



**SOL2012 The 9th Solanaceae Conference**  
**August 26-30, 2012**  
**University of Neuchâtel, Switzerland**

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

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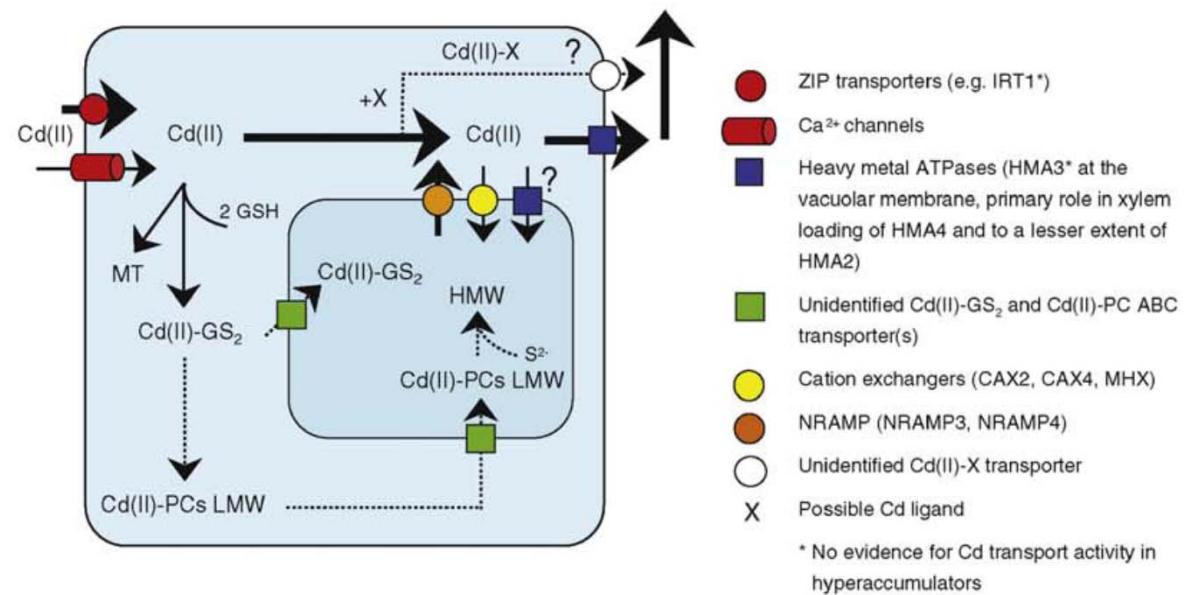
# Metals in plants



- Essential: iron (Fe), zinc (Zn), manganese (Mn), copper (Cu) are used as co-factors in enzymes
- Non-essential: cadmium (Cd), lead (Pb), mercury (Hg)...can enter the plant using the same transporters
- Different mechanisms of detoxification exists:
  - Transport to major storage organs/tissues
  - Sub-cellular compartmentalization
  - Chelation
  - Efflux from the plant

Verbruggen et al., 2009  
Current Opinion in Plant Biology

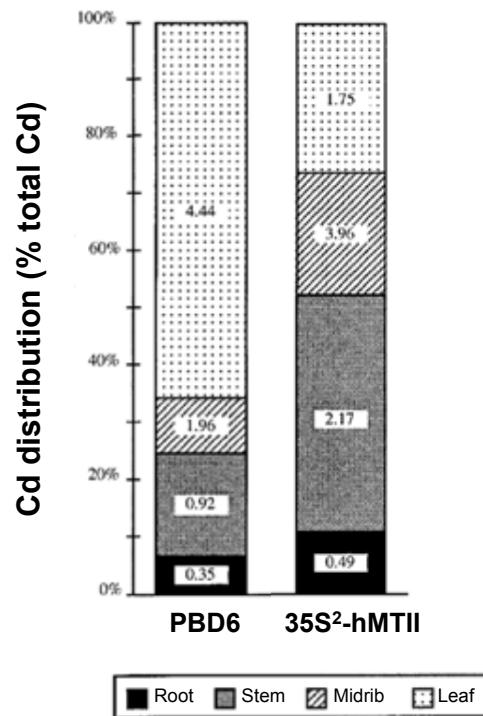
Cadmium in hyperaccumulators



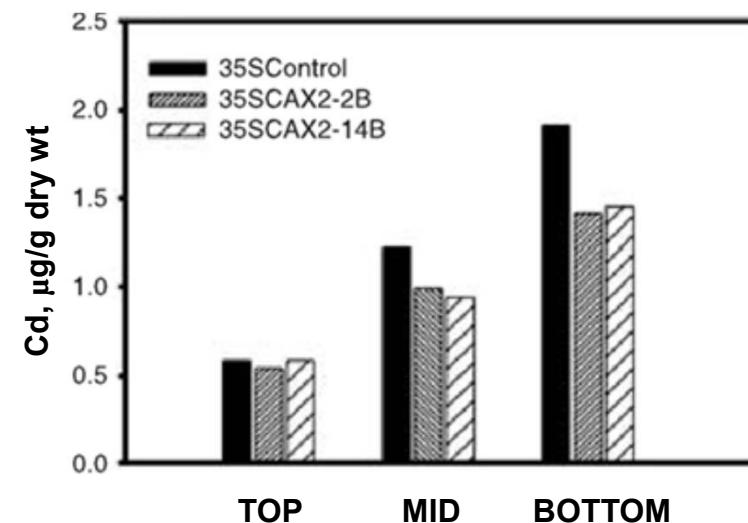
## GMO attempts to reduce cadmium in tobacco leaf



- Dorlhac et al., 1998: plants expressing a human metallothionein gene
- Korenkov et al., 2009: plants expressing AtCAX2 gene



