Potential use of Heavy Metal ATPases (HMA) mutants to reduce cadmium translocation from root to leaf in tobacco.

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Introduction

- Cadmium is present in the soil particularly in industrial areas, and can be brought by irrigation or fertilizers.

- Cadmium is extracted from the soil by the plant, and then accumulated in the tobacco leaves. No solution is available to avoid this transfer.

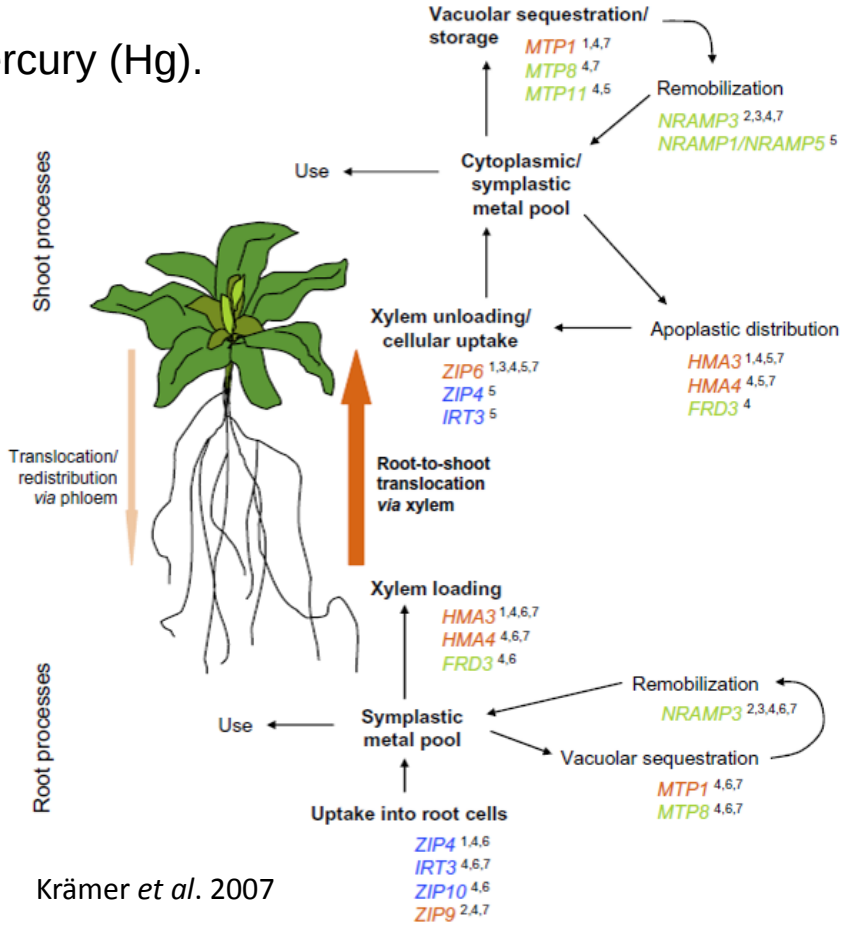
- 80% of cadmium is transferred from the leaf to the smoke during the burning process.

- Cadmium is classified in Group 1 (= known human carcinogens) by The International Agency for Research on Cancer (IARC).

Metals in plants

- Essential: iron (Fe), zinc (Zn), manganese (Mn), copper (Cu) are used as co-factors in enzymes.
- Non-essential: cadmium (Cd), lead (Pb), mercury (Hg).

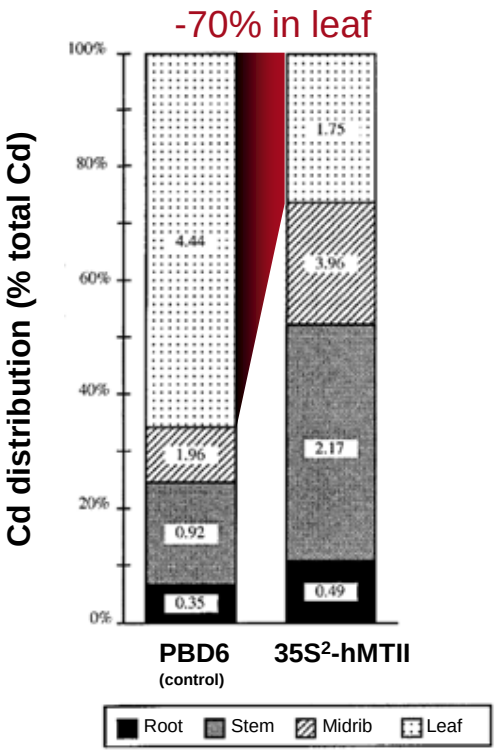
- Different mechanisms of transport exist:
  - Transport to major storage organs/tissues
  - Sub-cellular compartmentalization
  - Remobilization and redistribution.



