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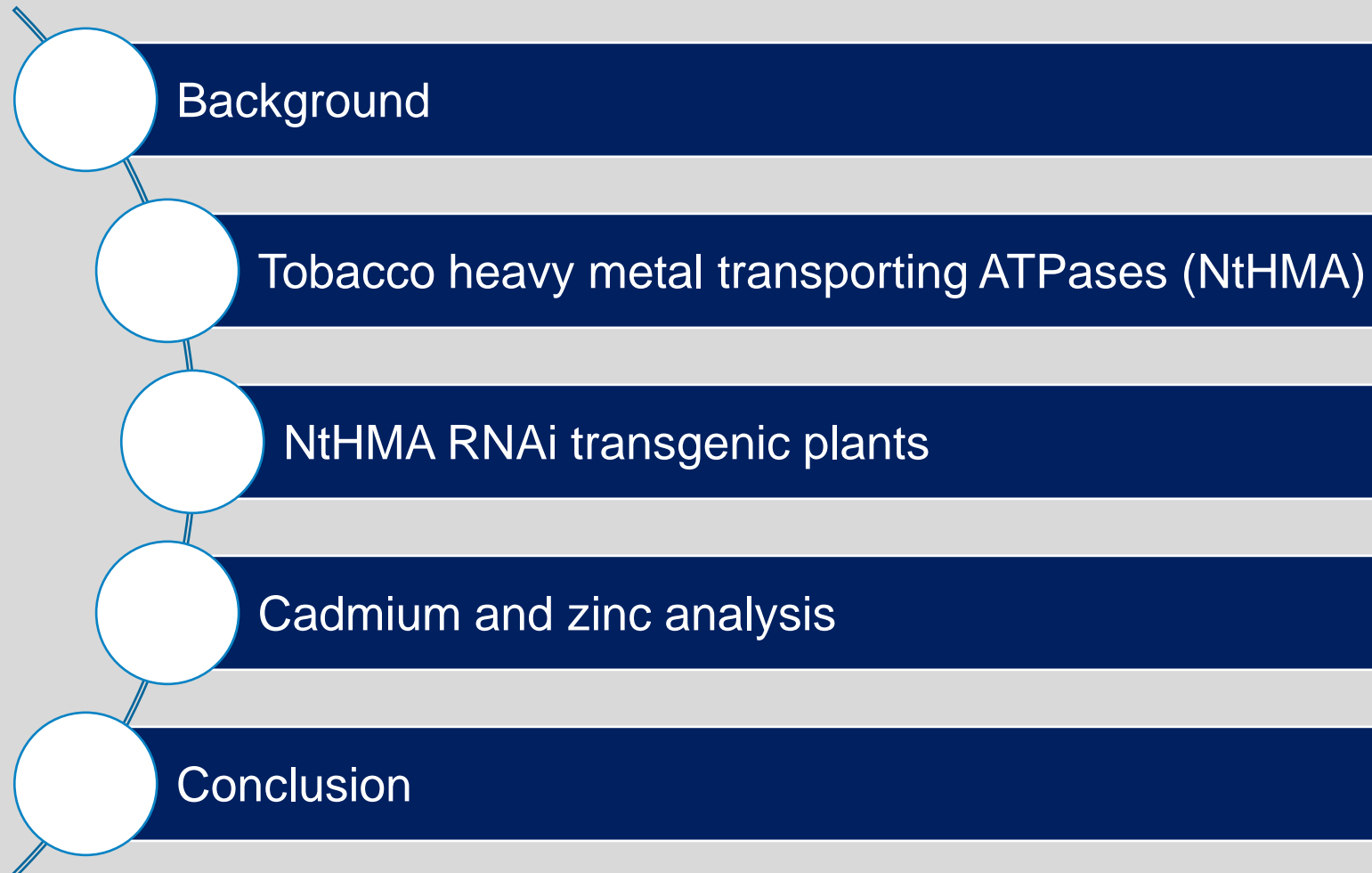
CADMIUM REDUCTION IN TOBACCO LEAVES

[Kudithipudi, C.](#) and [Morris, J.W.](#)

Altria Client Services LLC, Richmond, VA 23219

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BACKGROUND



- Cadmium is a heavy metal, classified as a Class 1 “known human carcinogen” by the International Agency for Research on Cancer (IARC) and included on the Food and Drug Administration (FDA) abbreviated Harmful and Potentially Harmful Constituent (HPHC) list for tobacco and tobacco smoke
- Plants accumulate zinc, an essential element. However, due to lack of specificity of some transport proteins, tobacco plants also accumulate cadmium with most of it being translocated to the leaves
- Specialized membrane transport proteins in the form of channels, carriers, or pumps mediate the movement of heavy metals through membranes
- Heavy metal P-type ATPases (HMAs) are a subgroup of transport proteins, P-type ATPases super family, that contribute to long distance translocation of heavy metals including zinc and cadmium

"Guidance for Industry, Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke Under Section 904(a)(3) of the Federal Food, Drug and Cosmetics Act (3/2012)."