70%



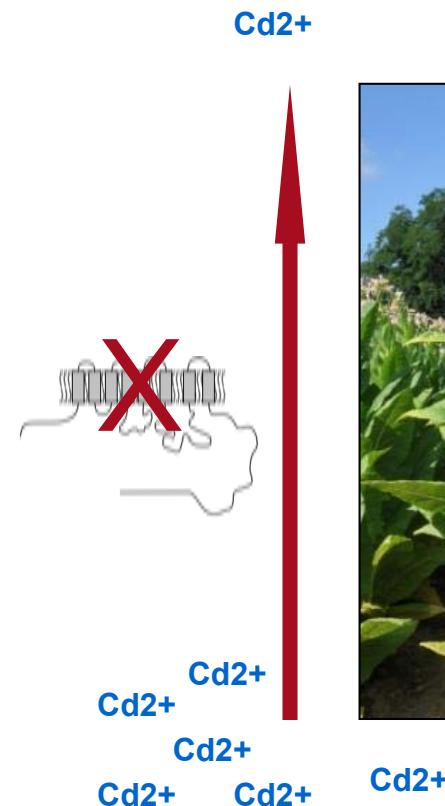
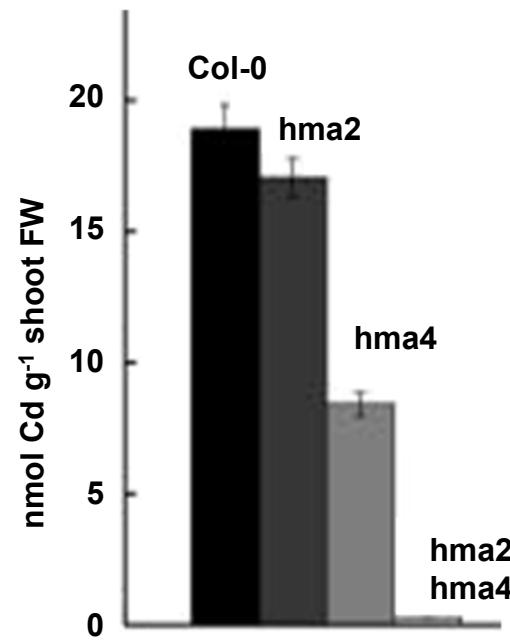
15-25%