Krämer *et al*. 2007

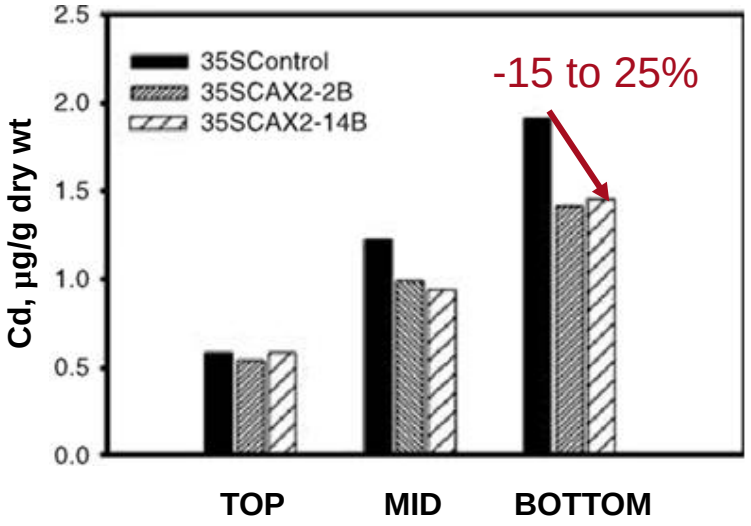
GMO attempts to reduce cadmium in tobacco leaf

- Tobacco plants expressing a human metallothionein gene.



Dorlhac *et al*. 1998

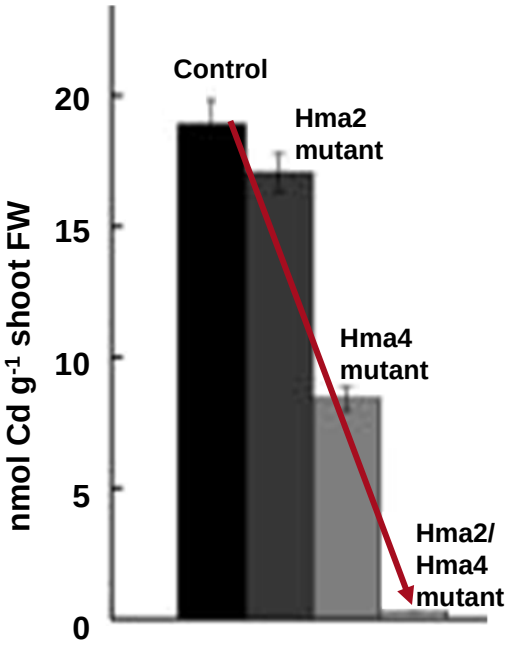
- Tobacco plants expressing *Arabidopsis thaliana* CAX2 gene.



