Supplementary Material

Efficacy of a Plant-Microbe System: *Pisum sativum* (L.) Cadmium-Tolerant Mutant and *Rhizobium leguminosarum* Strains, Expressing Pea Metallothionein Genes *PsMT1* and *PsMT2*, for Cadmium Phytoremediation

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Supplemental Table 1. Primers used in this study.

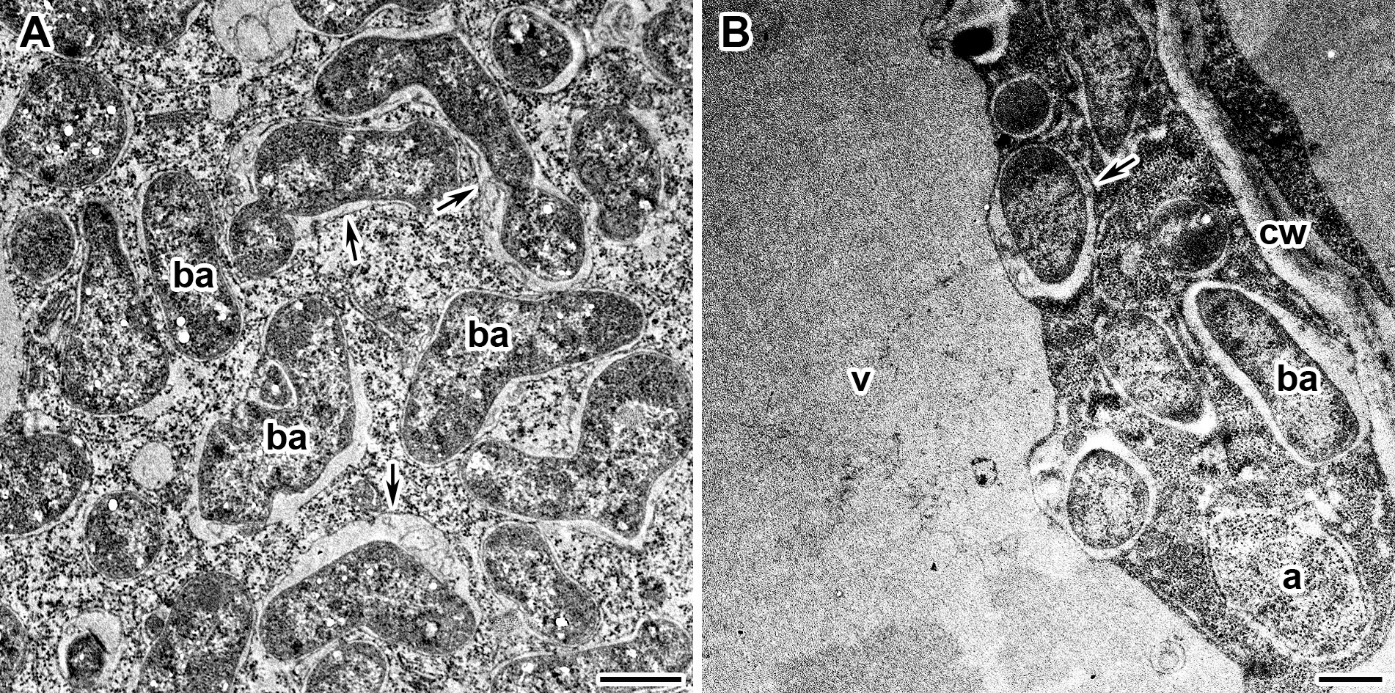
|  |  |
| --- | --- |
| **Primer name** | **Sequence (5’–3’)** |
| MT1-F | ATGTCTGGATGTGGTTGTGGA |
| MT1-R | GCCTCCAATATCTCTGCTTCA |
| MT2-F | ATGTCTTGCTGTGGTGGAAACT |
| MT2-R | ATCCTGCCACTAAACGGGG |
| nifH-F | GGATCCCGTCGTTGCCTGCTG |
| nifH-R | GTTTGGCGTTCCTTCATGTGTTC |
| PsMT-1F | ATGTCTGGATGTGGTTGTGGAAG |
| PsMT-1R | TCATTTGCAGTTGCAAGGGTCA |
| PsMT-2F | TGGAAACTGTGGTTGCGGTACTAG |
| PsMT-2R | TCCACATTTGCAGCCATCATTCTC |

