

Table S1. Primer sequences used in this study

| Name | Forward (5'- 3') | Reverse (5'- 3') | Applications |
|---------------------------------|--------------------------------------|--------------------------------------|--------------|
| <i>PcNRAMP1</i> | TGAAAATCCGCAAATCGC | TCCACAAAGATGTTCCGGTCAT | RT-qPCR |
| <i>PcNRAMP2</i> | AGGATTATTCCGATGAAGTAGC | GTTGCCCACAGTAAGAGCC | |
| <i>PcNRAMP3.1</i> | CTCCTGGTGCTGTCAATCTAA | GGCACGCCTGGAAAGAT | |
| <i>PcNRAMP3.2</i> | AGCTTTTCAAACATAACGGGAG | TCTGATGTCATCGCTTAGCAA | |
| <i>PcNRAMP4</i> | TGGGAATTATTGGCATCAACA | TCTGAACACCCCTCTATTATGGC | |
| <i>PcNRAMP5</i> | GCCCACTTGTCGCTTTACT | GTGGATTGACAACCCGAAG | |
| <i>PcNRAMP6.1</i> | ATGGCAATCTATATCCTAGCAG | CAAGTCCGTTTTCCATGTTA | |
| <i>PcNRAMP6.2</i> | CACCACTATCAAATCGAGGTT | TTTTCAAGGCAATAGCAAT | |
| <i>PcActin2/7</i> | CCCATTGAGCACGGTATTGT | TACGACCACTGGCATAACAGG | |
| <i>PcEF1α</i> | TCCCTTTGTCCCAATCTCTG | ACATCCTGAAGTGGGAGACG | |
| <i>PcNRAMP1</i> | ATGGCGGGTTCGTCTTCTGT | CAAATCATTTGCATTACTCCTC | Clone |
| <i>GW-PcNRAMP1</i> | GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGC | GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACAA | Gateway |
| | GGGTTCGTCTTCTGT | ATCATTTGCATTACTC | |

Table S1. Continued

| Name | Forward (5'- 3') | Reverse (5'- 3') | Applications |
|----------------------------------|--------------------------------------|--------------------------------------|-----------------------------------|
| <i>35S-PcNRAMP1</i> | TCCATTGCCCAGCTATCTGTCAC | ACTCATACTTGTATTGCGCTCCT | |
| <i>PcNRAMP1</i> -RT | GCACCATTGATTGAGAATTCAGA | TCATACTTGTATTGCGCTCCT | The selection of transgenic lines |
| <i>PcActin</i> | TCATCGGAATGGAAGCTGCTGGTA | TCATCGGAATGGAAGCTGCTGGTA | |
| <i>PcNRAMP1</i> ^{D61A} | TTGTTTCAATTGCATACATTGCCCCTGG | GCAATGTATGCAATTGAAACAAGGAACCCGGGA | |
| <i>PcNRAMP1</i> ^{G63A} | GCATACATTGACCCTGCAAATTTTGAAACTGACCT | GCAGGGTCAATGTATGCAATTGAAACAAGGA | |
| | GC | | Site-directed mutations |
| <i>PcNRAMP1</i> ^{M236A} | CACTTCTCGGTGCCATGGTTGCGCCGCACAATCTC | GAATGGAGATTGTGCGGCGCAACCATGGCACCGAGA | |
| | CATTC | AGTG | |
| <i>PcNRAMP1</i> ^{P405A} | CGATGATTCTATCTTTTGAGCTCGCTTTTGCTCTCG | CGAGCTCAAAAAGATAGAATCATCGATGCAATAATG | |

Table S2. Accession numbers of *NRAMP* genes presented in Figure. 1.

