

Characterization of genetic material aiming to selection of parents with traits of interest



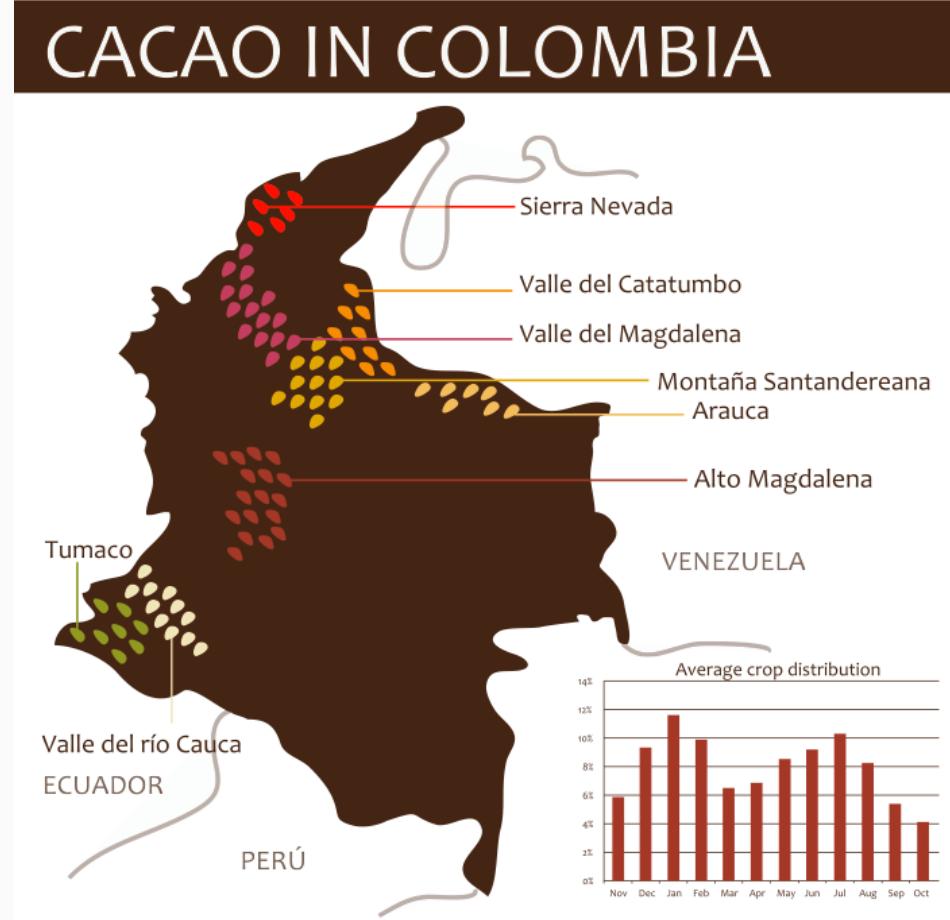
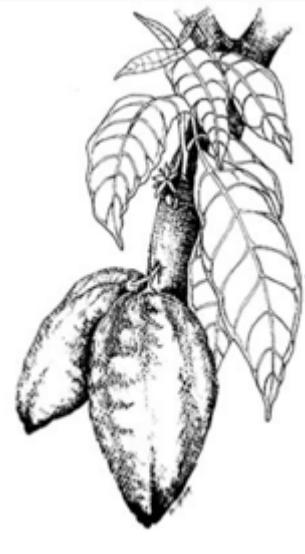
Roxana Yockteng
Investigadora PhD



Cacao

Theobroma cacao L.

Malvaceae – 22 species in genus *Theobroma*



Cacao plantation issues in Colombia



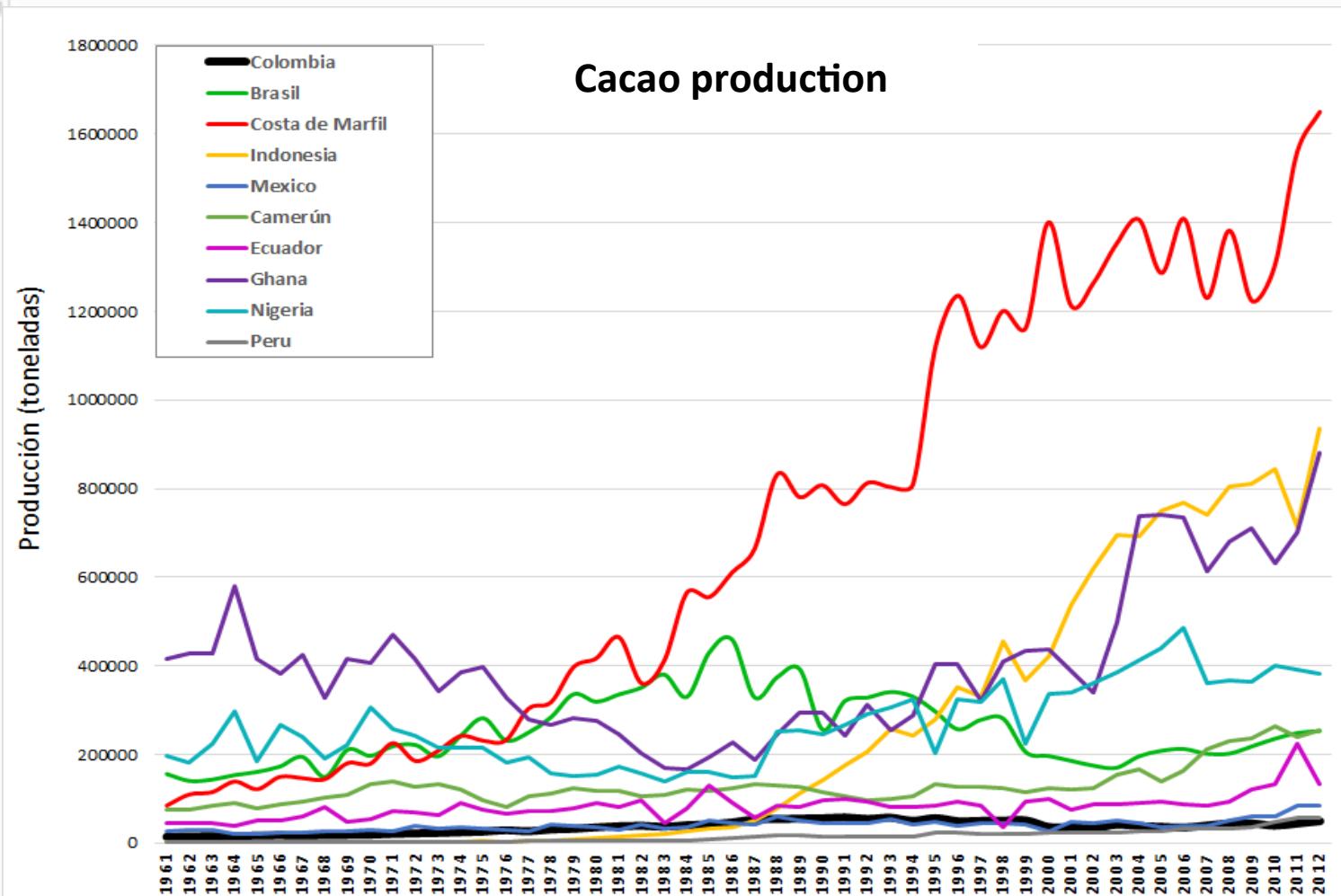
Monilia (*Moniliophthora roreri*)

Black pod
(*Phytophthora* sp)

Witches' broom
(*M. perniciosa*)

- Old plantations
- Diseases
- Low yield: 300-500 kg/ha
- Cadmium uptake
- Compatibility

