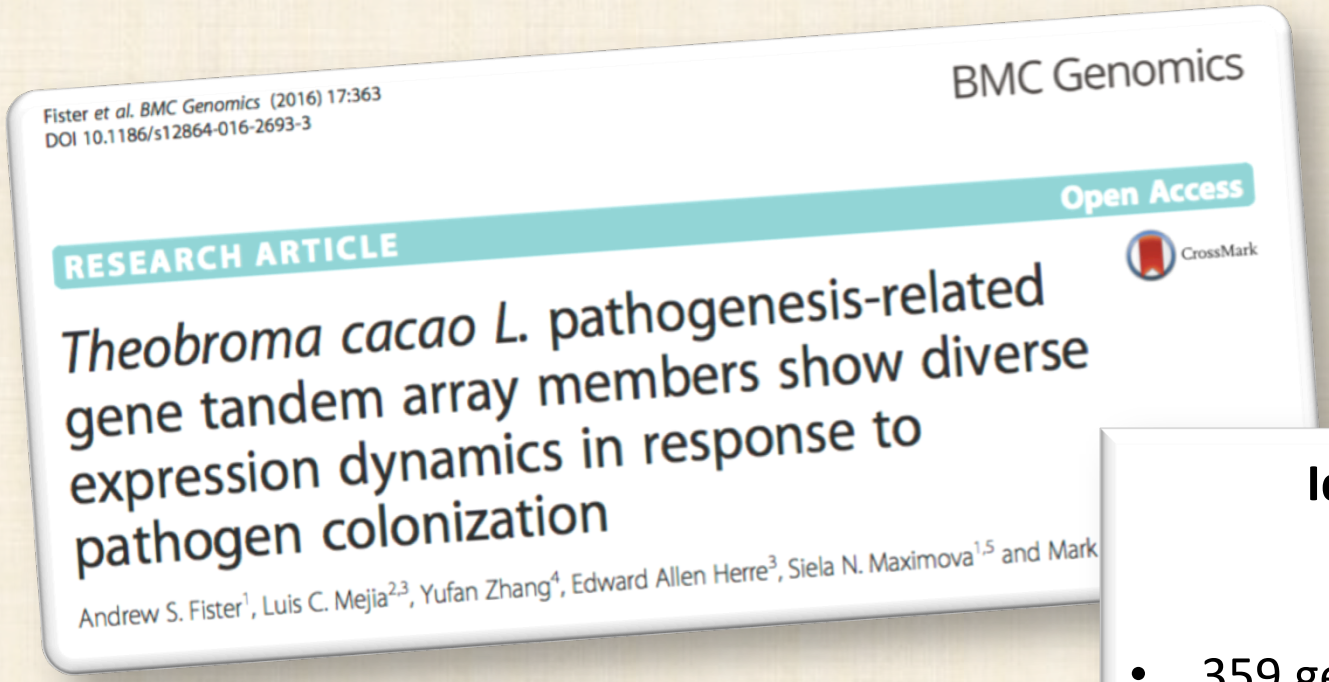
Andrew Fister



Fister et al. BMC Genomics (2016) 17:363
DOI 10.1186/s12864-016-2693-3

BMC Genomics

Open Access

RESEARCH ARTICLE

Theobroma cacao L. pathogenesis-related gene tandem array members show diverse expression dynamics in response to pathogen colonization

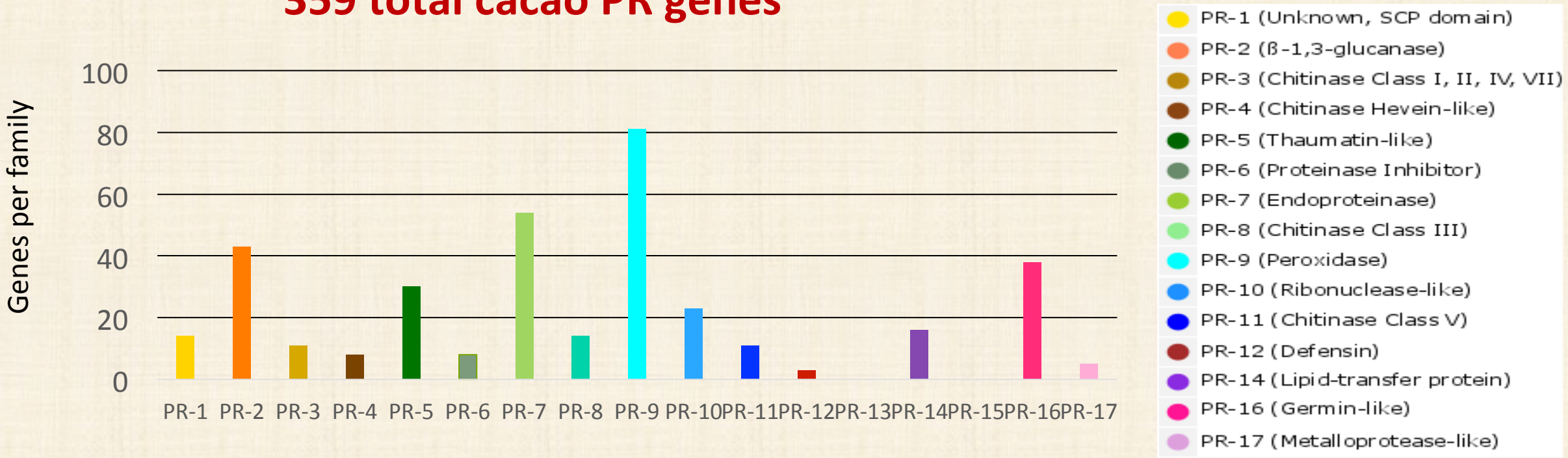
Andrew S. Fister[1], Luis C. Mejia[2,3], Yufan Zhang[4], Edward Allen Herre[3], Siela N. Maximova[1,5] and Mark

Identified and Annotated
All Cacao PR Genes

- 359 genes
- 45 Chitinases genes in 4 Multi-gene families
- Complex gene expression profiles
- Complex transcriptional response to *Phytophthora* infection

Fifteen PR families are present in cacao, and family sizes range from 3 to 81.

359 total cacao PR genes



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Functions of Pathogenesis-Related Proteins