## Non-GMO approach: HMA mutants to reduce Cd in tobacco



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

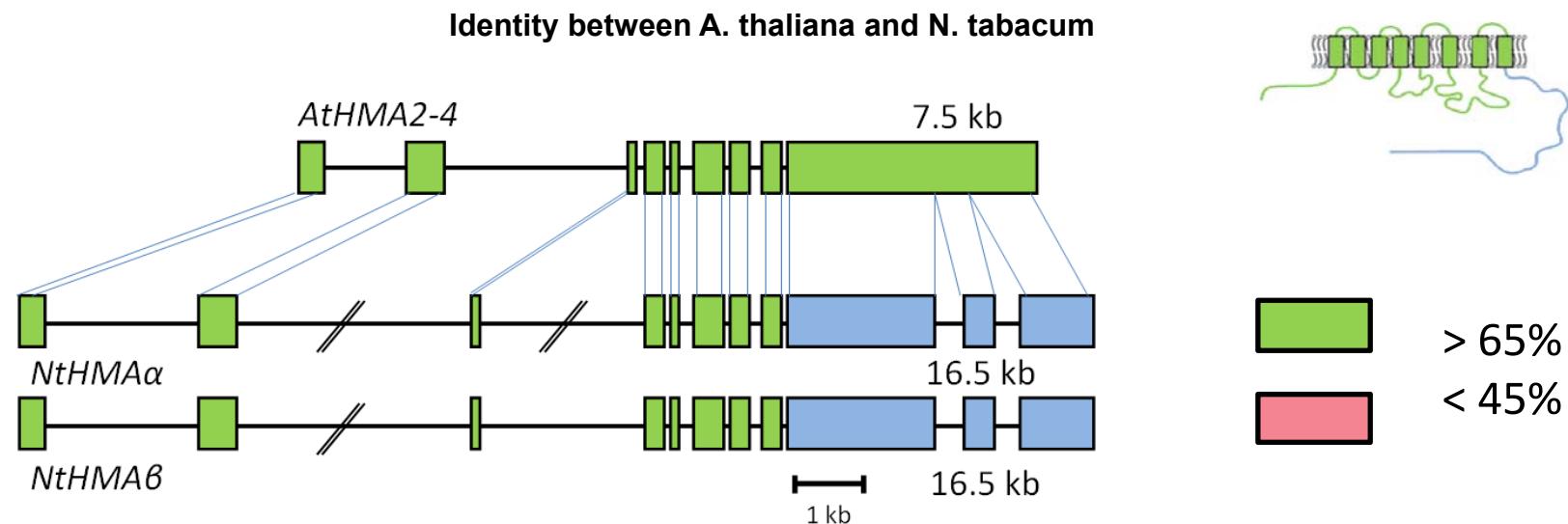
Wong et al., 2009



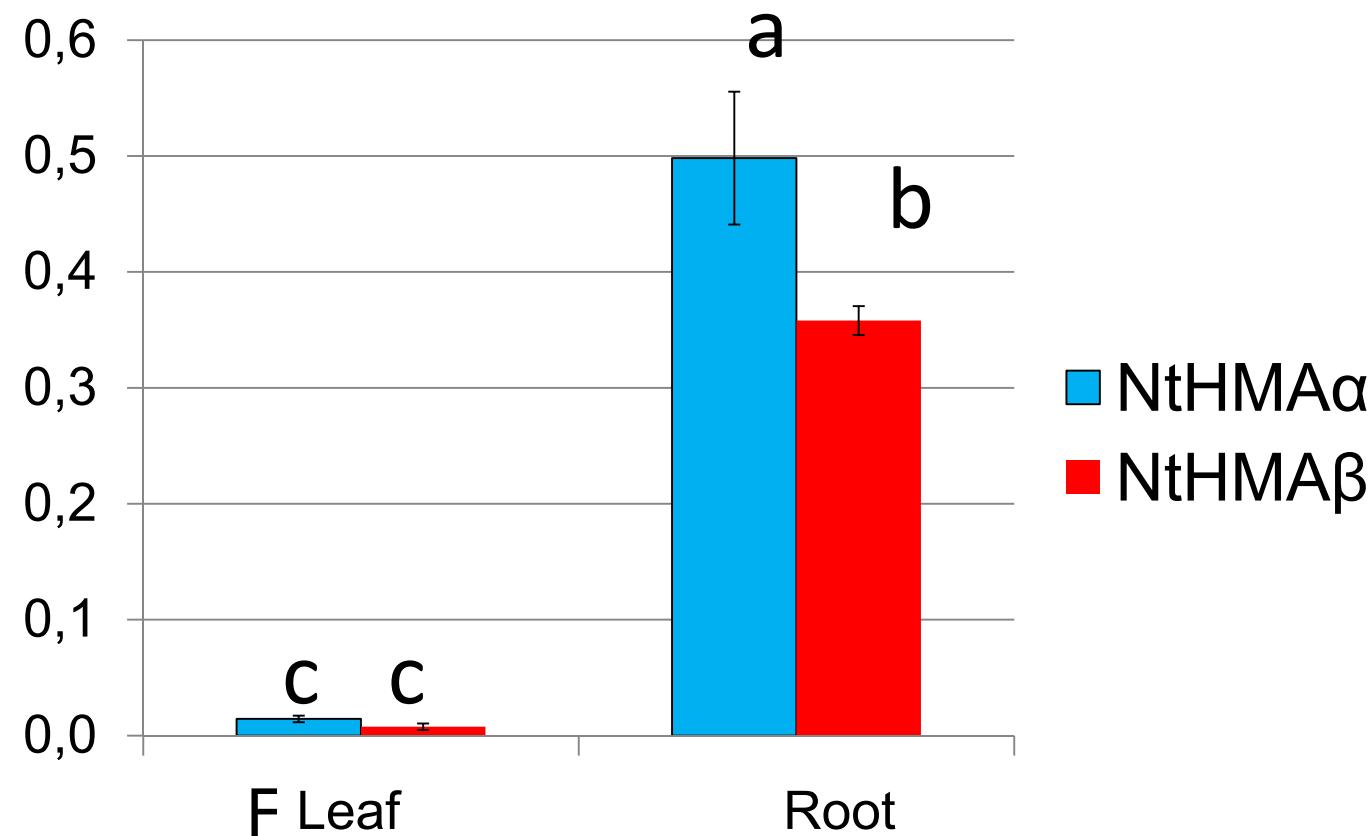
## Two HMA genes identified in tobacco



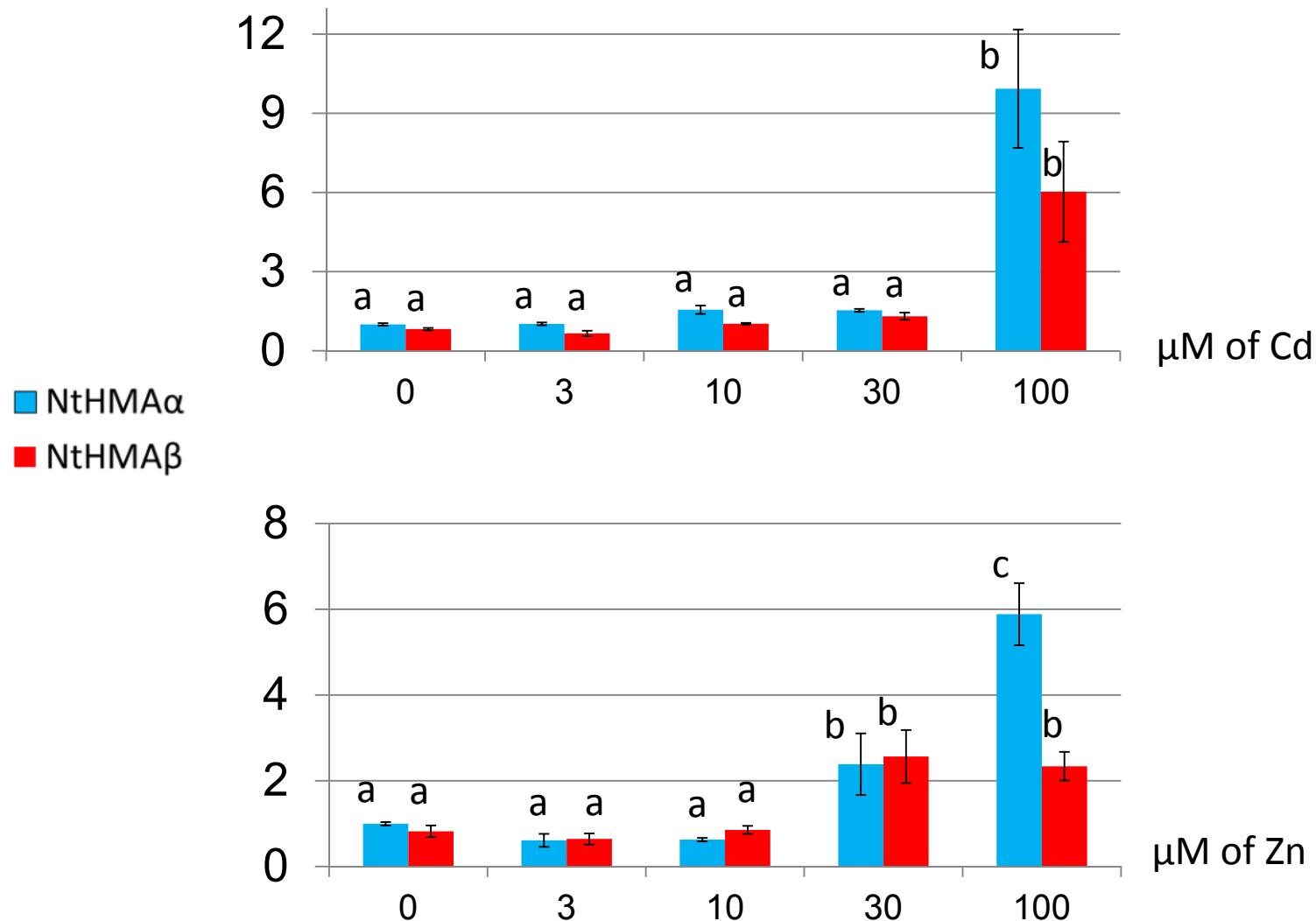
- Blast search with AtHMA4 protein: one contig identified in tobacco, corresponding to exons 4 to 8 in *A. thaliana*
- Cloning and sequencing on *N. tabacum* and its ancestors
  - *NtHMAα* from *N. sylvestris*
  - *NtHMAβ* from *N. tomentosiformis*
- BAC library (CNRGV-Toulouse) to get the full length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



