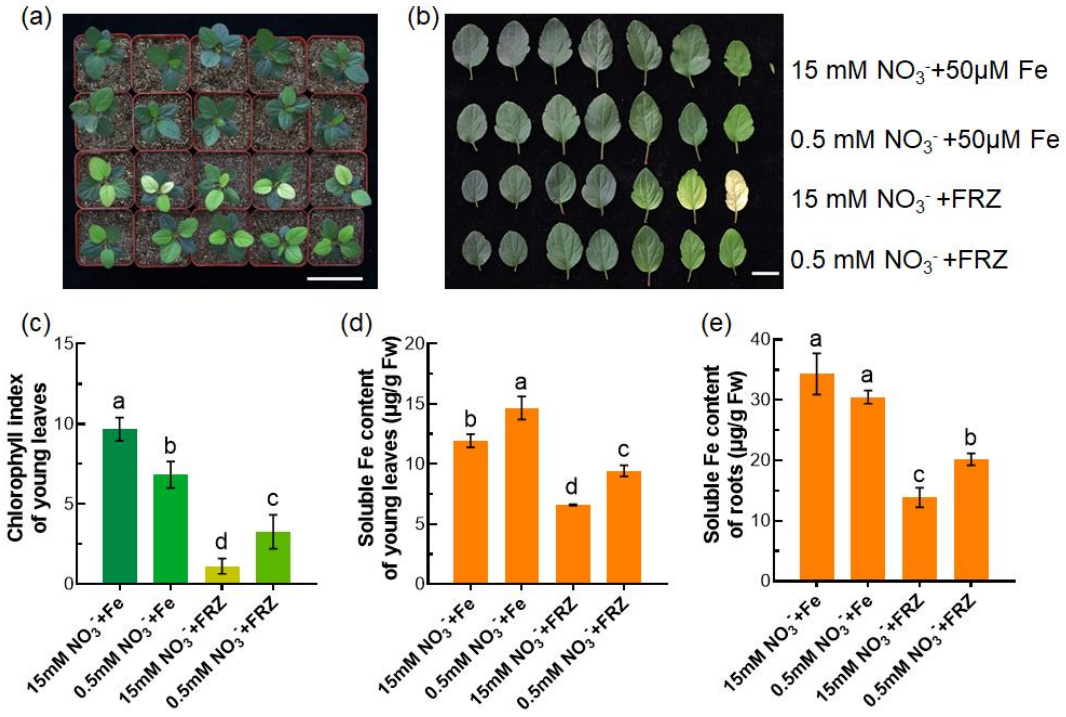

Figure 1. Effects of different nitrate treatment on chlorophyll and soluble Fe content. The phenotypes (a, b), the chlorophyll content of young leaves (c), and the soluble Fe content of young leaves (d) and roots (e) of 6-week-old seedlings grown in vermiculite treated with 15 mM KNO_3 + 50 μM Fe, 0.5 mM KNO_3 + 50 μM Fe, 15 mM KNO_3 +200 μM ferrozine (FRZ), 0.5 mM KNO_3 + 200 μM ferrozine (FRZ) for two weeks were showed. Error bars represent standard deviation (n≥3). Different letters represent significantly different values at $P<0.05$.