Cell Wall Degradation

43 11 8 54 14 11

PR-2, PR-3, PR-4, PR-7, PR-8, PR-11

Membrane Degradation

30 3 16

PR-5, PR-12, PR-14

Protein Inhibition/Degradation

8 54 5

PR-6, PR-7, PR-17

DNA and RNA Degradation

8 23

PR-4, PR-10

Reactive Oxygen Species Generation

81 38

PR-9, PR-16

Cell Wall Modification

81

PR-9

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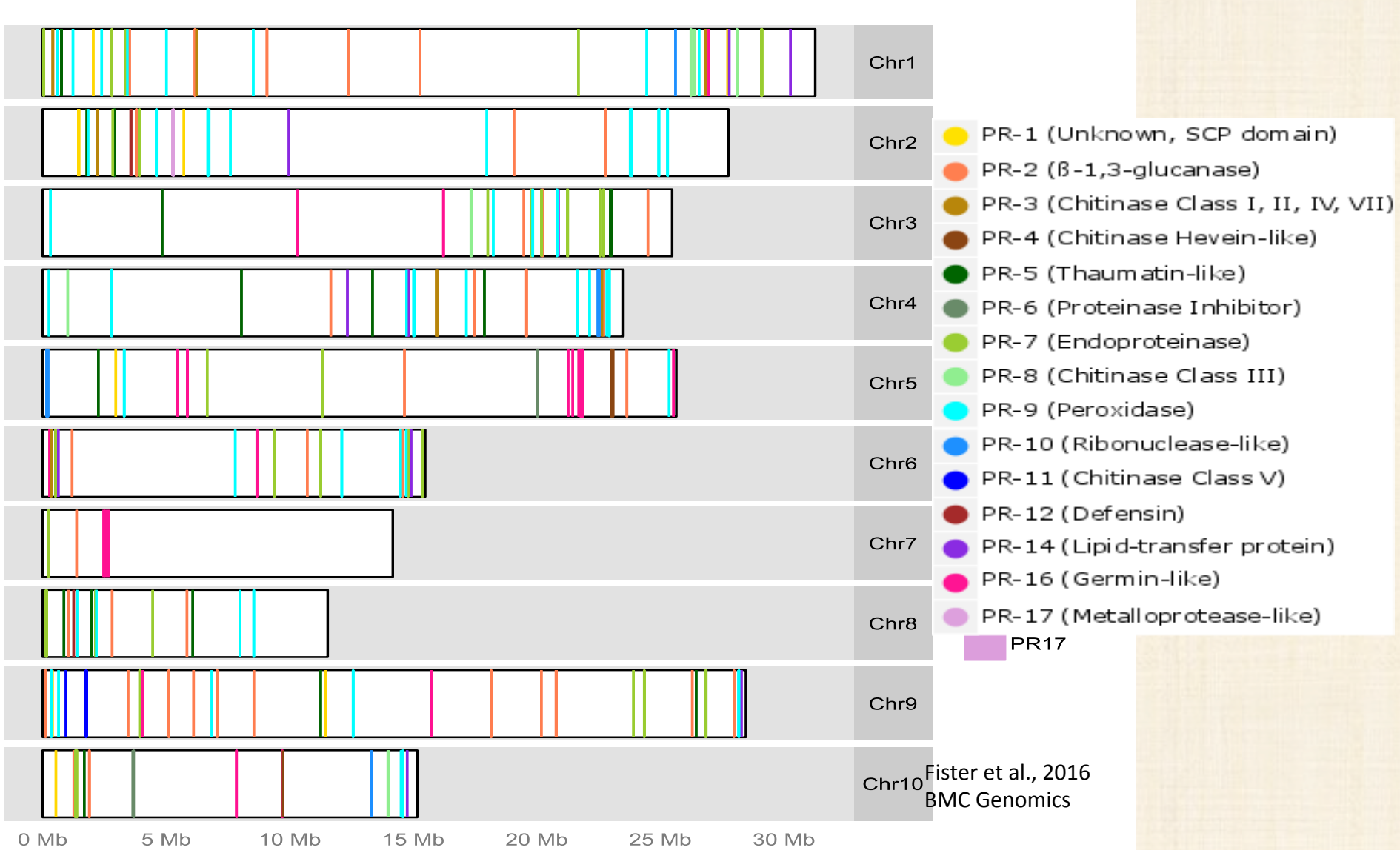
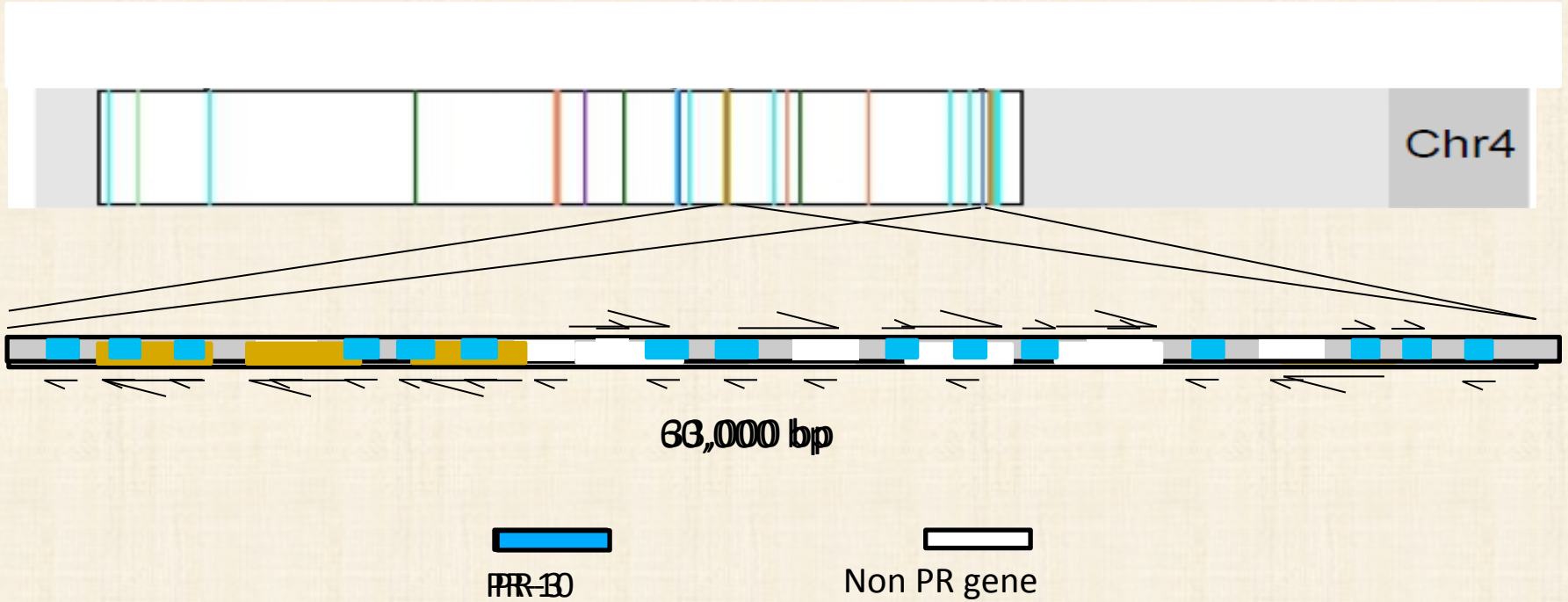
The genes and families are scattered throughout the genome



Fister et al., 2016
BMC Genomics

Tandem arrays are a common feature within PR gene families.



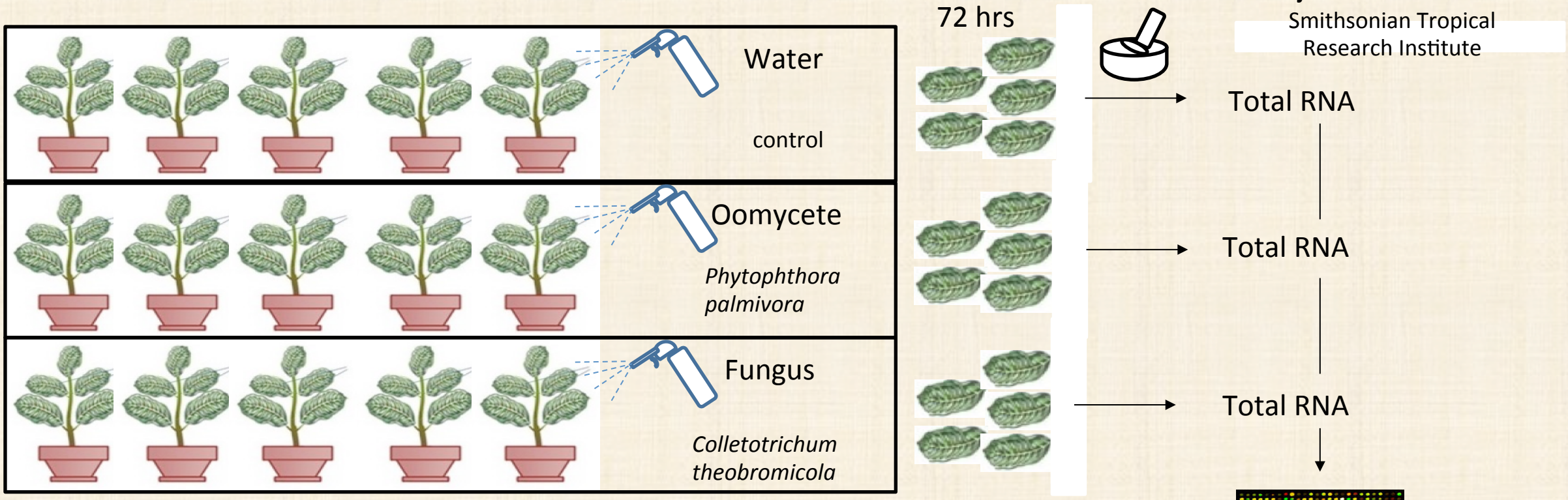
- PR-1 (Unknown, SCP domain)
- PR-2 (ß-1,3-glucanase)
- PR-3 (Chitinase Class I, II, IV, VII)
- PR-4 (Chitinase Hevein-like)
- PR-5 (Thaumatin-like)
- PR-6 (Proteinase Inhibitor)
- PR-7 (Endoproteinase)
- PR-8 (Chitinase Class III)
- PR-9 (Peroxidase)
- PR-10 (Ribonuclease-like)
- PR-11 (Chitinase Class V)
- PR-12 (Defensin)
- PR-14 (Lipid-transfer protein)
- PR-16 (Germin-like)
- PR-17 (Metalloprotease-like)

Throughout the genome, half of the PR genes are **densely physically clustered**.

Within large families, how diverse are expression profiles?

