

Natural genetic variation in zinc (Zn) accumulation by Brassicaceae



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BBSRC Crop Sciences Targeted Priority Studentship



The University of
Nottingham



Aims

- ◆ To test the hypothesis that Hheavy Metal Associated (*HMA*) genes are involved in altered Zn accumulation in Brassicaceae.

Methodology

- ◆ Comparative and functional genomic approaches.
 - *Brassica rapa* and *B. oleracea*
 - *Noccaea caerulescens*
 - *Arabidopsis thaliana*

Zn accumulation in *Brassica*

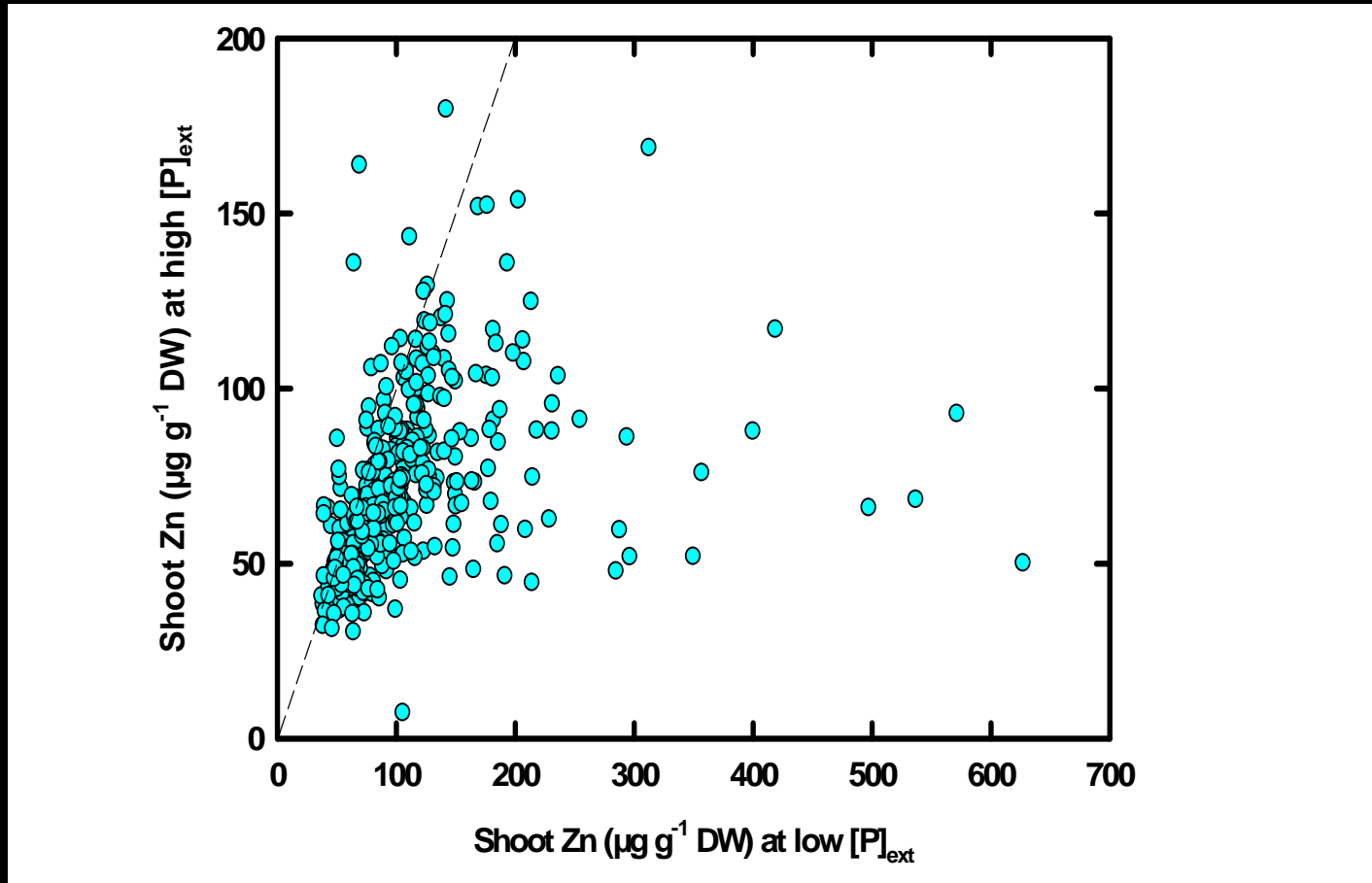
Variance component	Ca	Mg	K	P	Zn	Fe
Genotype (V_A)	36.0	37.7	22.2	7.4	18.5	7.1
$[P]_{\text{ext}}$	0.2	4.0	2.2	43.1	7.2	3.5
$[P]_{\text{ext}}$ / genotype	1.4	1.1	1.4	0.5	2.5	0.0
'other'	62.4	57.2	74.2	49.0	71.8	89.4