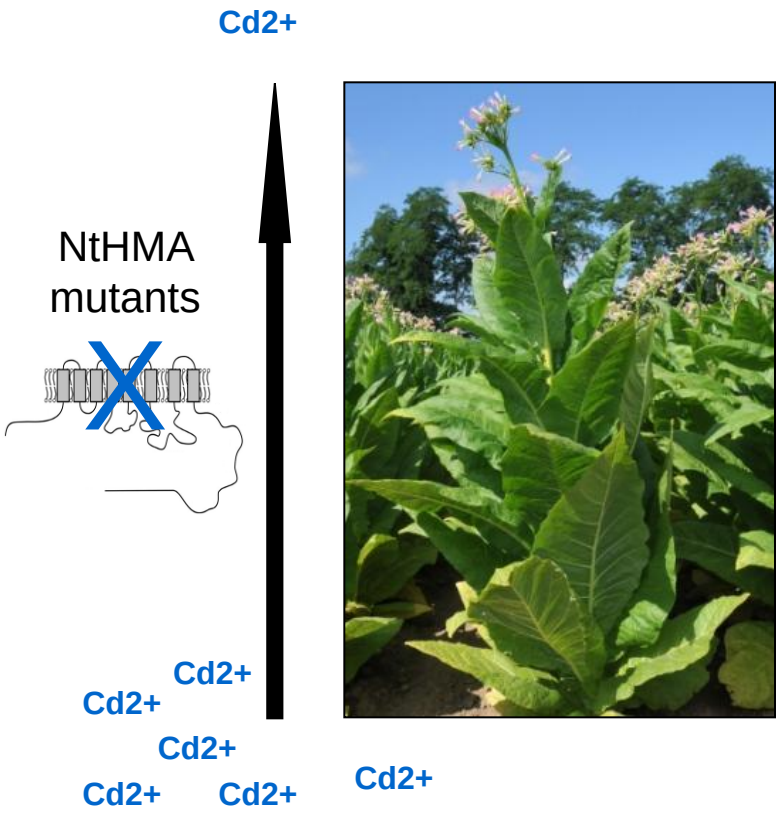
Korenkov *et al*. 2009

Non-GMO strategy: HMA mutants to reduce Cd in tobacco

- HMA ATPases are the major mechanism for root-to-shoot Cd translocation in *Arabidopsis thaliana.*