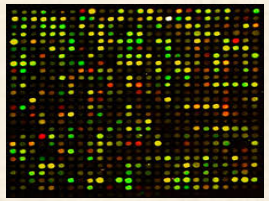

Dr. Luis Mejia Dr. Allen Herre
Smithsonian Tropical Research Institute

Experimental Design:



N = 5 seedlings for each treatment
Genotype: UF17-derived seeds

Hybridized to 46,000 probe whole genome microarray.
Analyzed with RMA and LIMMA.



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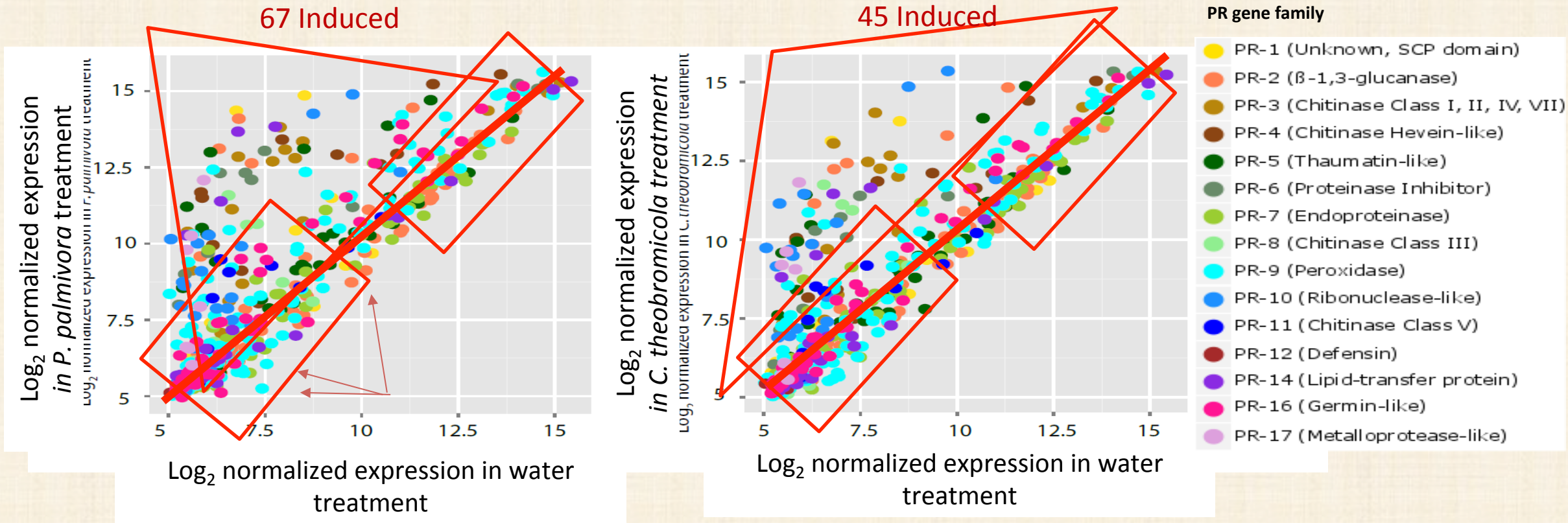
Across families, PR genes had diverse expression profiles.



Constitutive: 201 Low 84 High 224 Low 90 High

7 Repressed 0 Repressed

Fister et al., 2016 BMC Genomics

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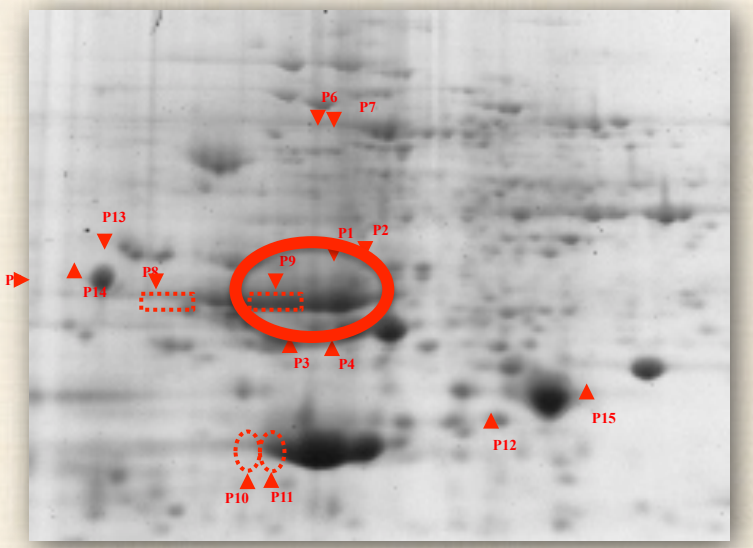
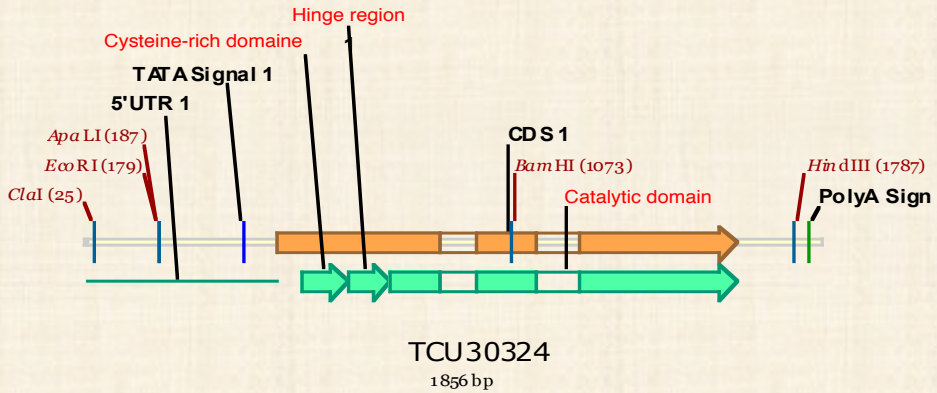
FUNCTIONAL GENOMICS CASE STUDY
Chitinase

Pod Proteins 2-D Gel



Acidic Endo-Chitinase
2-D Gel Electrophoresis Separation
LC-Mass Spec Protein Identification

1995 - Gene Cloned



Niemenak, N., Kaiser, E., Maximova, S. N., Laremore, T. & Guiltinan, M. J. (2015) Proteome analysis during pod, zygotic and somatic embryo maturation of *Theobroma cacao*. Journal of Plant Physiology 180, 49-60

Snyder-Leiby, T.E., and Furtek, D.B. (**1995**). A genomic clone (Accession No. U30324) from *Theobroma cacao* L. with high similarity to plant class I endochitinase sequences. Plant Physiol. 109, 338.

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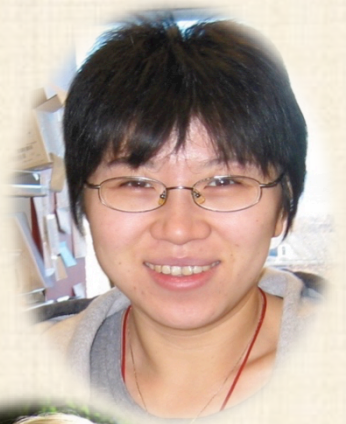
Functional Genomics of the Chitinase Gene