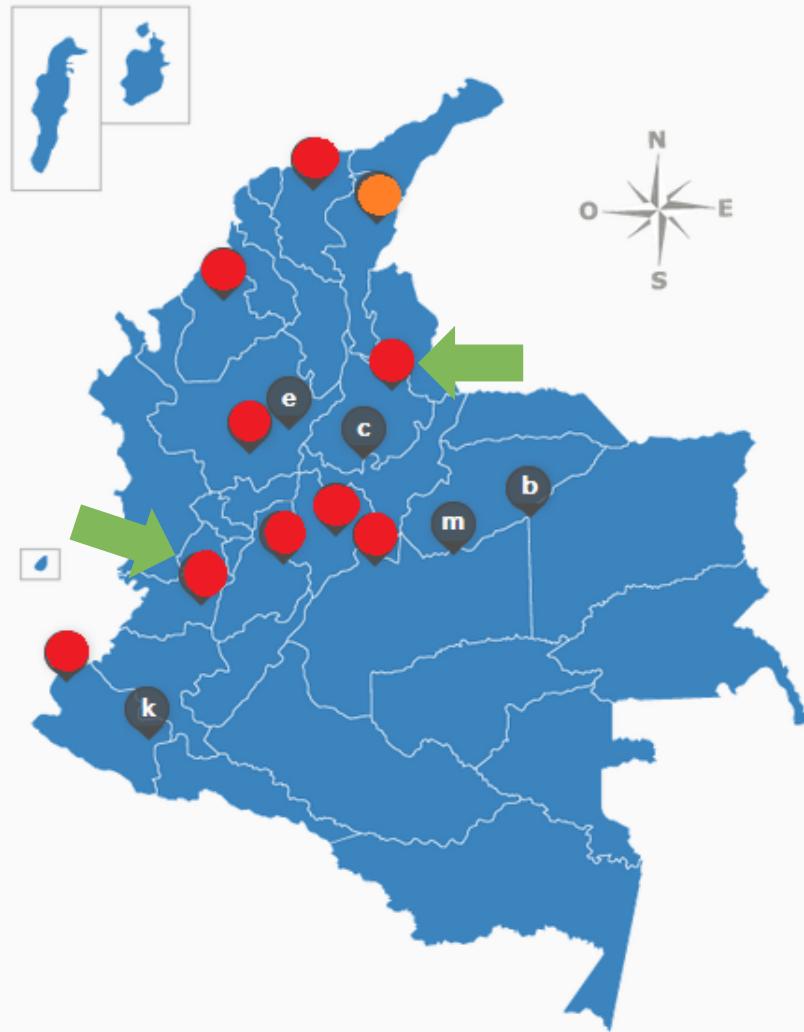
World production



Tomado de Fao-Stat, 2014



Corpoica's cacao Germplasm Bank

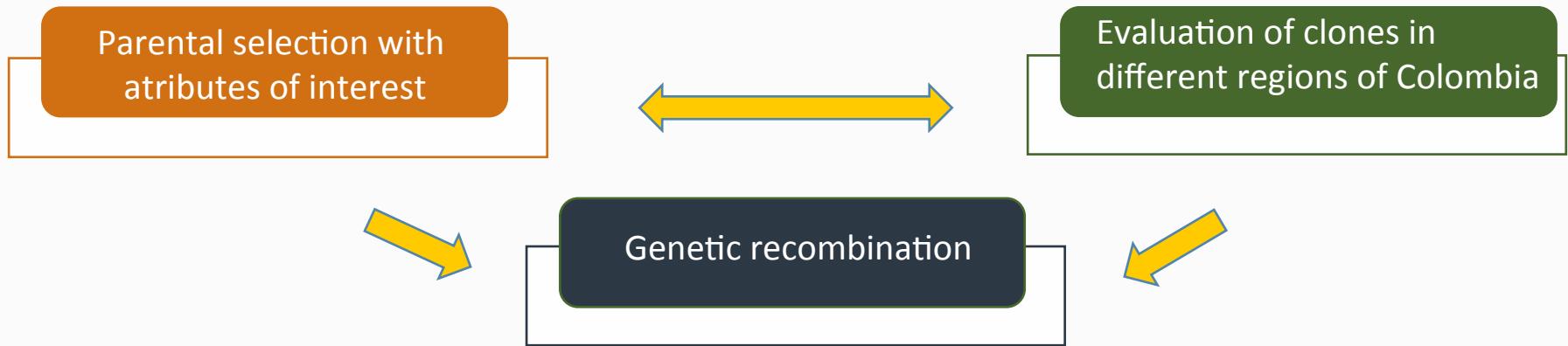


C.I. Palmira : 509 accessions
C.I. La Suiza: 455 accessions



Breeding program in Corpoica

Offer improved genetic materials: high productivity and disease resistance





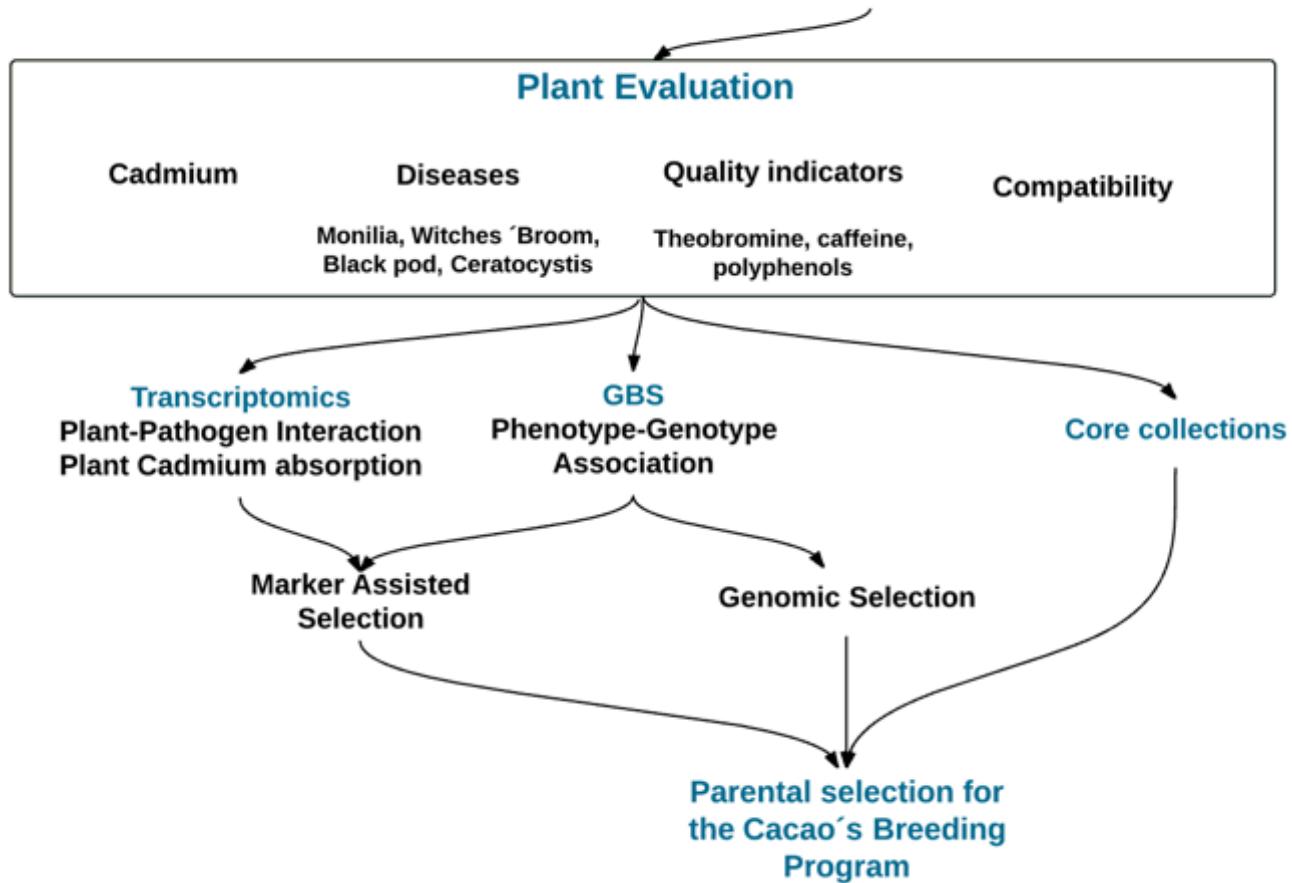
Cacao Breeding Program

Genotype selection by traits of interest



Yeirme Jaimes PhD
Caren Rodríguez PhD,
Leonora Rodríguez PhD,
Roberto Coronado,
Julian Mateus MSc

Daniel Bravo PhD,
Alejandro Caro PhD,
Jaime A. Osorio MSc,
Jhon Berdugo MSc,
Paola Delgadillo,
Roxana Yockteng PhD





Questions

Where are distributed in Colombia cacao and their wild relatives?