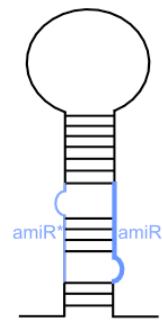
Transcripts accumulation



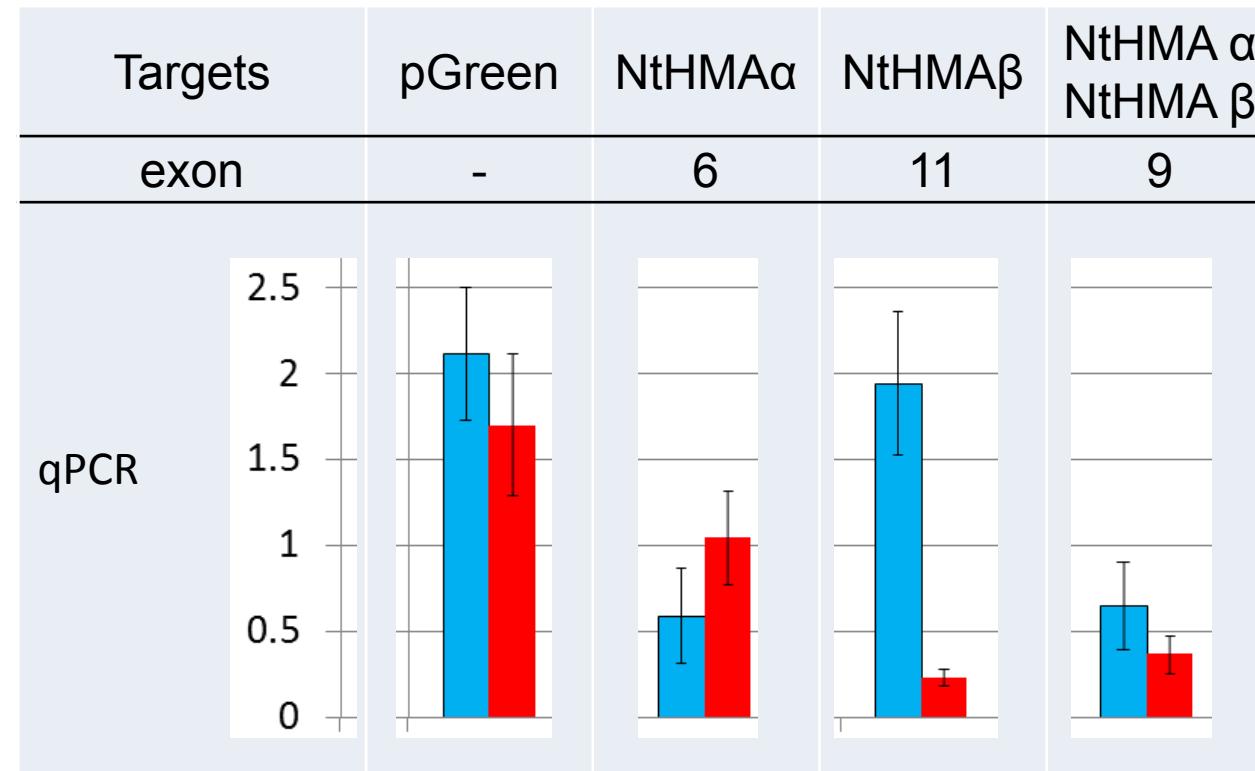
## Zinc and Cadmium effects on transcripts accumulation in roots