| Gene Name | Locus names or Accession nos. or AGI codes | Species |
|-------------------|--|-----------------------------|
| <i>AtNRAMP1</i> | AT1G80830 | <i>Arabidopsis thaliana</i> |
| <i>AtNRAMP2</i> | AT1G47240 | <i>A. thaliana</i> |
| <i>AtNRAMP3</i> | AT2G23150 | <i>A. thaliana</i> |
| <i>AtNRAMP4</i> | AT5G67330 | <i>A. thaliana</i> |
| <i>AtNRAMP5</i> | AT4G18790 | <i>A. thaliana</i> |
| <i>AtNRAMP6</i> | AT1G15960 | <i>A. thaliana</i> |
| <i>OsNRAMP1</i> | Os07g0258400 | <i>Oryza sativa</i> |
| <i>OsNRAMP2</i> | Os03g0208500 | <i>O. sativa</i> |
| <i>OsNRAMP3</i> | Os06g0676000 | <i>O. sativa</i> |
| <i>OsNRAT1</i> | Os02g0131800 | <i>O. sativa</i> |
| <i>OsNRAMP5</i> | Os07g0257200 | <i>O. sativa</i> |
| <i>OsNRAMP6</i> | Os01g0503400 | <i>O. sativa</i> |
| <i>OsNRAMP7</i> | Os12g0581600 | <i>O. sativa</i> |
| <i>PcNRAMP1</i> | Spta717-Potri.001G044900 | <i>Populus × canescens</i> |
| <i>PcNRAMP2</i> | Spta717-Potri.002G121000 | <i>P. × canescens</i> |
| <i>PcNRAMP3.1</i> | Spta717-Potri.007G050600 | <i>P. × canescens</i> |
| <i>PcNRAMP3.2</i> | Spta717-Potri.007G050700 | <i>P. × canescens</i> |
| <i>PcNRAMP4</i> | Spta717-Potri.002G080400 | <i>P. × canescens</i> |
| <i>PcNRAMP5</i> | Spta717-Potri.002G080500 | <i>P. × canescens</i> |
| <i>PcNRAMP6.1</i> | Spta717-Potri.005G181000 | <i>P. × canescens</i> |
| <i>PcNRAMP6.2</i> | Spta717-Potri.005G181100 | <i>P. × canescens</i> |
| <i>PtNRAMP1</i> | Potri.001G044900 | <i>Populus trichocarpa</i> |
| <i>PtNRAMP2</i> | Potri.002G121000 | <i>P. trichocarpa</i> |
| <i>PtNRAMP3.1</i> | Potri.007G050600 | <i>P. trichocarpa</i> |
| <i>PtNRAMP3.2</i> | Potri.007G050700 | <i>P. trichocarpa</i> |

Table S2. Continued

| Gene Name | Locus names or Accession nos. or AGI codes | Species |
|-------------------|--|-----------------------|
| <i>PtNRAMP4</i> | Potri.002G080400 | <i>P. trichocarpa</i> |
| <i>PtNRAMP5</i> | Potri.002G080500 | <i>P. trichocarpa</i> |
| <i>PtNRAMP6.1</i> | Potri.005G181000 | <i>P. trichocarpa</i> |
| <i>PtNRAMP6.2</i> | Potri.005G181100 | <i>P. trichocarpa</i> |

Table S3. Characteristics of the sequences of *PcNRAMP* genes and their proteins

| Gene name | Accession number | Chromosomal location | Length of CDS (bp) | Number of amino acids (aa) | Molecular weight (KDa) | Theoretical pI | Grand average of hydropathicity | Transmembrane helices |
|-------------------|------------------|----------------------|--------------------|----------------------------|------------------------|----------------|---------------------------------|-----------------------|
| <i>PcNRAMP1</i> | Potri.001G044900 | 1 | 1626 | 541 | 58.65 | 8.6 | 0.571 | 12 |
| <i>PcNRAMP2</i> | Potri.002G121000 | 2 | 1611 | 536 | 58.61 | 5.19 | 0.496 | 11 |
| <i>PcNRAMP3.1</i> | Potri.007G050600 | 7 | 1518 | 505 | 55.51 | 5.01 | 0.65 | 11 |
| <i>PcNRAMP3.2</i> | Potri.007G050700 | 7 | 1503 | 500 | 54.64 | 4.93 | 0.667 | 11 |
| <i>PcNRAMP4</i> | Potri.002G080400 | 2 | 1659 | 552 | 60.1 | 8.23 | 0.442 | 12 |
| <i>PcNRAMP5</i> | Potri.002G080500 | 2 | 1641 | 546 | 58.96 | 7.06 | 0.548 | 12 |
| <i>PcNRAMP6.1</i> | Potri.005G181000 | 5 | 1746 | 581 | 63.4 | 7.56 | 0.512 | 12 |
| <i>PcNRAMP6.2</i> | Potri.005G181100 | 5 | 1758 | 585 | 63.95 | 8.42 | 0.463 | 10 |

Table S4. Biomass of WT and transgenic lines (*PcNRAMP1*-OE5, *PcNRAMP1*-OE8, *PcNRAMP1*-OE9) of *P. × canescens* treated with either 0 (–Cd) or 100 (+Cd) μ M CdCl₂ for 2 weeks. Data indicate means \pm SE (n = 4). Different letters after the values indicate significant differences between the treatments. *P*-values of the two-way ANOVAs of genotype (G), Cd and their interactions (G \times Cd) are also indicated. *: *P*<0.05; **: *P*<0.01; ***: *P*<0.001; ****: *P*<0.0001; ns: not significant.