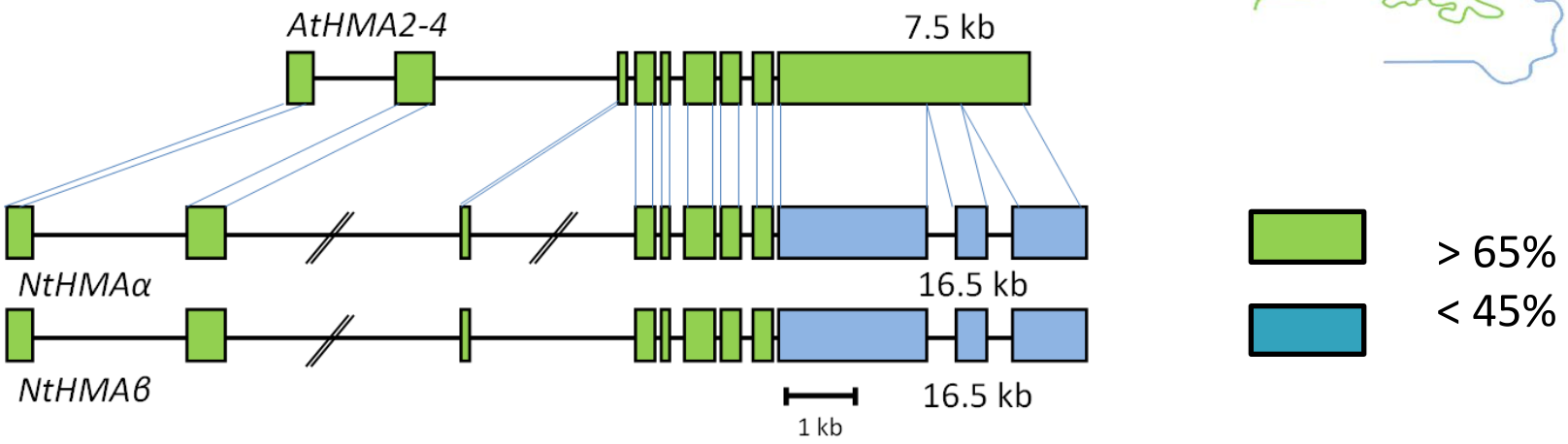
Wong *et al*. 2009



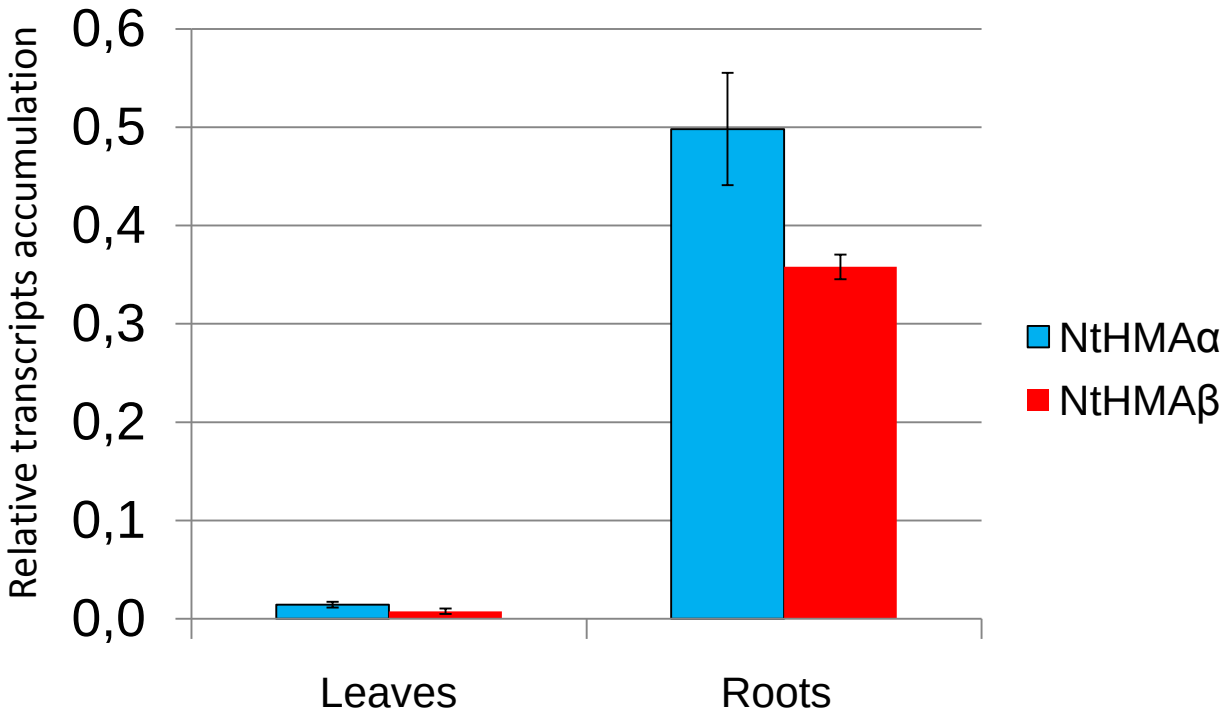
Two HMA genes identified in tobacco

- Blast search with AtHMA4 protein: one contig identified in tobacco, corresponding to exons 4 to 8 in *A. thaliana.*
- Cloning and sequencing on *N. tabacum* and its ancestors.
  - *NtHMA*α from *N. sylvestris*
  - *NtHMA*β from *N. tomentosiformis*
- BAC library (CNRGV-Toulouse) to get the full length sequences.