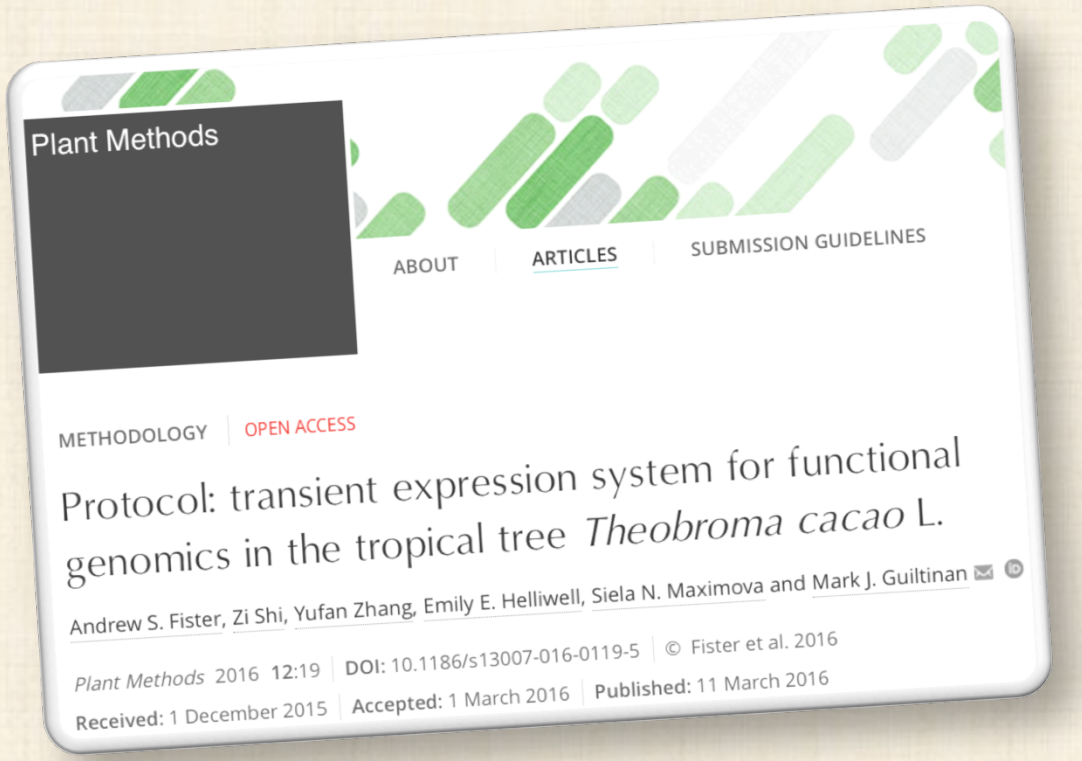


Approach:

Increase the level of expression of a gene
Perform Plant Disease Bioassay to measure effect

Techniques:

Transient Expression: 2-7 days, not in every cell
Stable Expression: Permanent and in all cells


Plant Methods

ABOUT ARTICLES SUBMISSION GUIDELINES

METHODOLOGY OPEN ACCESS

Protocol: transient expression system for functional genomics in the tropical tree *Theobroma cacao* L.

Andrew S. Fister, Zi Shi, Yufan Zhang, Emily E. Helliwell, Siela N. Maximova and Mark J. Guiltinan

Plant Methods 2016 12:19 | DOI: 10.1186/s13007-016-0119-5 | © Fister et al. 2016

Received: 1 December 2015 | Accepted: 1 March 2016 | Published: 11 March 2016

Transient Assay Team

Andrew Fister
Zi Shi
Emily Helliwell

NSF

PennState
College of Agricultural Sciences

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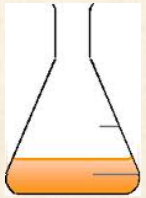
Transient Candidate Gene Expression Assay

Select, Clone and Verify Candidate Genes

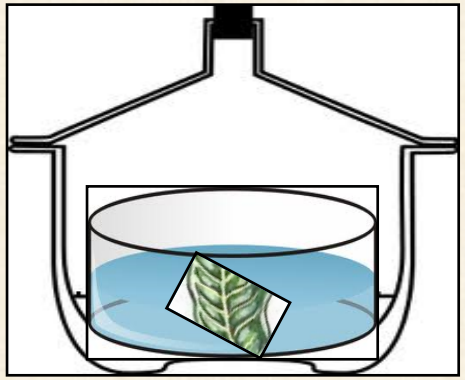
Construct Expression Vectors and Introduce them into Agrobacterium

Grow Agrobacterium Culture

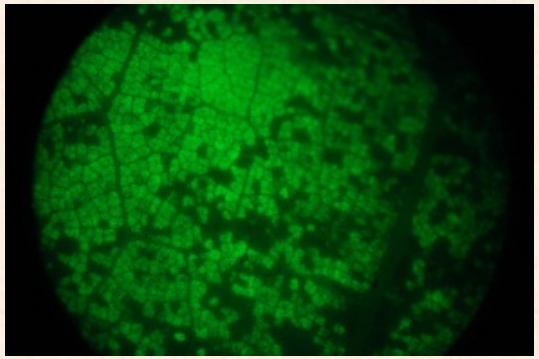


Cacao Leaf

Vacuum for 2 min



Screen for GFP coverage on 90% of leaf surface



Infect with Pathogen and Evaluate Infection

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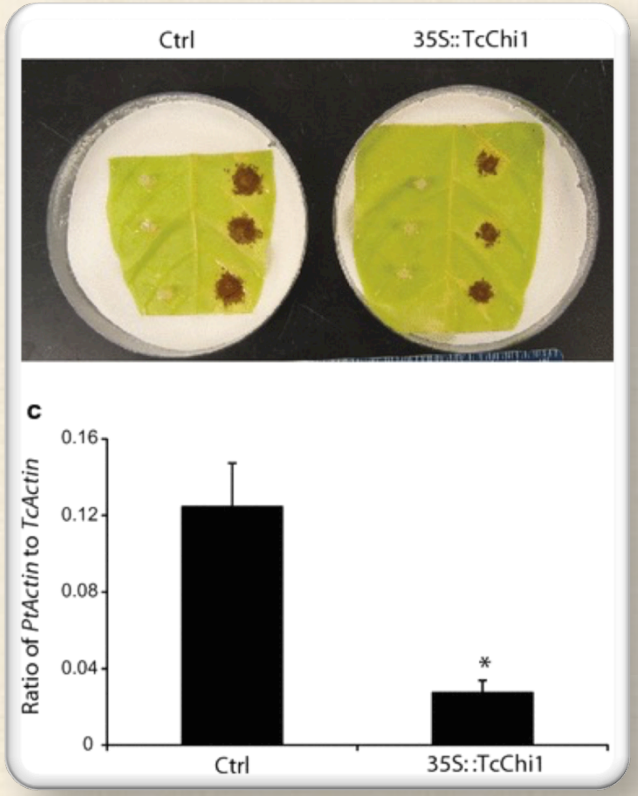
Increased Expression of Chitinase Gene Strongly Increases Disease Resistance

Transient Expression in Leaves



- 6-fold increase in expression
- 4-fold decrease in pathogen replication

High Level Stable Expression

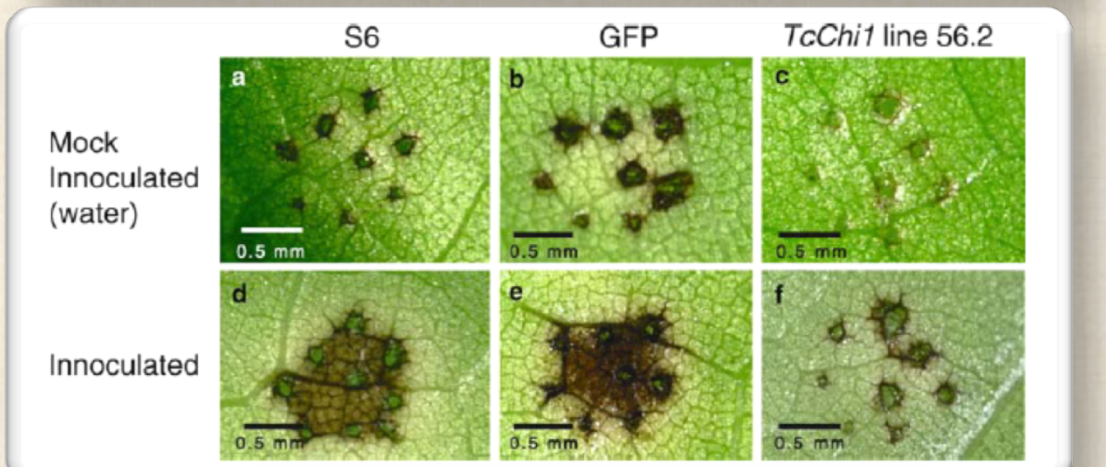
Planta (2005)
DOI 10.1007/s00425-005-0188-6

ORIGINAL ARTICLE

Siela N. Maximova · Jean-Philippe Marelli · Ann Young
Sharon Pishak · Joseph A. Verica · Mark J. Guiltinan

Over-expression of a cacao class I chitinase gene in *Theobroma cacao* L. enhances resistance against the pathogen, *Colletotrichum gloeosporioides*

Received: 1 September 2005 / Accepted: 16 November 2005



3-fold decrease in lesion size

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NEW PROJECT
Discovery and Functional Characterization of Genes Regulating Plant Immunity in Perennial Crops

Mark Guiltinan - Cacao Genomics

Siela Maximova - Functional Genomics

Claude dePamphillis - Evolutionary Genomics

James Marden - Evolutionary Genomics

Peter Tiffin, Univ. of Minnesota - Population Genomics

Dapeng Zhang, USDA ARS Beltsville - Genetic Diversity

Désiré Pokou, CNRA, Ivory Coast, West Africa - Breeding

Wilberth Phillips and Mariela Leandro, CATIE, Costa Rica - Germplasm Collection and Plant Pathology

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Main Goals

Selection of candidate genes for disease resistance employing a new genomics approach developed at PSU for tropical trees

1. Evaluate genomic diversity within cacao by resequencing the genomes with extreme resistance phenotypes and genetic background
2. Sequence of transcriptomes to corroborate gene models, identify splice variants, and characterize responses to pathogen infection
3. Identify genes most likely to be important in affecting quantitative resistance to pathogens
4. Verify the functions of these genes

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Somatic Embryogenesis

- Method for rapid, large scale multiplication of plants.
- Plants are generated without a fertilization and genetically identical to the parent.
- Performed in sterile culture.
- Can be used for production of disease free plants.
- Germplasm storage via cryopreservation.

