

Mechanisms of cadmium toxicity and tolerance in Populus

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Why Cadmium?

- ◆ Widespread contaminant
- ◆ Harmful effects on plants
- ◆ Diseases in humans
- ◆ Superfund sites

Cd effects on Plants

- **Protein structure**
- **Lipid composition**
- **Nutrient uptake and interference**
- **Photosynthesis**



Cadmium tolerance in plants

Cd uptake via micronutrient transporters

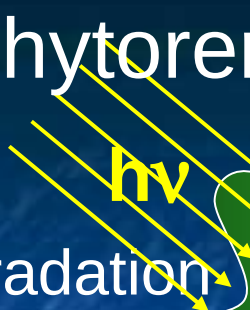
Synthesis of phytochelations (γ -Glu-Cys)_n-Gly)

Vacuolar sequestration

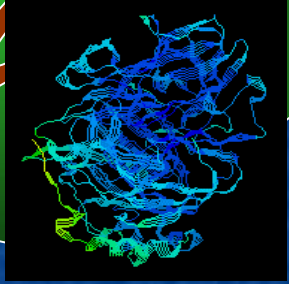
Oxidative stress tolerance mechanisms

Phytoremediation Mechanisms

Photodegradation



Phytotransformation
Sequestration



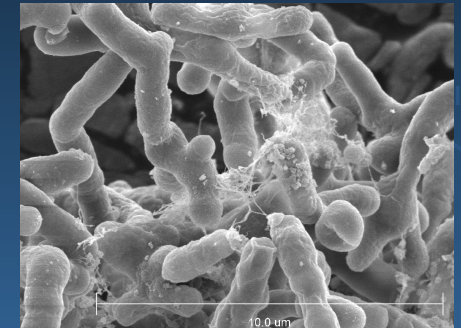
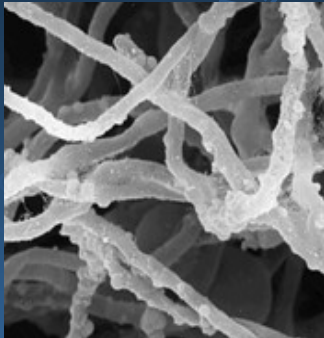
Phytovolatilization



Translocation

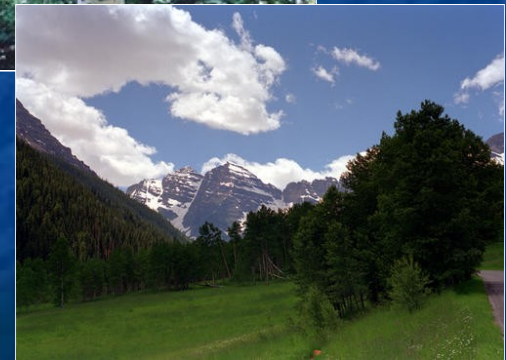
Uptake

Microbial Activity



Populus as a Model for Phytoremediation

- ***Fastest-growing deciduous tree in U.S.***
- ***Broad geographic range***
- ***Perennial and clonal***
- ***Large rooting volume and high transpiration rates***



Populus Genetic Assets

- *Small Genome (485 Mb)*
- *'Short' juvenile period: 4 yrs*
- *Available Genetic Resources*
 - *Pedigrees*
 - *Genetic maps*
 - *BAC libraries*
 - *Routine Transformation*
 - *Genome Sequence!*