Distribution of genus *Theobroma* in Colombia

Caren Rodríguez
Carlos González
Salvador Rojas
Jessica Moreno
Roxana Yockteng



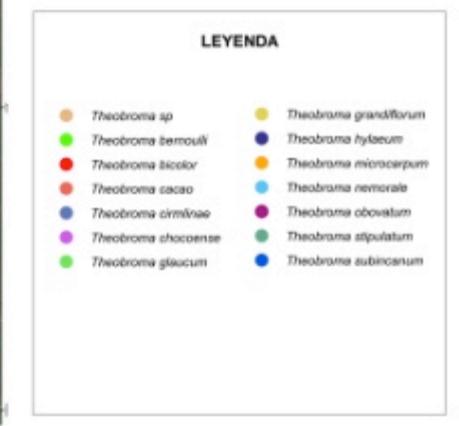
PARÁMETROS CARTOGRÁFICOS

- Proyección: Transversa de mercator
- Datum: Magna - Sirgas
- Origen: Magna - Colombia Bogotá
- Coordenadas planas: 1'000.000 mN/1'000.000 nE

FUENTES:

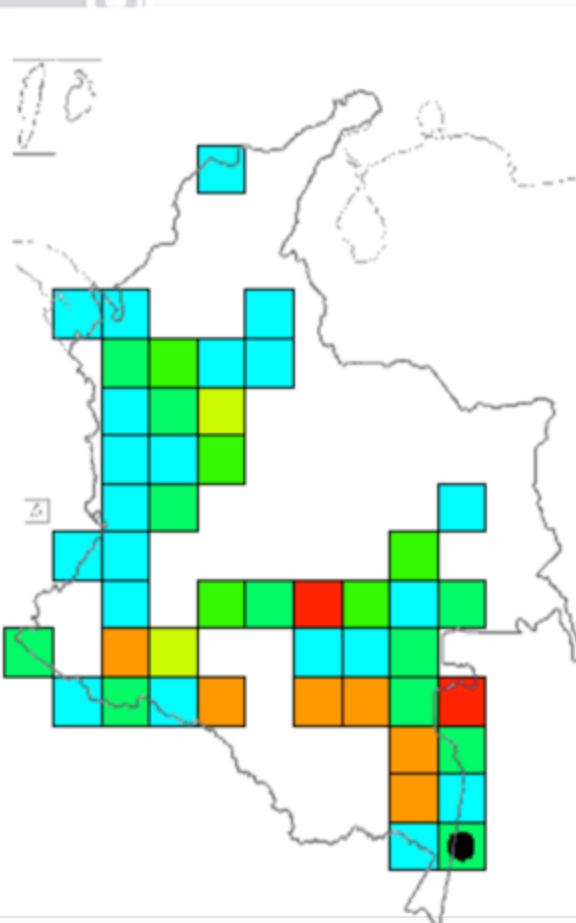
- Base de datos - GBIF.org
- Bases de datos herbarios nacionales

ELABORADO POR: Jessica Moreno B.
Diana Elisa Correa P.

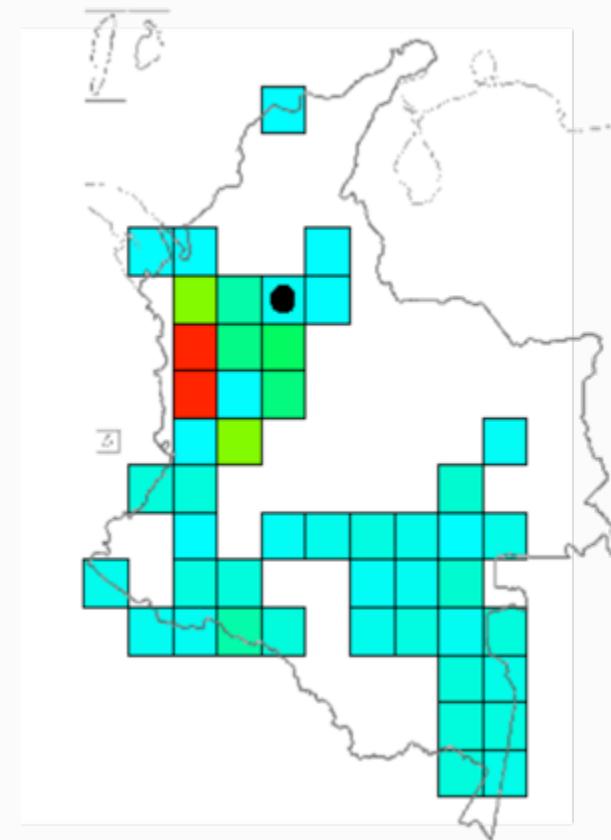




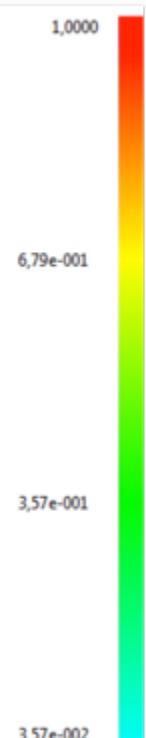
Theobroma Geographical Diversity



No sp



Endemism

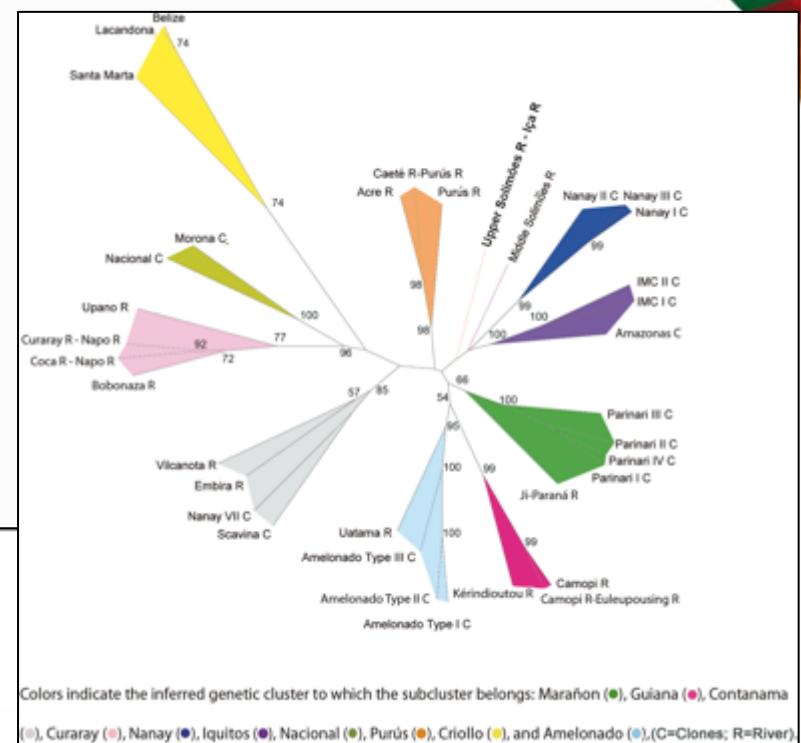
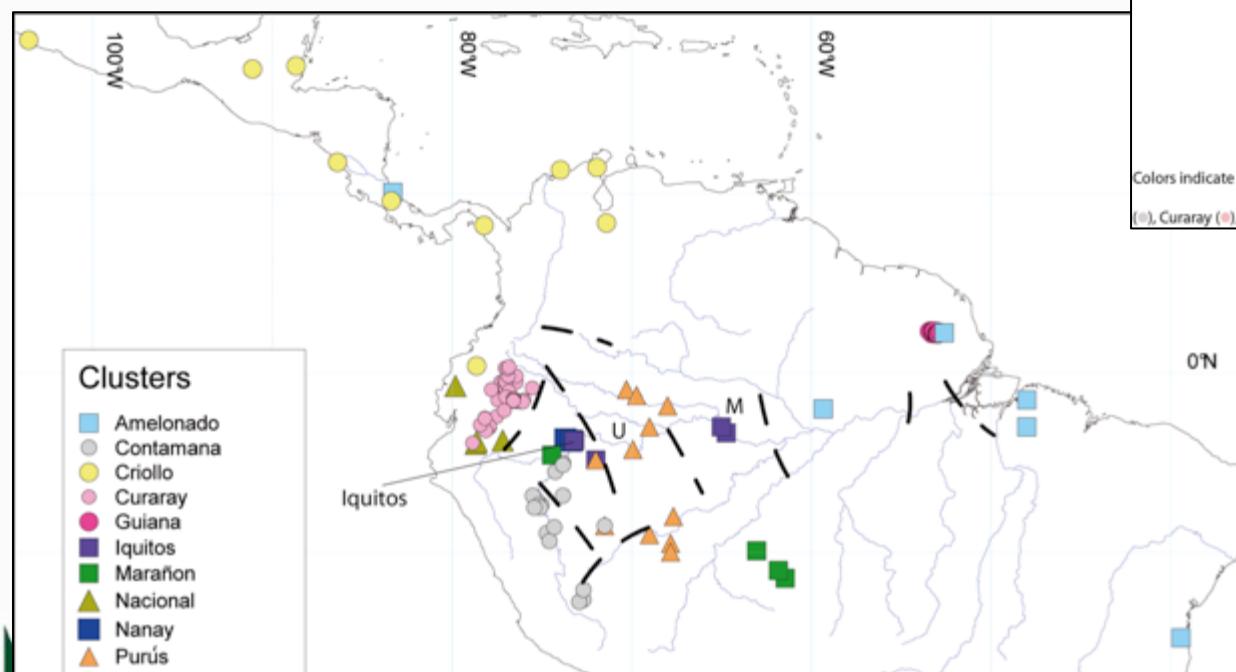




Questions

Does Corpoica's germplasm bank diverse?