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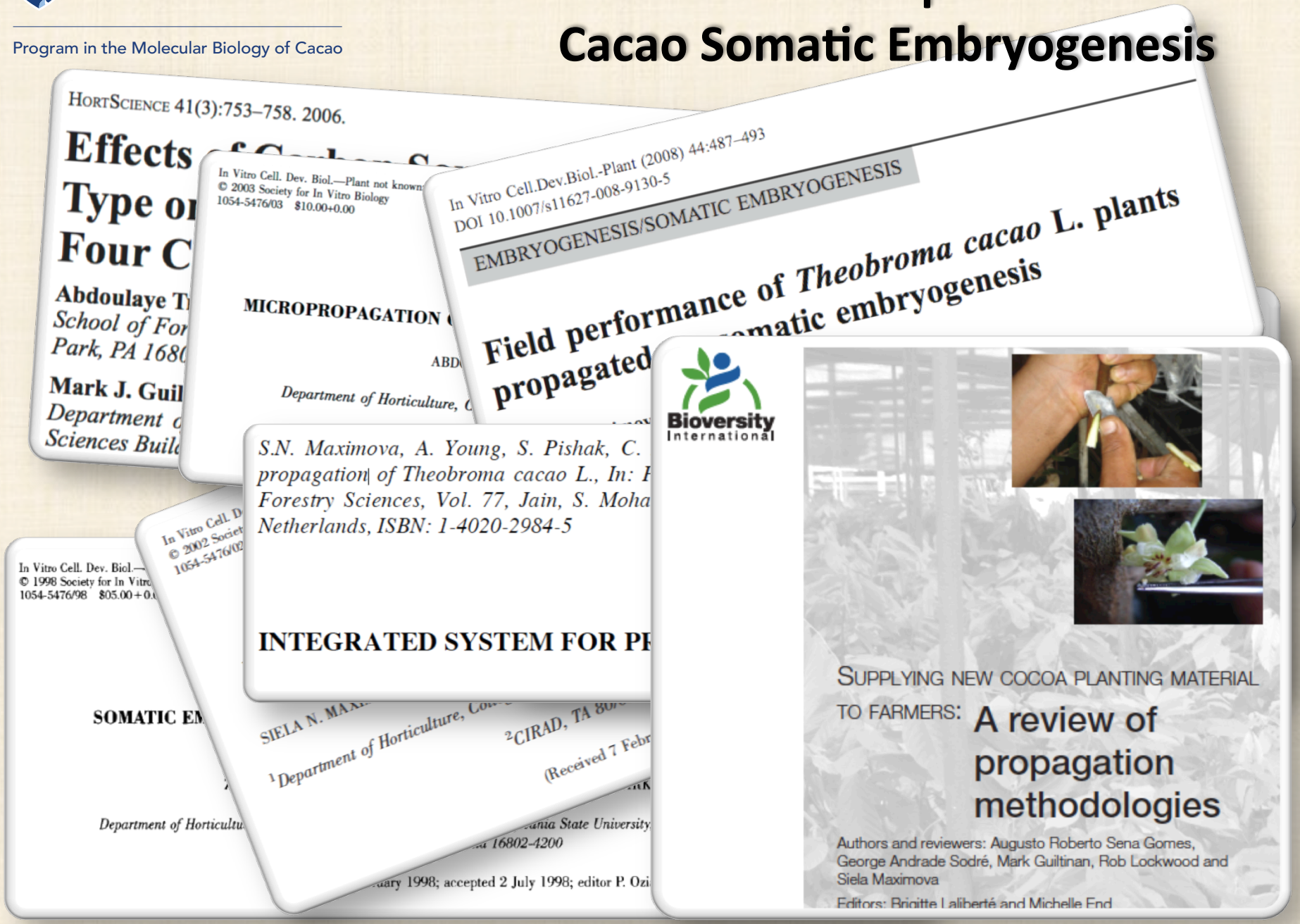
Manuscripts on Cacao Somatic Embryogenesis



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Field Test Sites for *In Vitro* Cloning Technology 1999 - 2016

Ecuador

Saint Lucia

Puerto Rico

Indonesia

Brazil

Ghana

Ivory Coast

Malaysia

Ecuador

Ghana West Africa

Brazil

Photos: Mark Guiltinan, PSU

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Nestlé Good Food, Good Life

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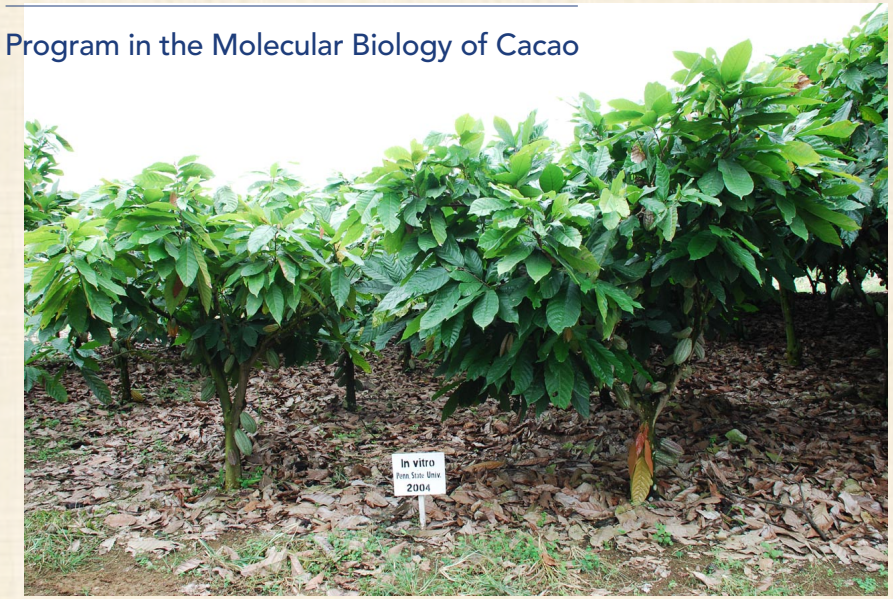

Field Test at The Nestle Farm, Ecuador

(National cacao tests since 1999)