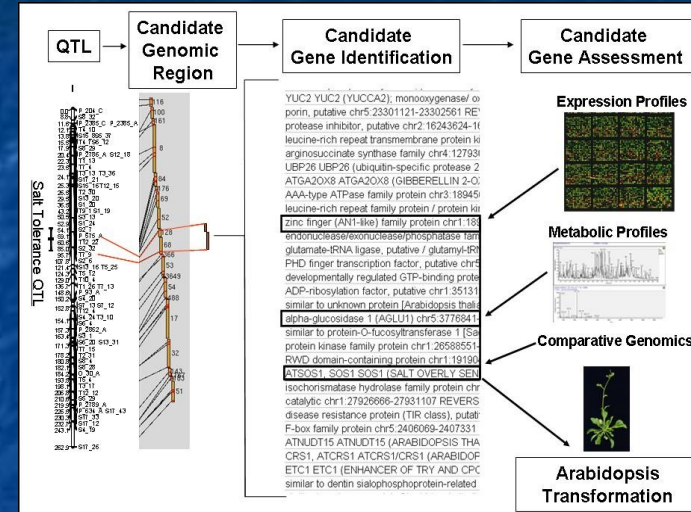
Project Objectives

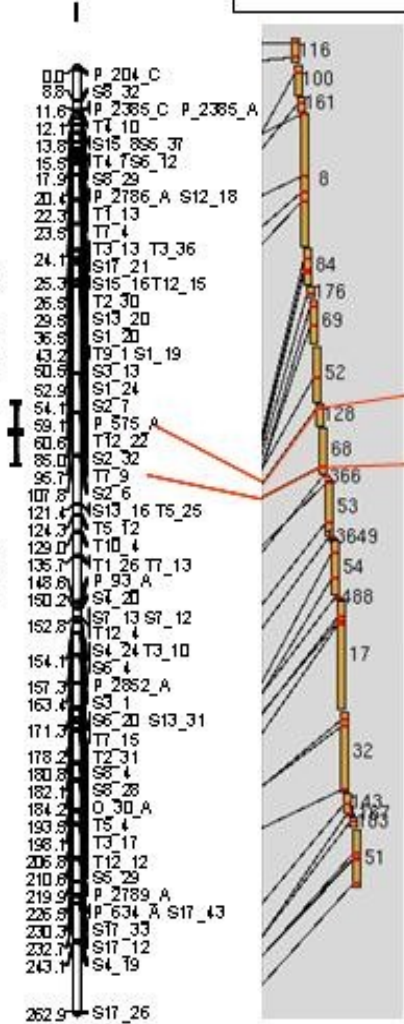
1. Identify *Populus* genotypes that are tolerant and susceptible to cadmium

2. Map QTL for Cd tolerance and accumulation in *Populus*

3. Perform whole-genome microarray analyses for resistant and susceptible genotypes of *Populus*

4. Functionally characterize candidate genes identified in QTL intervals

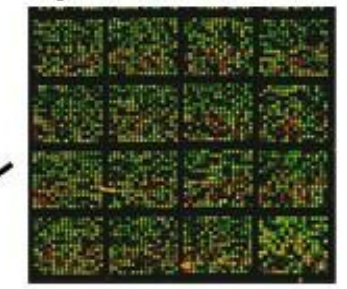




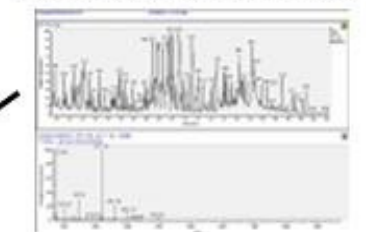
YUC2 YUC2 (YUCCA2); monooxygenase/ o
 porin, putative chr5:23301121-23302561 RE
 protease inhibitor, putative chr2:16243624-16
 leucine-rich repeat transmembrane protein ki
 arginosuccinate synthase family chr4:127931
 UBP26 UBP26 (ubiquitin-specific protease 2
 ATGA2OX8 ATGA2OX8 (GIBBERELLIN 2-O;
 AAA-type ATPase family protein chr3:189451
 leucine-rich repeat family protein / protein ki
 zinc finger (AN1-like) family protein chr1:189
 endonuclease/exonuclease/phosphatase fam
 glutamate-tRNA ligase, putative / glutamyl-tRN
 PHD finger transcription factor, putative chr5
 developmentally regulated GTP-binding prote
 ADP-ribosylation factor, putative chr1:35131
 similar to unknown protein [Arabidopsis thalia
 alpha-glucosidase 1 (AGLU1) chr5:3776841-
 similar to protein-O-fucosyltransferase 1 [Sa
 protein kinase family protein chr1:26588551-
 RWD domain-containing protein chr1:19190
ATSOS1, SOS1 SOS1 (SALT OVERLY SEN
 isochorismatase hydrolase family protein chr
 catalytic chr1:27926666-27931107 REVERS
 disease resistance protein (TIR class), putati
 F-box family protein chr5:2406069-2407331
 ATNUDT15 ATNUDT15 (ARABIDOPSIS THA
 CRS1, ATCRS1 ATCRS1/CRS1 (ARABIDOP
 ETC1 ETC1 (ENHANCER OF TRY AND CPC
 similar to dentin sialophosphoprotein-related

Candidate Gene Assessment

Expression Profiles



Metabolic Profiles



Comparative Genomics



Arabidopsis Transformation

Hydroponic Experimental System



- Interspecific backcross family: *P. trichocarpa* x *P. deltoides*
- 252 genotypes replicated twice per treatment
- Control and 25 μM Cd treatment