Relatively High heritability in AG population

Substantial $[P]_{\text{ext}}$ interaction effect

$[P]_{\text{ext}}$ and Zn interaction in *Brassica*



Brassica consensus contig formation using ContigExpress (Vector NTI)

The screenshot shows the ContigExpress software interface with a list of contigs on the left and their corresponding sequence alignments on the right. The contig list includes identifiers such as gi_151306052... through gi_156811162... and a consensus contig labeled 'Contig 702'. The alignment view shows sequence fragments with yellow highlights indicating matches. A red circle highlights the consensus contig sequence: `Contig 702 AGGCTAAAGGATGCCTCCTAGGGCACATTGACAAGGAAGCACCTGAATGGGAAAAGGATTGCAAATGAAGCTGCAAGGACATTACCTGGAAGAGAA`. A green arrow points from the text below to this highlighted sequence.

Consensus sequences were selected and aligned to *A. thaliana* sequences of interest using AlignX software (Vector NTI).

Brassica contig alignment to *A. thaliana* sequences of interest using AlignX (Vector NTI)

Align X HMA1 - AlignX

Project Edit View Align Analyses Assemble Tools Window Help

Active Pane:

Similarity

Absolute Complexity

Absolute Complexity (Contig 47)

	28669	28670	28680	28690	28700	28710	28720	28730	28740	28750	28760	28770
AthMA1	28276	ACAGTAGCTGAAGAGAGTAGATTACGAAAAGC	ATCAGTTCATACCTCACTTTTCAAATC	TGAAGATGAATCTAAACAGATCAAGGATGCT								
RCHMA1 genomic	3275	ACAGTAGCTGAAGAGAGTAGATTACGAAAAGC	ATCAGTTCATACCTCACTTTTCAAATC	TGAAGATGAATCTAAACAGATCAAGGATGCT								
rcContig 2,	563	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGC	ATCAGTTCATACCTCACTTTTCAAATC	TGAAGATGAATCTAAACAGATCAAGGATGCT								
Contig 1	165	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGC	ATCAGTTCATACCTCACTTTTCAAATC	TGAAGATGAATCTAAACAGATCAAGGATGCT								
Contig 47	516	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGC	ATCAGTTCATACCTCACTTTTCAAATC	TGAAGATGAATCTAAACAGATCAAGGATGCT								
Contig 23	1756	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Contig 61	1225	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
rcContig 26,	1242	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Contig 31	1002	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Contig 71	2014	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Contig 32	798	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
rcContig 3,	1063	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Contig 4	849	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Consensus	28669	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGC	ATCAGTTCATACCTCACTTTTCAAATC	TGAAGATGAATCTAAACAGATCAAGGATGCT								

Ready consensus positions: 99.1% identity positions: 0.0% aln: 28678

BAC end sequences within contigs, showing significant sequence similarity to *A. thaliana* HMAs were then selected.