| Genotype | Cd (μ M) | Root | Wood | Bark | Leaf | Whole plant |
|----------------------|------------------|--------------------|--------------------|-------------------|-------------------|--------------------|
| WT | 0 | 0.64 \pm 0.08 b | 1.83 \pm 0.48 c | 1.38 \pm 0.10 a | 7.50 \pm 0.29 a | 11.34 \pm 0.73 a |
| | 100 | 0.31 \pm 0.01a | 1.00 \pm 0.13 ab | 0.72 \pm 0.01 b | 5.62 \pm 0.27 b | 7.69 \pm 0.34 b |
| <i>PcNRAMP1</i> -OE5 | 0 | 0.58 \pm 0.17 ab | 1.97 \pm 0.48 c | 1.34 \pm 0.12 a | 7.58 \pm 1.2 a | 11.47 \pm 1.74 a |
| | 100 | 0.37 \pm 0.02 ab | 0.84 \pm 0.04 a | 0.73 \pm 0.07 b | 5.42 \pm 0.16 b | 7.34 \pm 0.06 b |
| <i>PcNRAMP1</i> -OE8 | 0 | 0.53 \pm 0.03 ab | 1.71 \pm 0.06 bc | 1.50 \pm 0.20 a | 7.31 \pm 0.62 a | 11.05 \pm 0.51 a |
| | 100 | 0.33 \pm 0.03 a | 0.95 \pm 0.05 ab | 0.61 \pm 0.04 b | 5.26 \pm 0.26 b | 7.15 \pm 0.21 b |
| <i>PcNRAMP1</i> -OE9 | 0 | 0.60 \pm 0.14 b | 2.26 \pm 0.22 c | 1.40 \pm 0.48 a | 7.42 \pm 0.23 a | 11.80 \pm 0.66 a |
| | 100 | 0.33 \pm 0.02 a | 0.77 \pm 0.08 a | 0.66 \pm 0.06 b | 5.33 \pm 0.28 b | 7.08 \pm 0.40 b |
| <i>P</i> -values | G | ns | ns | ns | ns | ns |
| | Cd | ** | **** | **** | **** | **** |
| | G x Cd | ns | ns | ns | ns | ns |

Table S5. Concentrations of Mn, Fe, Zn, Ca and Mg in different tissues of WT and transgenic lines (*PcNRAMP1*-OE5, *PcNRAMP1*-OE8, *PcNRAMP1*-OE9) of *P. × canescens* treated with either 0 (–Cd) or 100 (+Cd) μ M CdCl₂ for 2 weeks. Data indicate means \pm SE (n = 4). Different letters after the values indicate significant differences between the treatments. *P*-values of the two-way ANOVAs of genotype (G), Cd and their interactions (G \times Cd) are also indicated. *: *P*<0.05; **: *P*<0.01; ***: *P*<0.001; ****: *P*<0.0001; ns: not significant.