Cd vs Control



Variation in Cd Effects



Control roots



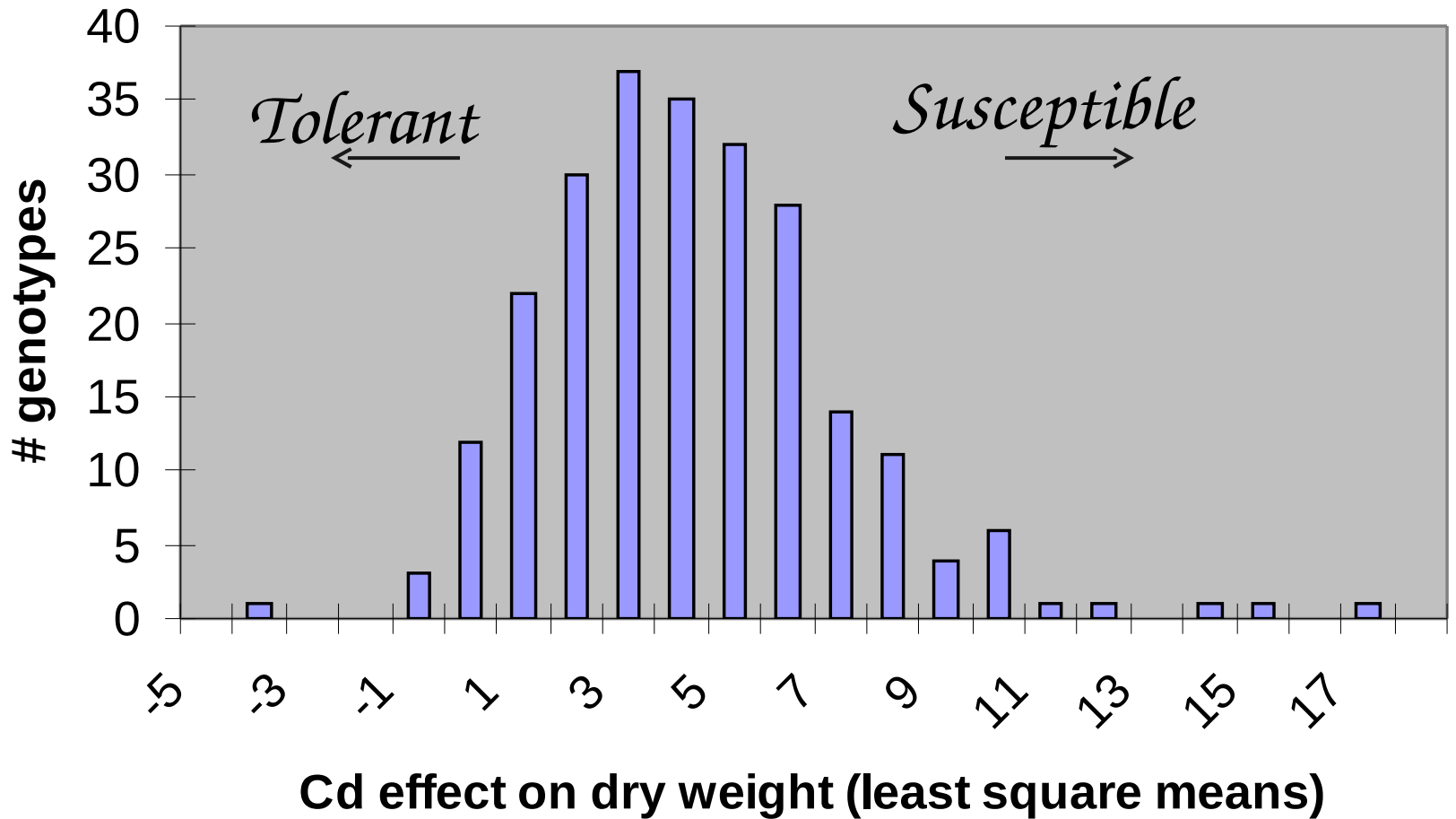
Treated roots

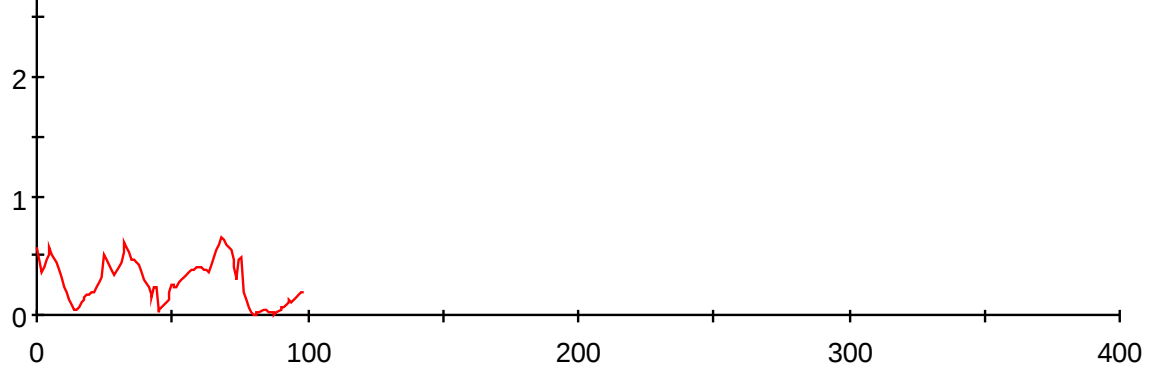


ANOVA of Dry Weights

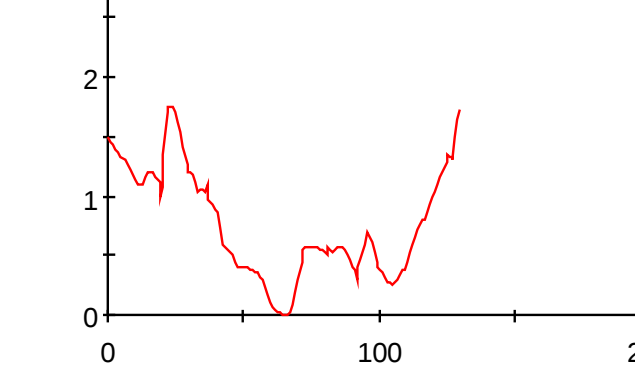
<i>SOURCE</i>	<i>DF</i>	<i>S.SQUARES</i>	<i>F-RATIO</i>	<i>PROB>F</i>
<i>Genotype</i>	239	2410.1632	1.9597	<0.0001
<i>Treatment</i>	1	3035.6440	589.9159	<0.0001
<i>PAR</i>	1	32.2535	6.2678	0.0126
<i>Cutting</i>	1	719.6326	139.8460	<0.0001
Geno*Trt.	239	1853.3381	1.5069	0.0001