Average yield of 2.6 t/ha from SE plants

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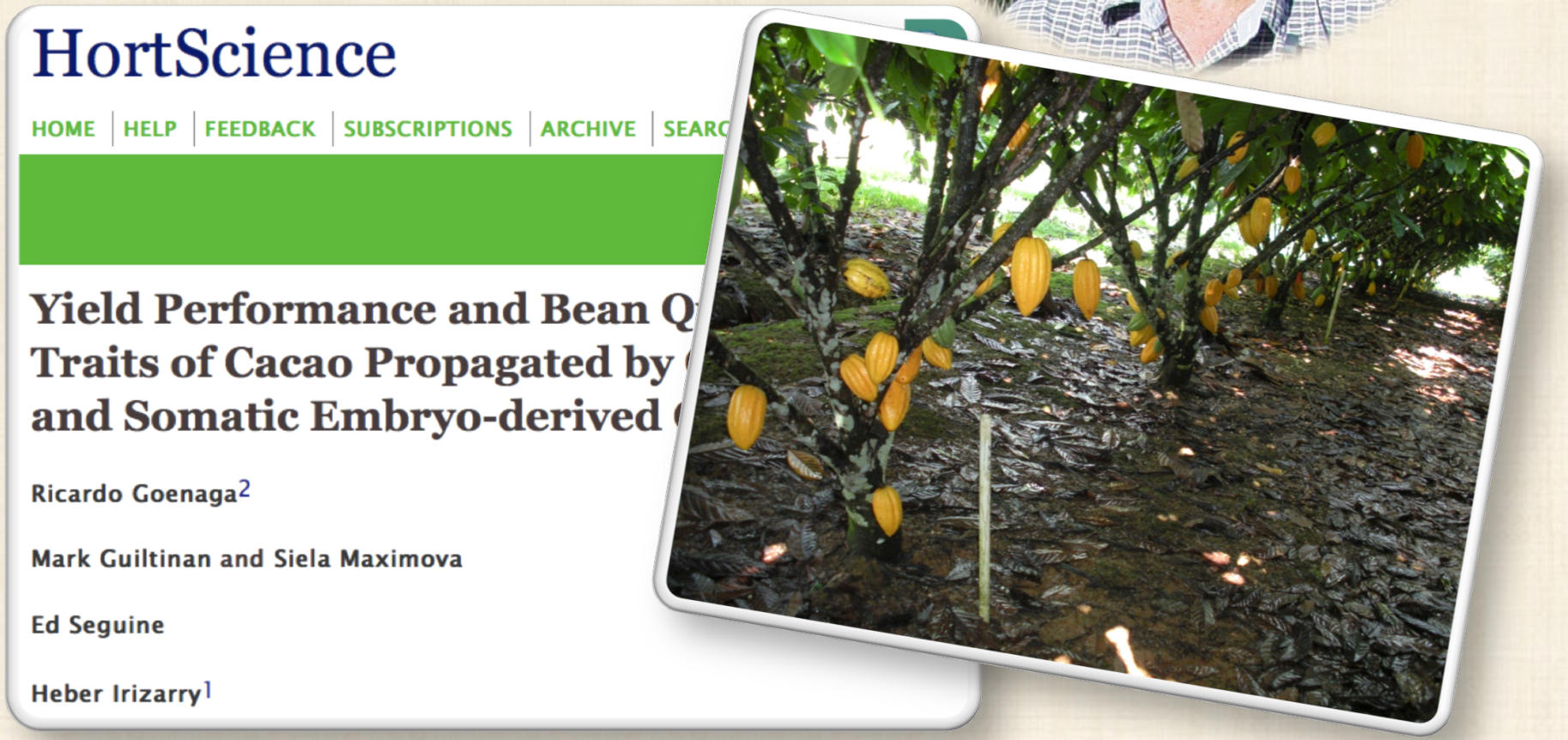
USDA ARS Agricultural Research Service
www.ars.usda.gov

Collaboration with USDA ARS Puerto Rico

Ricardo Goenaga



Large Scale Adoption of Somatic Embryogenesis in Indonesia
100 Million Cacao Somatic Embryos
Indonesia Coffee and Cocoa Research Institute (ICCRI)

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New Project

Cadmium Transporters in Cacao

Research Conducted by JAIME ANDRÉS OSORIO GUARÍN of CORPOICA

BORLAUG FELLOWSHIP

AUGUST 2016

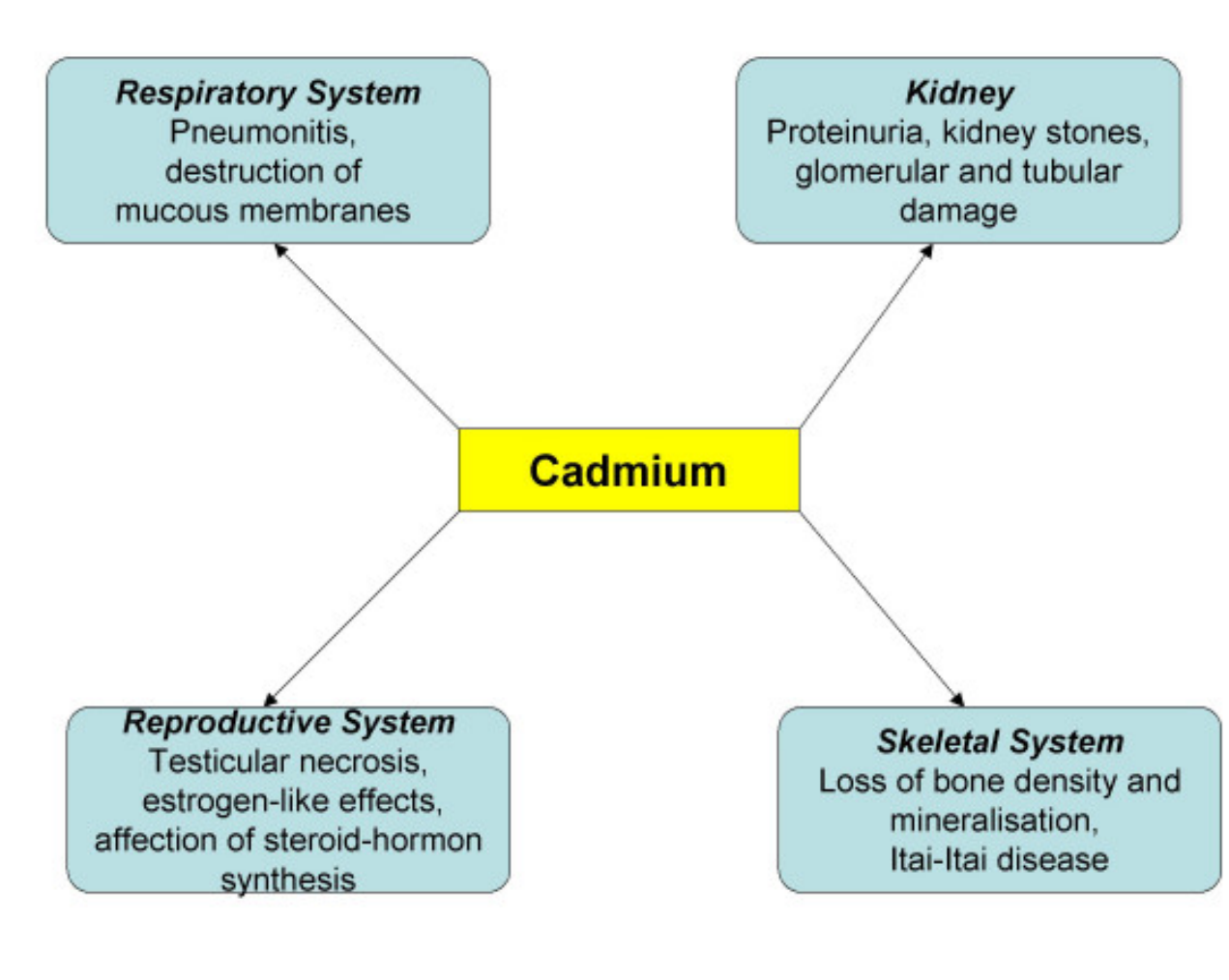
PENN STATE UNIVERSITY

USDA

Corpoica
Corporación Colombiana de Investigación Agropecuaria

World Cocoa Foundation

High levels of cadmium can affect different organs in humans



Godt et al., 2006
J Occup Med Toxicol. 2006; 1: 22.