PLANTS' RESPONSE TO CADMIUM STRESS



Immobilization

- Immobilization of cadmium by means of the cell wall and extracellular carbohydrates

Exclusion

- Preventing cadmium ions from entering the cytosol through the action of the plasma membrane

Chelation

- Detoxify nonessential trace metal(-loid)s by the synthesis of specific low-molecular-weight chelators to avoid binding to physiologically important proteins and to facilitate their transport into the vacuoles

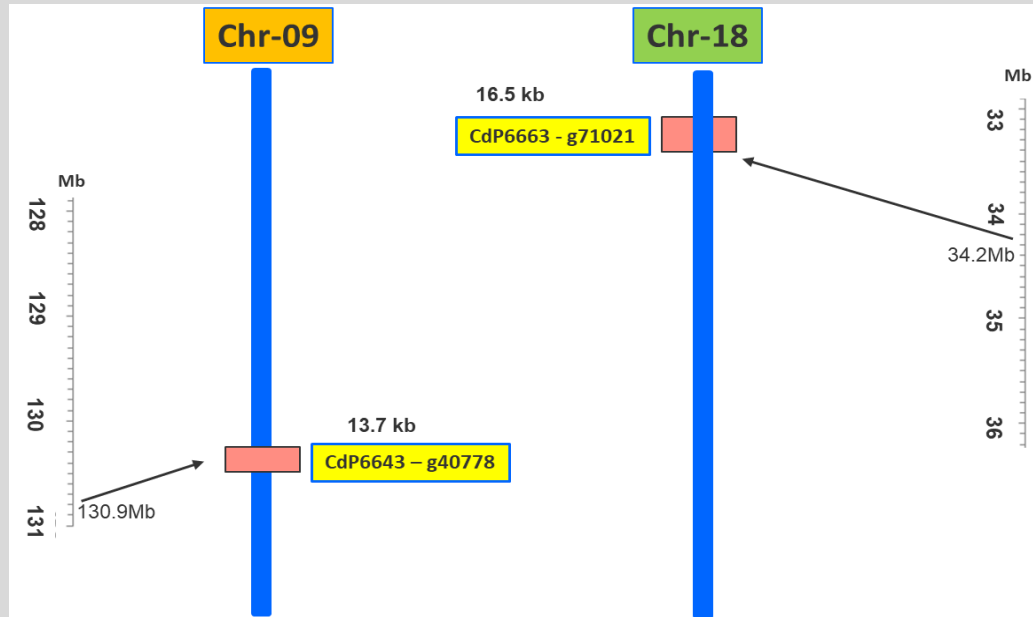
Compartmentalization

- Vacuolar compartmentalization prevents the free circulation of cadmium ions in the cytosol and forces them into a limited area

Transporters

- Short distance transport by Cd^{2+} /proton antiporters like CAX2 and CAX4
- Long distance root to shoot translocation by HMAs

