Supplementary data for the article:

SUPPLEMENTARY DATA

Table S1. Concentration of Fe in the xylem sap of cucumber plants after withdrawal of Fe from the nutrient solution.

Day after transfer to -Fe	Fe concentration in xylem sap (μM)	
	-Si	+Si
1	0.07 ± 0.02^b	0.18 ± 0.04^b
2	0.01 ± 0.00^a	0.03 ± 0.01^a
5	n.d	n.d.
10	n.d	n.d.

Iron deprived 7-day old plants were supplied with 10 μM Fe for 3 days and then transferred to Fe-free nutrient solution buffered with $CaCO_3$, (0.2 g l^{-1}; pH >7.0) with or without supply of 1.5 mM Si(OH)$_4$ for 11 days. Data shown are means (n = 4) ± SD. Significant differences (p<0.05) between treatments are indicated by different letters. n.d., not detected.

Table S2. Dry biomass of cucumber plants grown in the nutrient solution supplied with $CaCO_3$.

Treatment	Dry biomass (g per plant)			
	Root		Shoot	
	-Si	+Si	-Si	+Si
Control (+Fe)	1.05 ± 0.09^b	1.33 ± 0.26^b	4.5 ± 0.7^d	4.5 ± 0.8^d
-Fe	0.52 ± 0.04^a	0.58 ± 0.07^a	3.0 ± 0.3^c	4.3 ± 0.2^d

Iron deprived 7-day old plants were supplied with 10 μM Fe for 3 days and then transferred to Fe-free nutrient solution for 11 days (-Fe). The control plants where supplied with 10 μM Fe throughout the entire experiment (+Fe). The pH of nutrient solution was kept high (> 7.0) by buffering with 0.2 g l^{-1} $CaCO_3$. Data shown are means (n = 4) ± SD. Significant differences (p<0.05) between treatments are indicated by different letters.
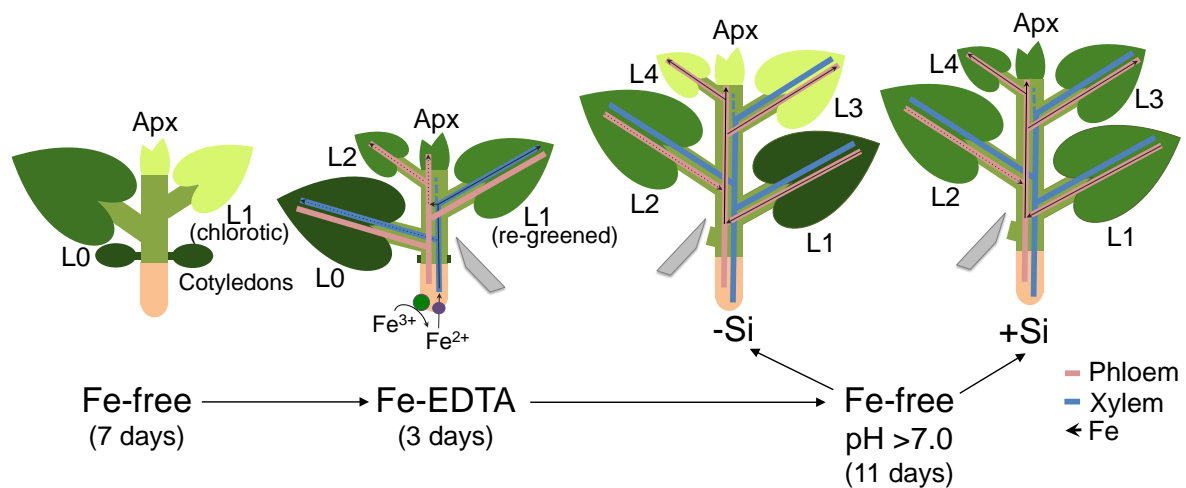
Fig. S1. Schematic presentation of experimental setup and movement of Fe within cucumber plants.
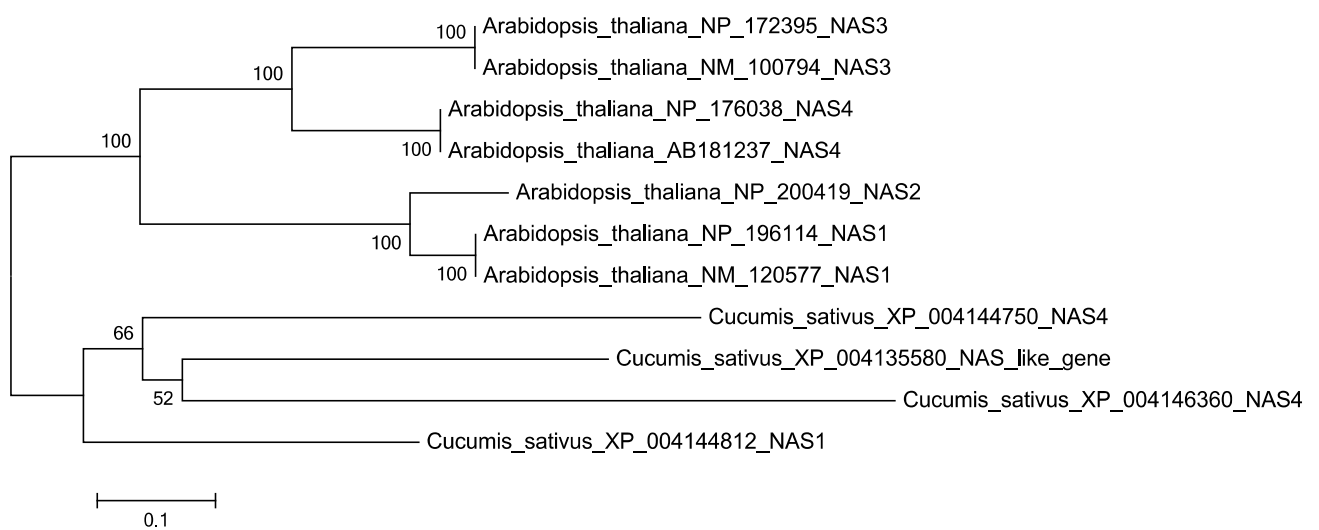

