

# 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*

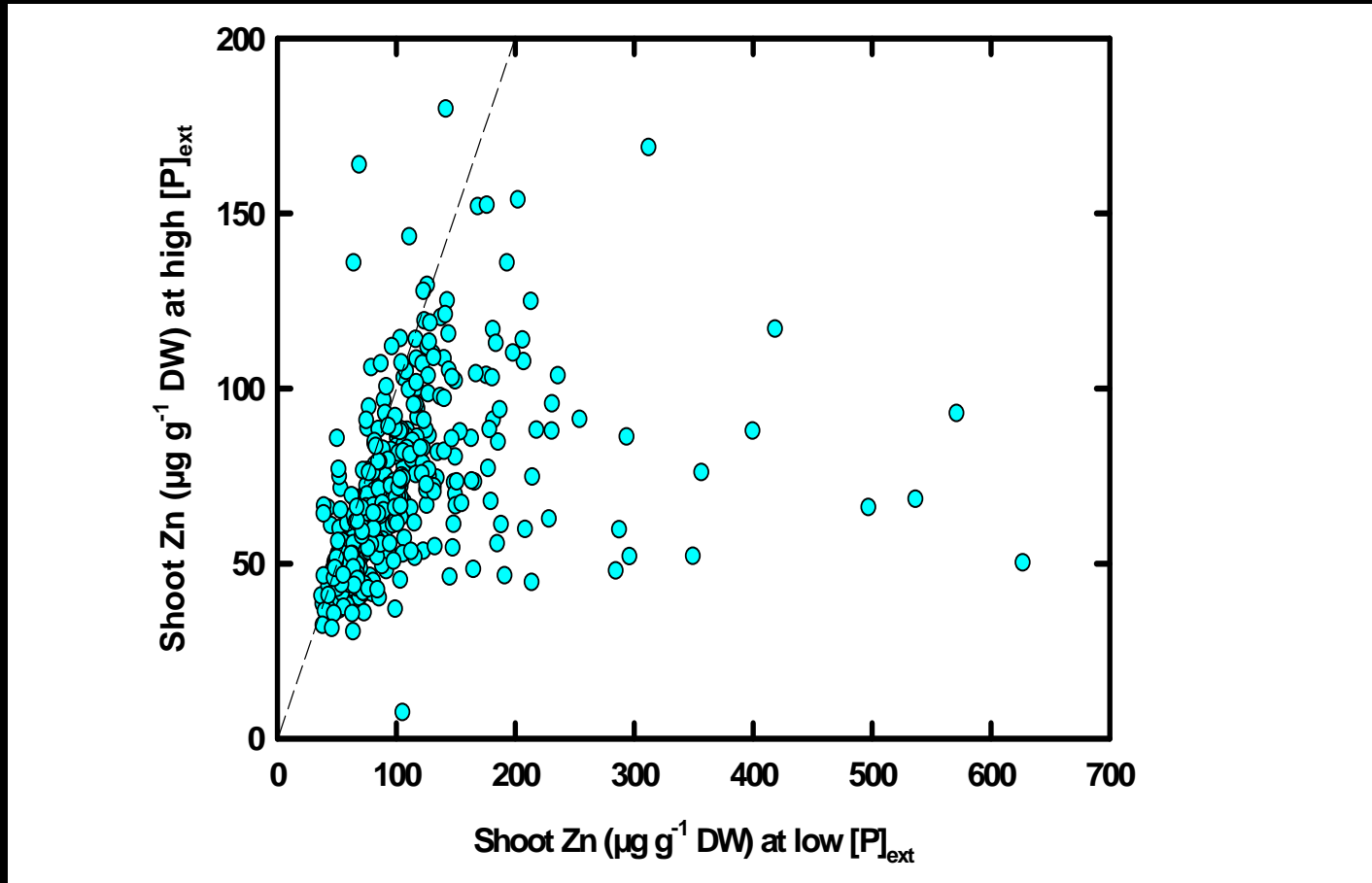
Variance component	Ca	Mg	K	P	Zn	Fe
Genotype ( $V_A$ )	36.0	37.7	22.2	7.4	18.5	7.1
$[P]_{\text{ext}}$	0.2	4.0	2.2	43.1	7.2	3.5
$[P]_{\text{ext}}$ / genotype	1.4	1.1	1.4	0.5	2.5	0.0
'other'	62.4	57.2	74.2	49.0	71.8	89.4