| Genotype | Cd | Root | | | | | Wood | | | | |
|----------------------|---------------|-------------------------------|-------------------------------|-------------------------------|-------------------------|-------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------|-------------------------|
| | | Mn | Fe | Zn | Ca | Mg | Mn | Fe | Zn | Ca | Mg |
| | | (μ g g ⁻¹ DW) | (μ g g ⁻¹ DW) | (μ g g ⁻¹ DW) | (mg g ⁻¹ DW) | (mg g ⁻¹ DW) | (μ g g ⁻¹ DW) | (μ g g ⁻¹ DW) | (μ g g ⁻¹ DW) | (mg g ⁻¹ DW) | (mg g ⁻¹ DW) |
| WT | 0 | 14.11 \pm 1.94 a | 275.80 \pm 48.88 a | 30.87 \pm 4.03 ab | 1.75 \pm 0.53 a | 6.50 \pm 0.28 c | 2.18 \pm 0.22 a | 152.73 \pm 17.52 a | 12.60 \pm 0.94 a | 0.75 \pm 0.01 ab | 0.46 \pm 0.06 a |
| | 100 | 11.28 \pm 4.09 a | 570.98 \pm 31.70 b | 48.05 \pm 5.02 cd | 4.20 \pm 0.14 c | 1.03 \pm 0.08 ab | 0.46 \pm 0.07 a | 136.65 \pm 3.52 a | 13.69 \pm 3.08 ab | 0.65 \pm 0.01 a | 0.36 \pm 0.06 a |
| <i>PcNRAMP1</i> -OE5 | 0 | 156.19 \pm 9.49 b | 691.88 \pm 117.55 b | 40.63 \pm 3.29 bc | 1.62 \pm 0.11 a | 0.70 \pm 0.05 a | 47.62 \pm 12.58 b | 338.37 \pm 18.50 b | 18.42 \pm 1.36 cd | 0.97 \pm 0.03 c | 0.33 \pm 0.03 a |
| | 100 | 9.62 \pm 0.89 ab | 1041.06 \pm 61.22 c | 27.00 \pm 2.98 a | 3.09 \pm 0.16 b | 0.71 \pm 0.01 a | 1.92 \pm 0.2 a | 126.93 \pm 9.01 a | 14.36 \pm 0.94 ab | 0.87 \pm 0.04 bc | 0.42 \pm 0.08 a |
| <i>PcNRAMP1</i> -OE8 | 0 | 183.90 \pm 8.66 bc | 708.94 \pm 33.50 b | 48.62 \pm 0.7 cd | 1.62 \pm 0.05 a | 0.94 \pm 0.03 ab | 46.24 \pm 0.99 b | 298.96 \pm 19.18 b | 21.75 \pm 0.50 de | 1.16 \pm 0.05 d | 0.34 \pm 0.03 a |
| | 100 | 4.24 \pm 1.19 a | 927.35 \pm 18.83 c | 37.5 \pm 0.47 ab | 3.23 \pm 0.16 b | 0.73 \pm 0.02 a | 0.8 \pm 0.14 a | 124.11 \pm 0.23 a | 18.24 \pm 0.78 bc | 0.91 \pm 0.01 c | 0.44 \pm 0.05 a |
| <i>PcNRAMP1</i> -OE9 | 0 | 171.68 \pm 7.65 c | 713.26 \pm 61.33 b | 56.61 \pm 2.18 d | 1.62 \pm 0.18 a | 1.17 \pm 0.01 b | 58.01 \pm 2.62 b | 303.47 \pm 8.10 b | 24.95 \pm 1.35 e | 1.36 \pm 0.12 e | 0.35 \pm 0.04 a |
| | 100 | 9.80 \pm 0.98 ab | 1106.96 \pm 71.60 c | 47.99 \pm 3.63 cd | 3.37 \pm 0.18 b | 0.74 \pm 0.04 a | 1.16 \pm 0.4 a | 124.89 \pm 1.97 a | 19.88 \pm 0.61 d | 0.96 \pm 0.03 c | 0.47 \pm 0.02 a |
| <i>P</i> -values | G | **** | **** | ** | * | ** | **** | **** | **** | **** | ns |
| | Cd | **** | **** | ns | **** | *** | **** | **** | * | **** | ns |
| | G \times Cd | **** | ns | ** | ns | ** | **** | **** | ns | * | ns |