Cacao diversity



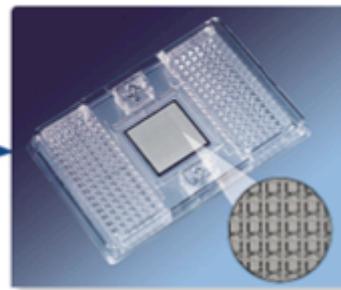
(Motamayor et al. 2008)

Genotyping Corpoica's Germplasm

Jaime A. Osorio
Jhon Berdugo
Roxana Yockteng

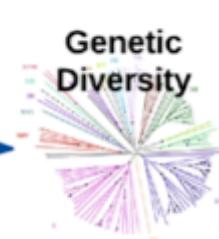


Markers SNPs (96)
FLUIDIGM



CIAT
Constanza Quintero
Patricia Zapata
Gerardo Gallego

Genetic
fingerprint

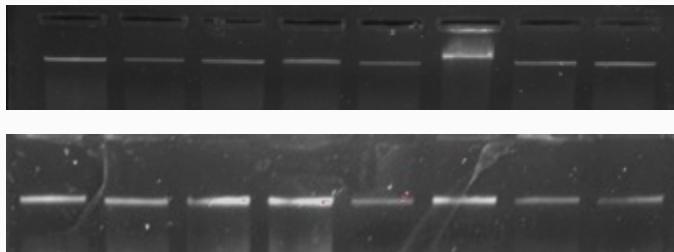


Selection of
accessions

Genetic Diversity Analyses

526 accessions

DNA extraction



DNA 20 ng/ μ L

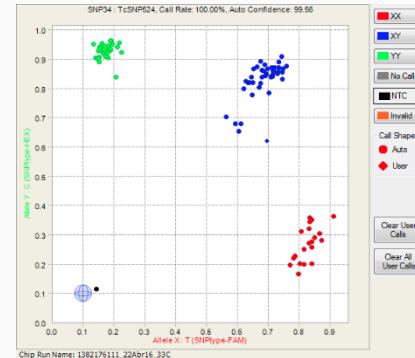


Genotyping for Fluidigm



96 SNPs

Data Analyses

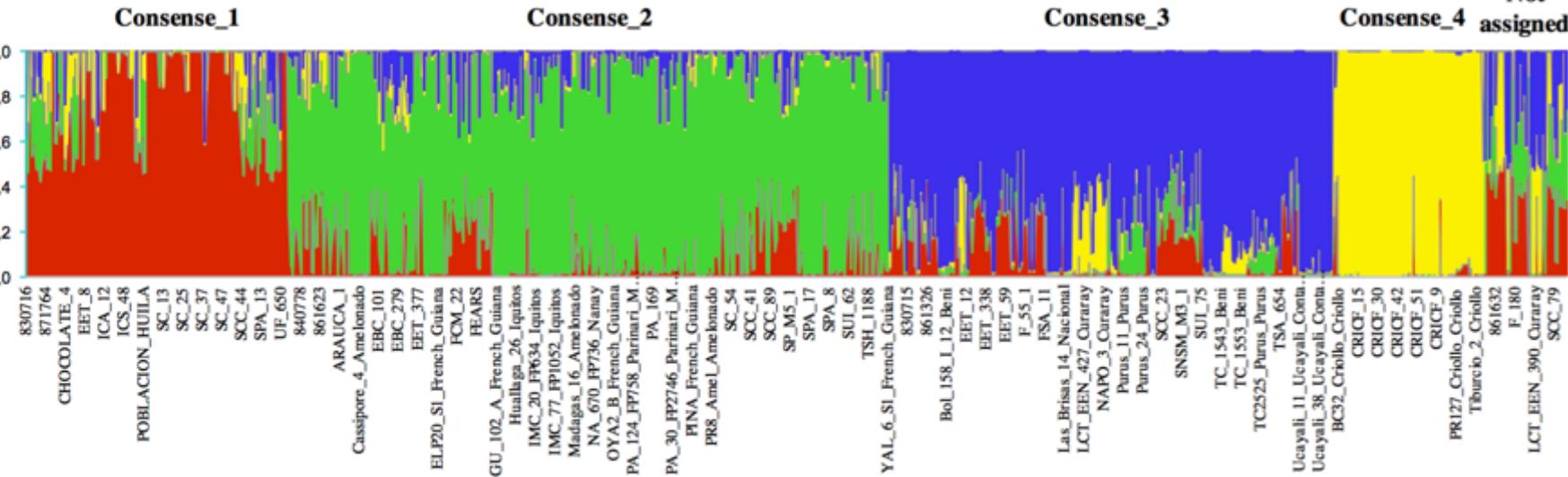


Genotype assignment

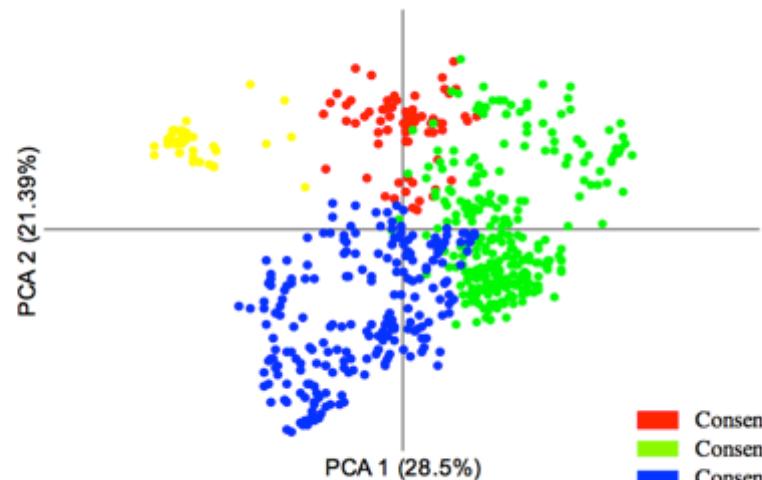
Michiels et al., (2003) modificado

Population structure

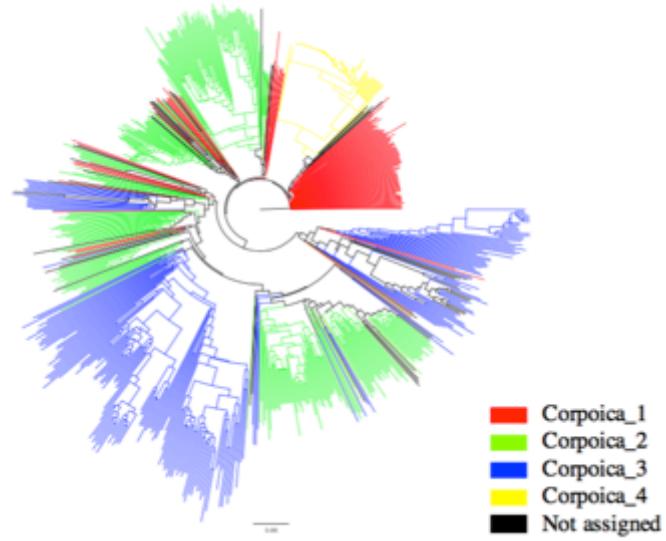
A



B



C

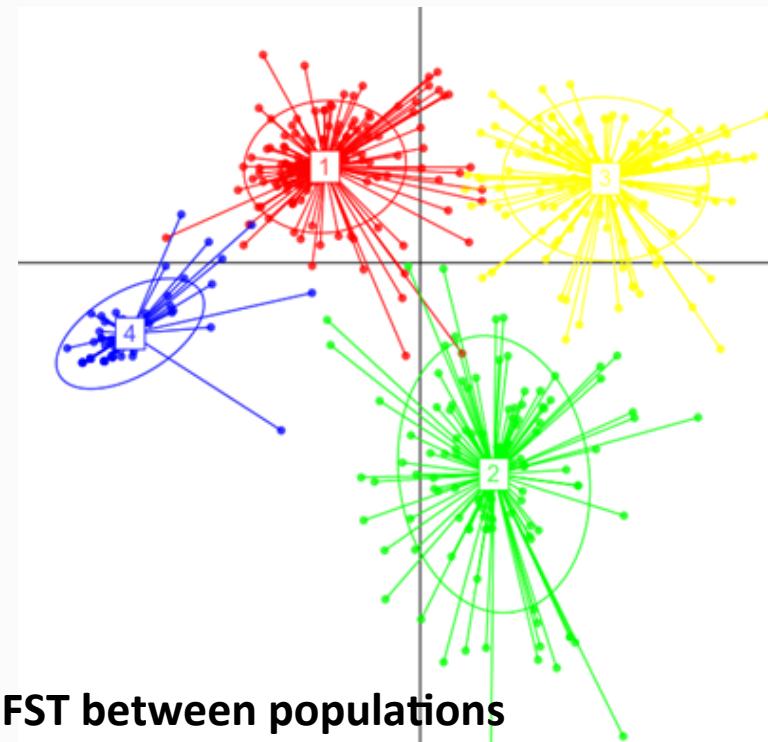


Genetic diversity

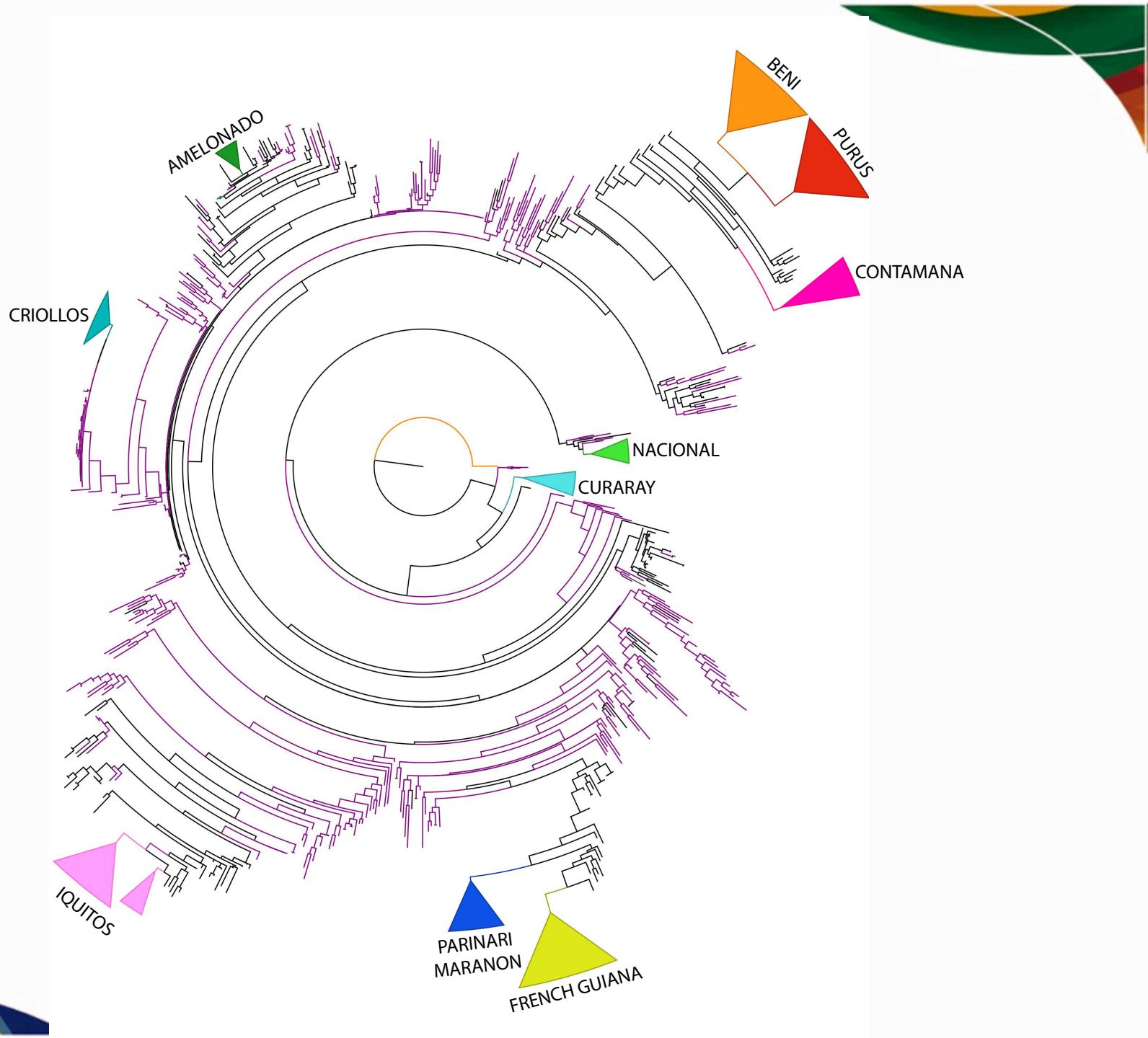
Analysis	Subpopulations	N ^A	H _O ^B (Mean +/- SD)	H _E ^C (Mean +/- SD)
Corpoica	Corpoica_1	119	0.559 (0.042)	0.338 (0.024)
	Corpoica_2	190	0.275 (0.014)	0.329 (0.017)