Distribution of Cadmium Effects Among Genotypes

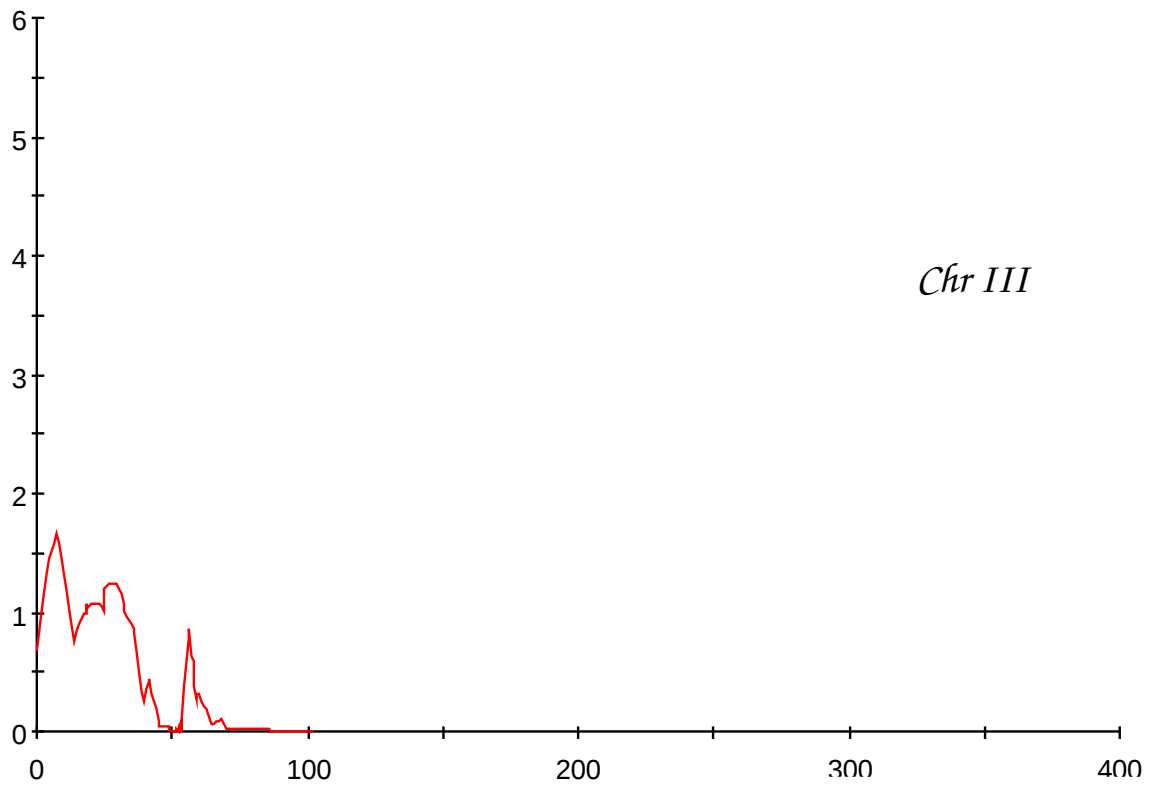




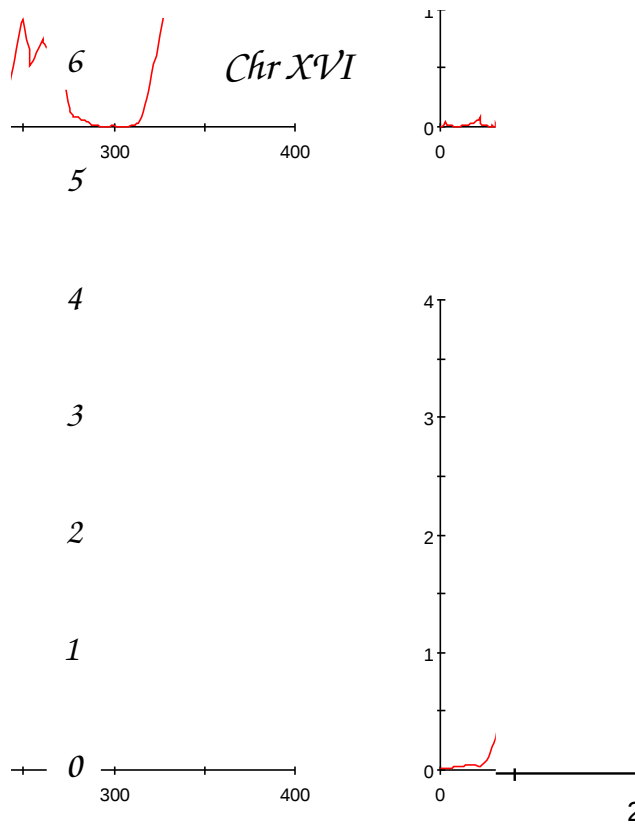
Group 15



Group



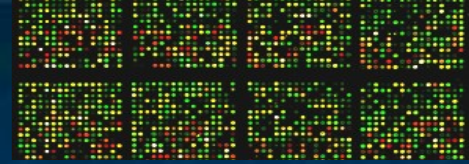
Chr III



Chr XVI

Position (cM)

Whole-genome microarray analyses for resistant and susceptible genotypes of Populus

