SUPPLEMENTARY MATERIAL

Determination of nicotianamine contents

Nicotianamine was extracted by using a modified protocol based on Neumann et al. (1999). Plant tissue (0.1 g) was grinded to a fine powder in liquid nitrogen and extracted in 1 ml H_2O for 20 min at 80 °C, followed by sonification for 15 min in a waterbath (Bandelin, Berlin, Germany). Cell debris was removed by two centrifugation steps for 20 min at 15000 x g. 25 µL of the resulting supernatant was diluted with 75 µL of 0.5 M sodium-borate buffer (pH 7.7) containing 50 mM EDTA and derivatized for 45 s by the addition of 50 µL 12 mM 9-fluorenylmethyl chloroformate (FMOC, Sigma) according to Gustavsson and Betner (1990). Excess of FMOC was precipitated by the addition of 50 µL 40 mM adamantan-1-amine hydrochoride (ADAM, Sigma) in acetone:water (3:1, v/v) and removed by two centrifugation steps for 30 min at 15,000 x g and 4 °C. The derivatisation-assay (10 µl) was injected on a Nucleosil 100-5 C18, 250/4 column (Macherey-Nagel, Düren, Germany) that was connected to a Waters 600E HPLC system. After equilibration of the column for 8 min in buffer A (20 % (v/v) acetonitrile in 50 mM sodium acetate buffer pH 4.2) nicotianamine was separated by using the following binary gradient with a flow rate of 1 mL per minute: 0-5 min (0 % B), 5-15 min (20 %B, linear), 15-20 min (100 % B, linear), 20-22 min (100 % B). 80 % (v/v) acetonitrile in 50 mM sodium acetate buffer pH 4.2 served as buffer B.

Fluorescence of FMOC-derivatives was detected by using a fluorescence detector (Jasco FP 920, excitation: 263 nm, emission: 313 nm, Gain: 10, Response: Fast) and quantified by external NA standards (T. Hasegawa Co., Tokyo, Japan). Data were collected and processed with the Millenium32 software (Waters). The identity of nicotianamine was evidenced by spiking of chemically synthesized nicotianamine to plant samples. The nicotianamine-free tomato mutant *chloronerva* was used as a control. In leaves of Arabidopsis, a recovery rate of 98% ± 7% was achieved after addition of external standard to the plant sample prior to nicotianamine extraction (Suppl. Fig. 7).

Figure legends to supplementary files

Suppl. Fig. 1: Nicotianamine (NA) contents of single and triple *nas* mutants compared to wild type. Analyzed were rosette leaves from six week-old plants grown upon vegetative conditions. (A) Single mutants, n= 5 (B) triple mutants, n= 5.

Suppl. Fig. 2: Quantitative reverse transcription-PCR analysis of *NAS* gene expression in single and triple *nas* mutants. Plants were grown under + Fe on Hoagland agar plates. (A) NAS1, (B) NAS2, (C) NAS3, in roots gene expression was not detectable (n.d.), (D) NAS4 .

Suppl. Fig. 3: Quantitative reverse transcription-PCR analysis of *NAS* gene expression during the vegetative (SD= short days) and reproductive growth (LD= long days). (A) roots, (B) leaves.

Suppl. Fig. 4: Effect of Fe deficiency treatment in *nas4x-1* mutants. Shoot appearance of 6 week-old wild type and *nas4x-1* plants grown in a hydroponic system and exposed for 5 days to 0 Fe (- Fe) or 50 µM Fe (+ Fe). Note more severe intercostal leaf chlorosis of *nas4x-1* plants.

Suppl. Fig. 5: Zn and Cu contents of *nas4x-1* and wild type plants grown upon Zn (- Zn) and Cu (- Cu) deficiency or control conditions (named + Zn and + Cu), respectively. (A) Zn, n= 4; (B) Cu, n= 4

Suppl. Figure 6: Quantitative reverse transcription-PCR analysis of *NAS* gene expression in Ni-treated Col-0 plants.