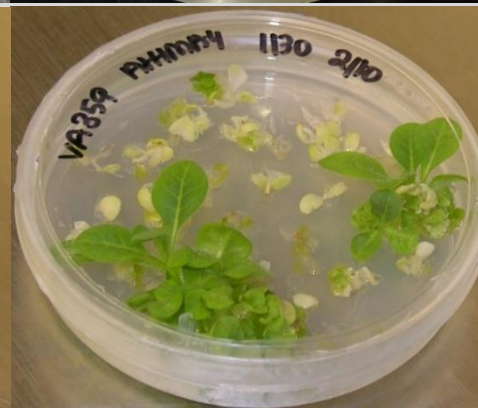
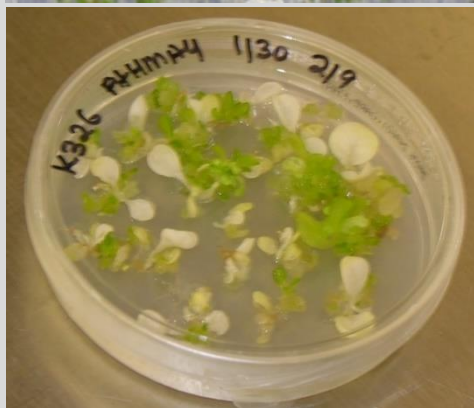
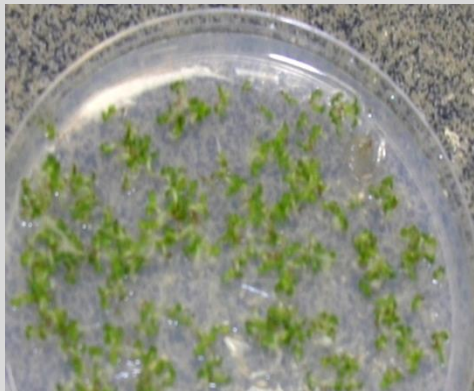
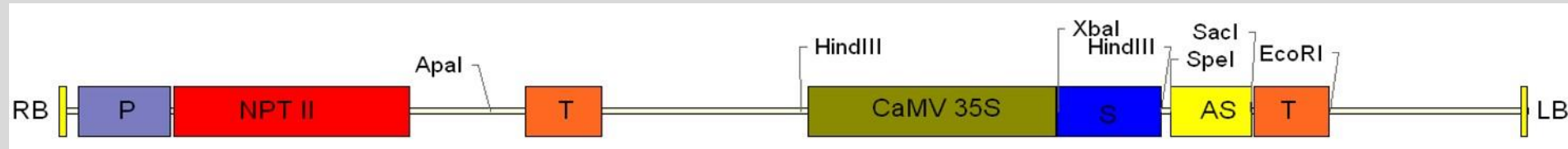
TOBACCO HEAVY METAL TRANSPORTING ATPASES GENE MODEL



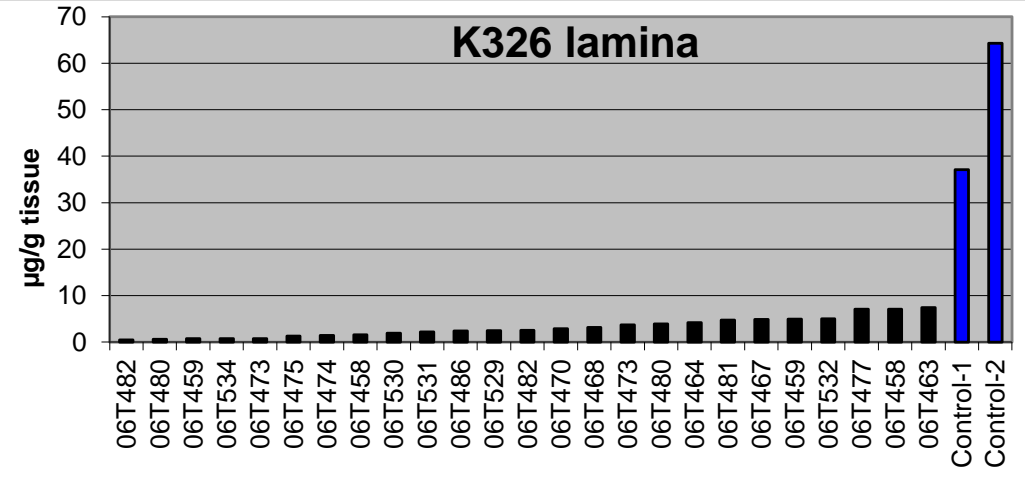
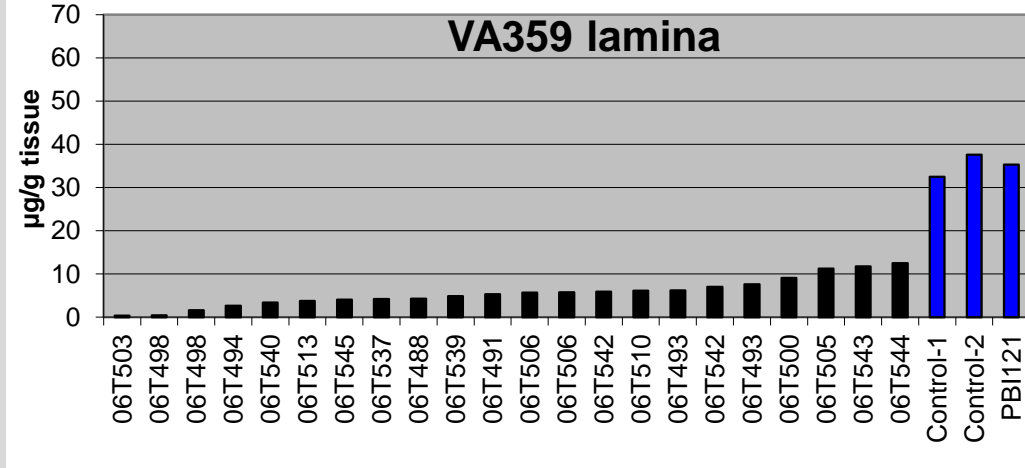
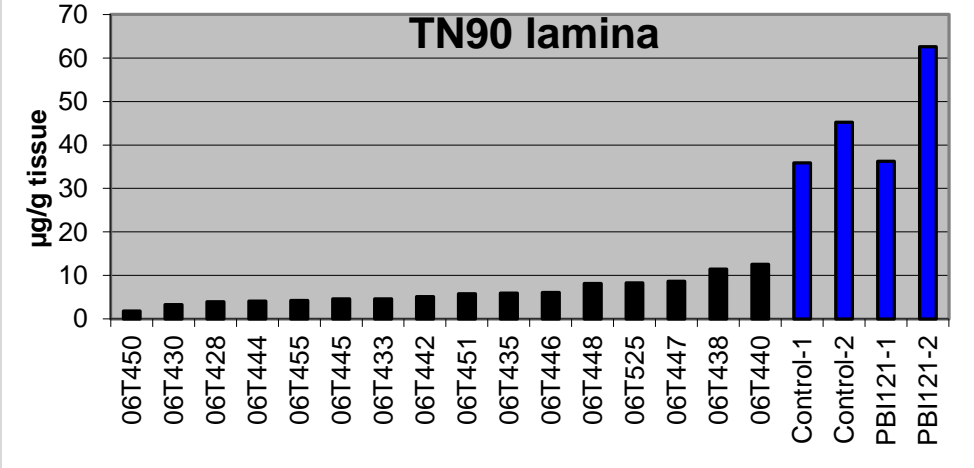
NtHMAs	Homology
Nucleotide	92%
Protein	89%