	Corpoica_3	131	0.494 (0.013)	0.427 (0.008)
	Corpoica_4	59	0.040 (0.005)	0.077 (0.008)
	Not assigned	37	0.393 (0.018)	0.402 (0.014)
Consense	Total_Corpoica	536	0.353 (0.013)	0.314 (0.009)
	Consense_1	134	0.447 (0.045)	0.336 (0.024)
	Consense_2	307	0.226 (0.013)	0.314 (0.018)
	Consense_3	226	0.293 (0.014)	0.363 (0.014)
	Consense_4	77	0.048 (0.008)	0.055 (0.009)
	Not assigned	43	0.293 (0.017)	0.403 (0.013)
	Total_Consense	787	0.261 (0.012)	0.294 (0.010)

^ANumber of samples, ^BObserved Heterozygosity, ^CExpected Heterozygosity,
SD: Standard deviation.

DAPC



	1	2	3	4
1	0.00000			
2	0.15613	0.00000		
3	0.22677	0.20068	0.00000	
4	0.37851	0.54223	0.42912	0.00000



Genes associated with disease resistance

Leidy Paola Delgadillo

Yeirme Jaimes

Leonora Rodríguez

Mauricio Soto

Roxana Yockteng



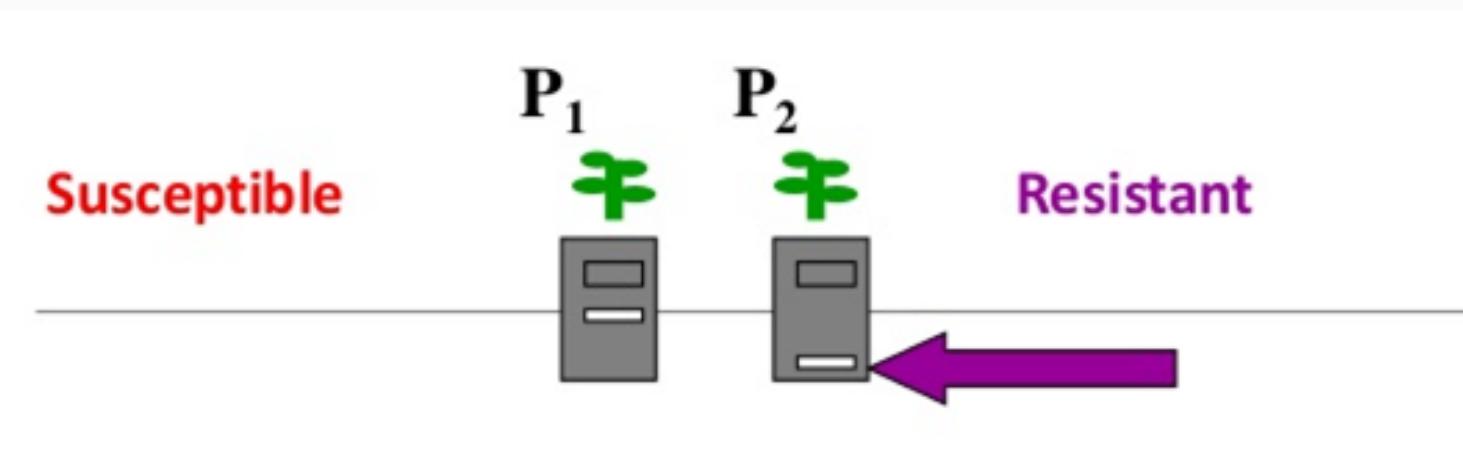
Phytophthora sp



Moniliophthora roreri

Question

- Which are the genes implicated in cacao plant defense?
- Are the genes activated the same in response to Phytophthora and Monilia?