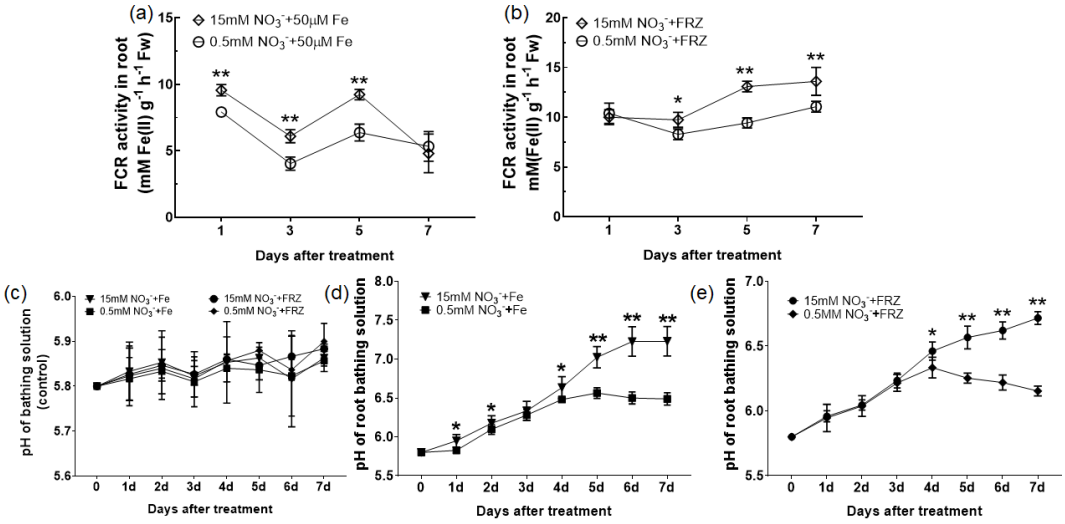

Figure 2. Effects of different nitrate treatment on Fe deficiency responses. FCR activity (a, b) and the pH of the treatment solution (c, d, e) of 6-week-old seedlings treated with 15 mM KNO_3 + 50 μM Fe, 0.5 mM KNO_3 + 50 μM Fe, 15 mM KNO_3 +200 μM ferrozine, 0.5 mM KNO_3 + 200 μM ferrozine for the indicated time are shown. Error bars represent standard deviation (n≥3). * represents significantly different values at $P<0.05$.