REGENERATION OF NtHMA RNAi TRANSGENIC TOBACCO PLANTS



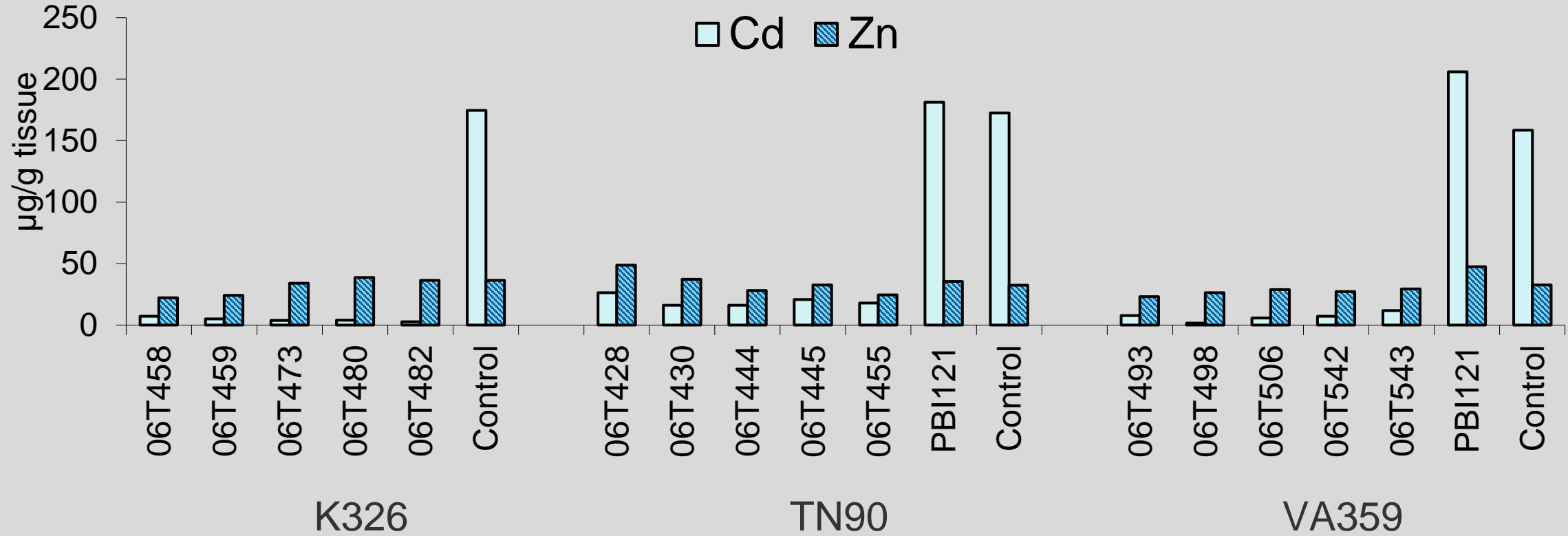
CADMIUM ANALYSIS OF NtHMA RNAi TRANSGENIC LAMINA AFTER 4 WEEKS OF TREATMENT WITH CADMIUM



