How provenance and cultivation method shape morphological and physiological traits in the nickel hyperaccumulator *Odontarrhena chalcidica*

Authors

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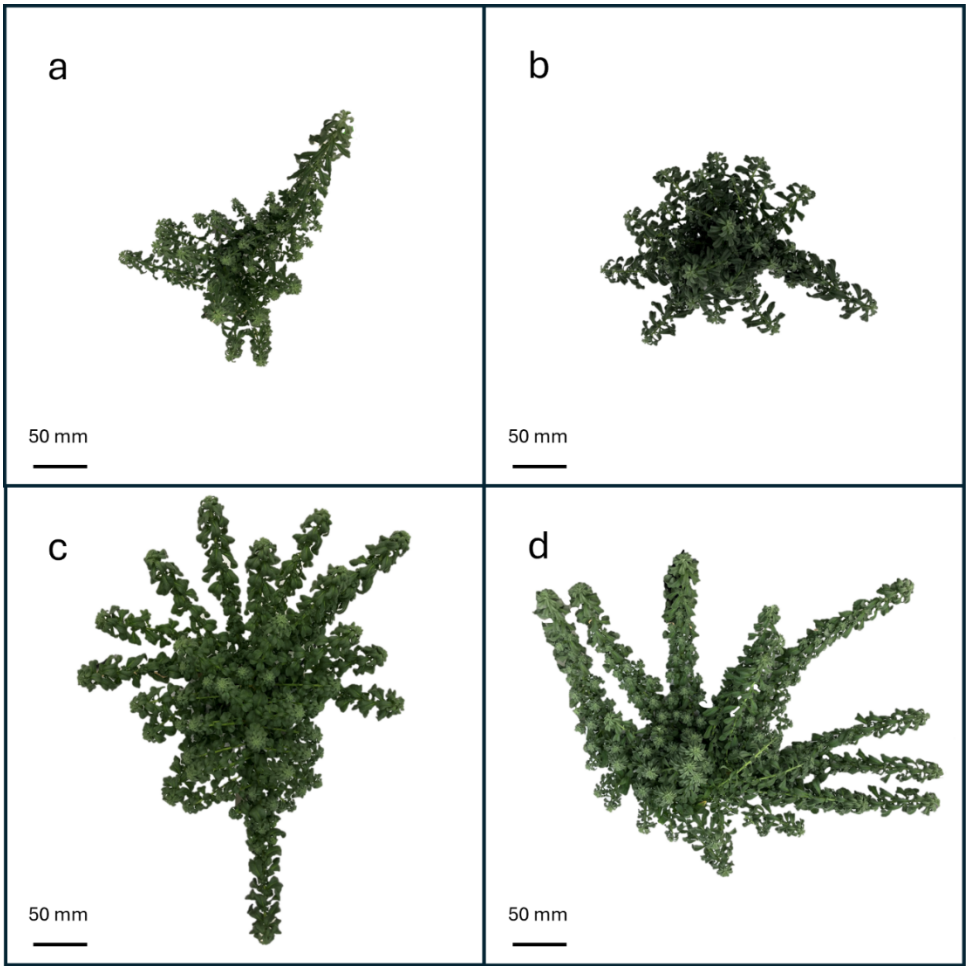
SUPPELEMENTARY MATERIALS



Supplementary Fig. S1. Plant architecture of the four genotypes of *Odontarrhena chalcidica*. EX (**a**); AO (**b**); KT (**c**); GR (**d**). The plant photos are representative for each genotype and were taken from a top-down perspective by B. Montanarini during hydroponic cultivation.

TABLES

Table S1. Nickel content in aeroponic and hydroponic nutrient solution and bioavailable nickel (DPTA-extractable) in pot soil along the cultivation period.

System (Timepoint)	Total nickel content (mg L^{-1})
Nutrient solution (day 10)	1.52 ± 0.49
Nutrient solution (day 24)	1.95 ± 0.02
Nutrient solution (day 38)	1.09 ± 0.14
Nutrient solution (day 52)	1.12 ± 0.05
Soil – DTPA-extractable Ni (day 0)	5.90 ± 0.96

Hydroponic and aeroponic systems shared a common nutrient solution tank and recirculation system. Data represent the average of the number of replicates ± SD. The number of replicates was for the nutrient solution (aeroponic and hydroponic) equal to three, while it was five for the soil. DPTA, diethylenetriaminepentaacetic acid.

Table S2. Specific Leaf Area (mm^2 mg DW^{-1}) of plants of the four genotypes of *Odontarrhena chalcidica* in aeroponic, hydroponic and pot cultivation regimes at the end of the cultivation period (day 52).

Genotype	Aeroponic	Hydroponic	Pot
EX	12.14 ± 0.32^{Ax}	11.39 ± 0.92^{Ax}	10.73 ± 2.31^{Ax}
AO	12.02 ± 2.01^{Ax}	11.35 ± 0.52^{Ax}	8.68 ± 0.50^{Bx}
KT	11.52 ± 2.96^{Ax}	14.44 ± 0.81^{Axy}	11.57 ± 3.15^{Ax}
GR	11.37 ± 2.38^{Ax}	12.92 ± 2.61^{Ax}	13.37 ± 5.36^{Ax}

Upper case letters and lowercase letters indicate statistically significant differences among cultivation systems within the same genotype and among different genotypes in the same cultivation system and were evaluated by the ANOVA/Kruskal-Wallis tests followed by Tukey HSD/Dunn's tests ($p < 0.05$). Data are are given as mean (n=4) ± SD.