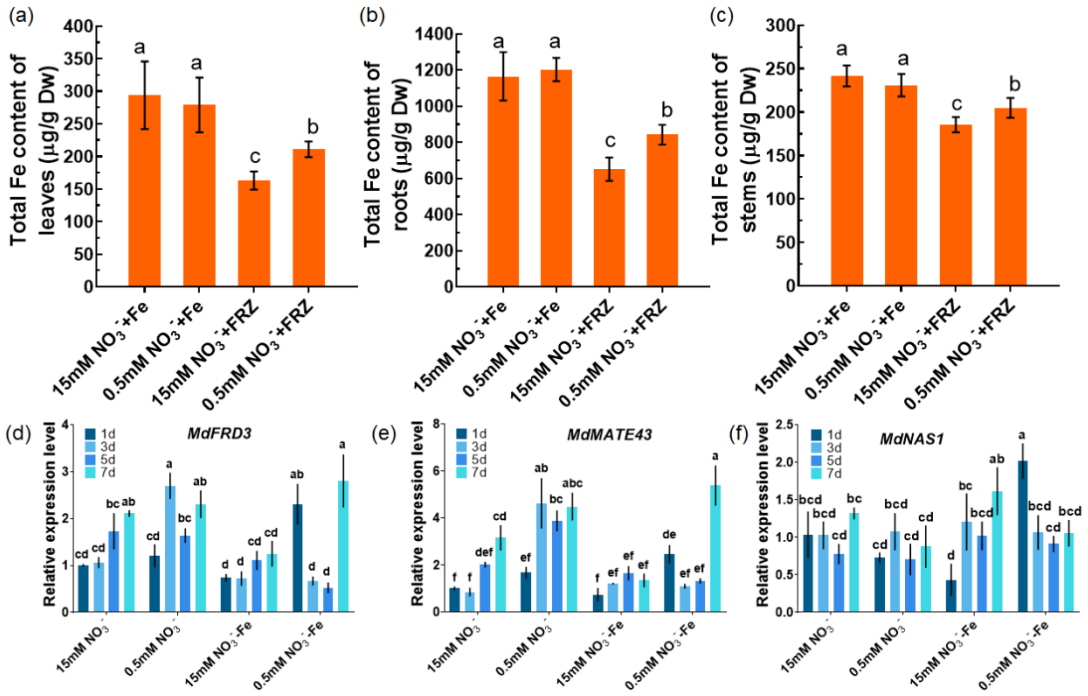

Figure 3. Effects of different nitrate treatment on Fe translocation from root to shoot. Total Fe content of leaves (a) and roots (b), and the Fe concentration in stems (c) of 6-week-old seedlings treated with 15 mM KNO_3 + 50 μM Fe, 0.5 mM KNO_3 + 50 μM Fe, 15 mM KNO_3 +200 μM ferrozine, 0.5 mM KNO_3 + 200 μM ferrozine for two weeks. Error bars represent standard deviation (n≥6). Seedlings of same growth status were treated with 0.5 mM KNO_3 + 50 μM Fe, 15 mM KNO_3 + 50 μM Fe, 0.5 mM KNO_3 -Fe (-Fe solution supplemented with 200 μM ferrozine) or 15 mM KNO_3 -Fe (-Fe solution supplemented with 200 μM ferrozine) solutions for 1, 3, 5 and 7 days, respectively. Relative expression level of *MdFRD3* (d), *MdMATE43* (e), *MdNAS1* (f) were detected. Different letters represent significantly different values at $P<0.05$.