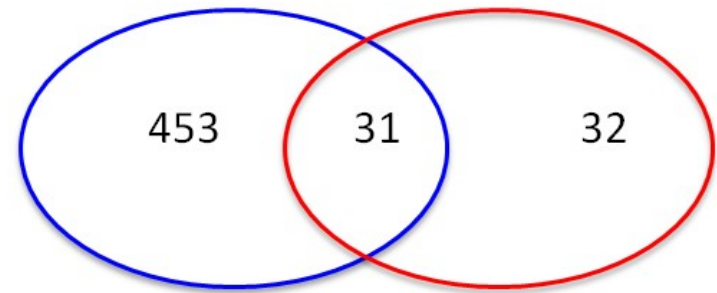
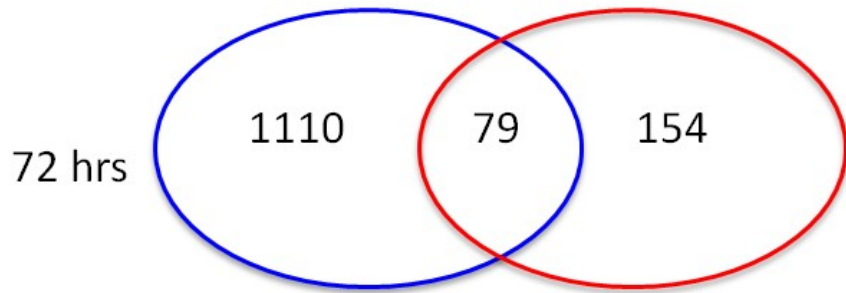
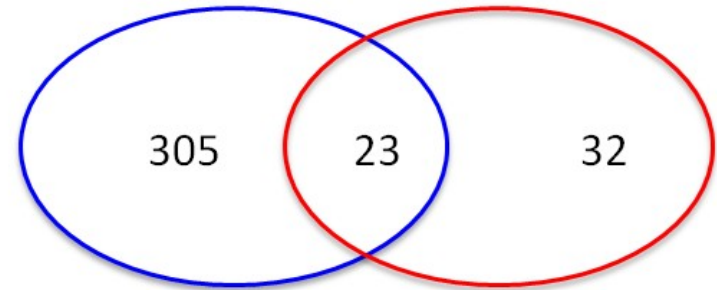
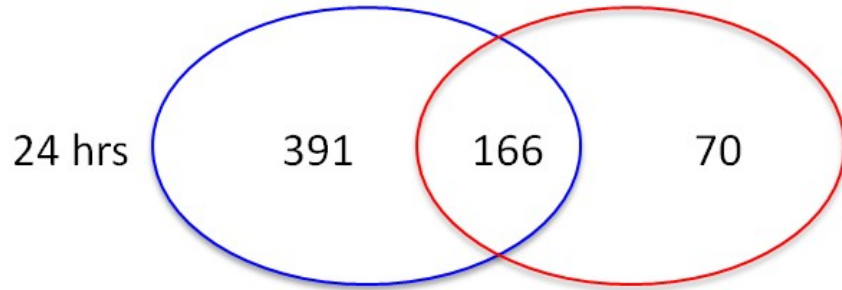




- ***Hydroponics experiment 25uM Cd***
- ***4 genotypes, 2-susceptible, 2-resistant***
- ***3 plants/collection, 2 tissues/plant (root & leaves)***
- ***2 time points (24hrs, 72 hrs)***



Up

Down



-  Susceptible (182)
-  Tolerant (1-183)