Gene families related with cadmium transport

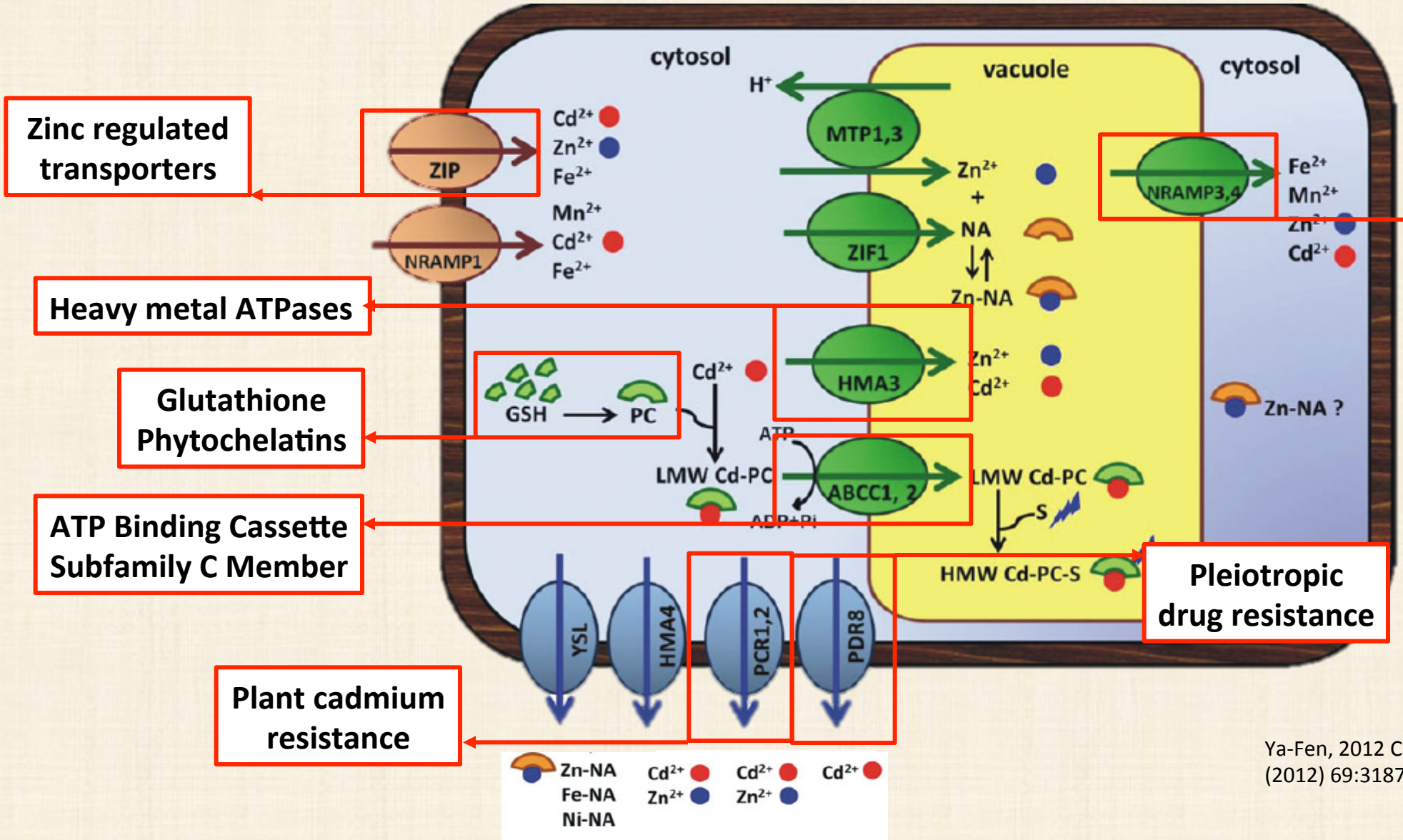
Zinc regulated transporters

Heavy metal ATPases

Glutathione Phytochelatins

ATP Binding Cassette Subfamily C Member

Plant cadmium resistance



Natural resistance associated with macrophage proteins

Pleiotropic drug resistance

Ya-Fen, 2012 Cell. Mol. Life Sci. (2012) 69:3187–3206

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OBJECTIVES

- Identify and select candidate genes involved in cadmium detoxification in *Theobroma cacao*.
- Develop a functional assay to evaluate the function of the cadmium related genes in *Theobroma cacao*.

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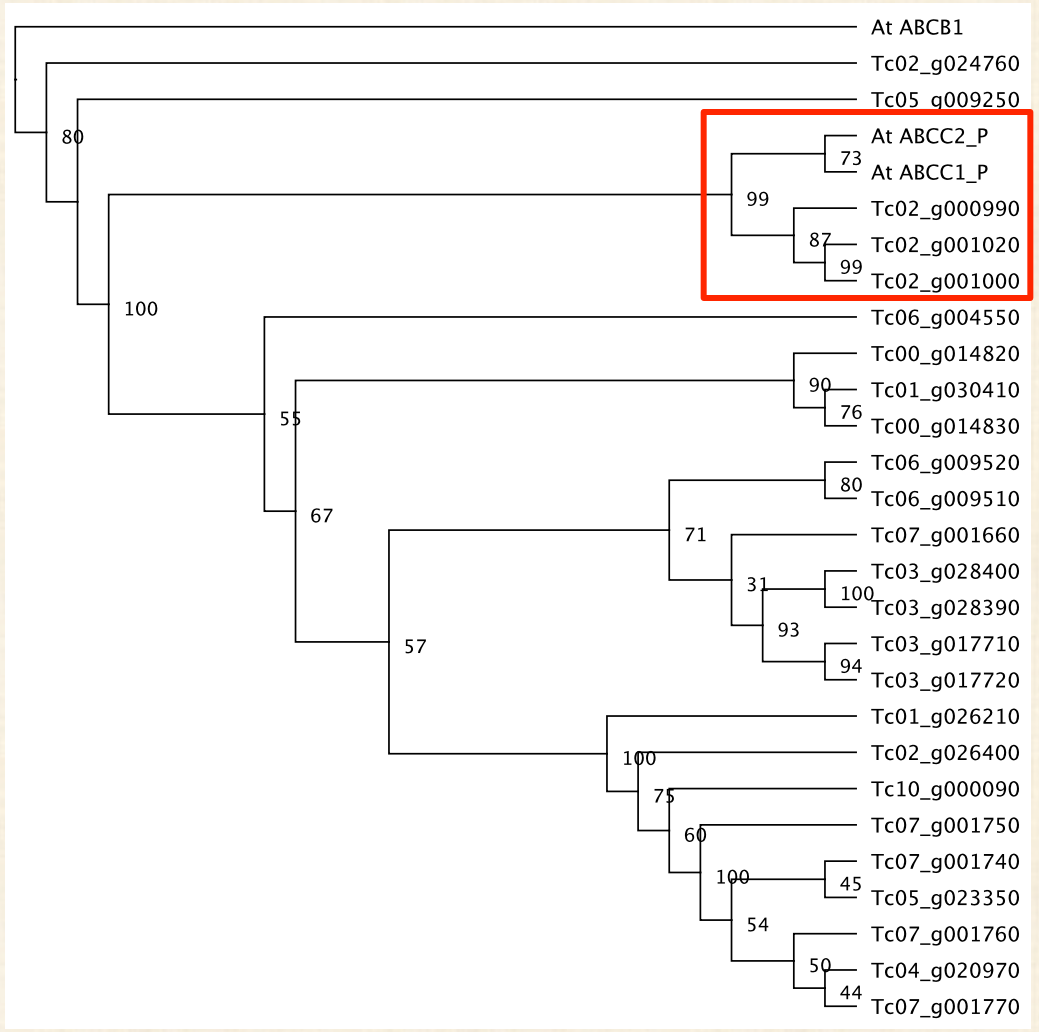
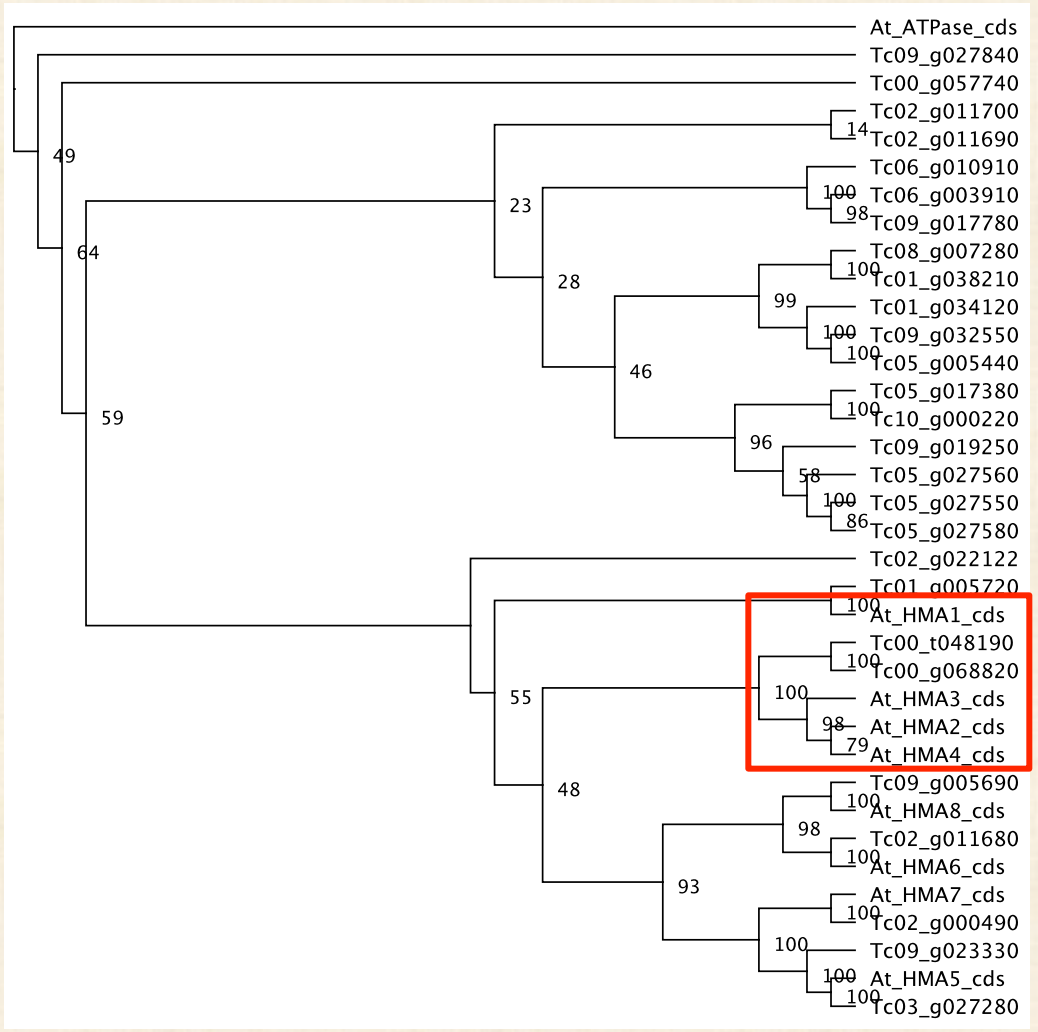
Phylogenetic trees showed orthology with the arabidopsis genes