Table S5. Continued

| Genotype | Cd | Bark | | | | | Leaf | | | | |
|----------------------|-------------------|----------------------------|----------------------------|----------------------------|--------------------------|--------------------------|----------------------------|----------------------------|----------------------------|--------------------------|--------------------------|
| | | Mn | Fe | Zn | Ca | Mg | Mn | Fe | Zn | Ca | Mg |
| | (μM) | ($\mu\text{g g}^{-1}$ DW) | ($\mu\text{g g}^{-1}$ DW) | ($\mu\text{g g}^{-1}$ DW) | (mg g^{-1} DW) | (mg g^{-1} DW) | ($\mu\text{g g}^{-1}$ DW) | ($\mu\text{g g}^{-1}$ DW) | ($\mu\text{g g}^{-1}$ DW) | (mg g^{-1} DW) | (mg g^{-1} DW) |
| WT | 0 | 13.8 \pm 1.56 a | 140.21 \pm 9.05 a | 136.48 \pm 16.39 a | 12.85 \pm 1.57 b | 2.73 \pm 0.55 b | 28.21 \pm 1.77 b | 240.54 \pm 3.77 bc | 53.24 \pm 1.20 ab | 7.04 \pm 0.96 d | 3.48 \pm 0.43 d |
| | 100 | 3.75 \pm 0.15 a | 108.72 \pm 7.92 a | 114.49 \pm 11.36 a | 6.82 \pm 0.169 a | 2.37 \pm 0.52 ab | 7.97 \pm 0.91 a | 228.60 \pm 9.76 b | 37.49 \pm 2.58 a | 2.60 \pm 0.04 a | 1.42 \pm 0.26 ab |
| <i>PcNRAMP1</i> -OE5 | 0 | 332.66 \pm 36.57 b | 775.91 \pm 31.92 b | 155.03 \pm 11.01 ab | 11.84 \pm 0.92 b | 1.78 \pm 0.03 ab | 51.91 \pm 12.71 c | 283.50 \pm 24.19 cd | 71.94 \pm 9.51 cd | 7.91 \pm 0.74 d | 2.25 \pm 0.35 c |
| | 100 | 16.58 \pm 1.71 a | 155.24 \pm 0.09 a | 121.34 \pm 16.22 a | 7.75 \pm 0.34 a | 1.51 \pm 0.16 a | 21.15 \pm 1.57 ab | 171.94 \pm 20.98 a | 40.74 \pm 4.42 a | 3.56 \pm 0.07 ab | 1.26 \pm 0.01 a |
| <i>PcNRAMP1</i> -OE8 | 0 | 337.68 \pm 6.96 b | 778.16 \pm 43.45 b | 193.79 \pm 7.05 bc | 11.87 \pm 0.48 b | 1.75 \pm 0.04 ab | 54.03 \pm 7.15 c | 298.65 \pm 4.06 d | 82.7 \pm 4.02 de | 7.38 \pm 0.65 d | 2.13 \pm 0.24 bc |
| | 100 | 7.94 \pm 1.94 a | 145.22 \pm 5.73 a | 120.39 \pm 10.85 a | 7.93 \pm 0.06 a | 1.46 \pm 0.13 a | 12.35 \pm 1.27 a | 171.62 \pm 13.36 a | 66.7 \pm 2.23 bc | 4.22 \pm 0.19 ab | 1.27 \pm 0.02 a |
| <i>PcNRAMP1</i> -OE9 | 0 | 359.33 \pm 20.97 b | 715.89 \pm 19.65 b | 226.96 \pm 11.50 c | 11.90 \pm 0.15 b | 1.72 \pm 0.05 ab | 51.38 \pm 5.22 c | 308.29 \pm 18.45 d | 93.45 \pm 6.70 e | 6.86 \pm 1.32 cd | 2.01 \pm 0.20 b |
| | 100 | 9.35 \pm 0.45 a | 150.20 \pm 9.51 a | 119.45 \pm 6.72 a | 8.45 \pm 0.30 a | 1.41 \pm 0.15 a | 14.31 \pm 0.57 ab | 180.62 \pm 21.12 a | 85.69 \pm 8.68 de | 4.88 \pm 0.40 bc | 1.28 \pm 0.06 a |
| <i>P</i> -values | G | **** | **** | ** | ns | * | * | ns | **** | ns | * |
| | Cd | **** | **** | **** | **** | ns | **** | **** | *** | **** | **** |
| | G \times Cd | **** | **** | ** | ns | ns | ns | ** | ns | ns | ns |

