Metal transporters involved in Cd uptake and distribution in cacao: gene function, evolution, and expression

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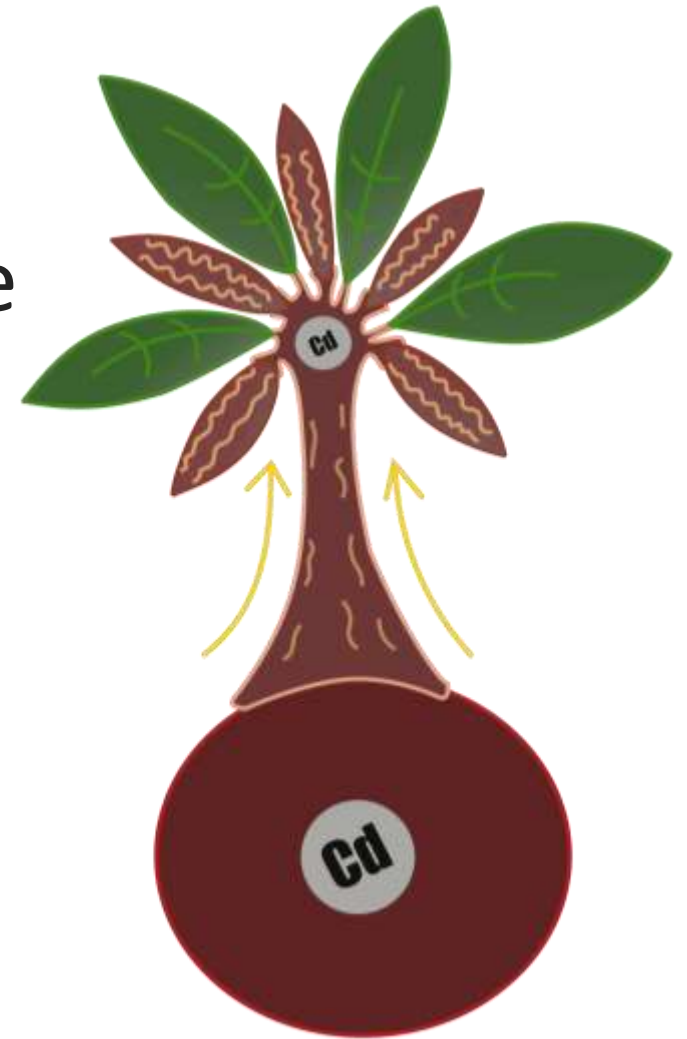
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2022 International Symposium on Cocoa Research (ISCR), Montpellier, France
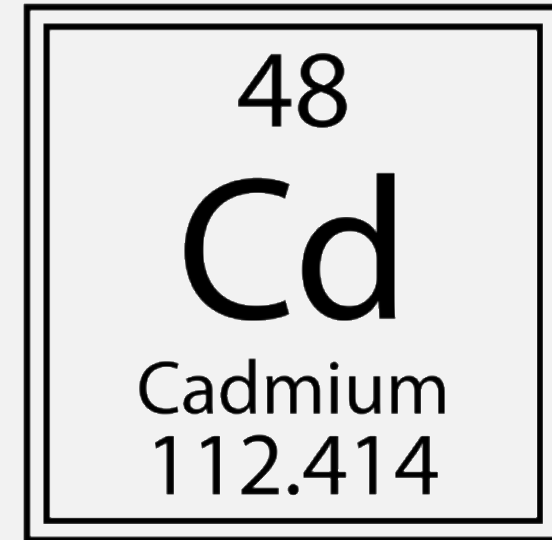
Outline

- Problem statement

- Background on metal uptake in plants

- HMA gene family in cacao

- Functional study using yeast assay

- Conclusions and summary

- Potential impacts

Cadmium and cacao: Why is it an issue?

- Cd consumed in high concentrations could lead to kidney damage, bone disease, and cancer in humans

- Cd is present in soils, especially in volcanic soils

- Cacao plants uptake Cd from soils and store it in different tissues

- Farmers from Latin America are most affected because of the continent's recent volcanic origin

Cadmium and plants

- Cd in non-essential element

- Enters plants mimicking Zn or Fe

- Abiotic factors influencing Cd uptake:
  - Concentration of Cd in the soil
  - Soil pH
  - Zn or Ca deficiency in soil

- Biotic factors influencing to Cd uptake :
  - Microbiome
  - Cacao production system
  - Plant genetics

48
Cd
Cadmium
112.414

Limited knowledge on Cd genetics and associated gene function in cacao!