Identity between *A. thaliana* and *N. tabacum*

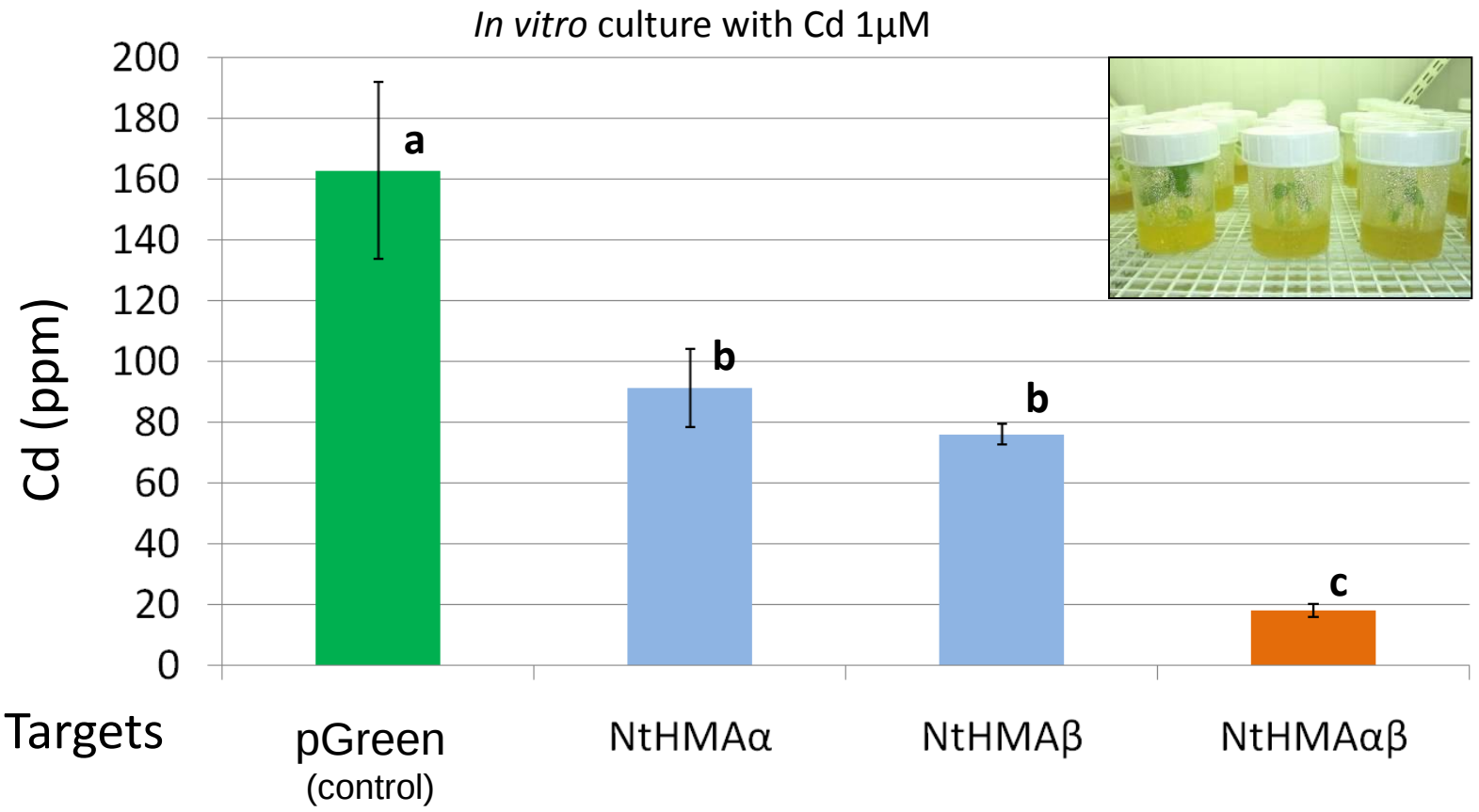


HMA genes expression in tobacco



- HMA genes are preferentially expressed in roots.

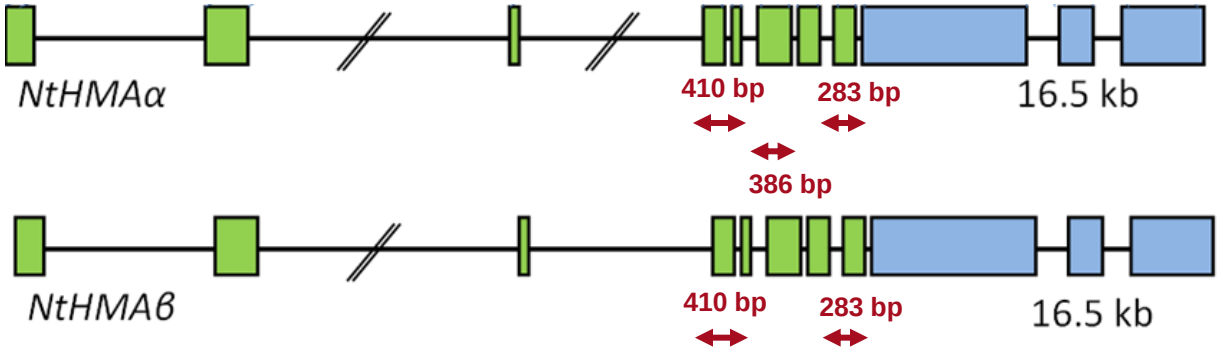
Cadmium in upper parts of miRNA lines

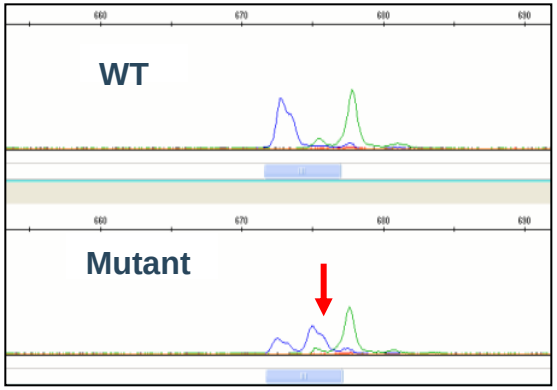


- HMA silencing of both genes induces a dramatic decrease of cadmium content in upper parts.