Suppl. Figure 7: Determination of the recovery rate of nicotianamine (NA) quantification by HPLC. Nicotianamine was extracted from wild type plant samples without (Col-0) and with the addition of nicotianamine standard (Col-0 + NA standard) prior to extraction. The samples were derivatised in parallel with non-extraction treated nicotianamine standard (NA standard), containing the same amount of nicotianamine as added to the plant samples prior to extraction. Quantification by HPLC yielded approx. 15 pmol nicotianamine per injection of both pure plant sample (14.8 pmol NA) and non-extraction treated nicotianamine standard (15.4 pmol NA). Addition of nicotianamine standard prior to extraction yielded 29.7 pmol nicotianamine per injection. From this result a recovery rate of 98% ± 7% was calculated. Thus, it can be concluded that nicotianamine was not lost during the extraction procedure. n= 3 technical repetitions.

Suppl. Table 1: Gene-specific primers used for real-time qPCR analysis.

Sequence of the oligonucleotides	Gene (AGI code)
F 5'-ATCTTCCACACAACGGACG-3' R 5'-ATCTTCCACACAACGGACG-3'	*NAS1* (At5g04950)
F 5'-AGATCGGACGGTGTGTGG-3' R 5'-CCTCGATCAAATTCTTCTCCAT-3'	*NAS2* (At5g56080)
F 5'-CAATTGGGAATGTTGGTGG-3' R 5'-TGTTCCTCCCTAGCTCCG-3'	*NAS3* (At1g09240)
F 5'-TGTAATCTCAAGGAAGCTAGGTG-3' R 5'-CAGTTACACGCGAGATCCG-3'	*NAS4* (At1g56430)
F 5'-TTCTTAGCTTCATAGGATCAGTCAA-3' R 5'-GTTCTTGTTCAGAAGTCTACCTGTT-3'	*YSL1* (At4g24120)
F 5'-TGTATCGGGAGCTTAGTGGTATA-3' R 5'-GCCTTAATGAGCCGCAGT -3'	*YSL2* (At5g24380)
F 5'-CTTGGAATATGAGAGATCGAGTTAA-3' R 5'-CGAATATTTACTCGGCATGAA-3'	*YSL3* (At5g53550)
F 5'-GGAGAAGGTGTTGCTCCATC-3' R 5'-TCCGGAGAAGGAGAGCTTAG-3'	*FIT* (At2g28160)
F 5'-AAGTCAGAGGAAGGGGTTACA-3' R 5'-GATGCATAGAGTAAAAGAGTCGCT-3'	*BHLH100* (At2g41240)
F 5'-AAGCTTTGATCACGGTTGG-3' R 5'-TTAGGTCCCATGAACTCCG-3'	*IRT1* (At4g19690)
F 5'-CTTGGTCATCTCCGTGAGC-3' R 5'-AAGATGTTGGAGATGGACGG-3'	*FRO2* (At1g01580)
F 5'-ACGCACTCTCGTCTTTCACC-3' R 5'-GAAAGGCTGGAACACGACTC-3'	*FER1* (At5g01600)
F 5'-GATTCTACTGGCTTCTCTTGGATC-3' R 5'-CCTAATCCGGCCTTCACTAAC-3'	*FRO3* (At1g23020)
F 5'-ACTTGTACCAGTTGGTTATGGG-3' R 5'-CTGGATGTACTCGTTGTTAGGC-3'	*EF1Balpha2* (At5g19510) cDNA
F 5'-TCCGAACAATACCAGAACTACG-3' R 5'-CCGGGACATATGGAGGTAAG-3'	*EF1Balpha2* (At5g19510) genomic DNA
F 5'-GAAAGTGGATTACCCGCTG-3' R 5'-CTCTAAGTTTCTGGCGAGGAG-3'	*UBP6* (At1g51710)