Genetic variation of Cd uptake in cacao

- Cacao is NOT Cd hyperaccumulator, but accumulates Cd: Cd usually doesn't exceed 10ppm in LATAM farms

- Cd uptake and accumulations varies within the species

- Cd content is the soil is highly correlated with accumulations in cacao leaves

Lewis, 2018, *Sci. Tot. Env.*; *Sci. Tot. Env.,* Engbersen, 2019

Overview of metal uptake in plants
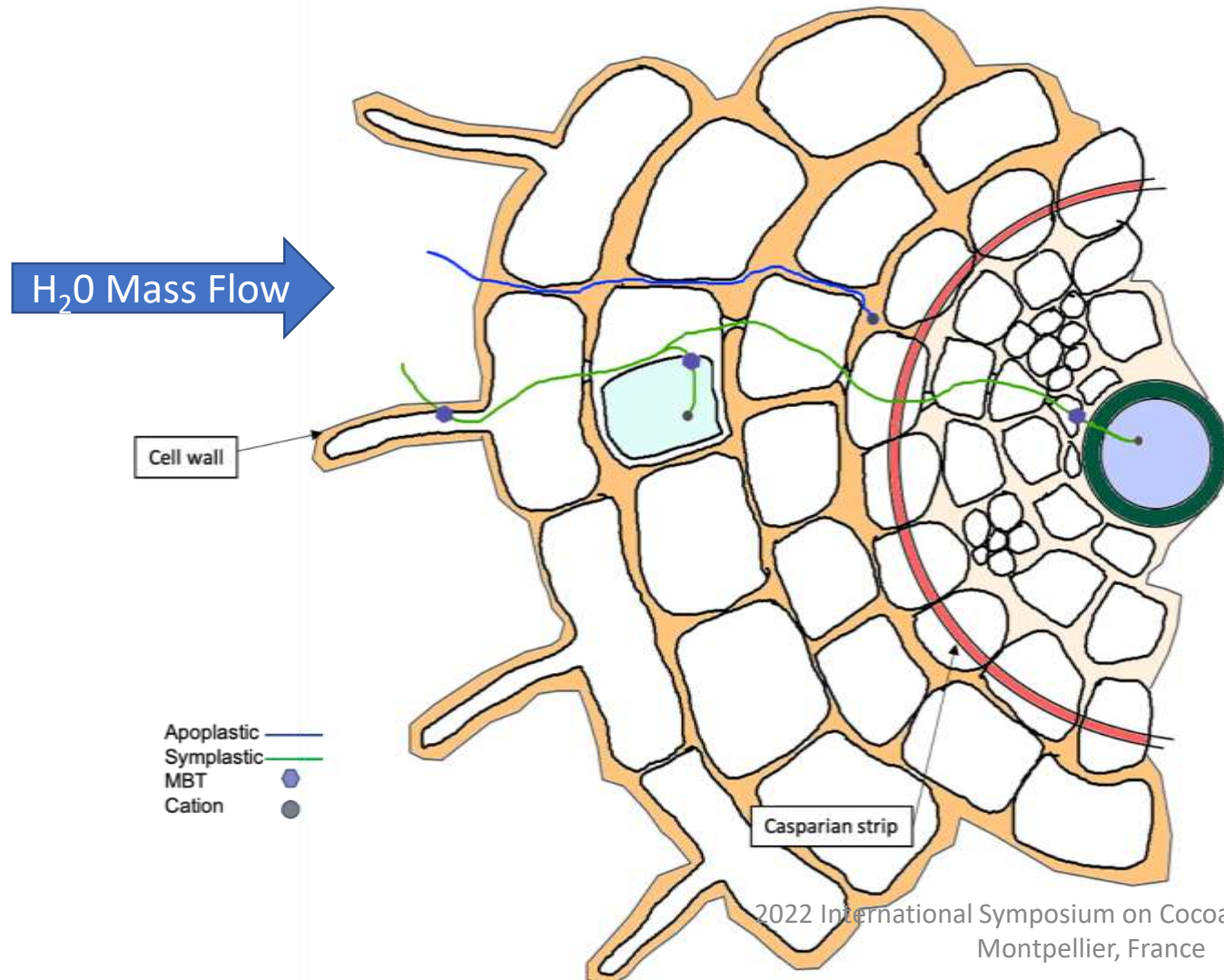
- Could first enter the apoplast

- Metal transporters mediate movement to symplast (NRAMP, ZIP, CAX).