Identification of NtHMA mutants

Five different targets for mutation screening in both genes:



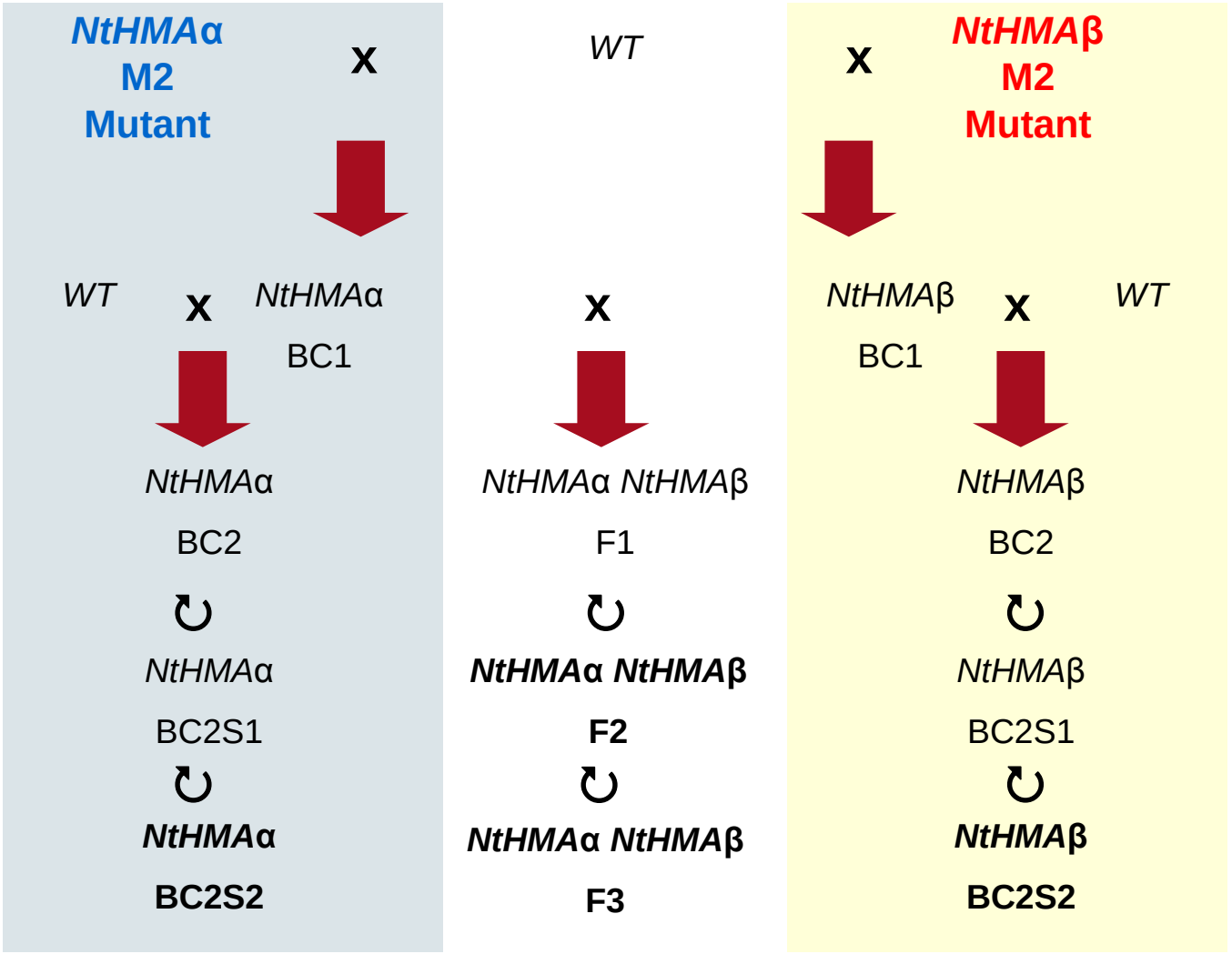


CE-SSCP profile obtained on ABI3130 (Applied Biosystems)