Supplemental Table 2. Content of nutrient elements in shoots of pea genotypes SGE and SGECdt grown in nutrient solution and inoculated with *R. leguminosarum* bv. *viciae* strains 3841, 3841-PsMT1 or 3841-PsMT2, respectively.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatments | B,  ng g-1 DW | Ca,  mg g-1 DW | Co,  µg g-1 DW | Cu,  µg g-1 DW | Fe,  µg g-1 DW | K,  mg g-1 DW | Mg,  mg g-1 DW | Mn,  µg g-1 DW | Mo,  µg g-1 DW | N,  mg g-1 DW | Na,  µg g-1 DW | Ni,  µg g-1 DW | P,  mg g-1 DW | Zn,  µg g-1 DW |
|  | **Untreated plants** | | | | | | | | | | | | | |
| SGE + 3841 | 28 ± 0.8cd | 8,0 ± 0.2abc | 4,4 ± 0.1ab | 84 ± 2d | 73 ± 8cd | 30 ± 0.2cd | 5,1 ± 0.1a | 64 ± 1a | 46 ± 2b | 18.1 ± 0.6abc | 288 ± 12d | 3,6 ± 0.1c | 70 ± 2c | 107 ± 4cd |
| SGECdt + 3841 | 27 ± 0.8cd | 8,6 ± 0.2c | 4,4 ± 0.1ab | 73 ± 3bc | 68 ± 5bc | 30 ± 0.6cd | 5,1 ± 0.2a | 67 ± 3a | 37 ± 1a | 17.8 ± 0.5abc | 262 ± 20cd | 3,5 ± 0.1bc | 58 ± 3b | 102 ± 2c |
| SGE + 3841-PsMT1 | 26 ± 0.8bc | 8,3 ± 0.2bc | 4,4 ± 0.1ab | 72 ± 2ab | 72 ± 5cd | 28 ± 0.7b | 5,2 ± 0.1a | 66 ± 2a | 32 ± 2a | 17.4 ± 0.7ab | 248 ± 12bcd | 3,4 ± 0.1bc | 59 ± 2b | 86 ± 3bc |
| SGECdt + 3841-PsMT1 | 26 ± 0.6bc | 8,4 ± 0.3bc | 4,2 ± 0.1ab | 72 ± 3ab | 69 ± 8c | 28 ± 0.9b | 5,0 ± 0.2a | 65 ± 2a | 38 ± 3a | 17.7 ± 0.9abc | 246 ± 19bcd | 3,2 ± 0.1bc | 58 ± 3b | 91 ± 3bc |
| SGE + 3841-PsMT2 | 33 ± 1.6e | 7,3 ± 0.4ab | 4,4 ± 0.1a | 79 ± 1bcd | 58 ± 4abc | 28 ± 0.3b | 5,4 ± 0.1ab | 68 ± 3ab | 36 ± 2a | 19.7 ± 0.7bc | 273 ± 7cd | 3,4 ± 0.1bc | 62 ± 1bc | 93 ± 3bc |
| SGECdt + 3841-PsMT2 | 36 ± 1.0e | 7,3 ± 0.4ab | 4,4 ± 0.2ab | 75 ± 2bc | 55 ± 7abc | 29 ± 0.6bc | 5,6 ± 0.3bc | 83 ± 3c | 37 ± 2a | 19.0 ± 1.4bc | 294 ± 32d | 3,4 ± 0.3bc | 59 ± 3b | 96 ± 2bc |
|  | **Plants treated with 0.5 µM CdCl2** | | | | | | | | | | | | | |
| SGE + 3841 | 27 ± 1.7bcd | 7,5 ± 0.5ab | 4,5 ± 0.1bc | 75 ± 2bcd | 86 ± 5d | 31 ± 0.5cd | 6,0 ± 0.2cd | 105 ± 4de | 36 ± 3a | 19.8 ± 0.4c | 252 ± 15bcd | 3,3 ± 0.1bc | 60 ± 2b | 106 ± 7cd |
| SGECdt + 3841 | 24 ± 1.3abc | 8,0 ± 0.5a | 4,1 ± 0.1ab | 70 ± 4ab | 58 ± 3abc | 27 ± 1.2ab | 5,6 ± 0.2bc | 101 ± 5de | 34 ± 3a | 19.9 ± 1.0c | 171 ± 15a | 2,7 ± 0.1ab | 56 ± 4ab | 97 ± 7c |
| SGE + 3841-PsMT1 | 18 ± 1.1a | 7,2 ± 0.5a | 4,3 ± 0.2ab | 65 ± 4ab | 56 ± 5abc | 28 ± 1.2b | 6,1 ± 0.3cd | 90 ± 6cd | 38 ± 3a | 18.6 ± 0.7bc | 198 ± 15ab | 2,8 ± 0.2ab | 51 ± 4ab | 73 ± 4a |