Fig. S2. The NJ phylogenetic tree of the NAS family in cucumber (*Cucumis sativus*) and Arabidopsis (*Arabidopsis thaliana*).
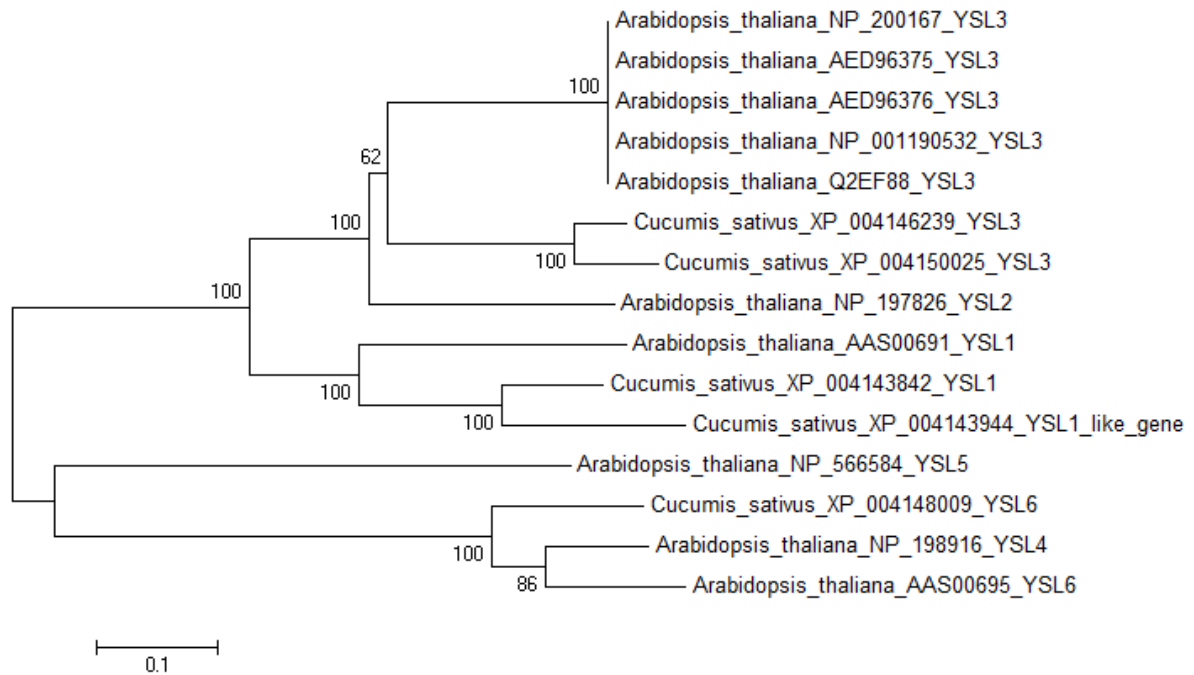
Fig. S3. The NJ phylogenetic tree of cucumber (*Cucumis sativus*) and Arabidopsis (*Arabidopsis thaliana*) YSL proteins.