Table S3. Stomata density on leaves of the four genotypes of *Odontarrhena chalcidica* in aeroponic, hydroponic and pot cultivation regimes measured by Environmental Scanning Electron Microscopy (ESEM) at the end of the cultivation period (day 52).

	Abaxial leaf surface (number mm^{-2})			**Adaxial leaf surface (number mm^{-2})**		
Genotype	Aeroponic	Hydroponic	Pot	Aeroponic	Hydroponic	Pot
EX	288.51 ± 64.36^{Ax}	301.14 ± 42.31^{Ax}	299.87 ± 145.86^{Ax}	91.54 ± 31.24^{Ax}	95.01 ± 22.81^{Ax}	116.48 ± 52.16^{Ax}
AO	339.65 ± 41.51^{Ax}	257.58 ± 29.47^{Ax}	186.87 ± 83.52^{ABx}	67.87 ± 21.44^{Ax}	100.06 ± 17.93^{Ax}	84.60 ± 30.62^{Ax}
KT	382.42 ± 65.51^{Ax}	406.57 ± 33.71^{Axy}	472.22 ± 76.02^{Axy}	146.94 ± 26.37^{Axy}	166.35 ± 20.16^{Ay}	167.30 ± 20.89^{Ax}
GR	381.31 ± 46.92^{Ax}	428.98 ± 49.46^{Axy}	350.38 ± 93.89^{Ax}	129.42 ± 52.17^{Ax}	165.40 ± 42.07^{Ay}	150.25 ± 15.97^{Ax}

Upper case letters and lowercase letters indicate statistically significant differences among cultivation systems within the same genotype and among different genotypes in the same cultivation system and were evaluated by the ANOVA/Kruskal-Wallis tests followed by Tukey HSD/Dunn's tests ($p < 0.05$). Data are given as mean (n=4) ± SD.

Table S4. Trichomes density on leaves of the four genotypes of *Odontarrhena chalcidica* in aeroponic, hydroponic and pot cultivation regimes measured by Environmental Scanning Electron Microscopy (ESEM) at the end of the cultivation period (day 52).

	Abaxial leaf surface (number mm^{-2})			**Adaxial leaf surface (number mm^{-2})**		
Genotype	Aeroponic	Hydroponic	Pot	Aeroponic	Hydroponic	Pot
EX	52.00 ± 13.45^{Ax}	48.22 ± 5.79^{Ax}	48.13 ± 5.16^{Ax}	16.75 ± 10.48^{Ax}	9.45 ± 0.93^{Ax}	10.24 ± 4.44^{Ax}
AO	68.39 ± 3.32^{Ax}	61.93 ± 1.37^{Ay}	55.20 ± 8.79^{ABx}	17.01 ± 2.46^{Ax}	19.52 ± 1.55^{Ay}	21.91 ± 6.98^{Ay}
KT	57.75 ± 12.39^{Ax}	46.81 ± 5.16^{Ax}	62.41 ± 11.55^{Ax}	6.15 ± 0.92^{Axy}	4.53 ± 0.33^{Az}	7.52 ± 1.97^{ABx}
GR	51.95 ± 4.37^{Ax}	53.80 ± 3.76^{Axy}	55.03 ± 7.59^{Ax}	12.57 ± 2.23^{Ax}	10.46 ± 2.10^{Ax}	13.01 ± 2.60^{Axy}

Upper case letters and lowercase letters indicate statistically significant differences among cultivation systems within the same genotype and among different genotypes in the same cultivation system and were evaluated by the ANOVA/Kruskal-Wallis tests followed by Tukey HSD/Dunn's tests ($p < 0.05$). Data are given as mean (n=4) ± SD.

Table S5. Photosynthetic parameters of the four genotypes of *Odontarrhena chalcidica* in aeroponic, hydroponic and pot cultivation regimes measured by LI-COR infrared gas analyzer on three mature leaves per individual at day 52.