- Metal transporters mediate the loading into the xylem parenchyma (HMA2 & HMA4, IRT1)

- Metal transporters mediate Elements the cortex cell can be trapped in vacuole (CAX, HMA)

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Functions of HMA proteins in plants

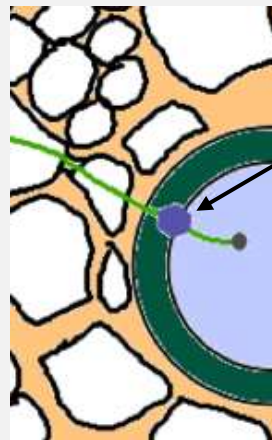Vacuolar sequestration
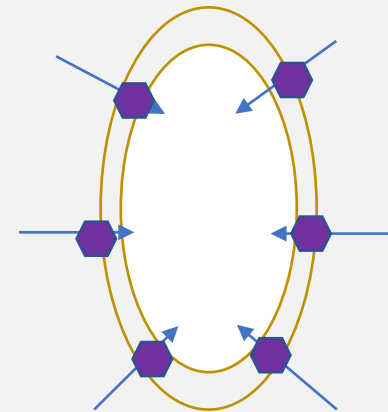(Morel, 2003)

Roots
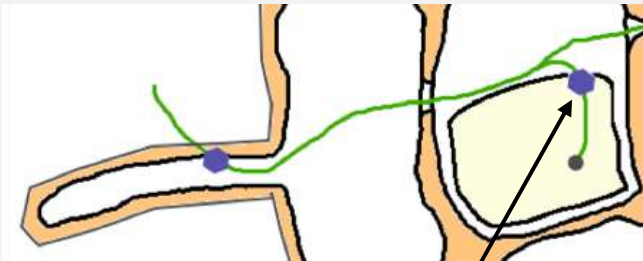
AtHMA3

Xylem Loading
(Eren, 2004 & 2006)

Stele

AtHMA2/AtHMA4

Seed development
(Olsen, 2016)

Bean

AtHMA2/AtHMA4

Eight HMA genes identified in the cacao genome

Gene Name/ID	Accession number	Length (residues)	Localization (DeepLoc)	TM (THMM2)
Tc01v2_t005580.1	Tc01v2_t005580.1	813	Plastid	6
TcHMA7	Tc02v2_t000500.1	814	Lysosome/Vacuole	8
TcHMA6	Tc02v2_t011530.1	814	Plastid	8
TcHMA5.2	Tc03v2_t023170.1	814	Mitochondrion	8
TcHMA5.1	Tc09v2_t019080.2	814	Cell membrane	8
TcHMA3.1	Tc09v2_t015490.1	1058	Cell membrane	6
TcHMA3.2	Tc09v2_t015500.1	1058	Cell membrane	6
TcHMA1	Tc01v2_t018910.1	586	Nucleus	0