Relatively High heritability in AG population

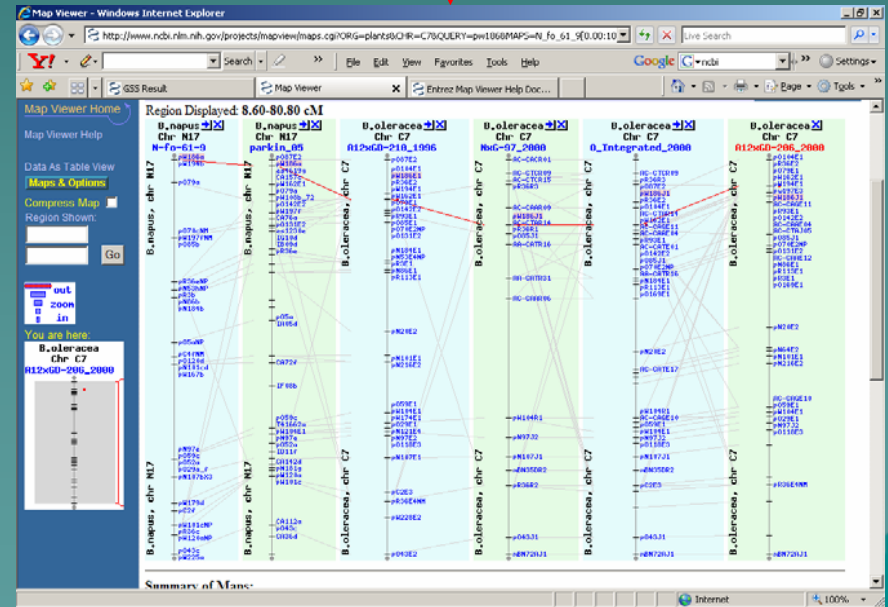
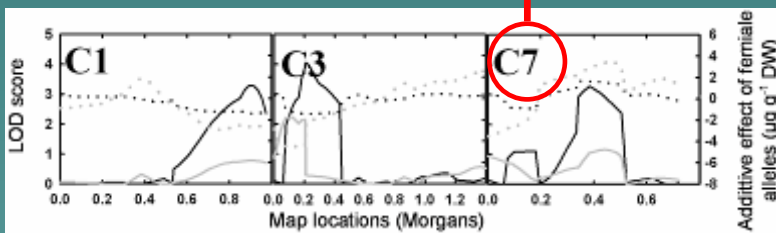
Substantial  $[P]_{\text{ext}}$  interaction effect

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



# Brassica HMAs and Zn accumulation

- ◆ Associations between  $[Zn]_{leaf}$  and 3 linkage groups of *B. oleracea* (C1, C3, C7).
- ◆ RFLP probes located within *Brassica* Zn QTL intervals show significant sequence similarity to *AtHMA 1, 2, 3* and 4.







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

The screenshot shows the AlignX HMA1 - AlignX software interface. The main window displays a project list on the left, similarity and complexity plots in the middle, and a dendrogram on the right. The bottom section displays a sequence alignment table with a red box highlighting a specific region and a green arrow pointing to it.