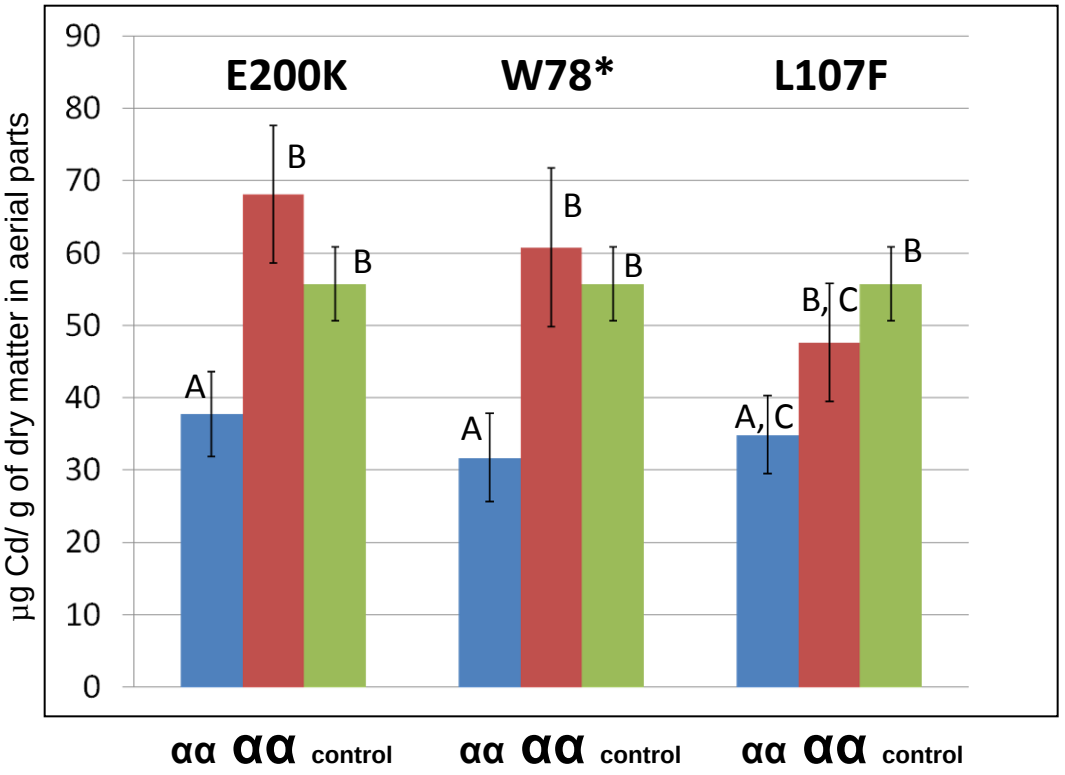
	Total	Silent	Missens	Nonsens	Intron	Splicing
*NtHMA*α	42	11	28	1	1	1
*NtHMA*β	29	5	21	1	2	0

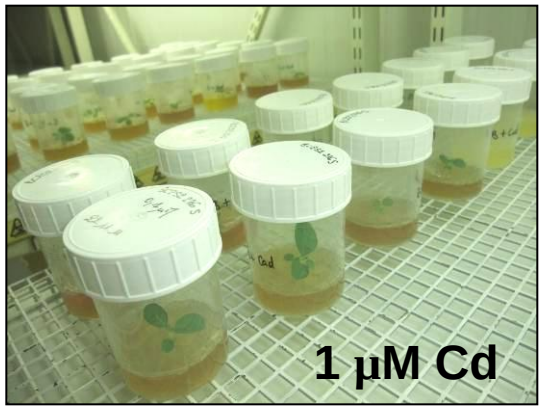
Mutations obtained in both genes

Cleaning mutants by backcrosses (BC) and pyramiding mutations in F2-F3 generation by crosses