Defense response to *Phytophthora palmivora*



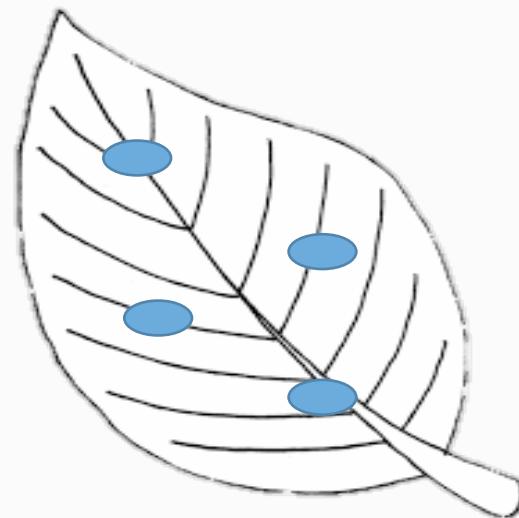
3 month after grafting



Phytophthora palmivora

Inoculation

Inoculum al 1×10^6
Agar-water 0.4%.



48 hours after inoculation



Quantitative scale



CCN 51



0h

24h

48h

96h

RNAseq experiment

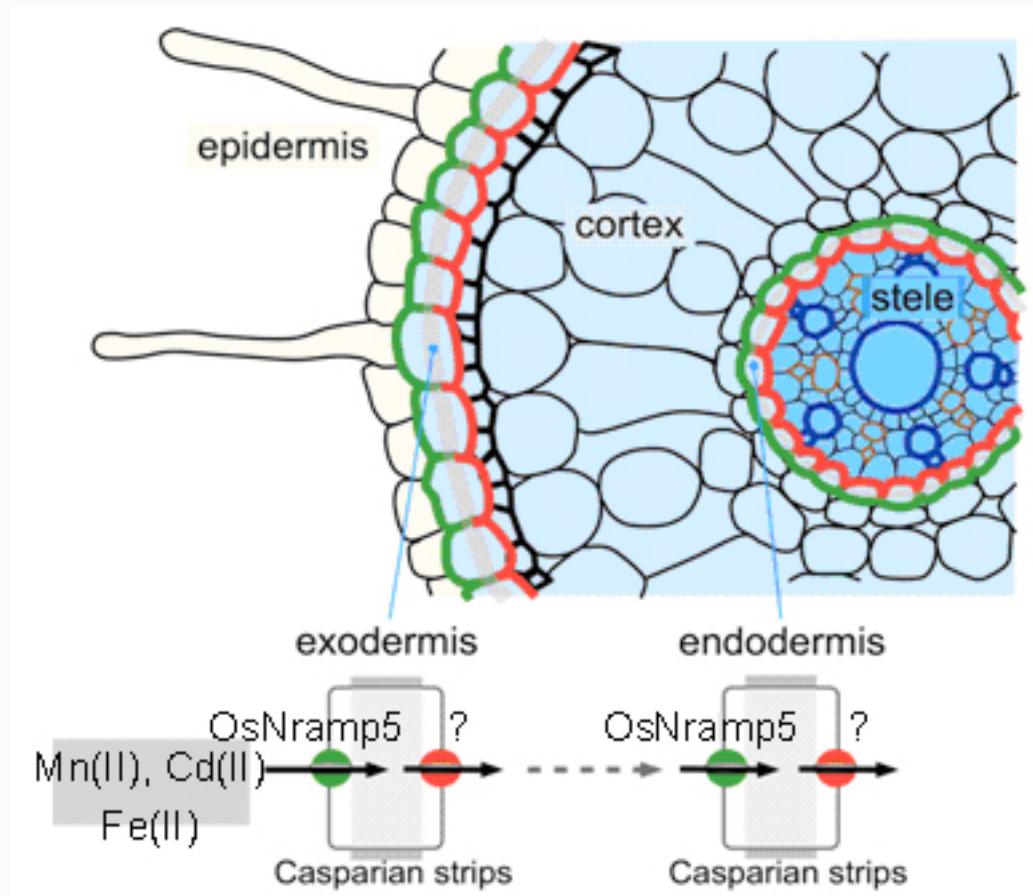
	Resistente						susceptible					
	Inoculada			Muestra			Inoculada			Muestra		
tiempo 0	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
tiempo 24 h	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
Tiempo 48h	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
tiempo 96h	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3

X 3



Cadmium uptake and genes implicated

Caren Rodríguez, Diego ?, Andrea Montenegro, Jaime Osorio, Jhon Berdugo, Paola Delgadillo, Roxana Yockteng



Questions

Which genotypes do absorb less cadmium?

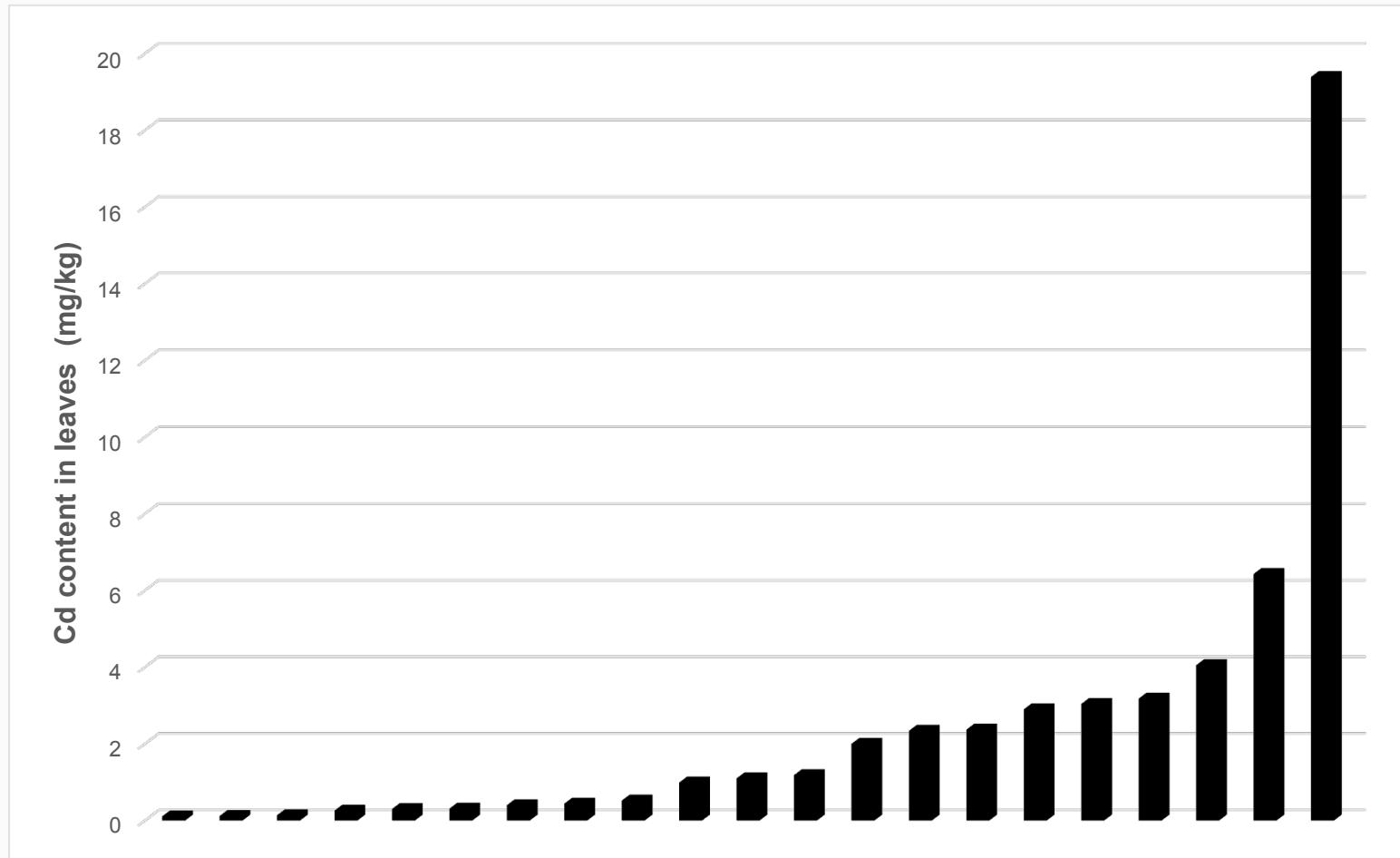
Which are the genes implicated in Cadmium uptake in cacao?