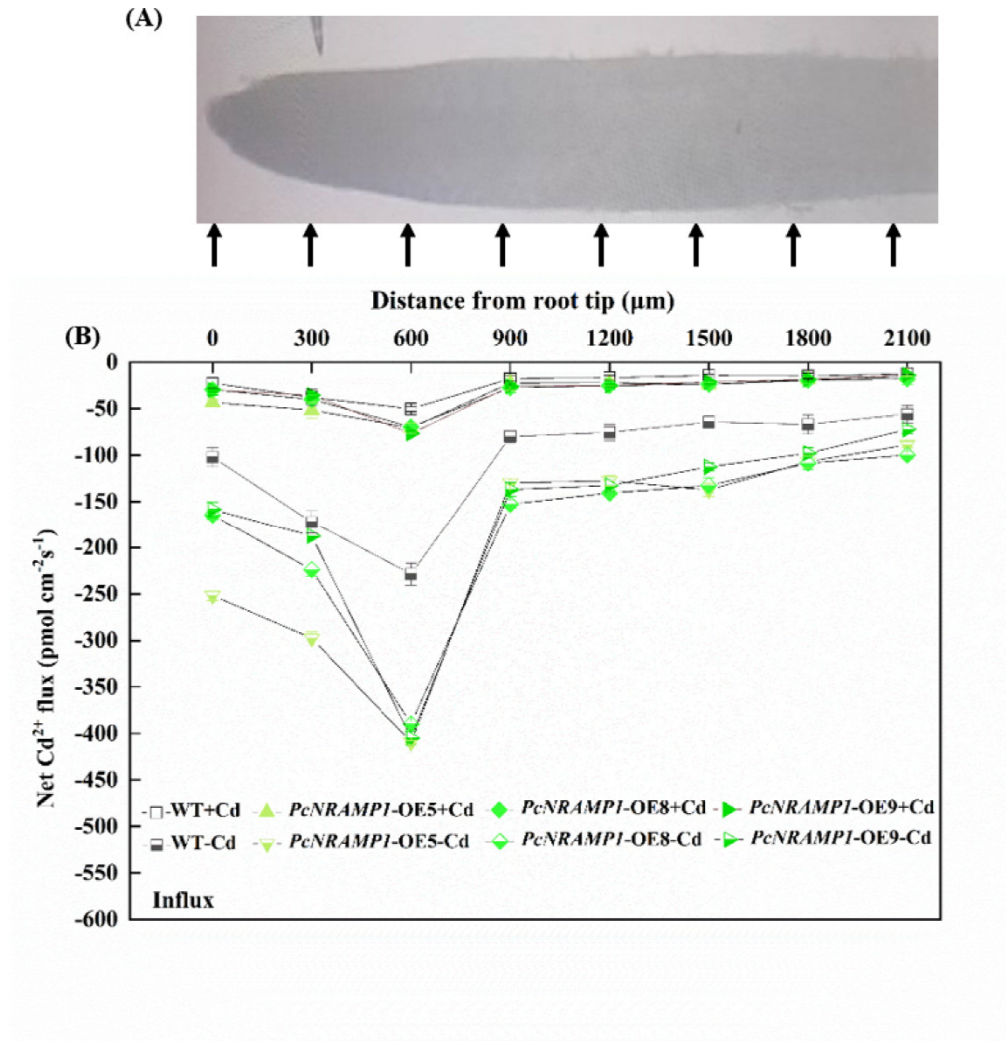


Figure S1. Root tip and net Cd^{2+} fluxes along root apices. (A-B) Root tip and net Cd^{2+} fluxes along root apices of WT and transgenic lines ($PcNRAMP1\text{-OE5}$, $PcNRAMP1\text{-OE8}$, $PcNRAMP1\text{-OE9}$) of *P. × canescens* exposed to either 0 (–Cd) or 100 (+Cd) μM CdCl_2 for 2 weeks. Data indicate means \pm SE ($n = 4$).

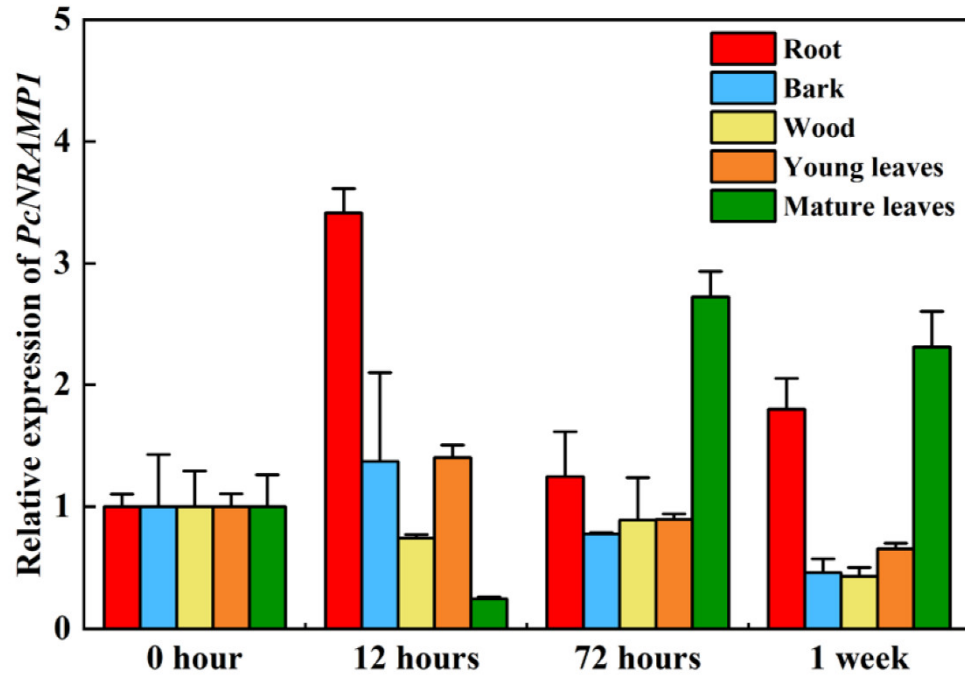


Figure S2. The expression levels of *PcNRAMP1* in different tissues of *P. × canescens* exposed to Cd for different times. Bars indicate means \pm SE (n = 4). The expression level of *PcNRAMP1* was set to 1 in different tissues at the beginning of the treatment (0 hour), and the corresponding fold changes were calculated at the other time points.