**Project List (Left Panel):**

- AthMA1 (50001)
- Contig 1 (1263)
- Contig 10 (586)
- Contig 100 (1081)
- Contig 101 (730)
- Contig 102 (896)
- Contig 103 (1404)
- Contig 104 (1172)
- Contig 105 (975)
- Contig 106 (1018)
- Contig 107 (1872)

**Similarity Plot (Middle Panel):**

Similarity: +0.311

Absolute Complexity: -0.787

Absolute Complexity (Contig 47): +10

**Dendrogram (Right Panel):**

- Contig 47 (-0.0949)
- rcContig 11, (-0.0367)
- rcContig 3, (-0.0237)
- rcContig 26, (0.0332)
- Contig 1 (-0.0332)
- Contig 4 (-0.0234)
- rcContig 178, (0.0420)
- rcContig 16, (-0.0420)
- Contig 32 (-0.0244)
- Contig 28 (0.0648)
- rcContig 164, (-0.0648)
- Contig 21 (-0.0262)
- rcContig 118, (0.0757)

**Sequence Alignment Table (Bottom Panel):**

	28669	28670	28680	28690	28700	28710	28720	28730	28740	28750	28760	28770
AthMA1	28276	ACAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTTTC	AAAATC	TGAAGATGA	ATCTAAACAGATCAAGGATGCT						
RCHMA1 genomic	3275	ACAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTTTC	AAAATC	TGAAGATGA	ATCTAAACAGATCAAGGATGCT						
rcContig 2,	563	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTTTC	AAAATC	TGAAGATGA	ATCTAAACAGATCAAGGATGCT						
Contig 1	165	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTTTC	AAAATC	TGAAGATGA	ATCTAAACAGATCAAGGATGCT						
Contig 47	516	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTTTC	AAAATC	TGAAGATGA	ATCTAAACAGATCAAGGATGCT						
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	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGCATCACTTGGTTCTATAGAGTTCAT	ACCTCACTTTC	AAAATC	TGAAGATGA	ATCTAAACAGATCAAGGATGCT						

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

**Status Bar:** Ready | consensus positions: 99.1% | identity positions: 0.0% |aln: 28678

# 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:

AthMA1 (50001)  
 Contig 1 (1263)  
 Contig 10 (586)  
 Contig 100 (1081)  
 Contig 101 (730)  
 Contig 102 (896)  
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Absolute Complexity: -0.787

Absolute Complexity (Contig 47): +10

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Contig 1	165	TCAGTAGCTGAAGAGAGTAGATTACGAAAAGC	ATCACTTGGTTCTATAGAGTTCAT	ACCTCACTTTC	CAAATCGAAGATGA	ATCTAAACAGATCAAGGATGCT						
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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'



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

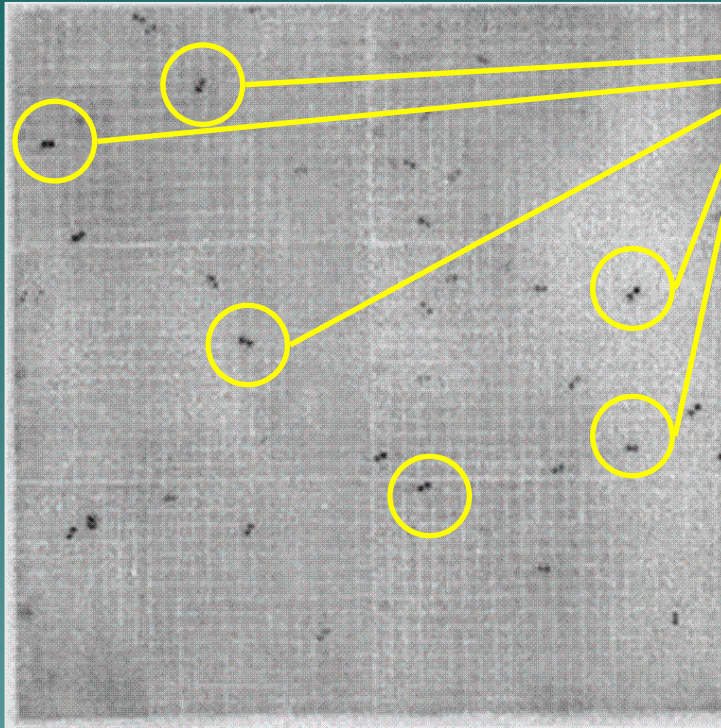
## Hyperaccumulates:

- 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*.