CADMIUM AND ZINC ANALYSIS IN SELECTED NtHMA RNAi TRANSGENIC LAMINA AFTER 8 WEEKS OF TREATMENT WITH CADMIUM



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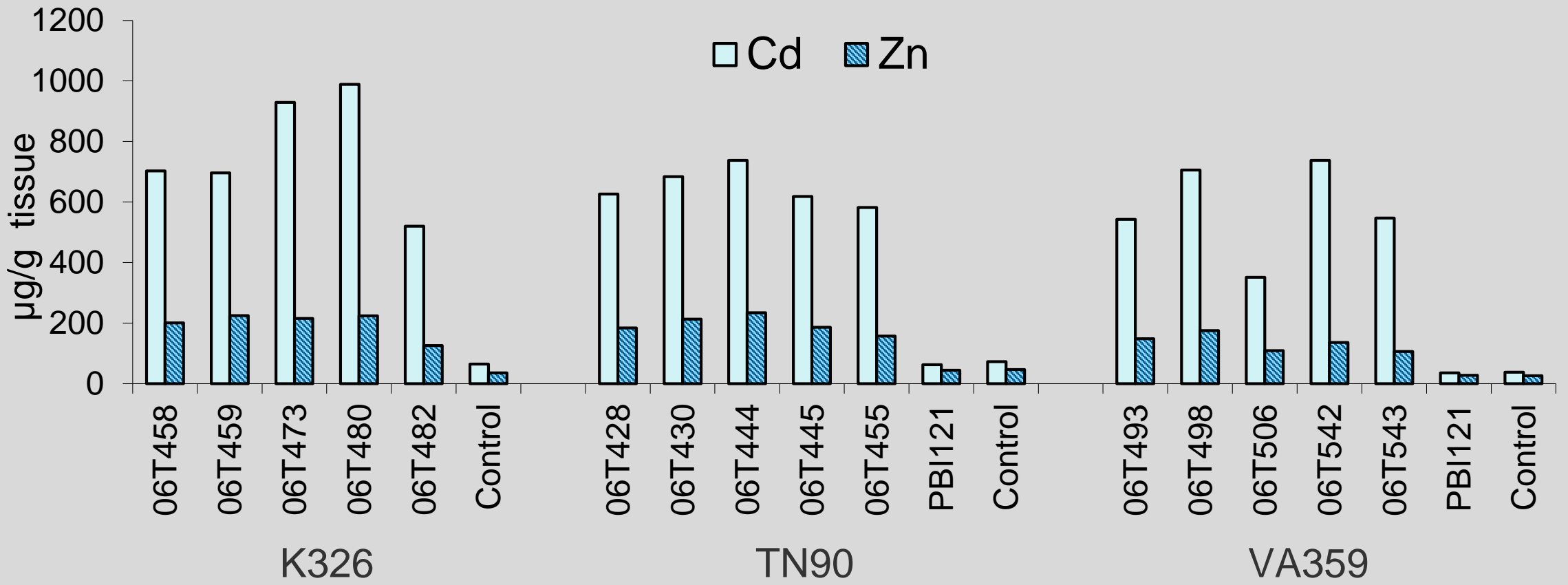


Leaf cadmium reduction varied among selected plants and ranged from 88 % (TN90 06T44) to 91 % (VA359 06T498) without substantially affecting zinc transport