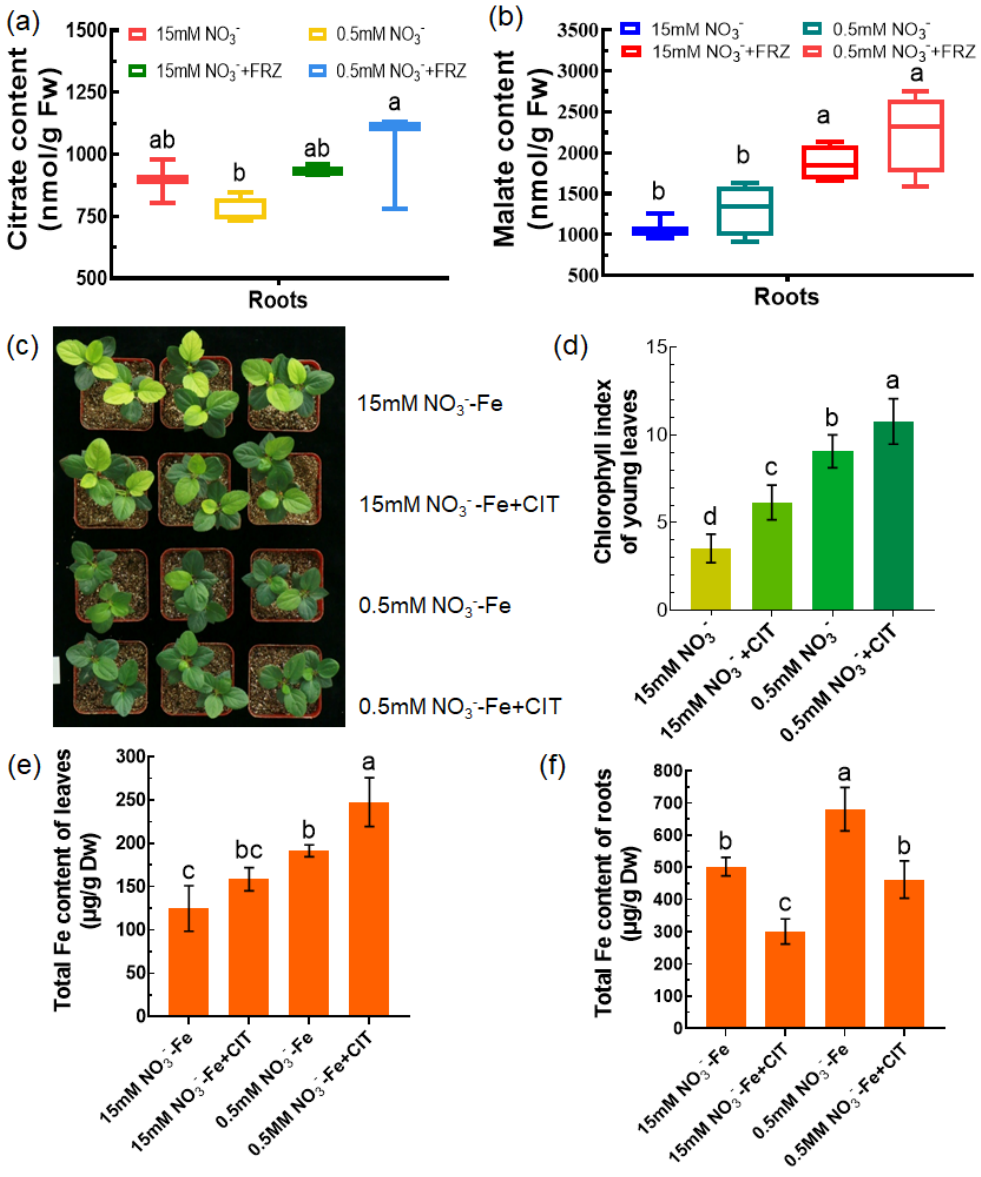

Figure 4.Nitrate alleviate iron deficiency partially through citrate. Citrate content (a) and malate content (b) in roots. 6-week-old seedlings were treated with 15 mM KNO_3 + 50 μM Fe, 0.5 mM KNO_3 + 50 μM Fe, 15 mM KNO_3 + 200 μM ferrozine, 0.5 mM KNO_3 + 200 μM ferrozine for two weeks. Error bars represent standard deviation (n≥3). Phenotype of exogenous 0.5 mM citrate treatment seedlings(c), chlorophyll content of young leaves (d), total Fe content of leaves (e) and roots (f) of 6-week-old seedlings treated with 15 mM KNO_3 + 200 μM ferrozine, 15 mM KNO_3 + 200 μM ferrozine +

0.5 mM citrate (CIT), 0.5 mM KNO_3 + 200 μM ferrozine, 0.5 mM KNO_3 + 200 μM ferrozine + 0.5 mM citrate (CIT) for 3 weeks. Error bars represent standard deviation (n≥3). Different letters represent significantly different values at *P<0.05*.

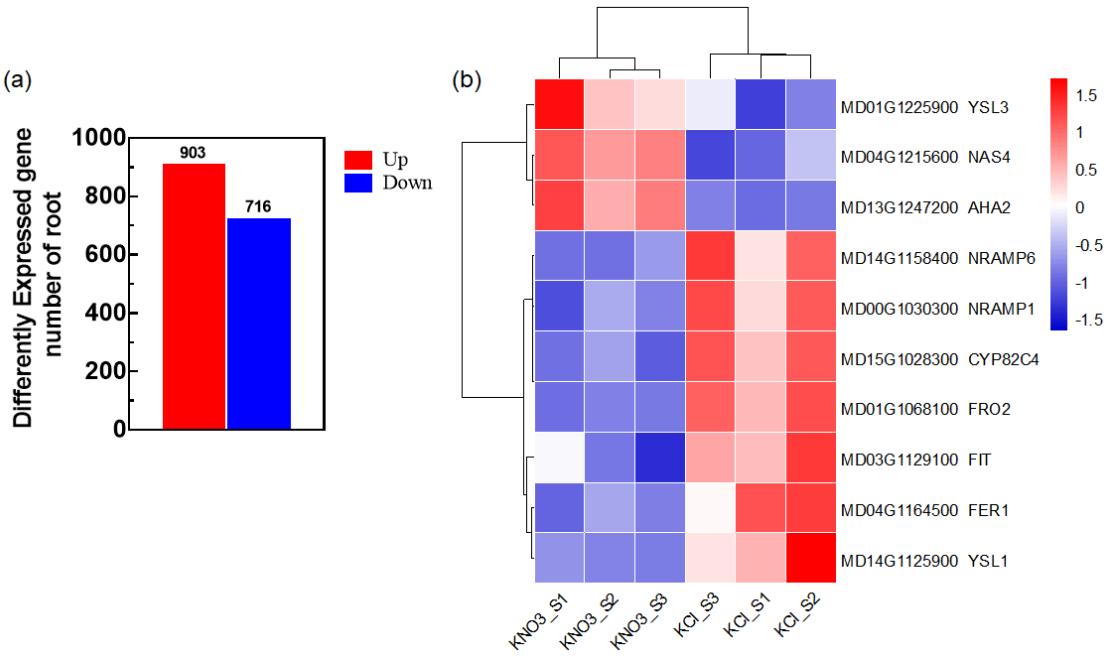

Figure 5. Differentially expressed genes analysis of different nitrate treatment using RNA-seq. Differently expressed gene number of roots (a) of 6-week-old seedlings treated with 15 mM KNO_3 and 15 mM KCl for 24 hours. Fe-related differently expressed genes of roots (b). S1, S2, S3 represent 3 biological repeats. The data were filtered at |Foldchange|≥1.5, *pval<0.05*.