ABCC FAMILY



HMA FAMILY

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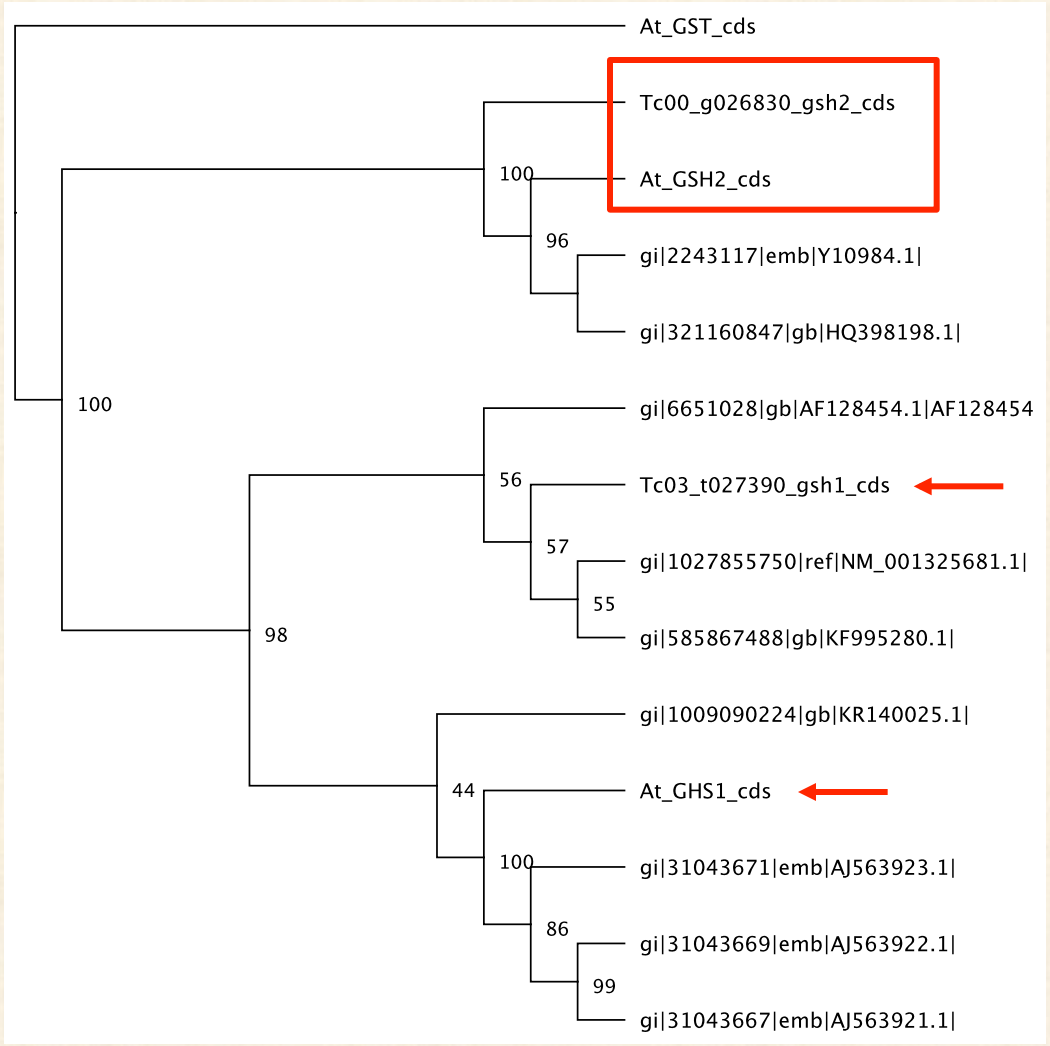
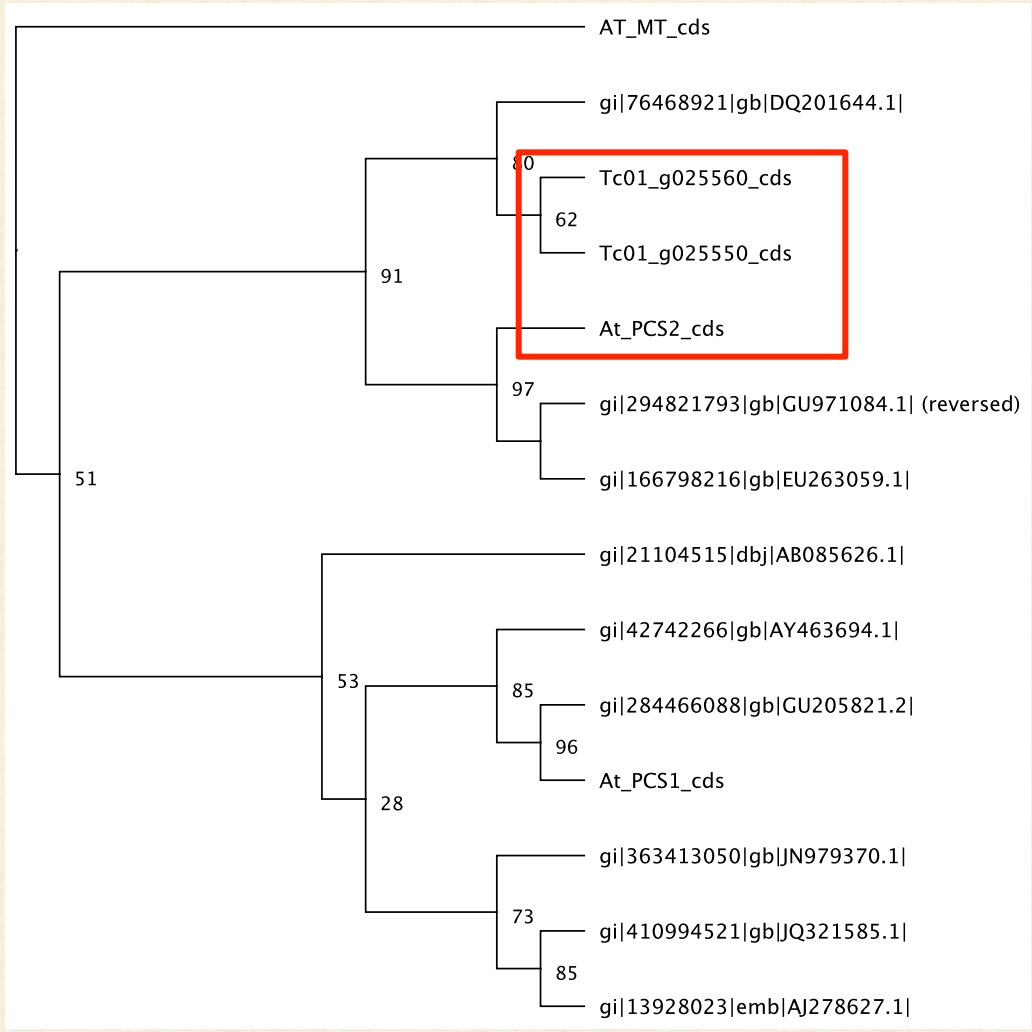
Phylogenetic trees showed orthology with the arabidopsis genes



GSH FAMILY



PCS FAMILY

Future Plan for Genomics of Cadmium in Cacao

- Gene Characterization
- Functional Tests of Gene Function
- Screening Germplasm for Natural Genetic Variation in Cd related Genes (expression and allelic variation)
- Develop Strategies for Genetic Approaches to Cd Problem in Cacao

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Thanks Very Much