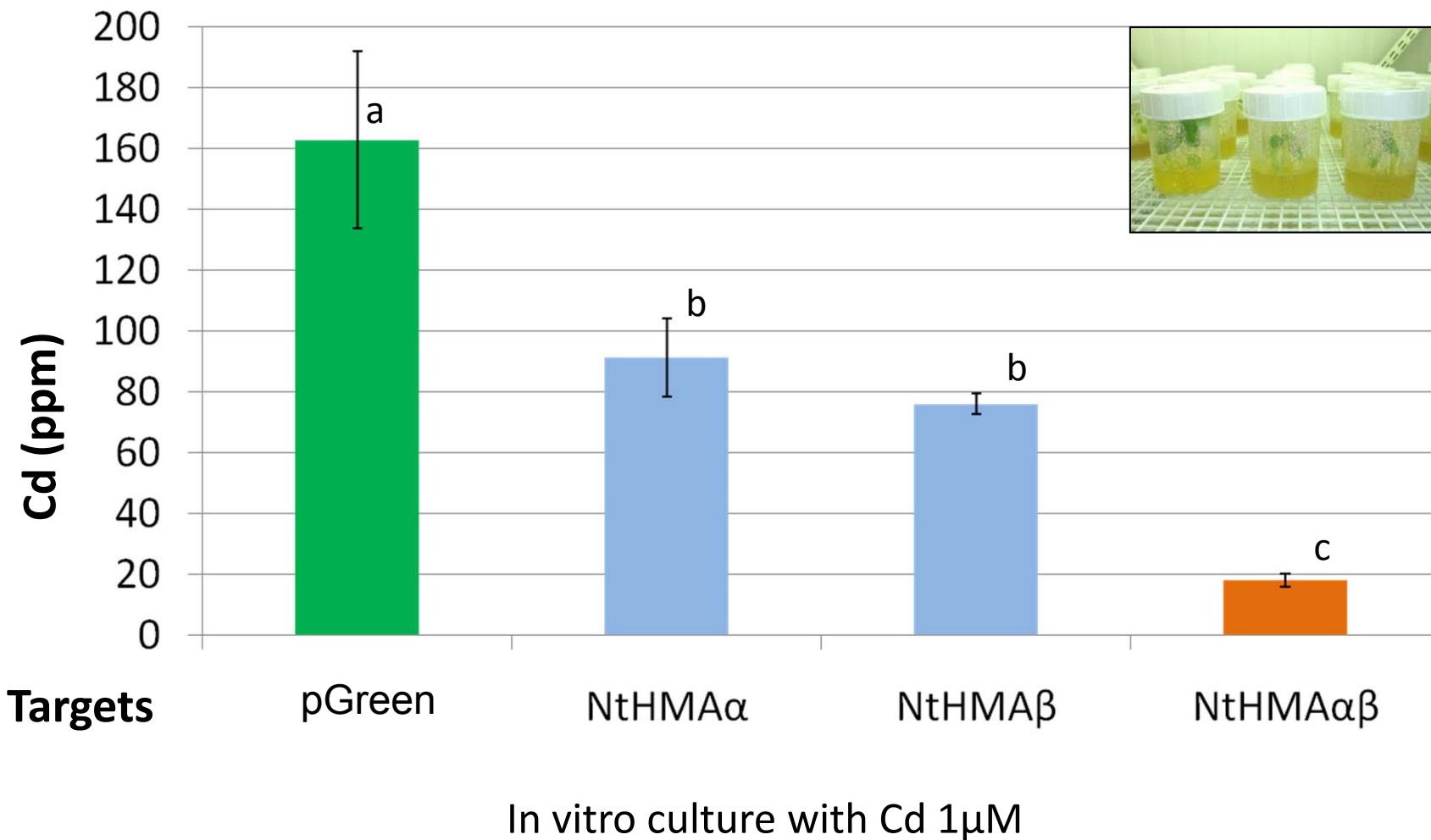
## HMA genes silencing by miRNA



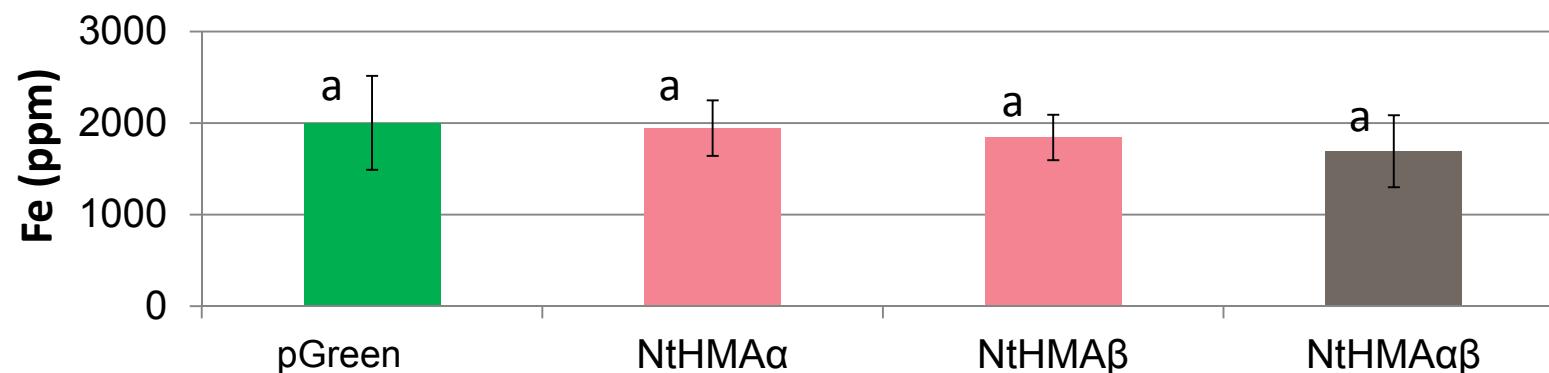
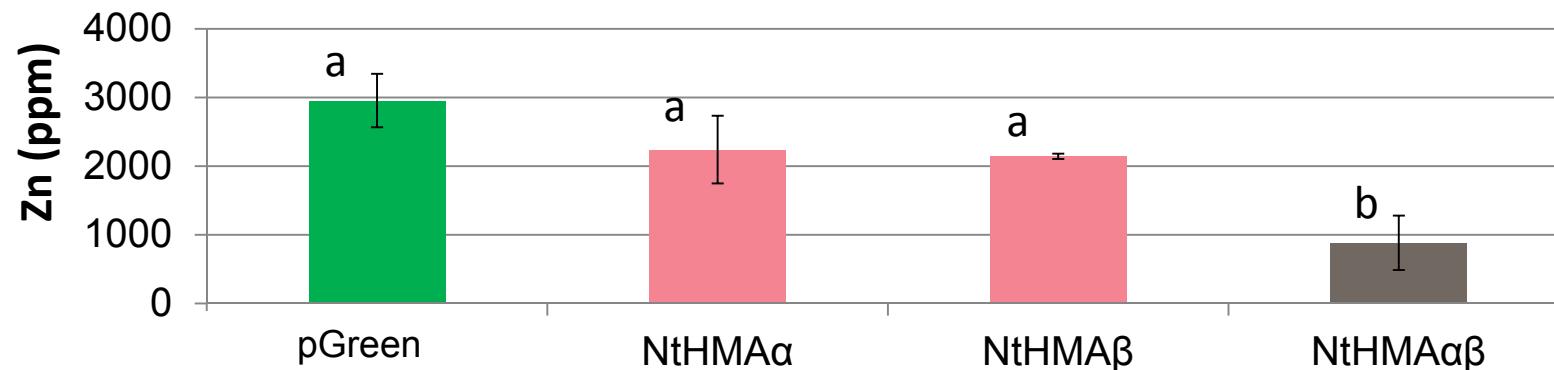
■ Alpha  
■ Beta



## Cadmium in aerial parts of miRNA lines

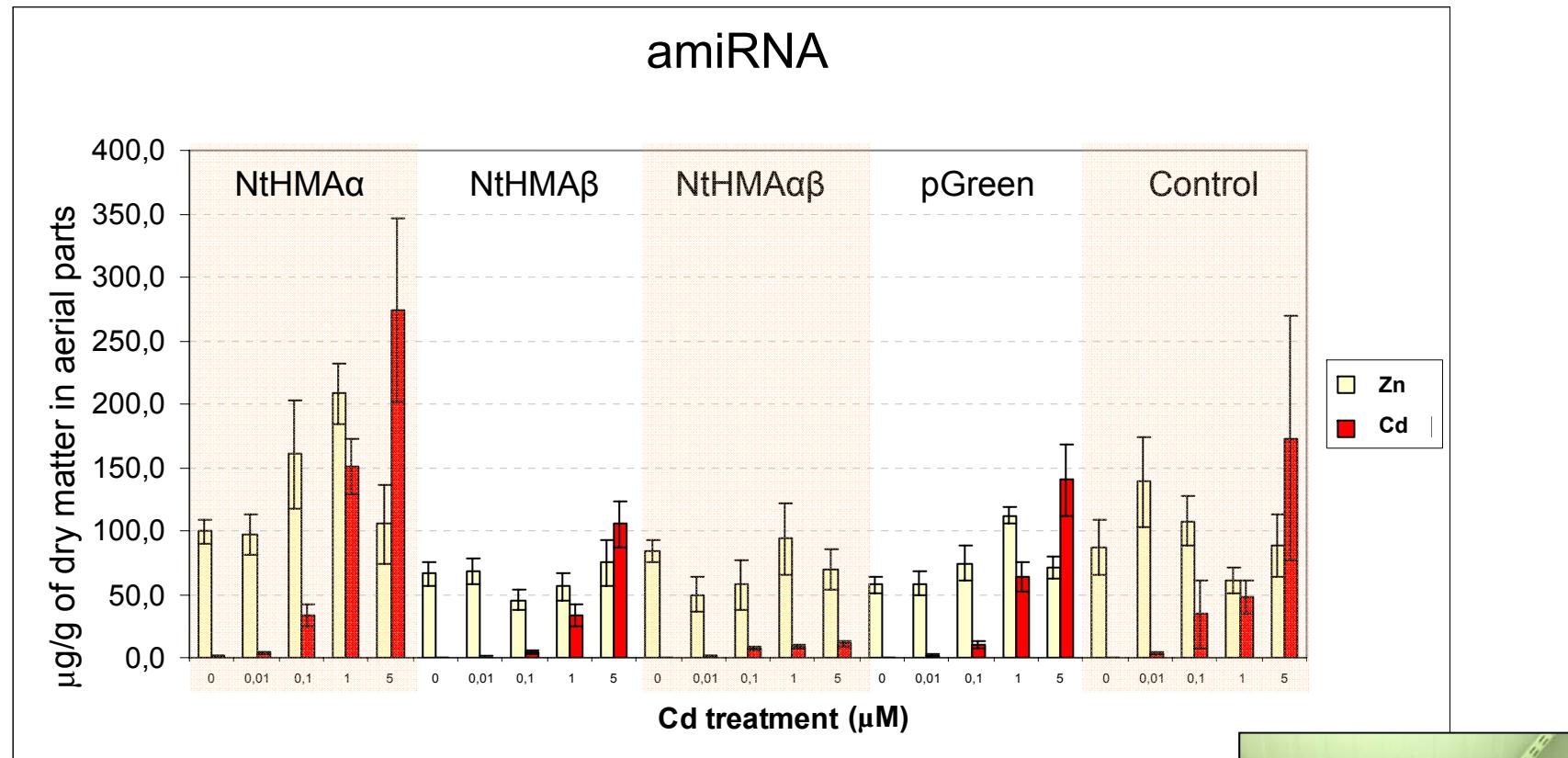


## Zinc and Iron in aerial parts of amiRNA lines

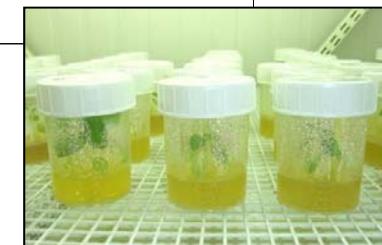


- Best results with both copies silenced
- Strong impact on Zinc with both copies