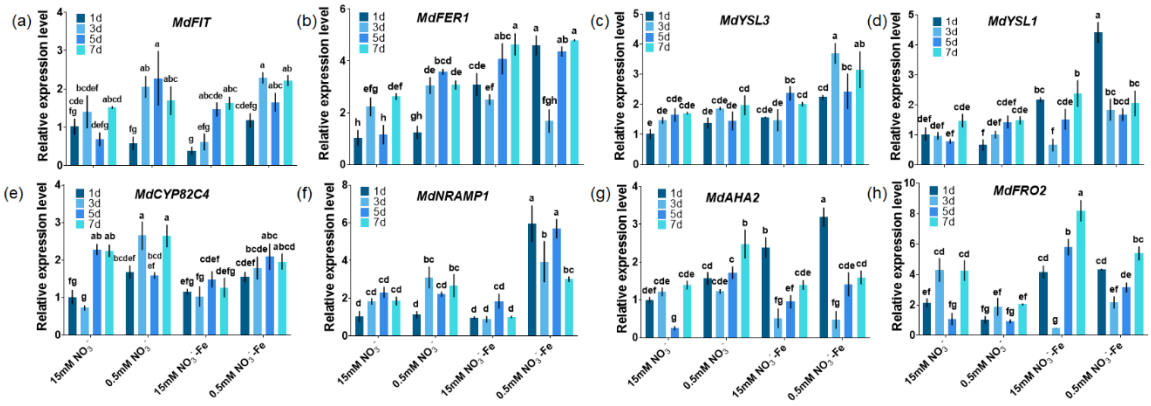

Figure 6. Relative expression level of Fe-related genes in response to LN and HN in roots. Seedlings of same growth status were treated with 0.5 mM KNO_3 + 50 μM Fe, 15 mM KNO_3 + 50 μM Fe, 0.5 mM KNO_3 -Fe (-Fe solution supplemented with 200 μM ferrozine) or 15 mM KNO_3 -Fe (-Fe solution supplemented with 200 μM ferrozine) solutions for 1, 3, 5 and 7 days, respectively. Relative expression level of *MdFIT* (a), *MdFER1* (b), *MdYSL3* (c), *MdYSL1* (d), *MdCYP82C4* (e), *MdNRAMP1* (f), *MdAHA2* (g) and *MdFRO2* (h) were detected. MdActin was selected as a control gene. Results were based on the average of three replicate experiments. Different letters represent significantly different values at $P<0.05$.