Conserved domains found in P-type proteins

PxxK
N-domain
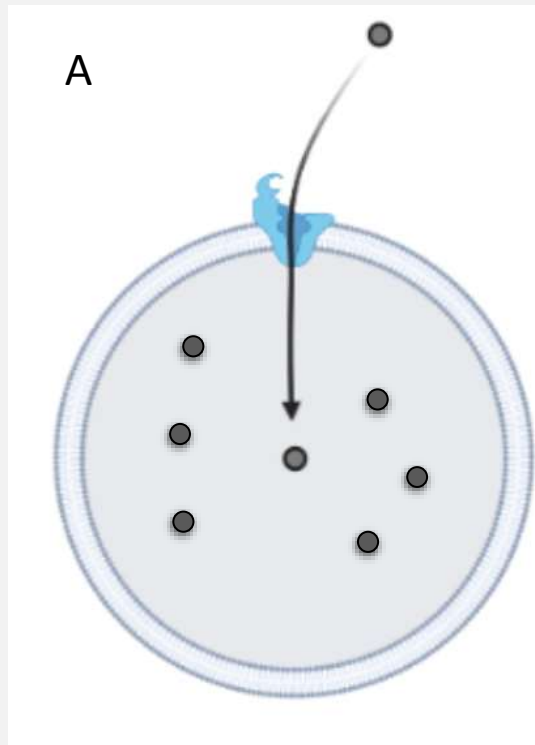
DKTGT
P-domain

GDGxNDxP
A-domain
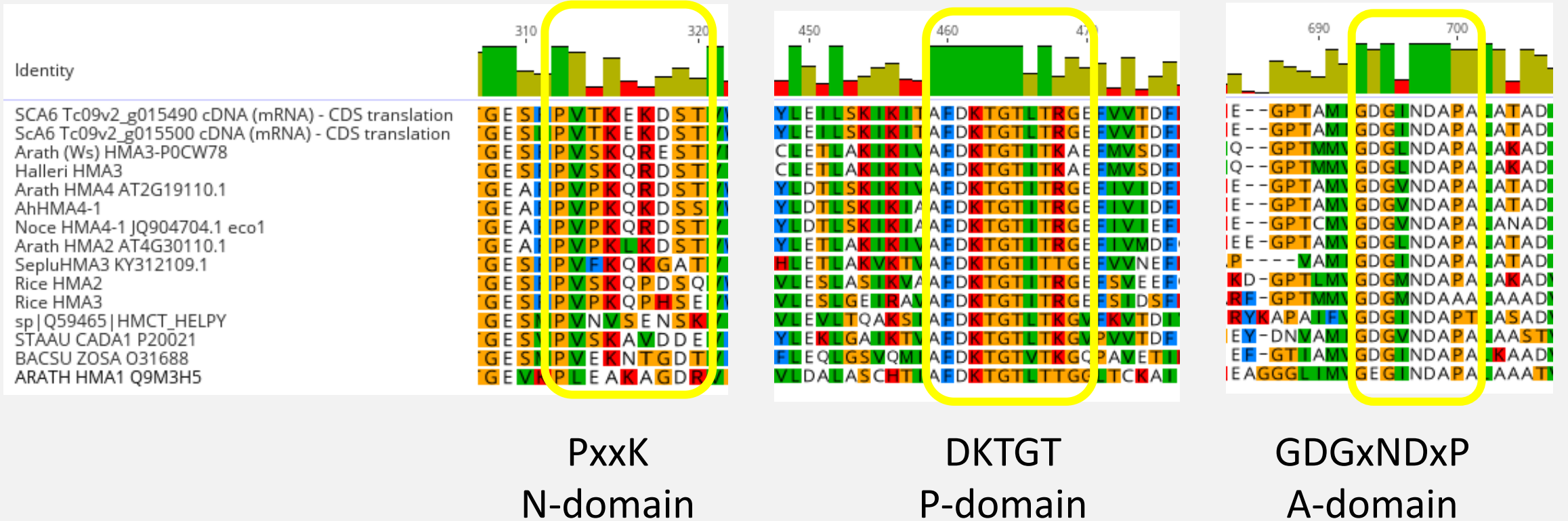
T. Cacao has one ortholog to Cd transporting HMAs in cacao
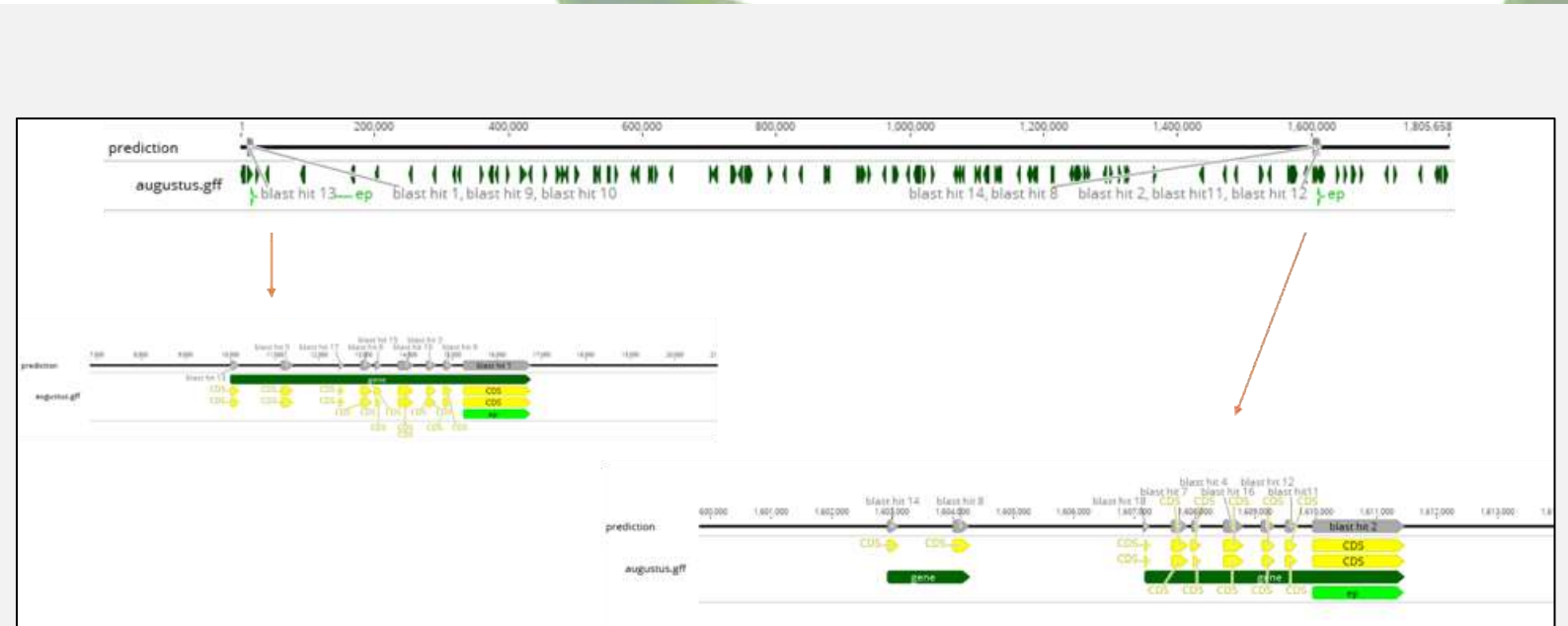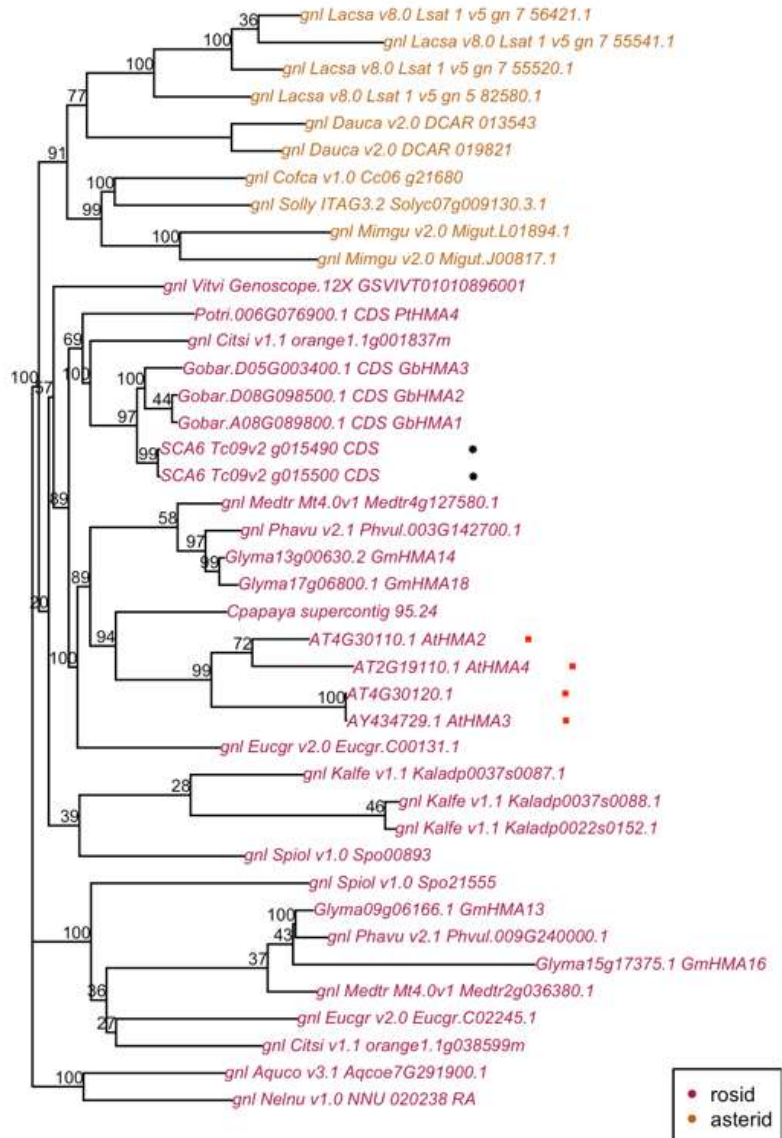
