

Regulation of calcium and magnesium concentrations in leafy *Brassica*

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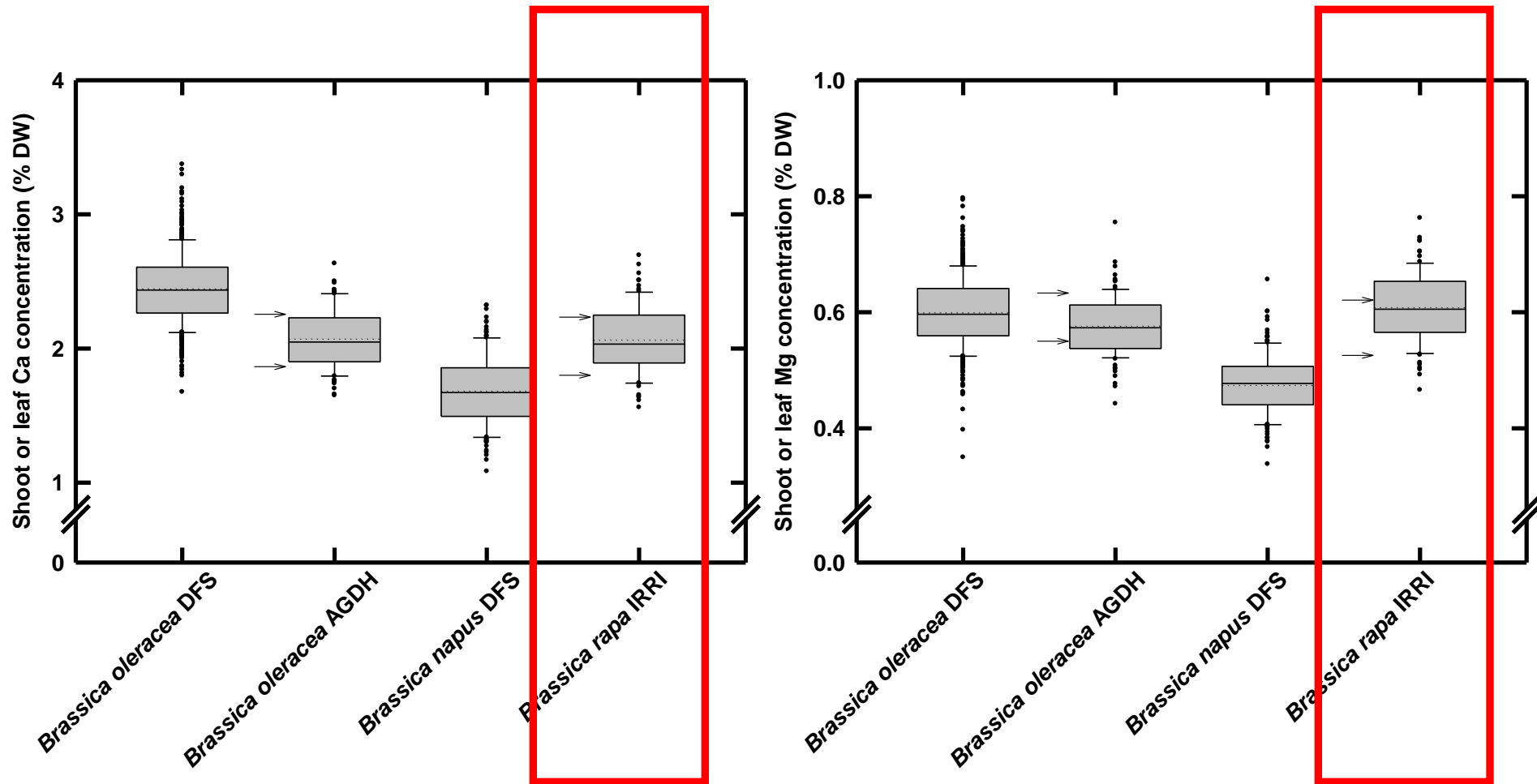
Aim

Identify genes affecting calcium (Ca) and magnesium (Mg) accumulation in leafy *Brassica*