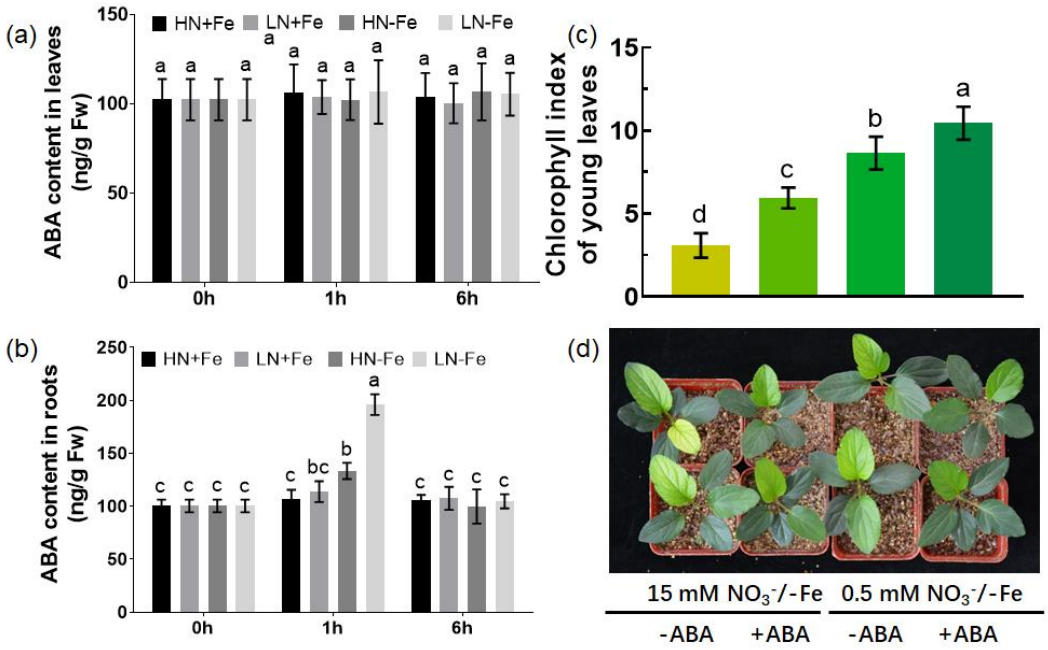

Figure 7. ABA alleviates nitrate-mediated Fe deficiency response. ABA content in young leaves (a) and roots (b) of 6-week-old seedlings treated with 15 mM KNO_3 + 50 μM Fe, 0.5 mM KNO_3 + 50 μM Fe, 15 mM KNO_3 +200 μM ferrozine, 0.5 mM KNO_3 + 200 μM ferrozine for the indicated time are shown. Chlorophyll content of young leaves (c) and phenotypes of exogenous 1μM ABA treatment (d). Error bars represent standard deviation (n≥4). Different letters represent significantly different values at $P<0.05$.