| SGECdt + 3841-PsMT1 | 23 ± 0.8b | 8,3 ± 0.5bc | 3,9 ± 0.2b | 64 ± 3a | 50 ± 3a | 25 ± 0.8a | 5,7 ± 0.3bcd | 98 ± 6de | 34 ± 4a | 16.2 ± 0.3a | 160 ± 12a | 2,5 ± 0.1a | 49 ± 3a | 73 ± 4a |
| SGE + 3841-PsMT2 | 24 ± 1.0b | 8,1 ± 0.5abc | 4,3 ± 0.1a | 72 ± 2b | 69 ± 5c | 32 ± 0.7d | 6,2 ± 0.2de | 106 ± 7e | 38 ± 1a | 17.9 ± 0.9abc | 155 ± 8a | 2,8 ± 0.1ab | 59 ± 2b | 89 ± 3bc |
| SGECdt + 3841-PsMT2 | 30 ± 1.1d | 7,6 ± 0.2ab | 5,0 ± 0.3c | 81 ± 5cd | 76 ± 6cd | 32 ± 0.8d | 6,7 ± 0.2e | 144 ± 7f | 47 ± 3b | 18.2 ± 0.7abc | 230 ± 38bc | 3,5 ± 0.3bc | 65 ± 5bc | 114 ± 4d |
|  | **Average for all inoculation treatments** | | | | | | | | | | | | | |
| Untreated SGE | 29 ± 1.0# | 7,9 ± 0.2\* | 4,4 ± 0.1\* | 78 ± 2# | 68 ± 4\* | 29 ± 0.4\* | 5,2 ± 0.1\* | 66 ± 1\* | 38 ± 2\* | 18.4 ± 0.4\* | 270 ± 7# | 3,5 ± 0.1# | 64 ± 2# | 95 ± 3\* |
| Untreated SGECdt | 30 ± 1.2# | 8,1 ± 0.2\* | 4,3 ± 0.1\* | 73 ± 2\*# | 64 ± 4\* | 29 ± 0.5\* | 5,3 ± 0.1\* | 72 ± 3\* | 37 ± 1\* | 18.1 ± 0.6\* | 267 ± 14# | 3,4 ± 0.1# | 58 ± 2\*# | 96 ± 2\* |
| Cd-treated SGE | 23 ± 1.2\* | 7,6 ± 0.3\* | 4,4 ± 0.1\* | 71 ± 2\* | 70 ± 4\* | 30 ± 0.6\* | 6,1 ± 0.1# | 101 ± 4# | 37 ± 1\* | 18.8 ± 0.4\* | 202 ± 13\* | 3,0 ± 0.1\* | 56 ± 2\* | 89 ± 4\* |
| Cd-treated SGECdt | 26 ± 1.0\* | 8,0 ± 0.2\* | 4,3 ± 0.2\* | 72 ± 3\* | 62 ± 4\* | 28 ± 0.9\* | 6,0 ± 0.2# | 114 ± 7@ | 38 ± 2\* | 18.2 ± 0.6\* | 187 ± 16\* | 2,9 ± 0.2\* | 57 ± 3\* | 94 ± 5\* |

Different letters show significant differences between treatments within sub columns for untreated and Cd-treated plants, whereas different symbols show significant differences between average values for all inoculation treatments (least significant difference test, *P* < 0.05, n = 5). De data are means ± SE. DW stands for dry weight.

**SUPPLEMENTAL FIGURE S1** Ultrastructural organization of untreated nodules of wild-type SGE inoculated with *R. leguminosarum* bv. *viciae* strain 3841. (**A**) An infected cell from the nitrogen fixation zone. (**B**) An infected cell from the infection zone. ba, bacteroid; cw, cell wall; v, vacuole; a, amyloplast; arrows indicate symbiosome membrane. Scale bar = 500 nm.



**SUPPLEMENTAL FIGURE S2** Accumulation of the electron dense crystals in vacuole in the cadmium treated nodules of wild-type SGE (**A,C,E**) and mutant SGECdt (**B,D,F**) inoculated with *R. leguminosarum* bv. *viciae* strain 3841 (**A,B**), 3841-MT1 (**C,D**) and 3841-MT2 (**E,F**). ba, bacteroid; b, bacterium; v, vacuole; triangles indicate electron dense crystals in vacuole. Scale bar = 500 nm.