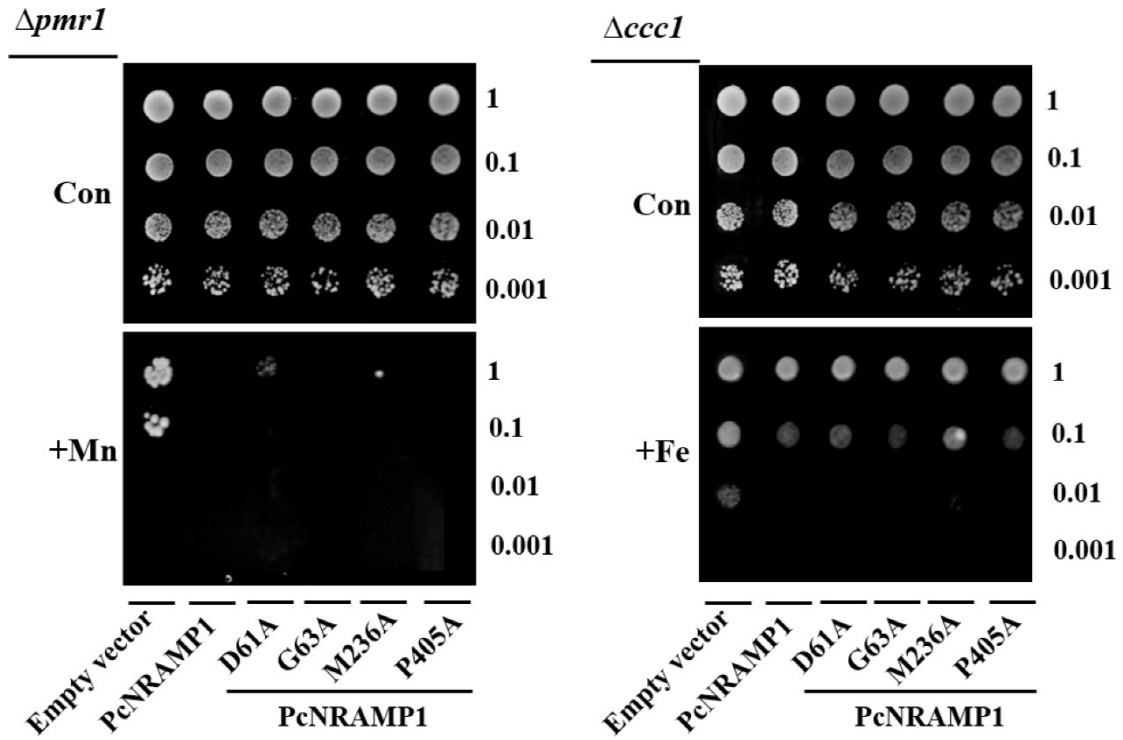


Figure S3. The growth status of yeast mutant strains $\Delta pmr1$ and $\Delta ccc1$ cells transformed with empty vector (pYES2), *PcNRAMP1*, or the mutated *PcNRAMP1*. The growth status of yeast mutant strains $\Delta pmr1$ and $\Delta ccc1$ hypersensitive to excess Mn and Fe, respectively, cells in which empty vector (pYES2), *PcNRAMP1*, or the mutated *PcNRAMP1* ($PcNRAMP1^{D61A}$, $PcNRAMP1^{G63A}$, $PcNRAMP1^{M236A}$ and $PcNRAMP1^{P405A}$) was transformed. The yeast cells diluted with OD600 nm of 1-0.001 were cultured in the plate with galactose under either control (Con) or other bivalent metals including 1 (+Mn) mM $MnSO_4$ and 8 (+Fe) mM $FeSO_4$ for 4 days.

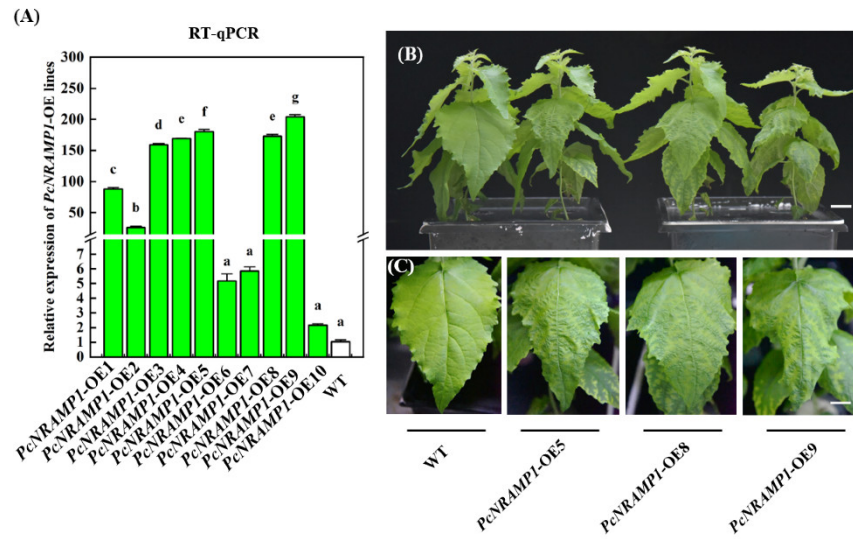


Figure S4. The selection of transgenic lines and the phenotypes of WT and poplars overexpressing *PcNRAMP1*. (A) The selection of transgenic lines. (B-C) The phenotypes of the aboveground plant and mature leaves (LPI=8) of WT and *PcNRAMP1*-overexpressed *P. × canescens* supplied with one-fourth Hoagland solution for 1 month. Scale bars in panels B and C indicate 5 and 2 cm, respectively.