In vitro assessment of cadmium transport in *NtHMAα* BC2S2 mutants



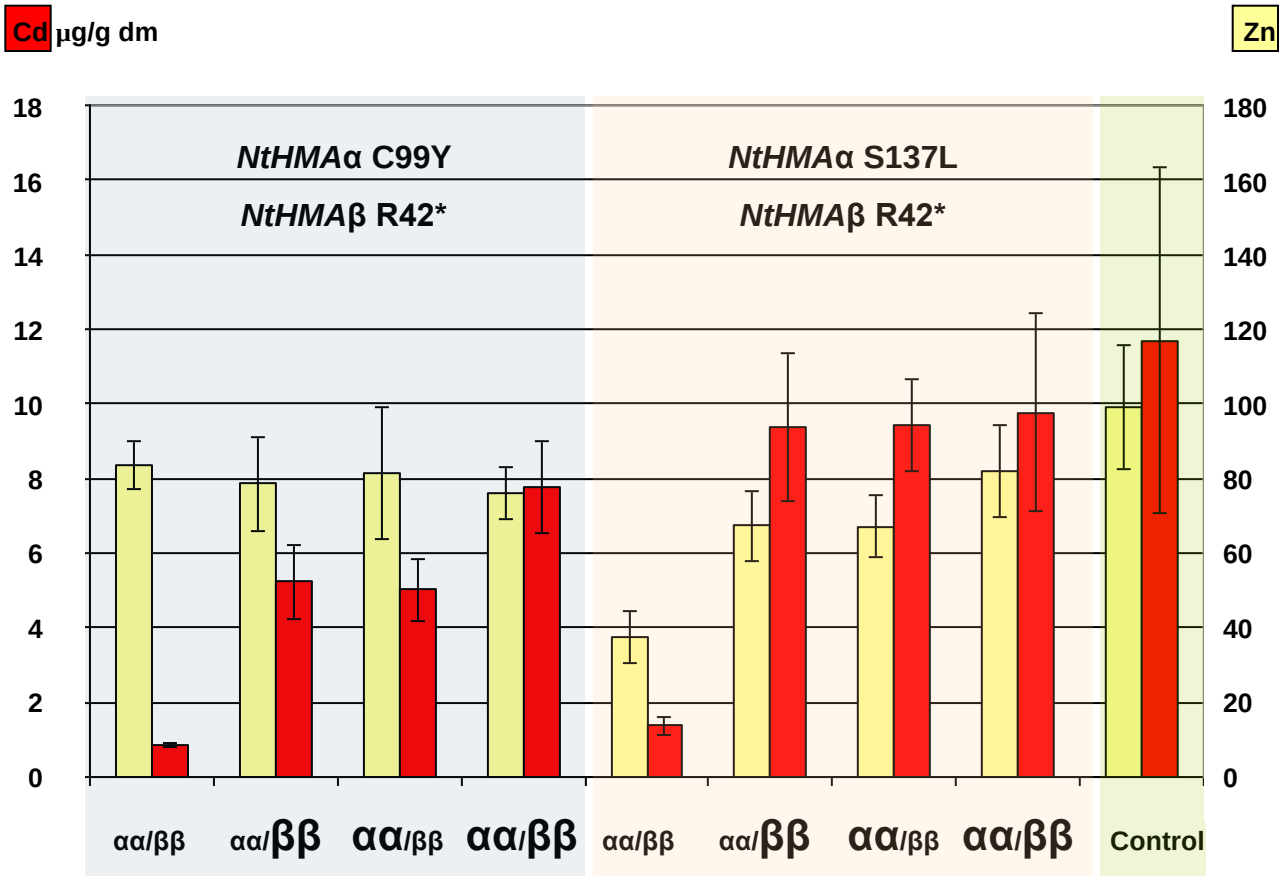


α = mutant in *NtHMAα*

α = wild type in *NtHMAα*

- Significant impact on Cd for E200K and W78*.
- No significant impact on Zn, Fe, Pb (not shown).

« Hydroponic » assessment of cadmium transport in F2 double mutants



α = mutant in *NtHMA*α

α = wild type in *NtHMA*α

β = mutant in *NtHMA*β

β = wild type in *NtHMA*β

- Significant impact on Cd for both crosses
- Significant impact on Zn for S137L x R42* family

Potential limitations with double HMA mutants

- Zinc is strongly affected in miRNA NtHMAα NtHMAβ plants
  - Impact on development, growth and fertility

- This phenotype is also observed in some F2 double mutants
  - Example of F2 (*NtHMA*α W78* *NtHMA*β R42*)

Expected segregation with 2 independant genes in 354 plants from a F2 population :

Genotype	αα/ββ	αα/ββ	αα/ββ	αα/ββ	αα/ββ	αα/ββ	αα/ββ	αα/ββ	αα/ββ
Expected ratio	1/16	1/16	1/16	1/16	2/16	2/16	2/16	2/16	4/16
Expected number	22	22	22	22	44	44	44	44	88
Observed number	6	25	20	17	61	41	42	45	97

P=0.0078, <0.01

A. thaliana hma2hma4

Hussain *et al.* 2004

Conclusion and perspectives

- **It is possible to decrease cadmium content in tobacco leaf with *NtHMA* mutants**.
- Double mutants show a dramatic reduction of cadmium in leaves.
- Severe mutations lead to an impact on zinc, with consequences on morphology and fertility.

- The best combination of mutations in *NtHMAα* and *NtHMAβ* must be defined to obtain the strongest Cd decrease in aerial parts, without affecting plant growth.

- F3 populations are now evaluated in area suspected to be cadmium-contaminated.

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