Brassica contig alignment to *A. thaliana* sequences of interest using AlignX (Vector NTI)

The screenshot displays the AlignX HMA1 software interface. The main workspace shows a list of contigs on the left, a similarity plot and absolute complexity plots in the middle, and a dendrogram on the right. The sequence alignment table at the bottom shows the alignment of various contigs to the HMA1 locus. A red box highlights the alignment of Contig 47, which shows a high degree of similarity to the HMA1 sequence. A green arrow points to a text box at the bottom of the image.

	28669	28670	28680	28690	28700	28710	28720	28730	28740	28750	28760	28770
AthMA1	28276	ACAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTCTTCAAATCAGAAGATGAGTCTAAACAGATCAAGGATGCT									
RCHMA1 genomic	3275	ACAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTCTTCAAATCAGAAGATGAGTCTAAACAGATCAAGGATGCT									
rcContig 2,	563	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTCTTCAAATCAGAAGATGAGTCTAAACAGATCAAGGATGCT									
Contig 1	165	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTCTTCAAATCAGAAGATGAGTCTAAACAGATCAAGGATGCT									
Contig 47	516	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTCTTCAAATCAGAAGATGAGTCTAAACAGATCAAGGATGCT									
Contig 23	1756	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Contig 61	1225	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
rcContig 26,	1242	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Contig 31	1002	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Contig 32	798	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
rcContig 3,	1063	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Contig 4	849	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Consensus	28669	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTCTTCAAATCAGAAGATGAGTCTAAACAGATCAAGGATGCT									

Ready consensus positions: 99.1% identity positions: 0.0%aln: 28678

Once locus specific *HMA* paralogues are confirmed we will screen a new *A*-genome TILLING population.

Noccaea caerulescens (J&C Presl.) FK Mey 'Saint-Laurent-Le-Minier'



Hyperaccumulates:

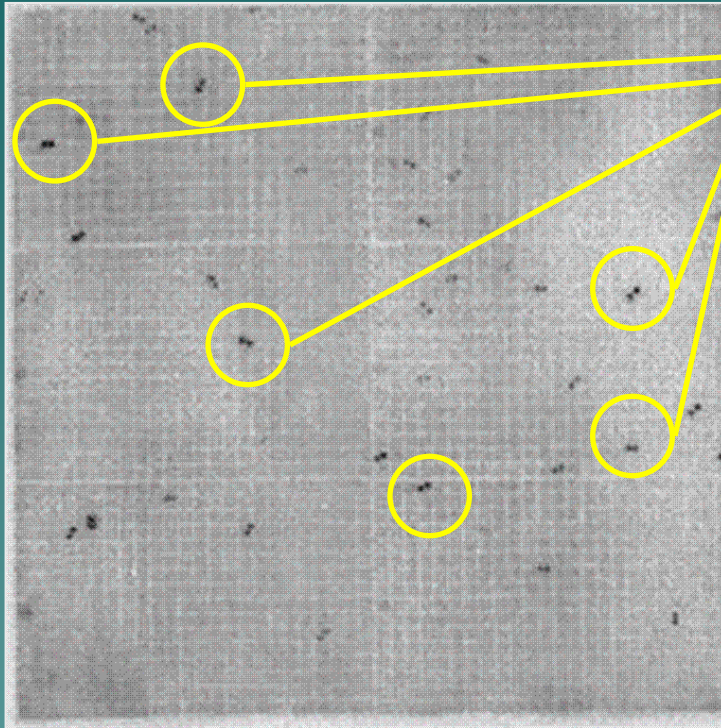
- Zn >3% SDW.
- Cadmium [Cd] up to 1.5% SDW.

Three experimental approaches for *NcHMA* functional analysis:

- ◆ We have created a 40 kb insert genomic Fosmid library.
- ◆ Attempting “floral dip” transformation in *Noccaea*.
- ◆ Functional characterisation of *NcHMAs* in *A. thaliana*.

Noccaea caerulescens (J&C Presl.) FK Mey 'Saint-Laurent-Le-Minier' previously *Thlaspi caerulescens* 'Ganges'.