Genes Overexpressed in Array Experiment That CoOccur in QTL Intervals

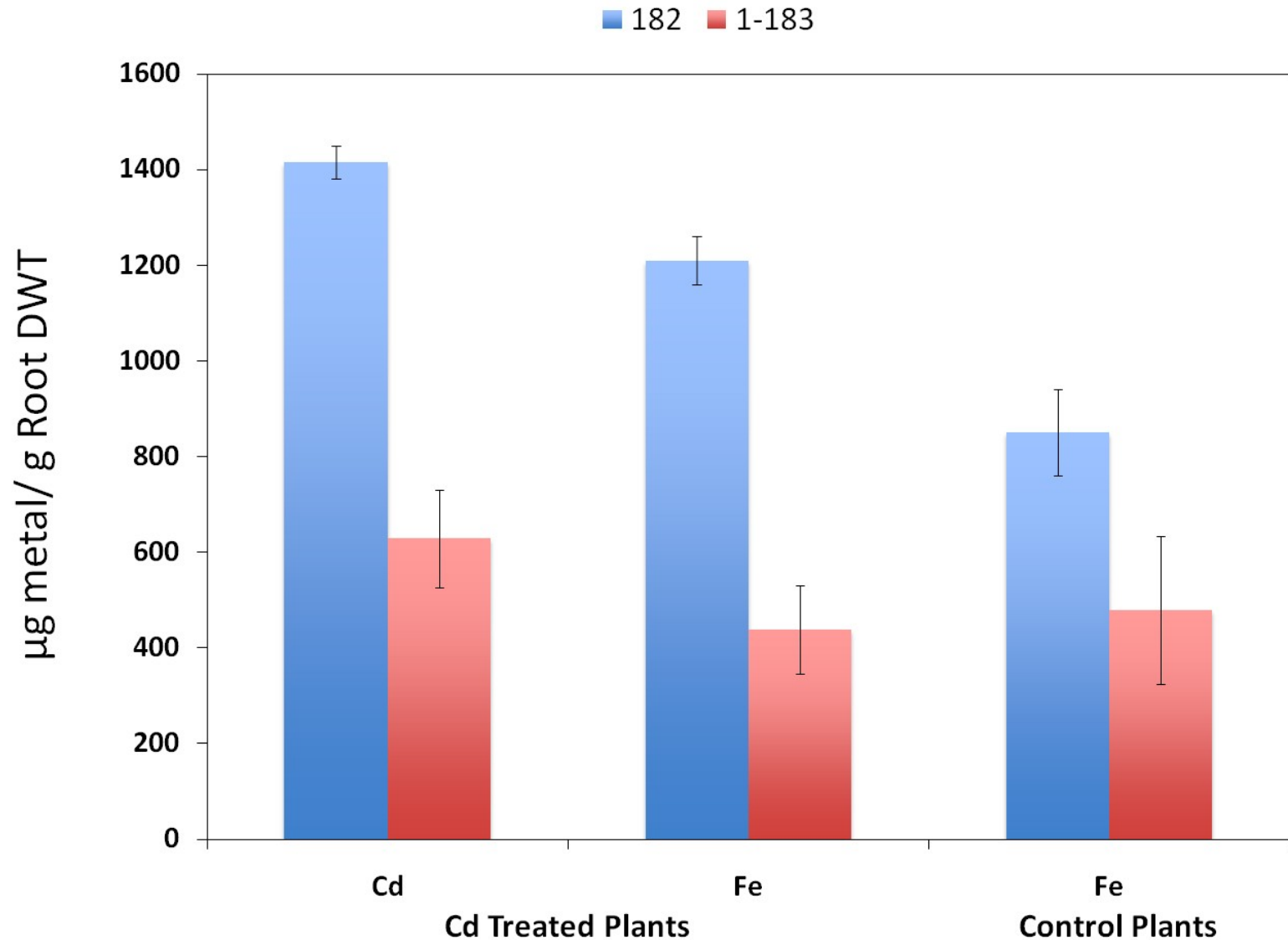
Gene	RP/Rsum	FC:(class1 /class2)	pfp	P.value	#Num	Hit Score Score Def
fgenesh4_pm.C_LG_XVI000330	96.0236	7.8854	0.0011	0	34016	29 kDa ribonucleoprotein, chloroplast, putative / RNA-binding protein cp29,
fgenesh4_pg.C_LG_III001134	320.9263	0.2134	0.0026	0	25103	ATMRP14 (Arabidopsis thaliana multidrug resistance-associated protein 14)
g1.XVI.2928.1	307.9748	0.1946	0.0025	0	51401	NHL repeat-containing protein
fgenesh4_pg.C_LG_III001060	560.9918	3.7564	0.0279	1.00E-04	25088	No Hits
g1.XVI.2910.1	828.1389	0.306	0.0134	1.00E-04	51396	similar to Conserved hypothetical protein 730 [Medicago truncatula]
eigene3.00031003	771.1612	0.2895	0.019	1.00E-04	7787	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G63520.1); contains domain Transcriptional factor tubby,
eExt_fgenesh4_pg.C_LG_III1085	829.6684	3.1391	0.0164	1.00E-04	3435	SP1L5 SP1L5 (SPIRAL1-LIKE5)
g1.XVI.2786.1	429.2525	4.2544	0.0174	1.00E-04	51386	zinc finger (C2H2 type) family protein
g1.XVI.2786.1	944.6035	3.1954	0.0212	1.00E-04	51386	zinc finger (C2H2 type) family protein
eExt_fgenesh4_pg.C_LG_III1187	694.3557	3.7735	0.0406	2.00E-04	3448	zinc finger (RING-H2 type) protein-related

Nature Precedings: doi:10.1038/npre.2010.5411.1 : Posted 17 Dec 2010

Iron/micronutrient genes up-regulated in susceptible genotype

	Putative Function (Arabidopsis)
NRAMP3	Fe export out of vacuole
YSL5	Fe-Nicotianamine Phloem transport
YSL8	Fe-Nicotianamine Phloem transport
HMA6/PAA1	Chloroplast Fe import
FRD3	Xylem citrate transporter/ transport of Fe from root to shoot
YSL8	Fe-Nicotianamine Phloem transport
YSL7	Fe-Nicotianamine Phloem transport
VIT1	Vacuolar Fe sequestration

Iron and Cd Contents are Significantly Different Between Susceptible and Resistant Genotypes