- Nine high quality genomes were analyzed
- Based on the DNA sequences we could not predict complete functional protein and verify the gene tandem duplication
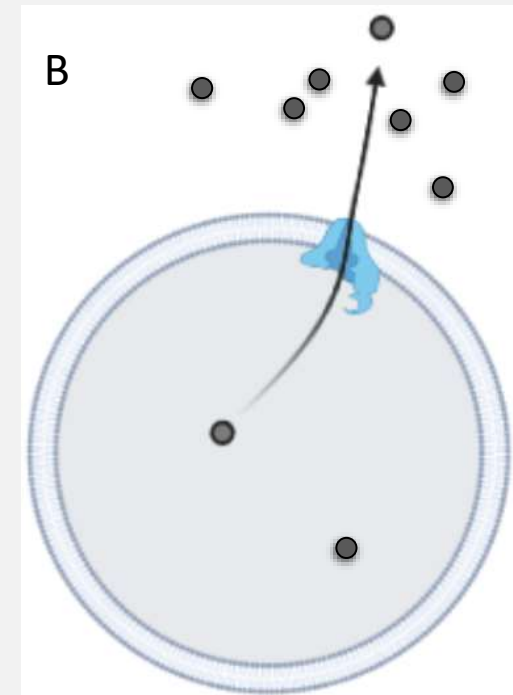
Cacao gene function assay in yeast cells