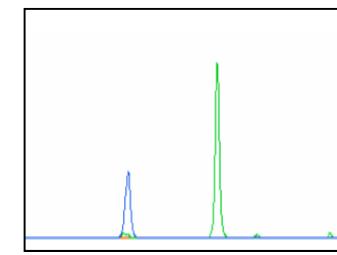
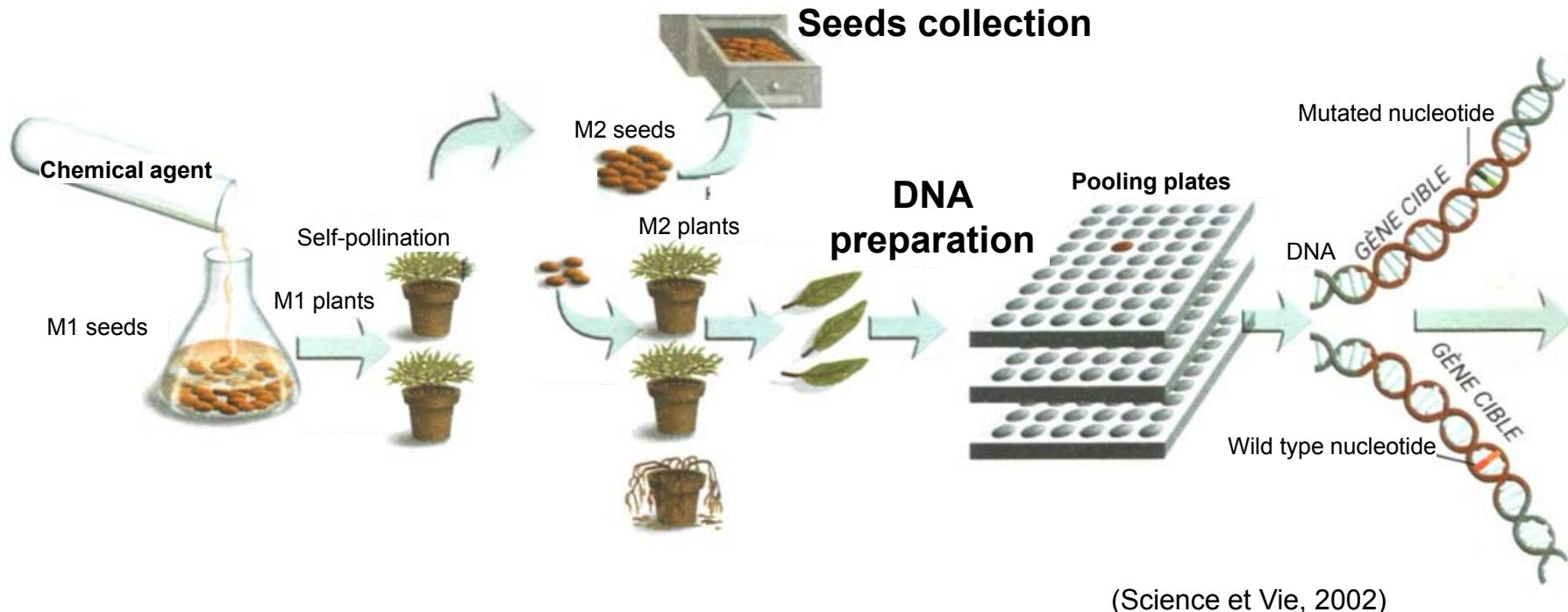
# In vitro assessment of Cd and Zn in aerial parts with differents Cd treatment



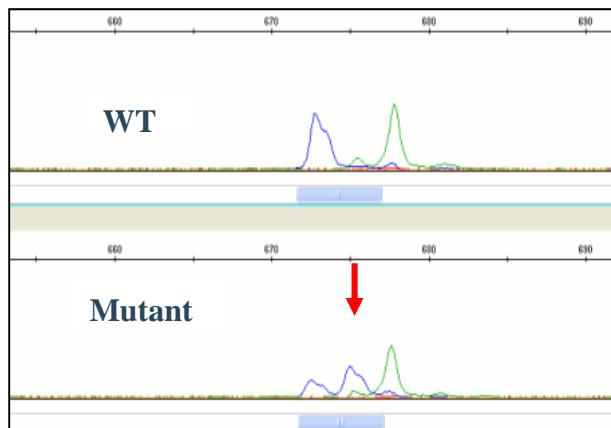
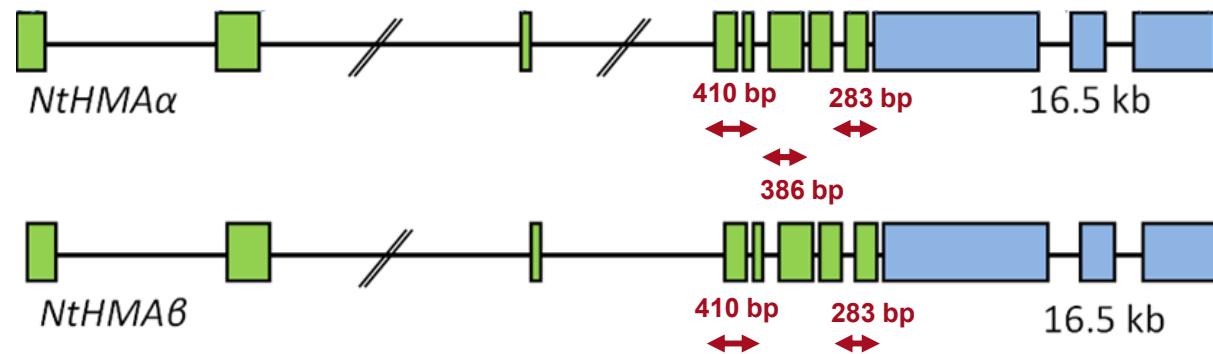
- Best results with both copies silenced
- Strong impact on Zinc with both copies