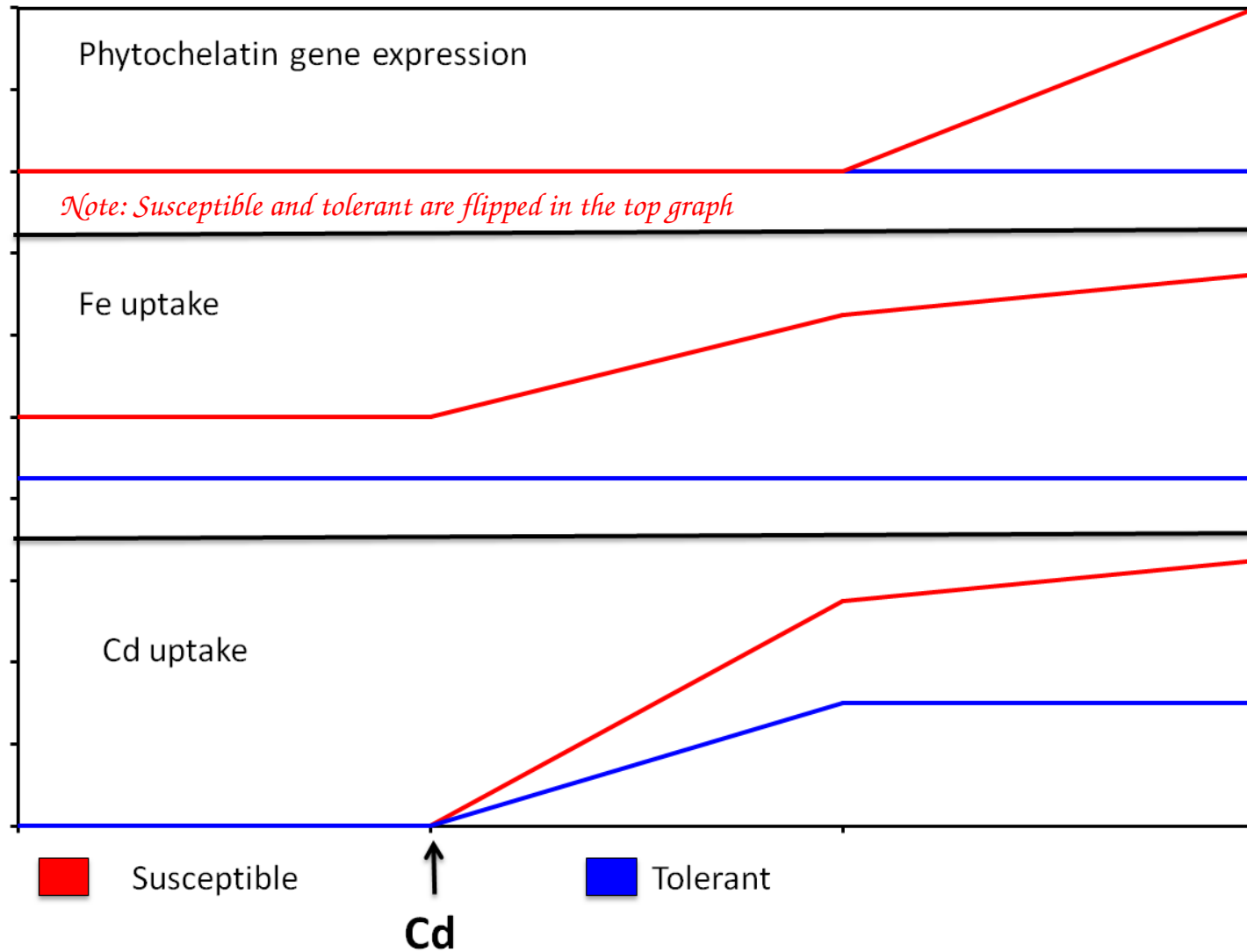


Hypothesis

The susceptible genotype has higher basal Fe uptake resulting in increased Cd uptake.

The resistant genotype has a higher “set point” for iron deficiency response induction.

Model for Interaction of Cd and Fe



Conclusions

- *Substantial variation exists for Cd tolerance in Populus, even within an interspecific full-sib pedigree*
- *Two major QTLs identified for Cd effects in Populus*
- *One mechanism for differential susceptibility may be due to differences in Fe metabolism*
- *Future research will focus on functional assessment of candidate genes that are differentially expressed and occur in QTL intervals*

Acknowledgements

❖ *Jonathan Cumming*

❖ *Jim Ervin*

❖ *Carina Barth*

❖ *Benoit Van Aken*

❖ *WVU Undergrads: Ryan Imperio, Chase Kempinski, Casey Michalski, Marshall Burns, Eryn Daugherty, Rachel McCormick, Andrew Nelson, Kystynne Aguirre*

Funding

- ❖ *U.S. Department of Energy Carbon Sequestration Program*
- ❖ *DOE EPSCOR Laboratory Partnership Program*
- ❖ *WVU Program to Stimulate Competitive Research*
- ❖ *WVU Awards for Research and Team Scholarship*
- ❖ *WVU Environmental Center*
- ❖ *WVU Research Corporation*
- ❖ *WVU Department of Biology*