

SUPPLEMENTARY INFORMATION

Article title:

Endosperm prevents toxic amounts of Zn from accumulating in the seed embryo – an adaptation to metalliferous sites in metal-tolerant *Biscutella laevigata*

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Table S1. Elemental composition ($\mu\text{g}\cdot\text{g}^{-1}$) of the individual *Biscutella laevigata* seed cross-sections. Errors of analysis are shown in brackets.

| Element | Population | Sample | Whole section | Region of interest (ROI) | | | | | |
|---------|------------|--------|---------------|--------------------------|-----------|------------|-------------|-----------|-------------|
| | | | | Testa | Hilum | Endosperm | Radicle | Hypocotyl | Cotyledon |
| Cr | M_PL2 | 1 | 3.7 (0.1) | 5.2 (0.8) | 4.5 (0.8) | 2.6 (0.7) | 4.1 (0.3) | 3.9 (0.6) | 3.8 (0.1) |
| | | 2 | 3.6 (0.1) | 3.1 (0.9) | 3.1 (0.7) | 2.8 (0.5) | 3.6 (0.5) | 3.4 (0.7) | 3.9 (0.1) |
| | M_PL6 | 1 | 3.7 (0.1) | 3.9 (0.7) | 4.9 (0.7) | 4.3 (0.8) | 3.3 (0.4) | 5.9 (0.7) | 4.0 (0.1) |
| | | 2 | 3.6 (0.1) | 6 (2) | 3.5 (0.7) | 4.1 (0.7) | 3.6 (0.3) | 4.1 (0.6) | 3.9 (0.1) |
| | NM_PL8 | 1 | 3.74 (0.1) | 5.8 (0.7) | 4.8 (0.7) | 5.4 (0.6) | 3.6 (0.3) | 4.6 (0.7) | 3.9 (0.1) |
| | | 2 | 3.75 (0.1) | 4.5 (0.6) | 3.7 (0.8) | 4 (1) | 3.7 (0.4) | 3.2 (0.7) | 4.06 (0.1) |
| | NM_SK14 | 1 | 4.1 (0.1) | 4.7 (0.6) | 3.8 (0.6) | 6.3 (0.8) | 4.6 (0.3) | 5.0 (0.7) | 4.2 (0.2) |
| | | 2 | 3.4 (0.1) | 6 (1) | 4.0 (0.6) | 3.8 (0.6) | 3.7 (0.3) | 3.6 (0.6) | 3.7 (0.1) |
| Ni | M_PL2 | 1 | 0.54 (0.05) | 1 (0.3) | <0.7 | <0.7 | 0.8 (0.1) | <0.6 | 0.60 (0.05) |
| | | 2 | 1.09 (0.06) | <0.8 | <0.7 | <1.1 (0.2) | 1.3 (0.2) | 1.4 (0.3) | 1.08 (0.07) |
| | M_PL6 | 1 | 0.55 (0.03) | <0.7 | <0.7 | 0.7 | 0.7 (0.2) | 0.9 (0.3) | 0.62 (0.04) |
| | | 2 | 0.92 (0.06) | <1.4 | <0.7 | <0.6 | 1.4 (0.1) | 1.0 (0.3) | 1.13 (0.07) |
| | NM_PL8 | 1 | 0.54 (0.04) | 0.7 (0.3) | <0.7 | <0.6 | 0.7 (0.1) | <0.6 | 0.66 (0.05) |
| | | 2 | 0.49 (0.04) | <0.6 | <0.7 | <1.2 | 0.5 (0.2) | 0.7 (0.3) | 0.58 (0.04) |
| | NM_SK14 | 1 | 0.79 (0.04) | 0.5 (0.2) | <0.6 | <0.7 | 1.1 (0.1) | 1.2 (0.3) | 0.90 (0.04) |
| | | 2 | 1.23 (0.05) | <1 | <0.6 | 1.4 (0.3) | 2.4 (0.1) | 2.8 (0.3) | 1.31 (0.04) |
| As | M_PL2 | 1 | 0.08 (0.03) | <0.4 | 0.5 (0.2) | <0.4 | 0.19 (0.08) | <0.3 | <0.07 |
| | | 2 | <0.05 | <0.5 | 0.4 (0.1) | 0.3 (0.1) | <0.2 | <0.4 | <0.07 |
| | M_PL6 | 1 | <0.04 | <0.4 | <0.4 | <0.4 | <0.2 | <0.4 | <0.06 |
| | | 2 | <0.05 | <0.9 | <0.4 | <0.3 | <0.19 | <0.4 | <0.07 |
| | NM_PL8 | 1 | <0.04 | <0.4 | <0.4 | <0.3 | <0.16 | <0.3 | <0.05 |
| | | 2 | <0.04 | <0.3 | <0.4 | <0.6 | <0.21 | <0.4 | <0.05 |
| | NM_SK14 | 1 | 0.10 (0.04) | 0.3 (0.1) | <0.3 | 0.5 (0.2) | <0.14 | <0.3 | 0.13 (0.04) |
| | | 2 | <0.04 | <0.6 | <0.3 | <0.3 | <0.16 | <0.3 | 0.06 (0.04) |
| Co | M_PL2 | 1 | <0.07 | <0.5 | <0.6 | <0.6 | <0.3 | <0.5 | <0.1 |
| | | 2 | <0.08 | <0.7 | <0.5 | <0.4 | <0.4 | <0.6 | 0.21 (0.08) |
| | M_PL6 | 1 | <0.06 | <0.6 | <0.6 | <0.6 | <0.3 | <0.6 | <0.08 |
| | | 2 | <0.07 | <1.2 | <0.6 | <0.5 | <0.3 | <0.6 | <0.1 |
| | NM_PL8 | 1 | 0.12 (0.05) | <0.6 | <0.6 | <0.5 | 0.5 ((0.2) | <0.5 | 0.17 (0.05) |
| | | 2 | <0.06 | <0.5 | <0.6 | 2 (1) | 1.1 (0.2) | 1.2 (0.3) | <0.08 |
| | NM_SK14 | 1 | <0.06 | <0.4 | <0.5 | <0.6 | <0.2 | <0.5 | <0.08 |
| | | 2 | <0.06 | <0.9 | 0.8 (0.5) | <0.5 | <0.2 | <0.5 | <0.07 |