A

Two main *predicted* scenarios:

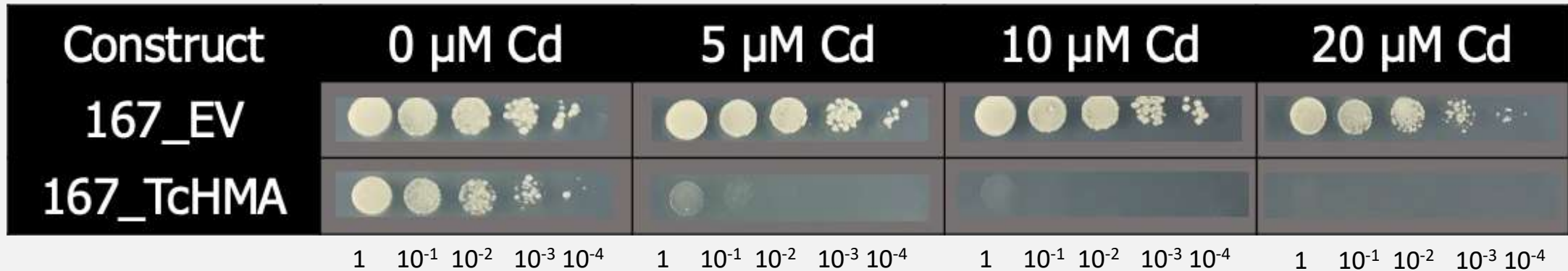A) A net ingress of Cd mediated by the HMA protein

→ Reduced yeast tolerance to Cd and reduced growth

B) A net egress of Cd mediated by the HMA protein

→ Increased yeast tolerance to Cd and better growth

B

Cd cation

Candidate gene Tc09v2_g015490 encodes for a Cd transporting protein

167_EV: Yeast cell cells containing the pYES2 vector with no gene insert
167_TcHMA: Yeast cells expressing the Tc09v2_t015490 gene driven by the GAL promoter

Conclusions and implications of this research for farmers

- Cacao has one orthologue to HMA3 with Cd transport capacity

- Knowledge of the role of this protein could lead to creating low accumulating genotypes

- Understanding the physiology of Cd uptake could help inform amendment strategies on the farms

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