Does the difference in cadmium uptake is correlated with the level of expression of certain heavy metal transporters?

First Platform to evaluate cadmium uptake



Differential Cd absorption



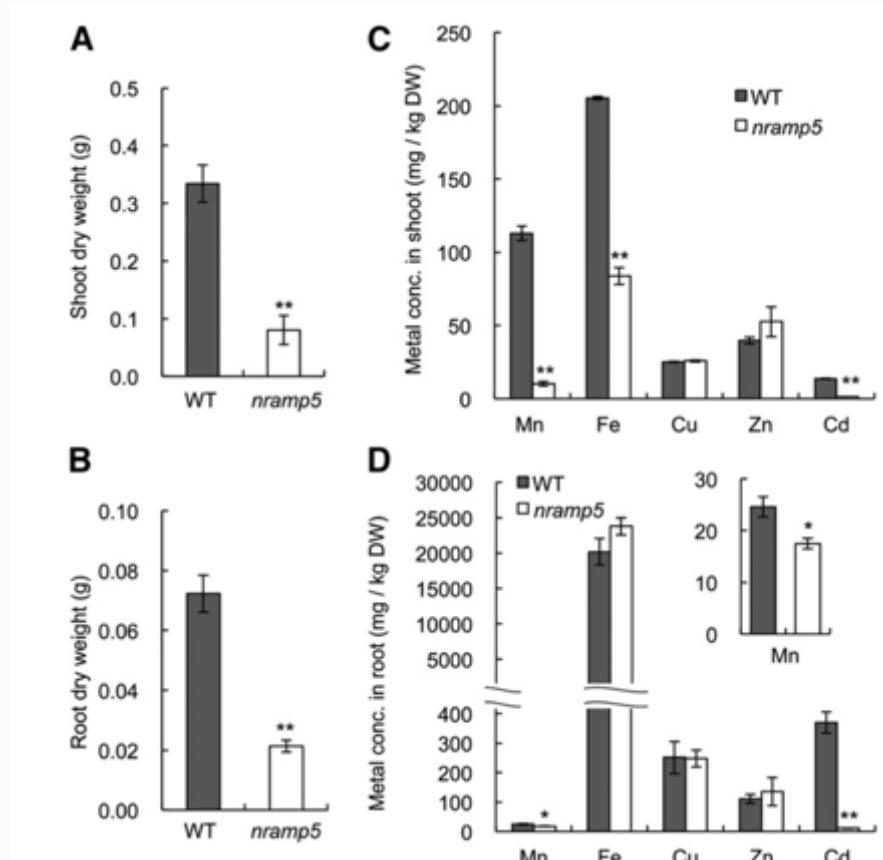
Second Platform to evaluate cadmium uptake



Heavy metal transporters

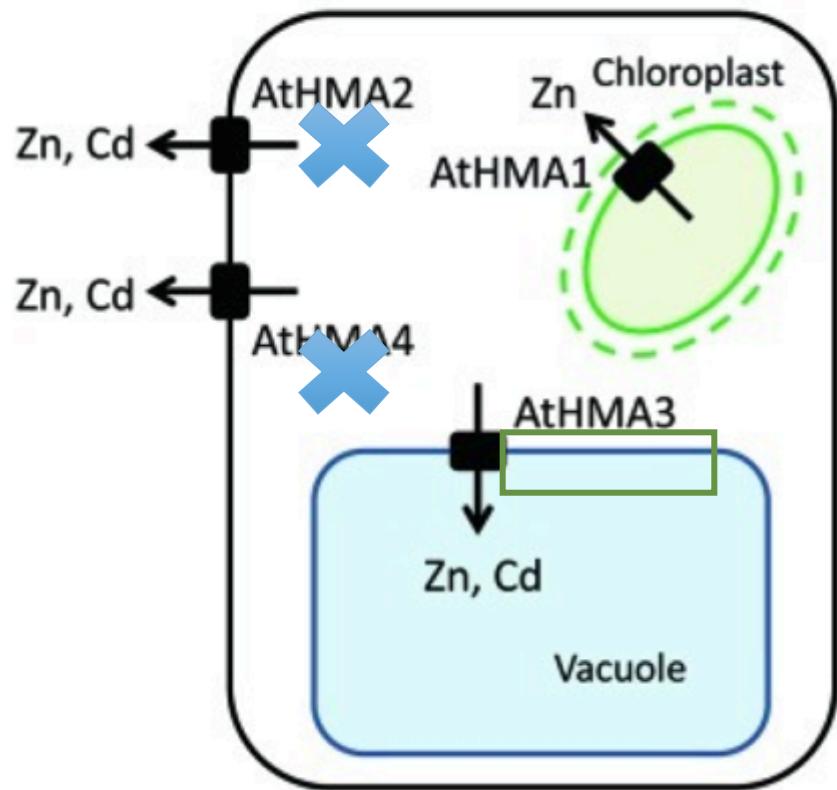
Two families of candidate genes

- *Nramp* (Natural resistance-associated macrophage protein)
- *HMA* (*Heavy Metal ATPases*)