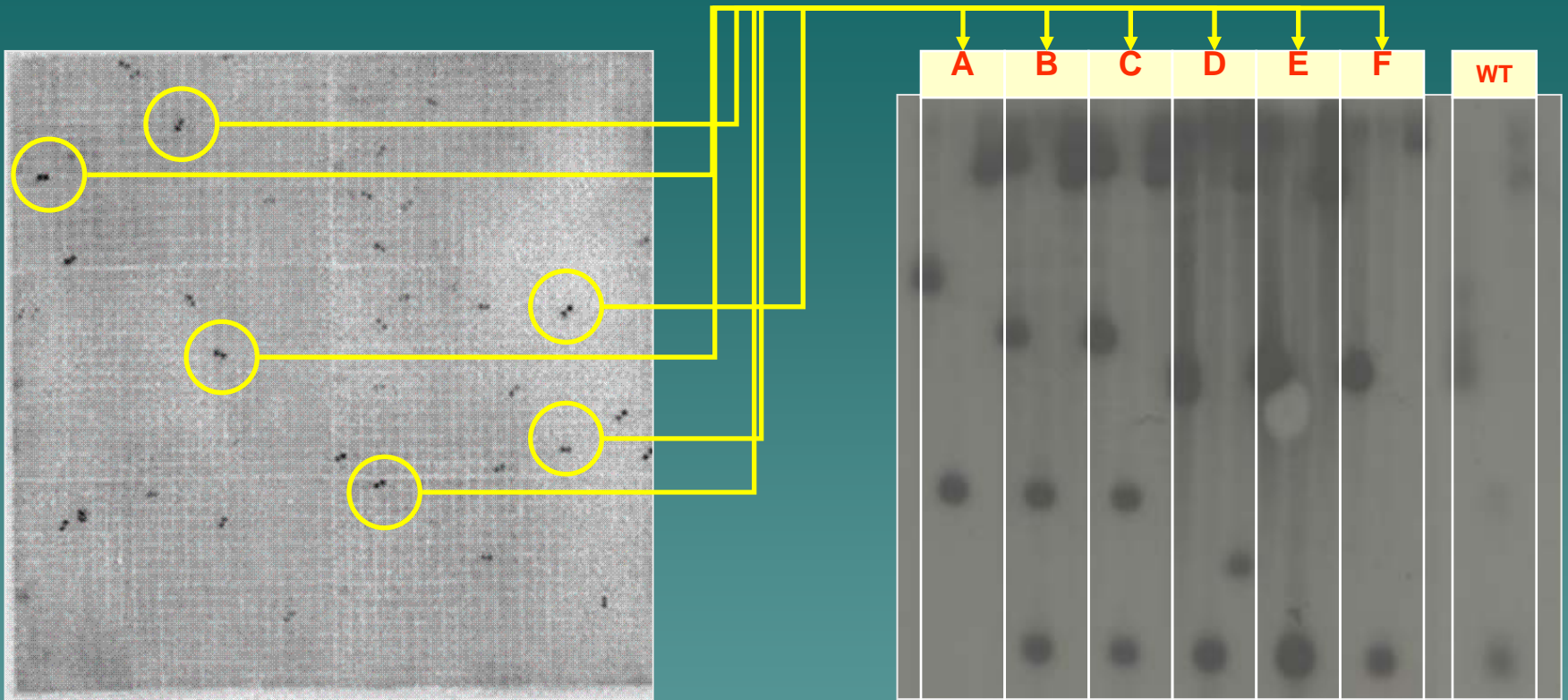
Identifying *NcHMAs*



AtHMA3_genomic_sequence	GCAAGAATTATTGATGATTTC AAGATCCAAGGCCAACAATGATGGTAGG	386
TcHMA3_library_probe_sequence	TCAAGAATCATCGATGAGTTTATGTCTCAAGGGCCTACAATGATGGTAGG	215
Fosmid_clone_H19P60	TCAAGAATCATCGATGAGTTTATGTCTCAAGGGCCTACAATGATGGTAGG	215
Fosmid_clone_D15P20	TCAAGAATCATCGATGAGTTTATGTCTCAAGGGCCTACAATGATGGTAGG	218
Fosmid_clone_C13P94	TCAAGAATCATCGATGAGTTTATGTCTCAAGGGCCTACAATGATGGTAGG	221
Fosmid_clone_E24P78	TCAAGAATCATCGATGAGTTTATGTCTCAAGGGCCTACAATGATGGTAGG	220
Fosmid_clone_05P59	TCAAGAATCATCGATGAGTTTATGTCTCAAGGGCCTACAATGATGGTAGG	217
	***** ** ***** * * * ***** *****	
AtHMA3_genomic_sequence	AGATGGGCTTAACGATGCACCGGCTTTAGCTAAAGCAGACATTGGTATAT	436
TcHMA3_library_probe_sequence	AGACGGGCTTAACGATGCGCCGGCCTTGCTAAAGCAGACATTGGTATTT	265
Fosmid_clone_H19P60	AGACGGGCTTAACGATGCGCCGGCCTTGCTAAAGCAGACATTGGTATTT	265
Fosmid_clone_D15P20	AGACGGGCTTAACGATGCGCCGGCCTTGCTAAAGCAGACATTGGTATTT	268
Fosmid_clone_C13P94	AGACGGGCTTAACGATGCGCCGGCCTTGCTAAAGCAGACATTGGTATTT	271
Fosmid_clone_E24P78	AGACGGGCTTAACGATGCGCCGGCCTTGCTAAAGCAGACATTGGTATTT	270
Fosmid_clone_05P59	AGACGGGCTTAACGATGCGCCGGCCTTGCTAAAGCAGACATTGGTATTT	267
	*** ***** ***** * ***** *****	
AtHMA3_genomic_sequence	CCATGGGGATCTCAGGGTCAGCACTTGCAACAGAGACAGGAGATCATT	486
TcHMA3_library_probe_sequence	CAATGGGGATCTCAGGGTCAGCACTTGCAACAGAGACAGGAGATCATT	315
Fosmid_clone_H19P60	CAATGGGGATCTCAGGGTCAGCACTTGCAACAGAGACAGGAGATCATT	315
Fosmid_clone_D15P20	CAATGGGGATCTCAGGGTCAGCACTTGCAACAGAGACAGGAGATCATT	318