	GasEx E (mol H_2O m^{-2} s^{-1})			**GasEx A (µmol CO_2 m^{-2} s^{-1})**			**GasEx gsw (mol H_2O m^{-2} s^{-1})**		
Genotype	Aeroponic	Hydroponic	Pot	Aeroponic	Hydroponic	Pot	Aeroponic	Hydroponic	Pot
EX	0.014 ± 0.001^{Ax}	0.011 ± 0.002^{Bx}	0.009 ± 0.002^{Bx}	23.059 ± 0.967^{Ax}	24.434 ± 3.330^{Bx}	17.711 ± 7.325^{Ax}	0.915 ± 0.078^{Ax}	0.676 ± 0.119^{Ax}	0.563 ± 0.063^{Ax}
AO	0.011 ± 0.001^{Ay}	0.011 ± 0.001^{Ax}	0.013 ± 0.003^{Ax}	19.974 ± 3.100^{Ax}	20.520 ± 3.907^{Ax}	26.994 ± 3.713^{Ax}	0.582 ± 0.085^{Ax}	0.700 ± 0.095^{Ax}	0.850 ± 0.179^{Ax}
KT	0.010 ± 0.001^{Ay}	0.010 ± 0.001^{Ax}	0.011 ± 0.002^{Ax}	21.399 ± 1.423^{Ax}	19.597 ± 1.591^{Ax}	22.345 ± 3.636^{Ax}	0.670 ± 0.025^{Ax}	0.685 ± 0.079^{Ax}	0.736 ± 0.158^{Ax}
GR	0.013 ± 0.003^{Ay}	0.011 ± 0.001^{Ax}	0.008 ± 0.003^{Ax}	22.949 ± 0.700^{Ax}	22.873 ± 0.774^{Ax}	18.404 ± 3.010^{Ax}	0.903 ± 0.220^{Ax}	0.637 ± 0.112^{Ax}	0.480 ± 0.160^{Ax}

GasEx E, transpiration rate; GasEx A, assimilation rate; GasEx gsw, stomatal conductance. Upper case letters and lowercase letters indicate statistically significant differences among cultivation systems within the same genotype and among different genotypes in the same cultivation system and were evaluated by the ANOVA/Kruskal-Wallis tests followed by Tukey HSD/Dunn's tests ($p < 0.05$). Data are given as mean (n=4) ± SD.

Table S6. Content of photosynthetic pigments in leaves of the four genotypes of *Odontarrhena chalcidica* in aeroponic, hydroponic and pot cultivation regimes measured with a spectrophotometer (day 52).

	Chlorophyll a (mg g FW^{-1})			**Chlorophyll b (mg g FW^{-1})**			**Carotenoids (mg g FW^{-1})**		
Genotype	Aeroponic	Hydroponic	Pot	Aeroponic	Hydroponic	Pot	Aeroponic	Hydroponic	Pot
EX	37.13 ± 1.37^{Ay}	15.43 ± 3.08^{By}	24.85 ± 0.62^{Cx}	11.99 ± 0.66^{Ay}	7.60 ± 0.84^{By}	8.54 ± 0.41^{ABx}	9.36 ± 0.42^{Ax}	3.29 ± 0.80^{Bx}	6.47 ± 0.10^{ABx}
AO	29.80 ± 2.41^{Ax}	37.44 ± 5.43^{Ax}	25.49 ± 6.09^{ABx}	9.88 ± 1.01^{Ax}	12.54 ± 1.97^{Ax}	7.85 ± 1.81^{ABx}	8.16 ± 0.82^{Ax}	10.86 ± 1.62^{Ay}	6.95 ± 1.77^{ABx}
KT	27.83 ± 2.83^{Ax}	31.85 ± 2.18^{Axy}	26.62 ± 1.30^{ABx}	8.28 ± 0.66^{Ax}	8.65 ± 0.29^{Axy}	7.97 ± 0.38^{Ax}	7.95 ± 1.14^{Ax}	10.44 ± 1.07^{By}	7.83 ± 0.26^{Ax}
GR	34.17 ± 3.53^{Axy}	30.92 ± 2.42^{Axy}	28.39 ± 4.07^{Ax}	11.37 ± 1.30^{Axy}	10.44 ± 0.81^{Axy}	9.67 ± 1.20^{Ax}	10.13 ± 1.05^{Ax}	9.60 ± 0.67^{Ay}	8.69 ± 1.30^{Ax}

Upper case letters and lowercase letters indicate statistically significant differences among cultivation systems within the same genotype and among different genotypes in the same cultivation system and were evaluated by the ANOVA/Kruskal-Wallis tests followed by Tukey HSD/Dunn's tests ($p < 0.05$). Data are given as mean (n=4) ± SD.

Table S7. Nickel Translocation Factor (TF, shoot metal concentration/root metal concentration) of plants of the four genotypes of *Odontarrhena chalcidica* in aeroponic, hydroponic and pot cultivation regimes (day 52).

Genotype	Aeroponic	Hydroponic	Pot
EX	5.71 ± 2.20^{Ax}	6.51 ± 0.94^{Ax}	6.31 ± 2.44^{Ax}
AO	6.93 ± 1.14^{Ax}	7.52 ± 2.01^{Ax}	11.38 ± 5.76^{Ax}
KT	3.15 ± 0.99^{Ax}	3.96 ± 0.56^{Ax}	6.88 ± 2.07^{Bx}
GR	6.71 ± 2.52^{Ax}	7.53 ± 0.82^{Ay}	16.69 ± 2.01^{Bxy}

Upper case letters and lowercase letters indicate statistically significant differences among cultivation systems within the same genotype and among different genotypes in the same cultivation system and were evaluated by the ANOVA/Kruskal-Wallis tests followed by Tukey HSD/Dunn's tests ($p < 0.05$). Data are given as mean (n=4) ± SD.