Genetic variation in zinc (Zn) accumulation by Brassicaceae

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Introduction

Zinc (Zn) is an essential plant nutrient. Most species have a leaf Zn concentration $[Zn]_{leaf}$ <0.1 mg Zn g^{-1} shoot dry weight (SDW). Some Brassicaceae e.g. *Noccaea caerulescens* (J&C Presl.) FK Mey and *Arabidopsis halleri* hyperaccumulate >10 mg Zn g^{-1} SDW[1]. Heavy Metal Associated (*HMA*) P_{1B}-type ATPases[2], implicated in xylem loading of Zn, are expressed highly in Zn hyperaccumulators[3,4,5]. Are these transporters potential markers for altered $[Zn]_{leaf}$ in Brassicaceae?

Materials and Methods

***Brassica rapa* R-O-18**

Rapid cycling (8 weeks) inbred *B. rapa* ssp. *trilocularis* 'Yellow Sarson' (2n=2x=20).

***Noccaea caerulescens* 'Ganges'**

Slow cycling (36 wks) vernal-obligate, Zn hyperaccumulator from S. France (2n = 2x = 14).

***BrHMA* Identification**

Alleles identified via *in silico* sequence analysis with other angiosperms.

***NcHMA* Identification**

Genomic fosmid library [36,864 EPI300-T1R cells, 8kb pCC1FOS™ vector 40 kb inserts], sequenced and assembled (GSFLX & Roche Newbler assembler V 1.1.02.15).

TILLing

TILLing (Targeted Induced Local Lesions IN Genomes) for *HMA4* Single Nucleotide Polymorphisms (SNP) in 9216 M_2 ethyl methane sulfonate (EMS) lines.

Promoter Analysis

HMA4 promoters studied in *A. thaliana* on increasing $[Zn]_{ext}$.

RNAi

Rapid cycling Fast Neutron (FN) lines (5500 seeds irradiated at 438 mGy min^{-1} at 10 MW) selected for efficient *in planta* reverse-genetic analyses.

Results

Novel *NcHMA4* tandem repeats revealed (Fig.1).

Rapid cycling selfed M_3 FN *Noccaea* fruit in 16 weeks, independent of vernalisation (Fig. 2).

BrHMA4s are currently being 'TILLed' for SNPs.

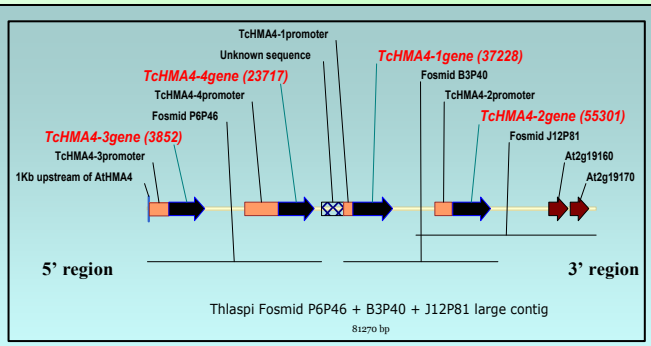

Figure 1. Tandem repeats of *NcHMA4* from *N. caerulescens*

Figure 2. Pre-vernal *Noccaea* WT (left) and FN (centre) after 16 weeks and post-vernal *Noccaea* WT (right) after 32 weeks.

References

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(4) Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, Motte P, Kroymann J, Weigel D, Krämer U 2008 Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of HMA4. *Nature* **453**: 391-395.

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Progress and Future work

Regulation of Brassicaceae *HMA4* promoters are being tested in *A. thaliana* on altered $[Zn]_{ext}$.

A transformation system for rapid cycling *N. caerulescens* is being developed to optimise molecular genetic analyses.

In planta functional analyses of *Brassica* / *Noccaea HMA*s.

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