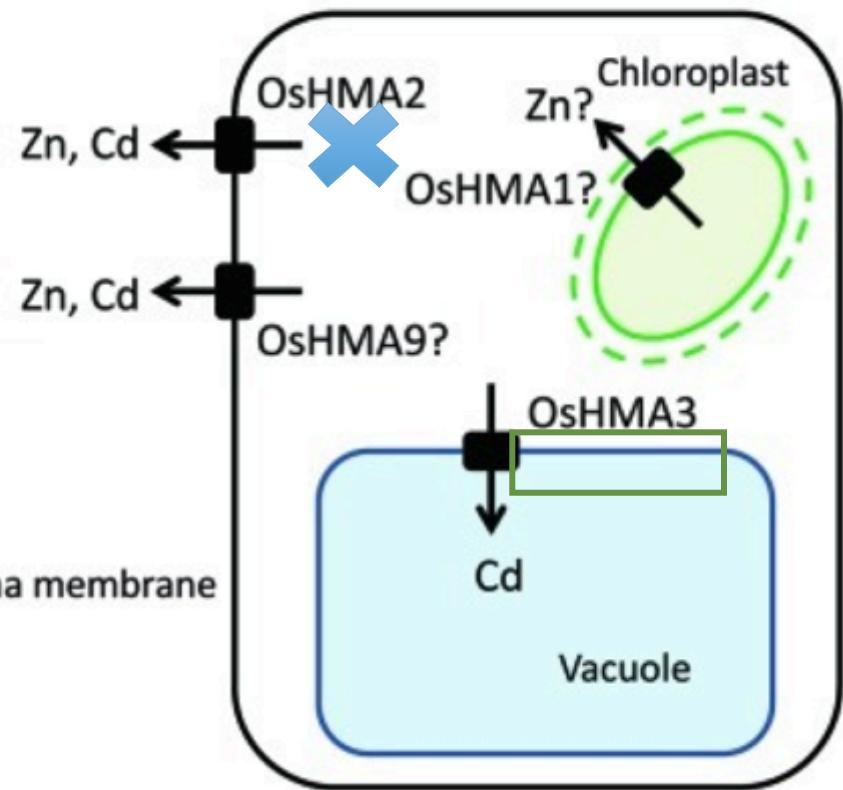


Sasaki et al. 2012
In rice

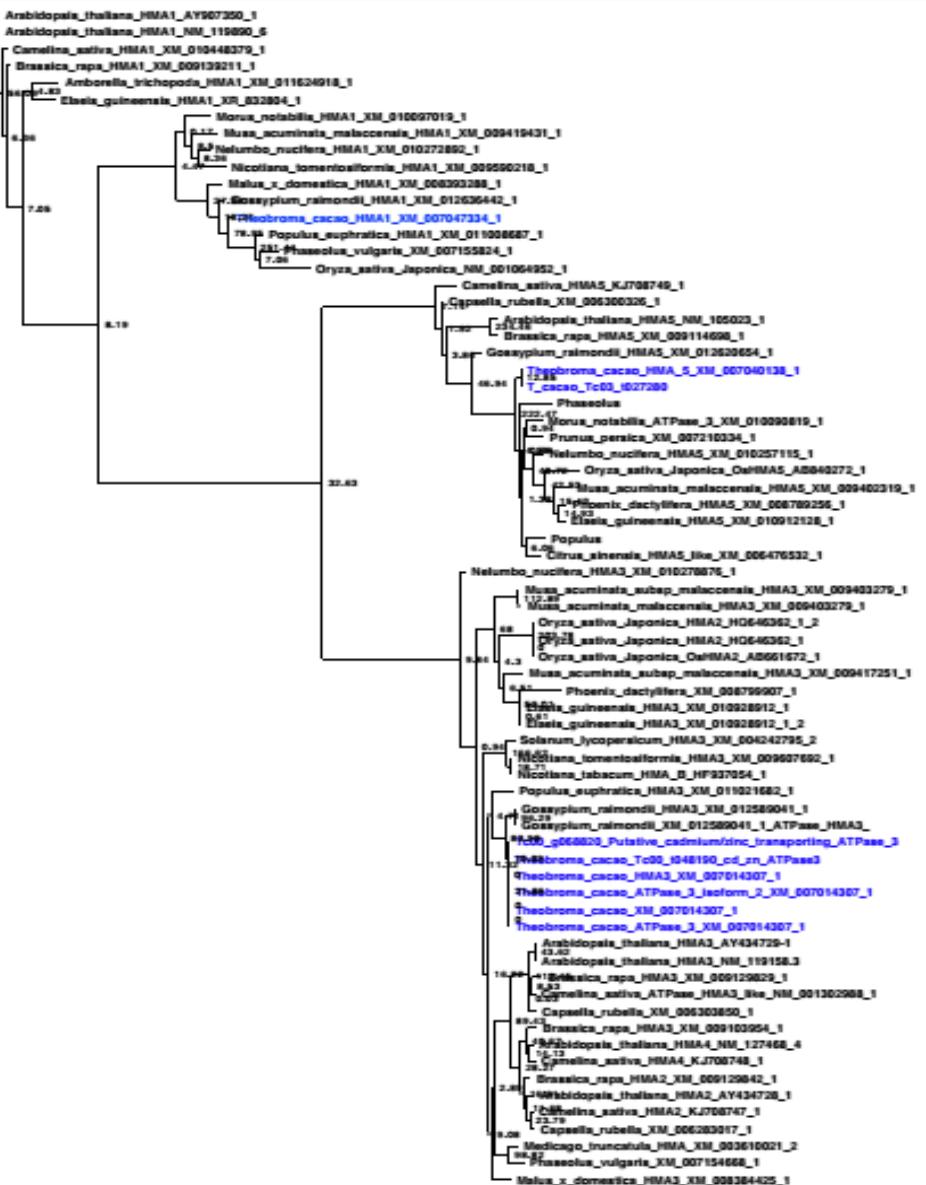
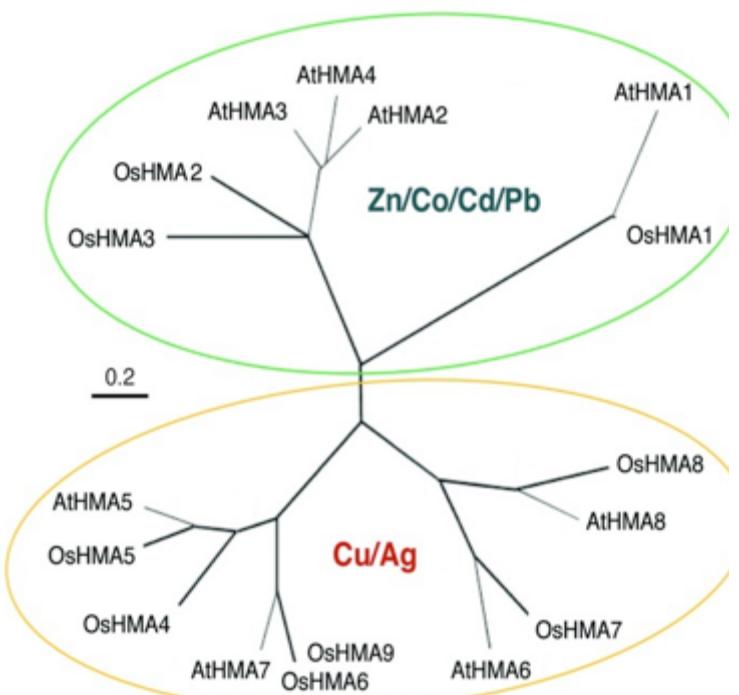
<Arabidopsis>



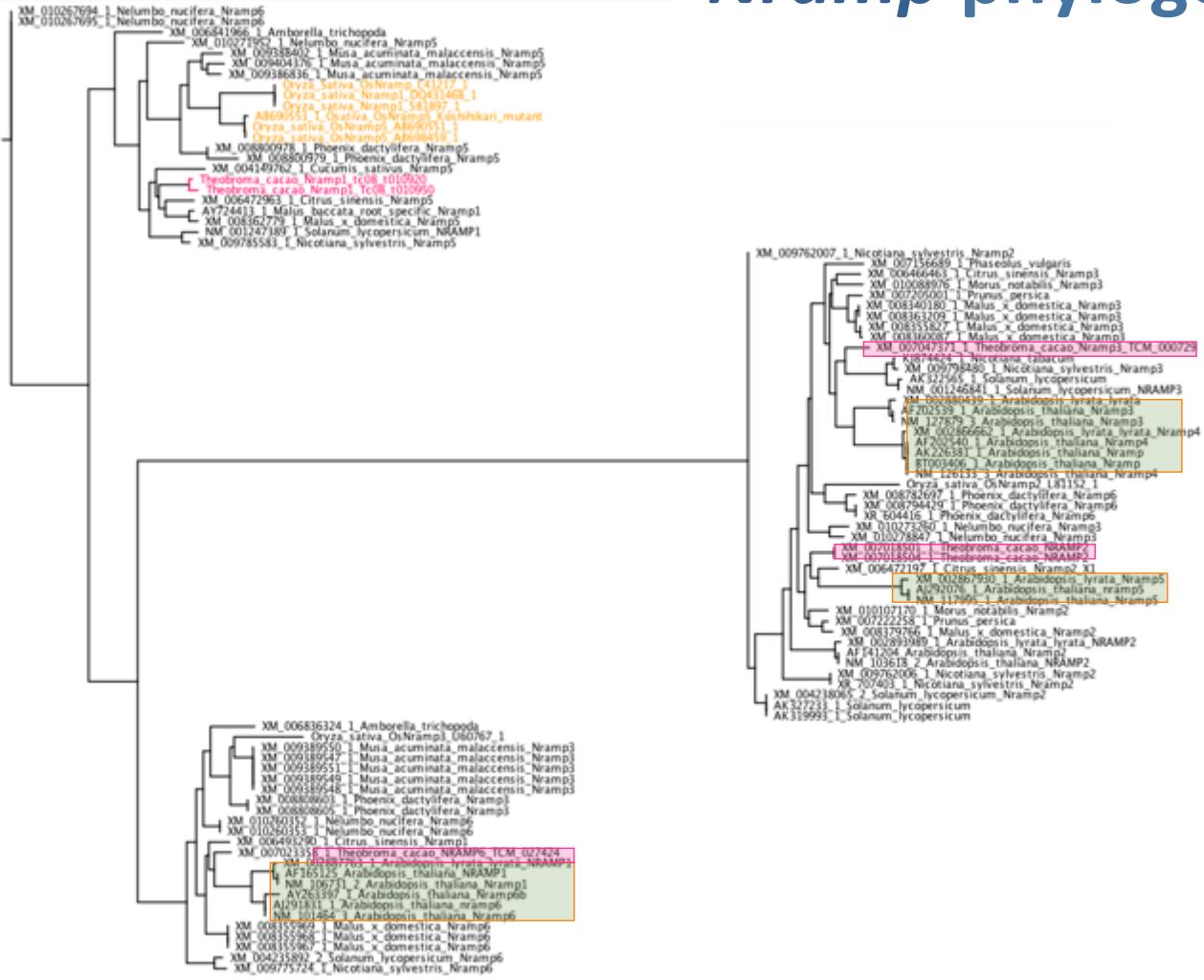
<Rice>



AtHMA2 and AtHMA4 are the major players in Cd translocation in *A. thaliana*. (Kim et al. 2009, Takahashi et al 2012)



Nramp phylogeny



Differential expression

RNA seq

	Genotype low Cd uptake		Genotype high Cd uptake	
	Leaves	Roots	Leaves	Roots
0H	R1, R2, R3	R1, R2, R3	R1, R2, R3	R1, R2, R3
8H	R1, R2, R3		R1, R2, R3	
24H	R1, R2, R3	R1, R2, R3	R1, R2, R3	R1, R2, R3



- Jaime A. Osorio
- Caren Rodríguez
- Andrea Montenegro
- Leidy Paola Delgadillo
- Jaime Andrés Osorio
- Ruth Angélica Rodríguez
- Jhon Berdugo
- Alejandro Caro