Methods

- . Comparative genomics
- . *Brassica rapa* TILLing

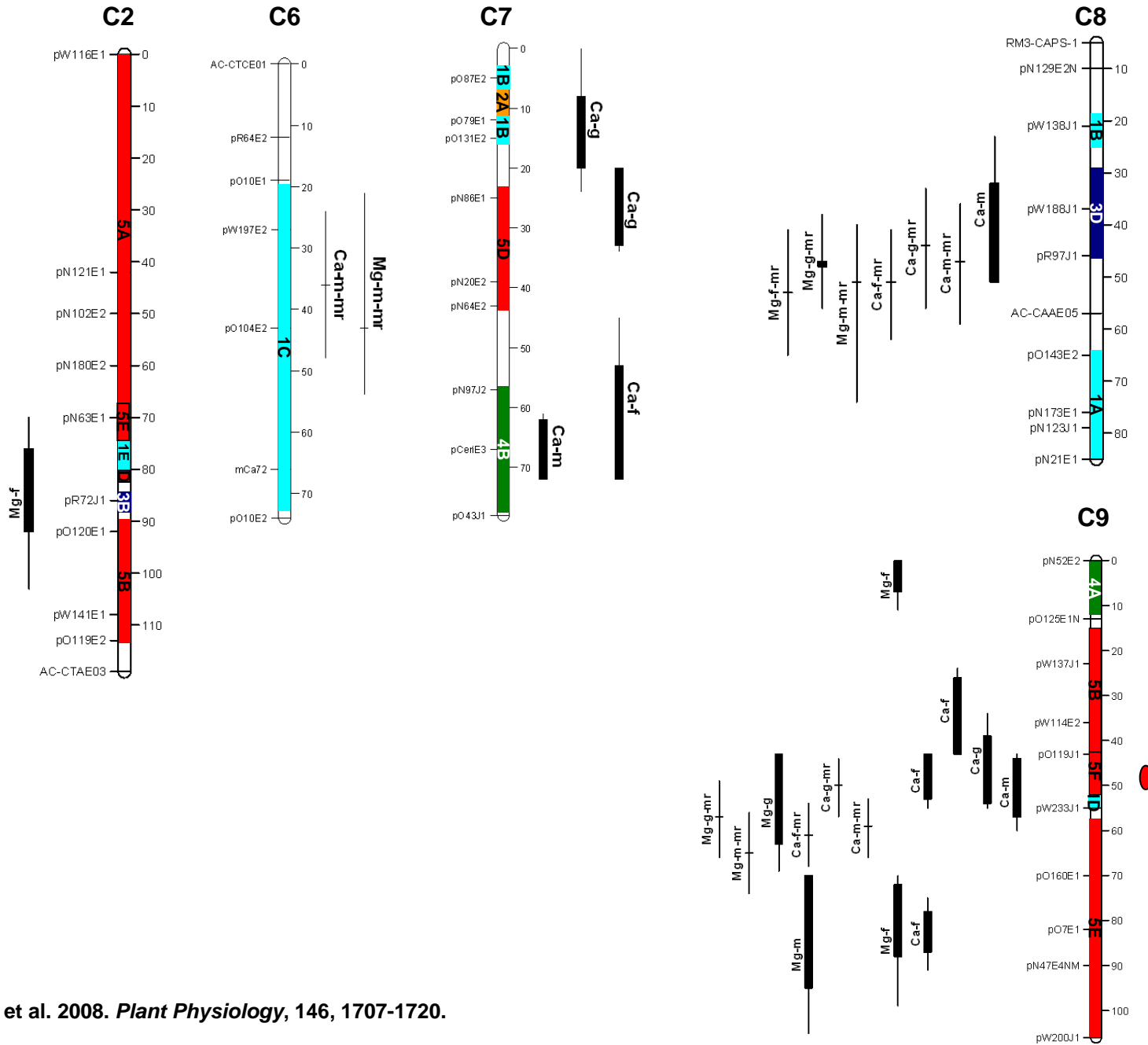
Variation in $[Ca]_{shoot}$ and $[Mg]_{shoot}$: A/C/AC Brassica



Broadley MR et al. 2008. *Plant Physiology*, 146, 1707-1720.