Methods

Phylogenetic analysis

Evolutionary relations of NAS and YSL amino-acid sequences of cucumber (*Cucumis sativus*) and Arabidopsis (*Arabidopsis thaliana*) available in GenBank (accessed on 20.09.2015) were assessed by constructing neighbor joining (NJ) trees in MEGA version 6.06. In cucumber, four NAS sequences (two sequences corresponding to NAS4, one to NAS1, and one to a NAS-like gene; accession numbers XP_004144750_NAS4, XP_004135580_NAS_LIKE GENE, XP_004146360_NAS4, XP_004144812_NAS1, respectively) and five YSL sequences (one sequence corresponding to YSL1, one to YSL1-like gene, two to YSL3 and one to YSL6; accession numbers XP_004143842_YSL1, XP_004143944_YSL1 LIKE, XP_004146239_YSL3, XP_004150025_YSL3, XP_004148009_YSL6, respectively) were used. In Arabidopsis, seven NAS sequences (two sequences corresponding to NAS1, one to NAS2, two to NAS3 and two to NAS4; accession numbers NP_196114_NAS1, NM_120577_NAS1, NP_200419_NAS2, NP_172395_NAS3, NM_100794_NAS3, NP_176038_NAS4, AB181237_NAS4, respectively) and ten sequences

of YSL (YSL1, YSL2, YSL4, YSL5 and YSL6 genes represented by one sequence each; accession numbers: AAS00691_YSL1, NP_197826_YSL2, NP_198916_YSL4, NP_566584_YSL5, AAS00695_YSL6, respectively and YSL3 represented by five sequences; accession numbers: Q2EF88_YSL3, NP_00190532_YSL3, AED96376_YSL3, AED96375_YSL3, NP_200167_YSL3) were used. The Bayesian Information Criterion (BIC) calculated in MEGA 6.06, used to evaluate models of evolution for both data sets, revealed that Whelan and Goldman (WG) and Dayhoff model with the gamma distribution of among-site rate variation (Dayhoff + G) had the best fit to the NAS matrix while Jones-Taylor-Thornton + G (JTT + G) model had the best fit to the YLS matrix. These models, along with the pairwise gap deletion option were used for phylogenetic reconstructions. Statistical support for nodes was based on 1000 non-parametric bootstrap replicates (BS), with ≥75 % considered good support and 50-75 % as moderate support.

Collection of xylem sap and determination of Fe

Xylem sap was obtained by exudation after plants were decapitated at the stem about 2 cm above the root base. Soft rubber tubes were fixed over decapitated stem and xylem sap was collected by a micropipette for 1 h, after discarding of the exudates obtained during the first few minutes. The concentration of Fe in freshly collected xylem sap was determined by ICP-OES immediately after the samples were diluted 5 times (v/v) with 1 M HNO_3.