Fosmid_clone_C13P94	CAATGGGGATCTCAGGGTCAGCACTTGCAACAGAGACAGGAGATCATT	321
Fosmid_clone_E24P78	CAATGGGGATCTCAGGGTCAGCACTTGCAACAGAGACAGGAGATCATT	320
Fosmid_clone_05P59	CAATGGGGATCTCAGGGTCAGCACTTGCAACAGAGACAGGAGATCATT	317
	* ***** ***** *****	

- Clones were initially grouped into *Noccaea* homologues of *AtHMA* 2, 3 and 4 through fragment PCR and sequencing.

Identifying *NcHMA*s



- ◆ They were then further grouped into *NcHMA* 2, 3 and 4 gene paralogues by Fosmid fingerprinting
 - each clone was digested with *EcoRI*, *HindIII* and *BamHI*, run in an agarose gel, blotted onto a nitrocellulose membrane and re-probed with *NcHMA* probes.

Work in Progress

Brassica.

- ◆ We have identified 50 *Brassica* BACs with homologues of *AtHMA1*, 2, 3 & 4.
- ◆ We are analysing 5 substitution lines from the AGDH mapping population for altered Zn_[leaf].

Noccaea caerulescens 'Ganges'.

- ◆ We have identified *NcHMAs* in 16 Fosmids from the genomic Fosmid library.
- ◆ These Fosmids are being sequenced using 454 ultra-high-throughput DNA pyrosequencing.
- ◆ We are screening 80,000 fast neutron mutagenised M₂ mutants for rapid cycling phenotypes to adapt to future molecular genetic approaches.

Arabidopsis thaliana.

- ◆ We have characterised Salk knockout lines for *AtHMA1*, 2, 3 and 4.

Acknowledgements



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Graham King



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Thank you