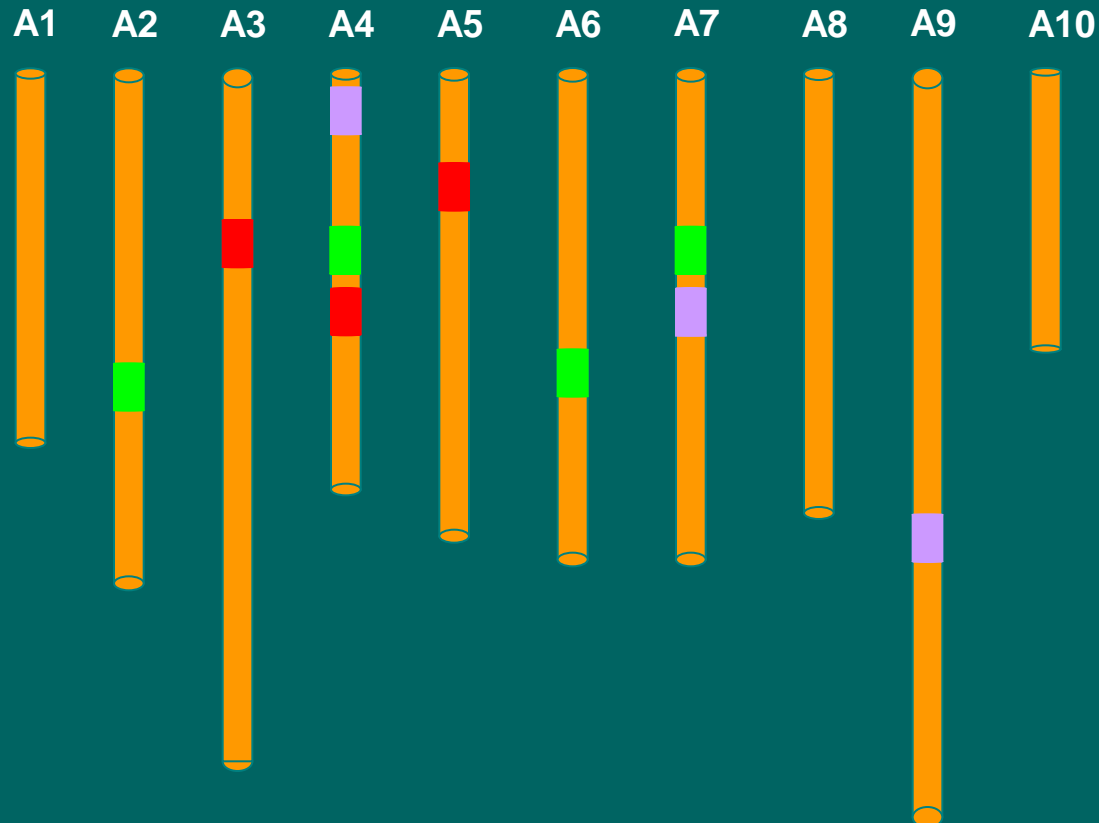
Broadley MR et al. 2009. *The Proceedings of the International Plant Nutrition Colloquium XVI*. Paper 1256.

C-Genome *Brassica*



A-Genome *Brassica*

Brassica rapa ĩ 7 \]] Zi Đ



BraESB1



Ca phenotype in *Atesb1* (Baxter I et al. 2009)