# Identification of NtHMA mutants

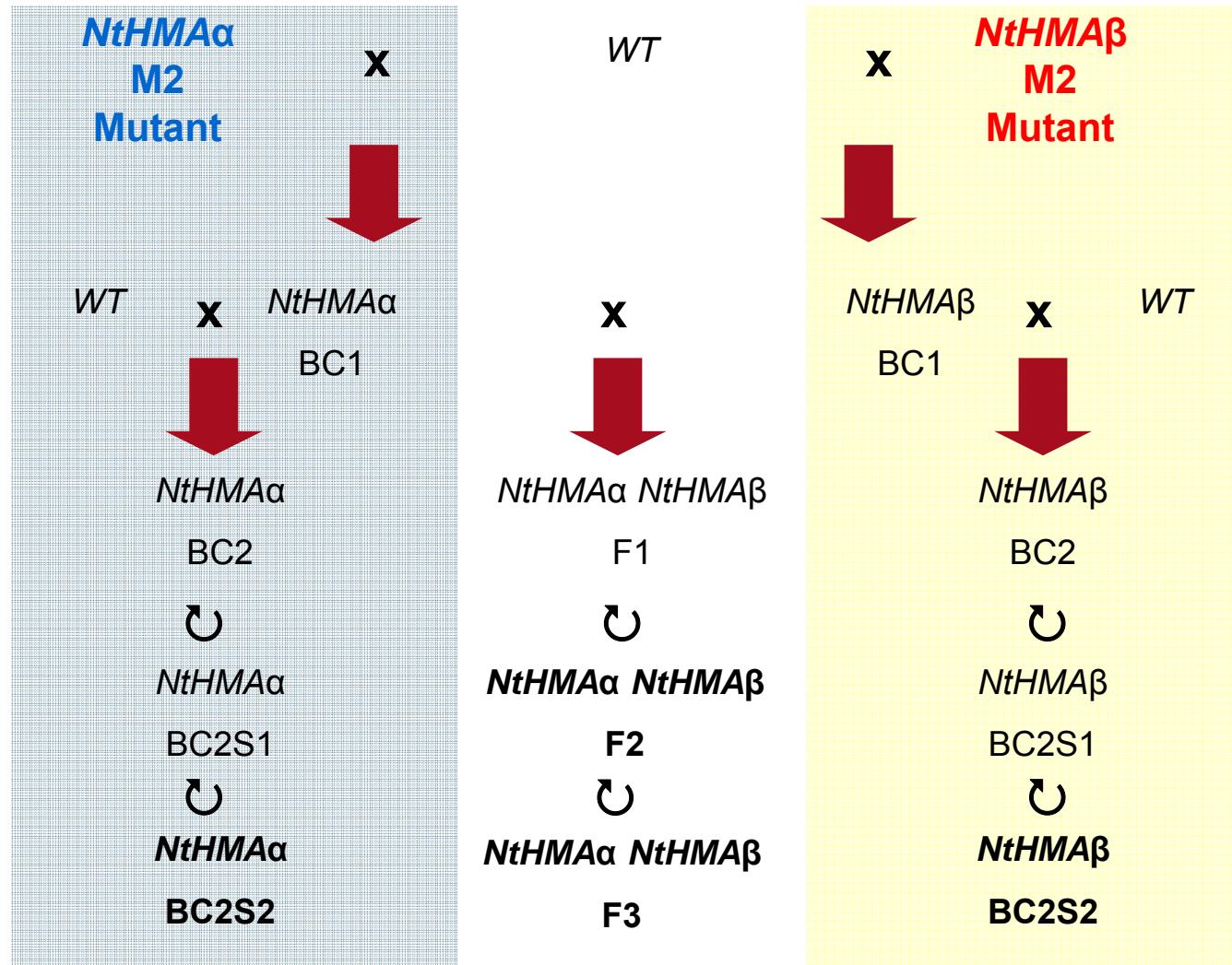


## Mutation screening on 4000 M2 families

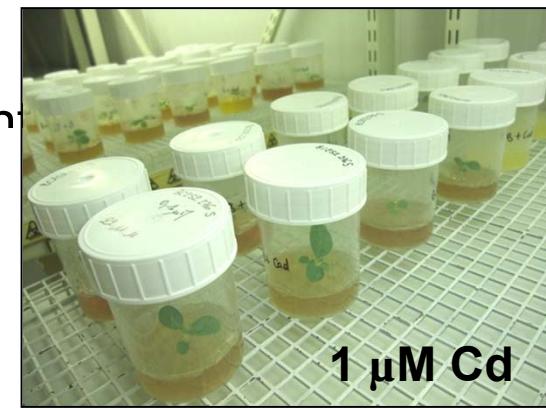
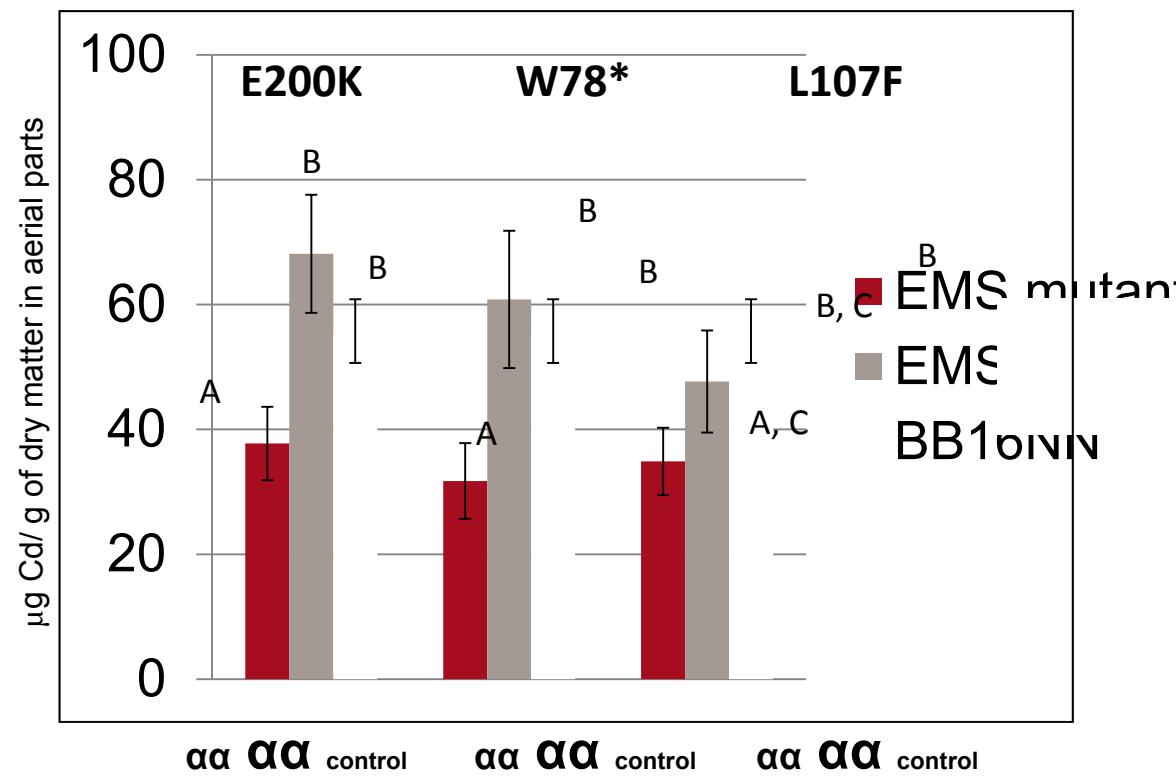