CADMIUM AND ZINC ANALYSIS IN SELECTED NtHMA RNAi TRANSGENIC ROOTS AFTER 8 WEEKS OF TREATMENT WITH CADMIUM

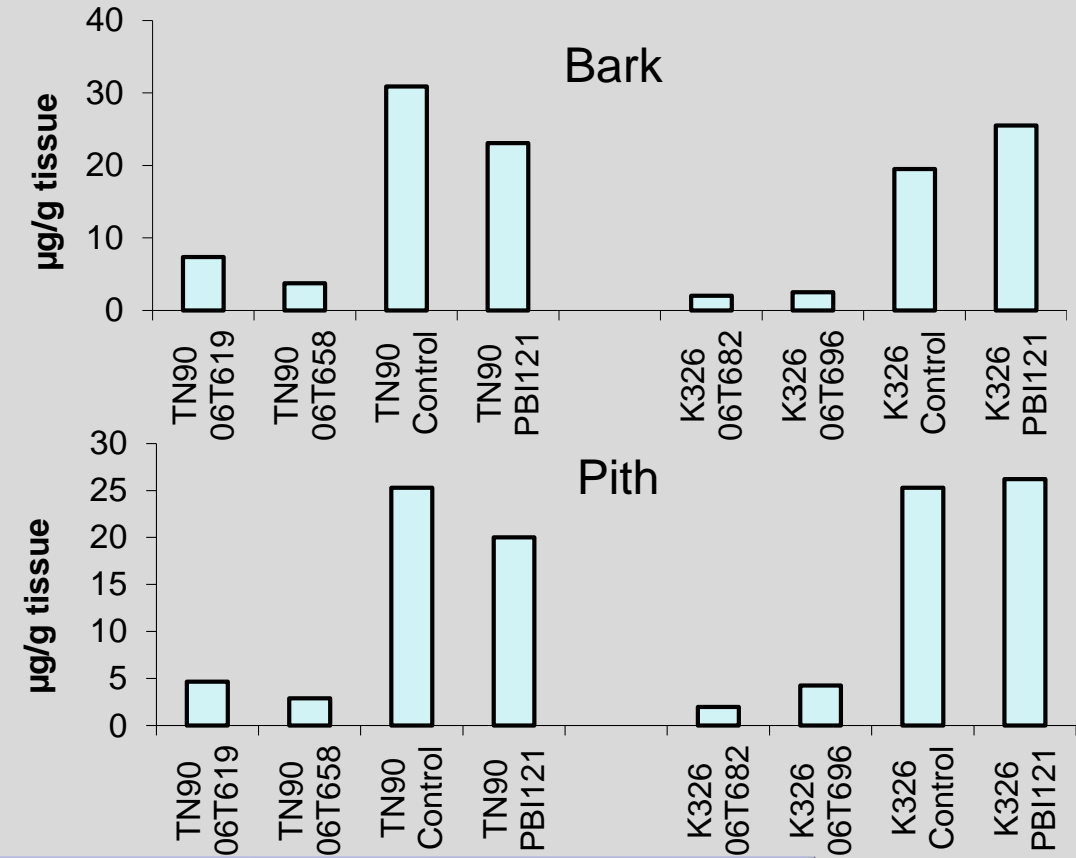
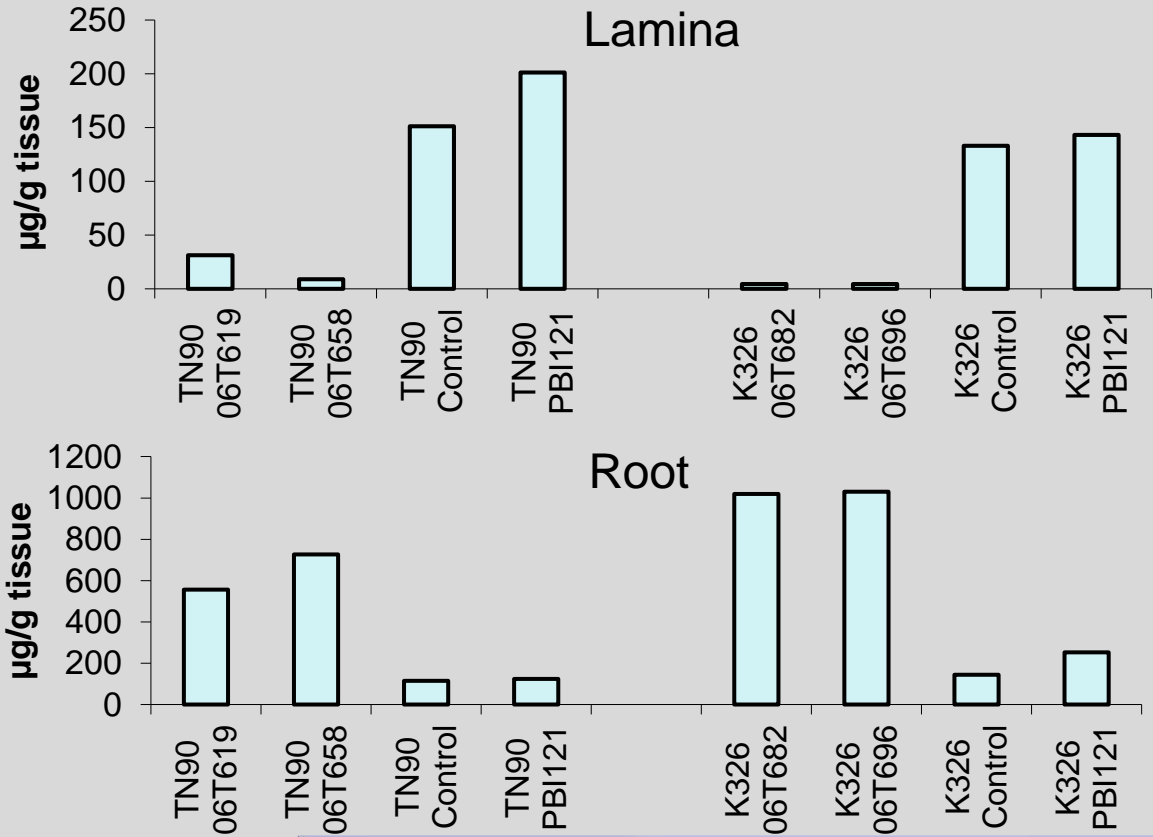