BraCAX1



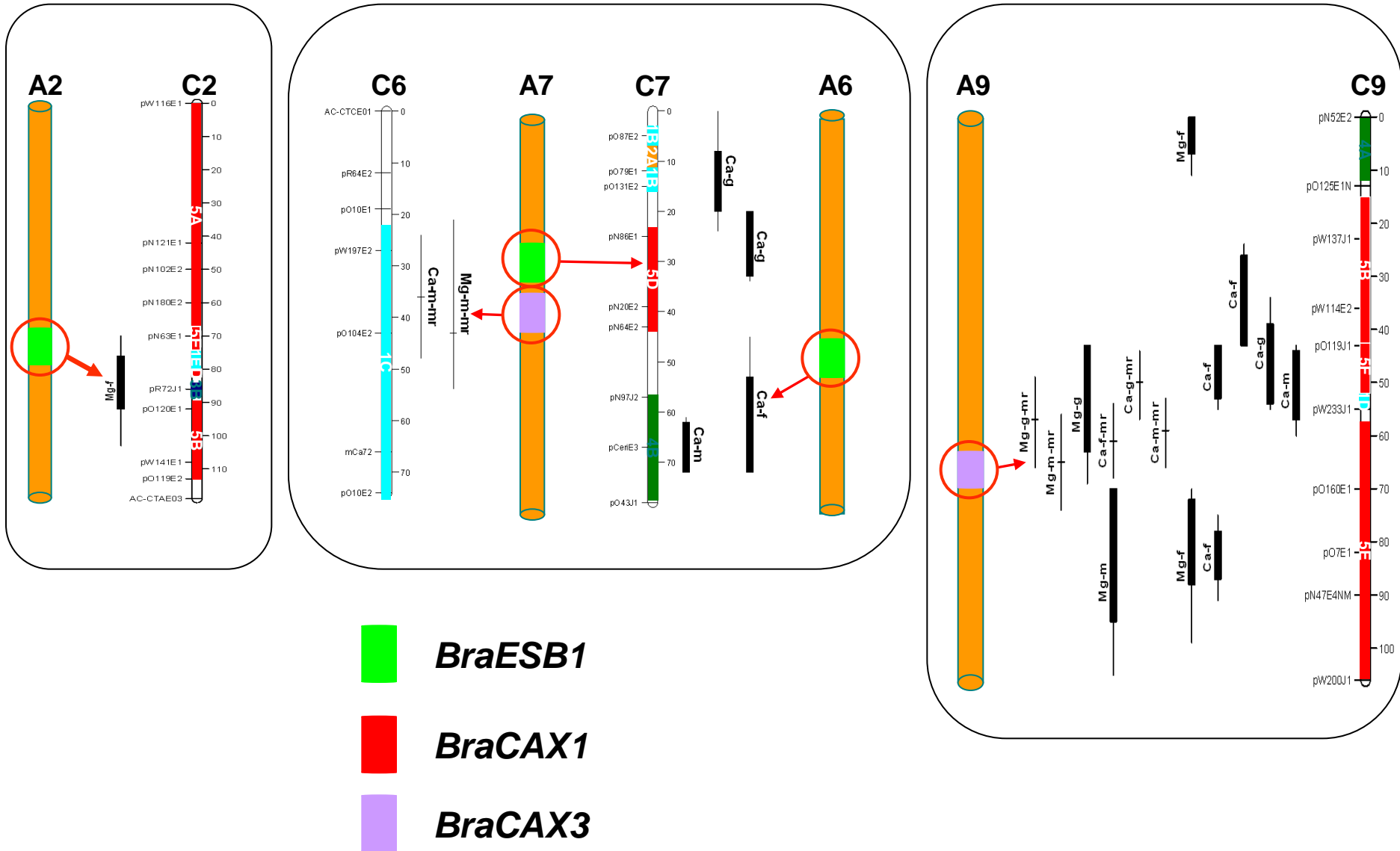
Mg & Ca phenotypes in *Atcax1cax3* (www.ionomicshub.org)