	Total	Silent	Missens	Nonsense	Intron	Splicing
<i>NtHMAα</i>	<b>42</b>	11	28	<b>1</b>	1	1
<i>NtHMAβ</i>	<b>29</b>	5	21	<b>1</b>	2	0

## Cleaning and pyramiding mutants



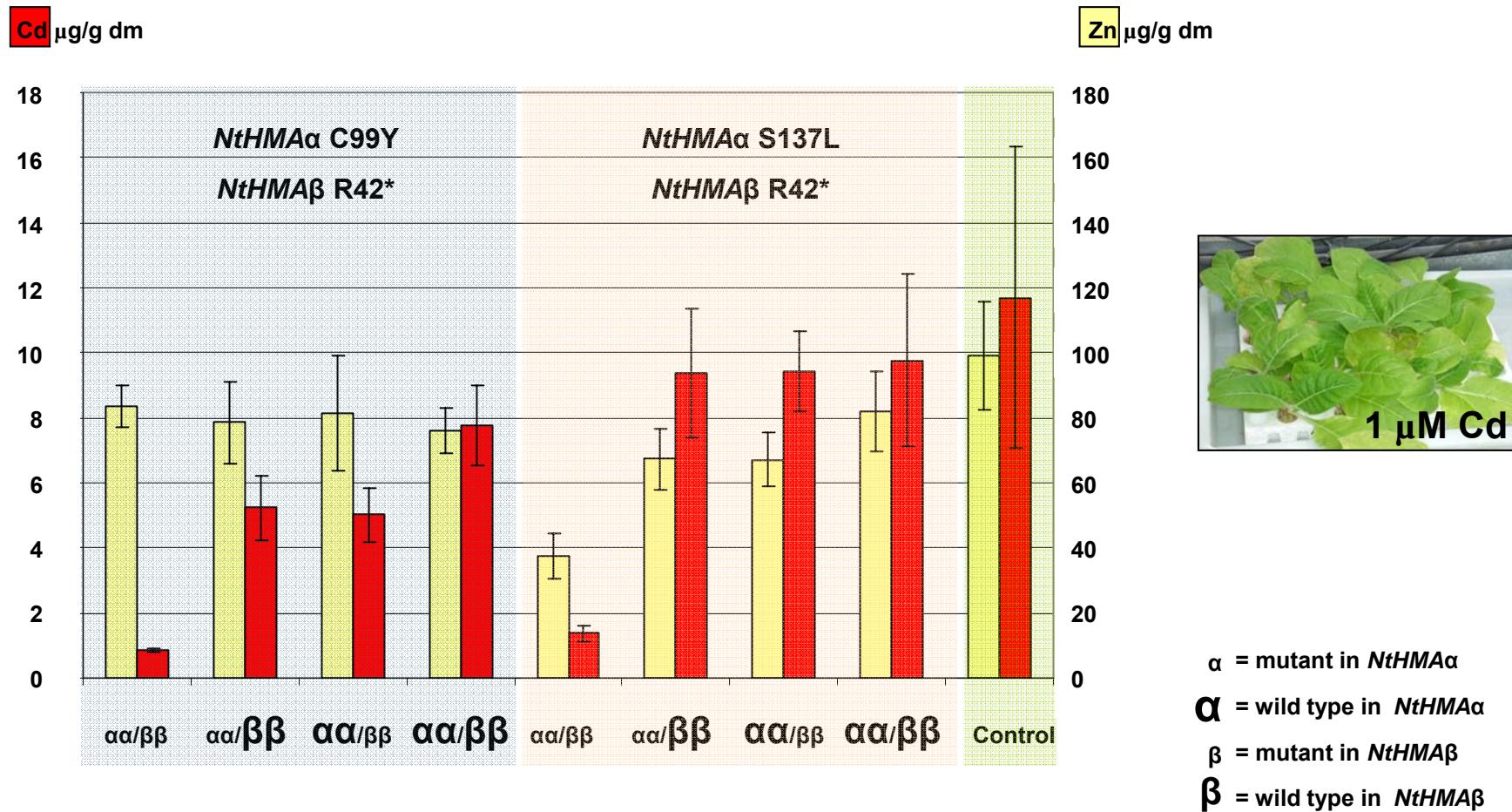
# In vitro assessment of cadmium transport in *NtHMAα* BC2S2 mutants



$\alpha$  = mutant in *NtHMAα*  
 $\alpha\alpha$  = 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



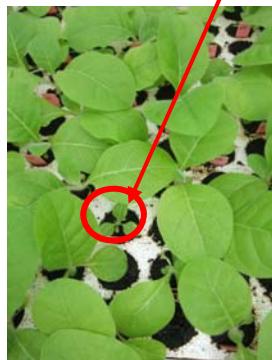
## Potential limitations with double HMA mutants



- Zinc is strongly affected in miRNA NtHMA $\alpha$  NtHMA $\beta$  plants :
  - Impact on development, growth and fertility
- This phenotype is also observed in some F2 double mutants:
  - Example of F2 (*NtHMA $\alpha$  W78\* NtHMA $\beta$  R42\**)

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

ratio	1/16	1/16	1/16	1/16	2/16	2/16	2/16	2/16	4/16
Genotype	$\alpha\alpha/\beta\beta$								
Expected	22	22	22	22	44	44	44	44	88
Observed	6	25	20	17	61	41	42	45	97



P=0.0078, <0.01



*A. thaliana*  
*hma2hma4*

Hussein 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 aerial parts.
  - Severe mutations lead to an impact on zinc, with consequences on morphology and fertility.
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- The best combination of mutations in *NtHMA $\alpha$*  and *NtHMA $\beta$*  must be defined to obtain the strongest Cd decrease in aerial parts, without affecting plant growth.
  - In vitro and hydroponic results must be confirmed into the field.
    - Some F3 populations are currently evaluated in the field in Bergerac



## The team



Victor Hermand (RNAi experiments and  
functional analysis of NtHMA)

Pierre Berthomieu  
Françoise Gosti

And the team of the Tobacco Institute of Bergerac

François Dorlhac de Borne, Julien Cotuchet, Christophe Decrops,  
Céline Sentenac and the others...





**Thank you for your attention !**

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