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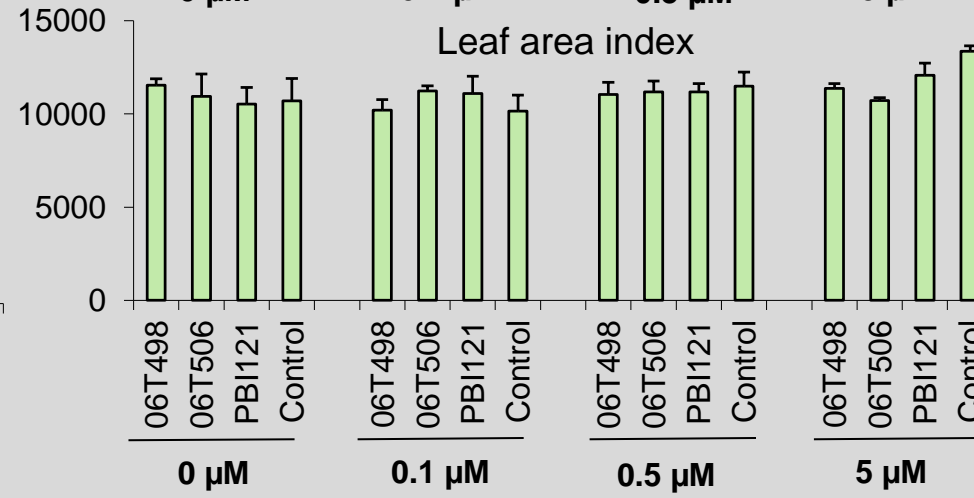
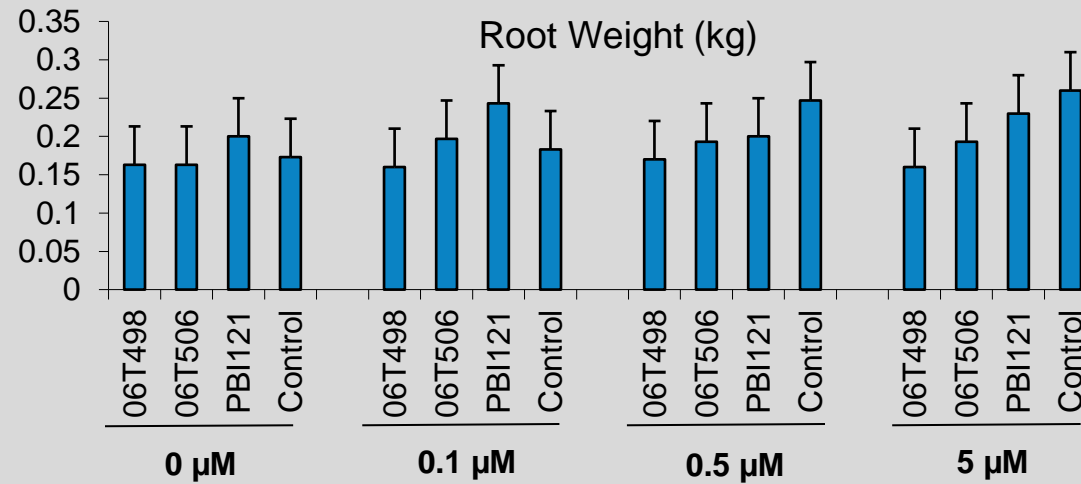
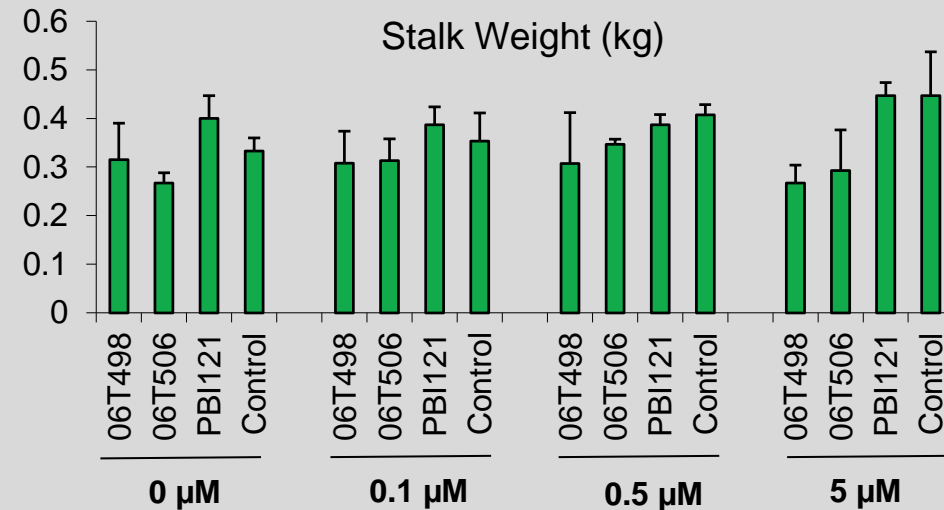
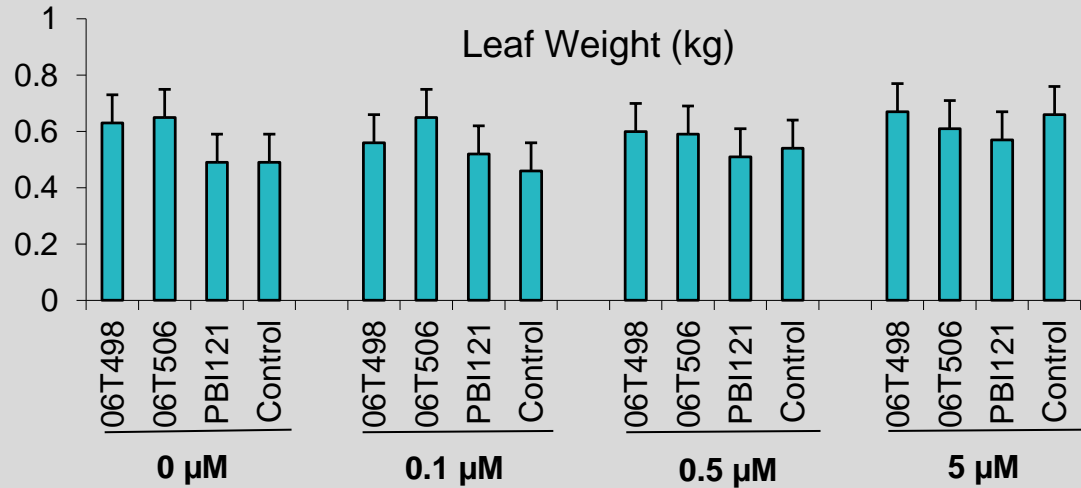
Cadmium was accumulated in VA359 06T542 roots as much as 20-fold compared to controls

CADMIUM ANALYSIS IN DIFFERENT TISSUES OF TRANSGENIC AND CONTROL PLANTS AFTER 8 WEEKS OF TREATMENT WITH CADMIUM



79 % to 91 % of cadmium levels were reduced in the leaf, bark, and pith of transgenic plants; whereas roots' cadmium increased 6-7 fold

GROWTH MATRICES OF VA359 NtHMA RNAi PLANTS UNDER GREENHOUSE CONDITIONS



CONCLUSIONS



- Two Qualitative Trait Loci (QTL) were identified for cadmium accumulation in tobacco roots
- Two heavy metal transporting ATPases are involved in transporting cadmium into the leaf
- Knocking out the function of HMA genes blocked the transport of cadmium to the above-ground parts of the plant
- Reduction of cadmium concentration in leaves ranged from 88% to 91% in selected plants, whereas cadmium accumulation in the roots was increased as much as 20-fold when compared to the controls
- No substantial differences in plant growth patterns were observed between transgenic plants and controls when grown under greenhouse conditions



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