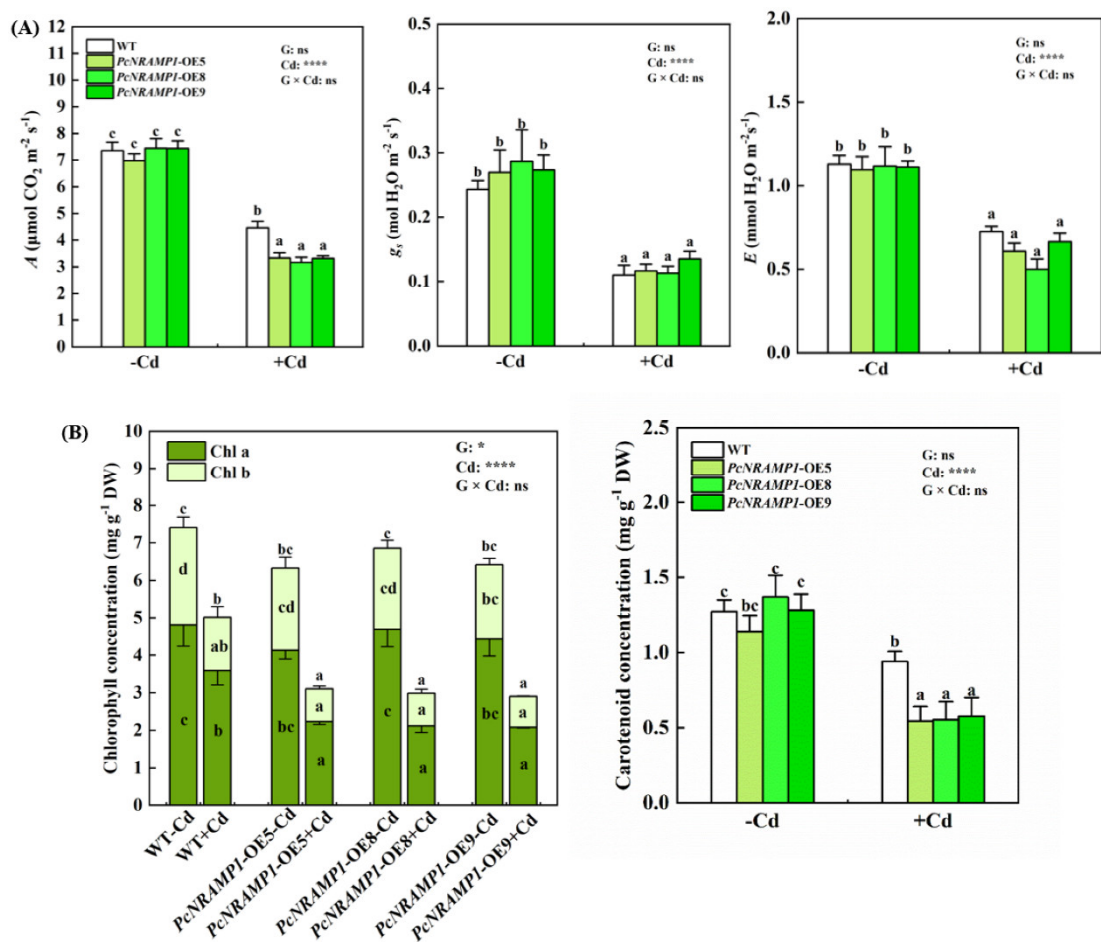


Figure S5. Photosynthesis and chlorophyll concentrations of WT and transgenic lines. (A-B) The photosynthetic characteristics and chlorophyll concentrations of WT and *PcNRAMP1*-OE5, *PcNRAMP1*-OE8, *PcNRAMP1*-OE9 of *P. × canescens* treated with either 0 (–Cd) or 100 (+Cd) μM CdCl_2 for 2 weeks. Data indicate means \pm SE ($n = 4$). Different letters on the bars indicate significant differences between the treatments. *P*-values of the two-way ANOVAs of genotype (G), Cd and their interactions ($G \times \text{Cd}$) are also indicated. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$; ns: not significant.