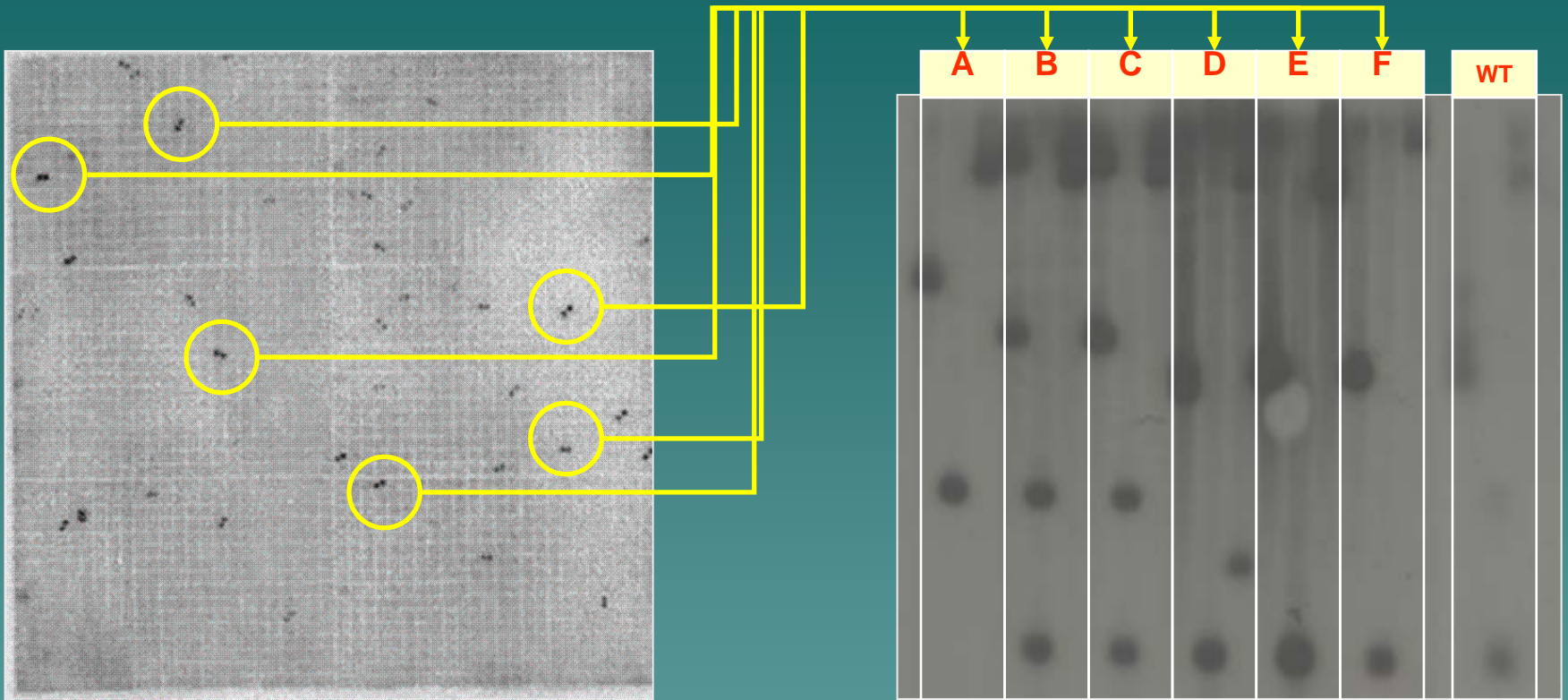
# Identifying *NcHMAs*



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 *Eco*RI, *Hind*III and *Bam*HI, 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  $M_2$  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



The University of  
Nottingham

**Martin Broadley**  
**Rupert Fray**



Warwick  
HRI

**John Hammond**  
**Helen Bowen**



**Philip White**



ROTHAMSTED  
RESEARCH

**Graham King**



**BBSRC Crop Sciences Targeted Priority Studentship**

**Thank you**