BraCAX3



A-Genome *Brassica*



: i f h \ Y f ' k c f _ '] b ' d f c

A-Genome *Brassica* E TILLING

| Plate Position | Plant Name | Sequence of WT | | | | | | | | | | | | | | Mutation Change | Het/Hom | Missense | Truncations | | Silent | | |
|----------------|------------|----------------|----|----|----|----|----|----|---|---|---|---|---|---|---|-----------------|---------|------------|-----------------|-----------------|-------------|-------------------|-----------------|
| | | -7 | -6 | -5 | -4 | -3 | -2 | -1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | G/A or C/T | Coding Region | Stop | Splice Site | Non-Coding Region | No Change in AA |
| 2:H11 | J130092-B | C | T | C | A | T | A | A | G | T | A | T | C | C | T | A | G/GA | HET | | | | 881G>GA | |
| 2:B4 | J130061-B | T | A | A | G | T | A | T | C | T | G | T | T | T | T | T | C/CT | HET | | | | 860C>CT | |
| 8:A3 | J130352-A | G | G | C | T | T | C | A | G | C | C | A | G | G | T | G | G/GA | HET | 826G>GA:77S>S/N | | | | |
| 8:G3 | J130355-A | A | G | A | A | A | T | G | G | C | G | G | G | G | A | T | G/GA | HET | | | | 545G>GA | |
| 4:E10 | J130183-A | G | A | G | A | G | C | T | G | A | G | A | C | A | T | G | G/A | HOM | | | | 626G>A:10L>L | |
| 4:G10 | J130184-A | C | T | G | C | C | G | C | C | A | T | T | A | T | C | G | C/CT | HET | | | | 800C>CT:68A>A/A | |
| 6:G12 | J130290-A | A | A | A | G | C | T | A | G | C | C | A | T | T | C | T | G/GA | HET | 771G>GA:59A>A/T | | | | |
| 1:D2 | J130006-B | T | A | T | A | A | T | A | C | C | A | A | G | A | G | C | C/CT | HET | | | | 494C>CT | |
| 5:B6 | J130214-B | A | A | A | C | A | T | A | G | C | T | T | T | C | A | T | G/GA | HET | | | | 517G>GA | |
| 8:A7 | J130368-A | T | C | G | T | T | G | A | G | G | A | A | G | A | A | G | G/GA | HET | 673G>GA:26R>R/K | | | | |
| 6:C11 | J130284-A | C | A | A | C | C | A | T | G | A | C | G | G | C | C | A | G/GA | HET | 599G>GA:1M>M/I | | | | |
| 2:A9 | J130081-A | A | A | G | A | A | C | A | G | C | T | C | A | C | A | G | G/GA | HET | 645G>GA:17A>A/T | | | | |
| 7:C5 | J130309-A | C | T | G | A | C | C | T | C | C | G | A | G | T | A | A | C/CT | HET | | | | | 689C>CT:31L>L/L |
| 14:F11 | J130690-B | T | T | T | C | T | C | T | C | T | A | A | T | C | T | C | C/CT | HET | 736C>CT:47S>S/F | | | | |
| 10:H8 | J130472-B | C | A | T | A | C | C | T | G | C | C | G | C | C | A | T | G/GA | HET | 795G>GA:67A>A/T | | | | |
| 16:E6 | J130766-A | G | A | A | G | G | T | T | C | C | A | T | A | C | A | A | C/CT | HET | 708C>CT:38P>P/S | | | | |
| 14:E6 | J130669-A | C | T | G | A | C | C | T | C | C | G | A | G | T | A | A | C/CT | HET | | | | | 689C>CT:31L>L/L |
| 21:F4 | J131000-B | T | T | T | C | C | G | G | C | C | A | T | A | C | C | T | C/CT | HET | 787C>CT:64A>A/V | | | | |
| 19:H2 | J130895-B | A | G | C | C | A | T | T | C | T | T | T | T | T | C | C | C/CT | HET | 777C>CT:61L>L/F | | | | |
| 22:D12 | J131079-B | A | T | G | T | C | T | T | C | T | T | C | T | T | C | G | C/T | HOM | 661C>T:22S>F | | | | |
| 18:C4 | J130853-A | A | G | C | A | T | G | T | C | T | T | C | T | T | C | T | C/T | HOM | 658C>T:21S>F | | | | |
| 28:B3 | J131331-B | C | T | G | T | T | C | A | C | T | A | T | T | T | A | A | C/CT | HET | | | | 421C>CT | |
| 29:C10 | J131408-A | G | A | A | C | A | G | C | G | T | T | C | A | A | A | A | G/GA | HET | | | | 926G>GA | |
| 26:H9 | J131262-B | G | T | T | A | T | T | G | C | G | G | C | T | T | C | A | C/CT | HET | | | | | 818C>CT:74C>C/C |
| 25:B4 | J131191-B | A | G | A | G | T | T | T | C | T | C | T | C | T | A | A | C/T | HOM | 732C>T:46L>F | | | | |
| 28:A11 | J131363-A | T | G | A | C | C | T | C | C | G | A | G | T | A | A | T | C/CT | HET | | 690C>CT:32R>R/X | | | |

Mutant Line

SNP site

Line Zygosity

Protein Truncations

Silent aa changes

A-Genome *Brassica* TILLING

- 19 M₃ and 30 M₂ *Bracax1* (n=149) being genotyped by Cleaved Amplified Polymorphisms markerS (CAPS) and sequencing.
- Backcross mutants with R-O-18 to clean background.
- Progeny analysed for mineral content through Inductively Coupled Plasma Mass Spectrometry (ICP-MS).



Acknowledgements



The University of
Nottingham

Martin Broadley



John Hammond

Helen Bowen



Philip White



Pauline Stephenson

Lars Østergaard



Fran Robson



Graham King



BBSRC Agri-Food Committee IPA



Thank you