Table S2. *P* values of the Wilcoxon test assessing the differences between the mean elemental composition of seeds from metallicolous and non-metallicolous *Biscutella laevigata*.

| Element | Whole Section | Region of Interest (ROI) | | | | | |
|---------|---------------|--------------------------|-------|--------------|--------------|-----------|--------------|
| | | Testa | Hilum | Endosperm | Radicle | Hypocotyl | Cotyledon |
| Zn | 0.114 | 0.686 | 0.343 | 0.029 | 0.486 | 0.486 | 0.343 |
| S | 1.000 | 0.486 | 0.486 | 0.200 | <i>0.057</i> | 0.343 | 0.886 |
| Cl | <i>0.057</i> | 0.343 | 0.114 | 0.114 | <i>0.057</i> | 0.114 | <i>0.057</i> |
| K | 0.486 | 0.686 | 1.000 | 1.000 | 0.200 | 0.686 | 0.343 |
| Ca | 0.343 | 1.000 | 0.686 | 0.343 | 0.886 | 0.561 | 0.486 |
| Mn | 0.486 | 1.000 | 0.663 | 0.029 | 0.886 | 0.886 | 0.884 |
| Fe | 0.886 | 0.686 | 0.686 | 0.686 | 0.686 | 0.686 | 0.686 |
| Cu | 0.114 | 1.000 | 0.343 | 0.309 | 0.343 | 0.200 | 0.029 |

Table S3 *P* values of the Kruskal-Wallis test assessing the differences between elemental composition of seed tissues within two groups of samples: metallicolous and non-metallicolous *Biscutella laevigata* ecotypes.

| Element | Metallicolous ecotype | Non- metallicolous ecotype |
|---------|-----------------------|----------------------------|
| Zn | 0.004 | 0.037 |
| Cl | 0.422 | 0.200 |
| Ca | 0.005 | 0.018 |
| Fe | 0.026 | 0.425 |
| S | 0.001 | 0.001 |
| K | 0.246 | 0.857 |
| Mn | 0.005 | 0.039 |
| Cu | 0.422 | 0.112 |