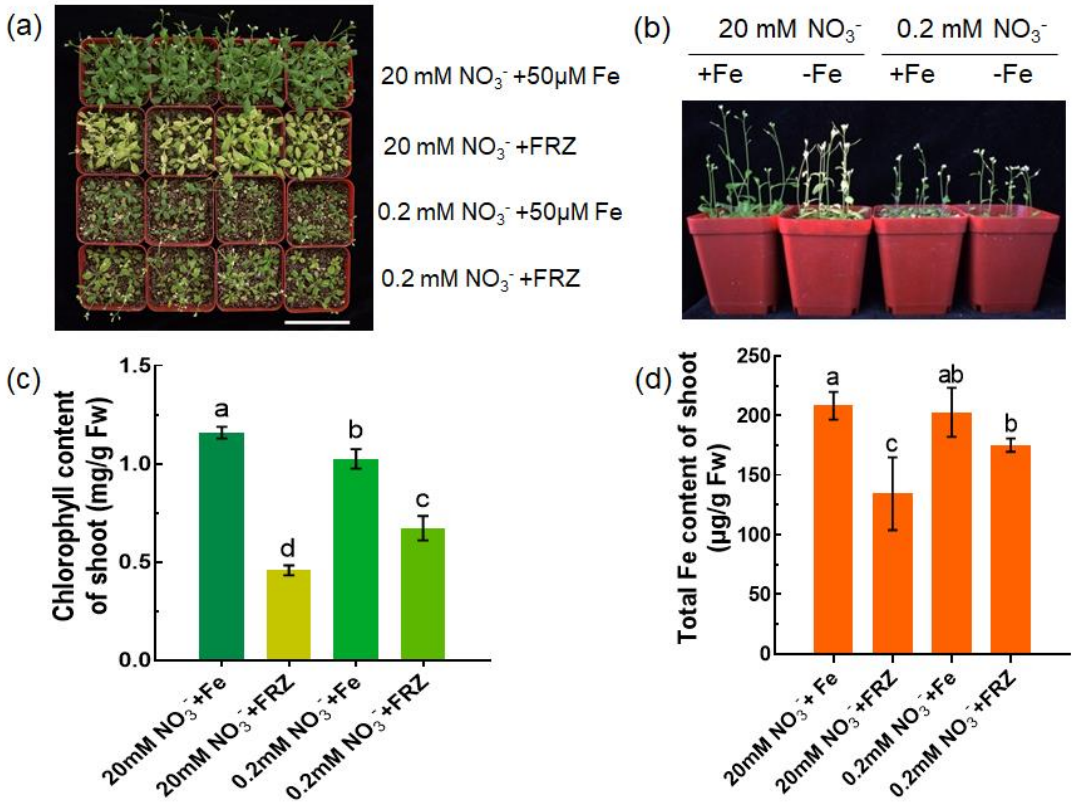

Figure 8. The regulatory mechanism on nitrate-mediated iron deficiency response is conserved in *Arabidopsis thaliana*. Phenotype (a, b), chlorophyll content (c) and total Fe content of shoot (d) of 2-week-old seedlings treated with 20 mM KNO_3 + 50 μM Fe, 0.2 mM KNO_3 + 50 μM Fe, 20 mM KNO_3 +200 μM ferrozine, 0.2 mM KNO_3 + 200 μM ferrozine for one week. Error bars represent standard deviation (n≥3). Different letters represent significantly different values at $P<0.05$.

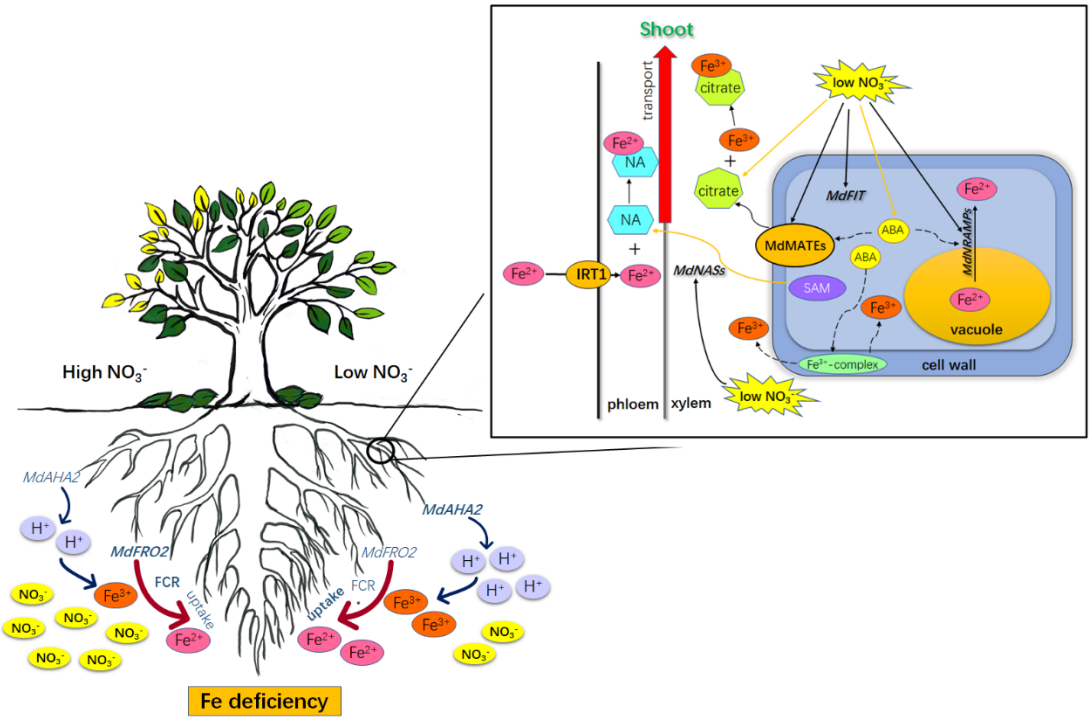

Figure 9. A model of nitrate in regulation of iron deficiency. NO_3^- is a member of substrates that affect plant iron deficiency response, both in direct and indirect ways. On the one hand, LN treatment helped to rhizosphere acidification and increase the solubility of Fe in rhizosphere. LN treatment increased the expression of genes including *MdFRD3*, *MdMATE43*, *MdNRAMP1*, *MdNRAMP6*, *MdNAS1*, *MdYSL1*, *MdYSL3* that are critical for Fe transport, and *MdFIT* which could activate expression of downstream genes to positively regulate Fe deficiency. On the other hand, LN treatment increased the citrate and ABA content in roots under Fe deprivation conditions, which contribute to Fe transportation and homeostasis. Dotted line represents the results of previous study (Haydon & Cobbett. 2007; Lei et al. 2014). Yellow arrows represent metabolism pathway.

Supplemental materials

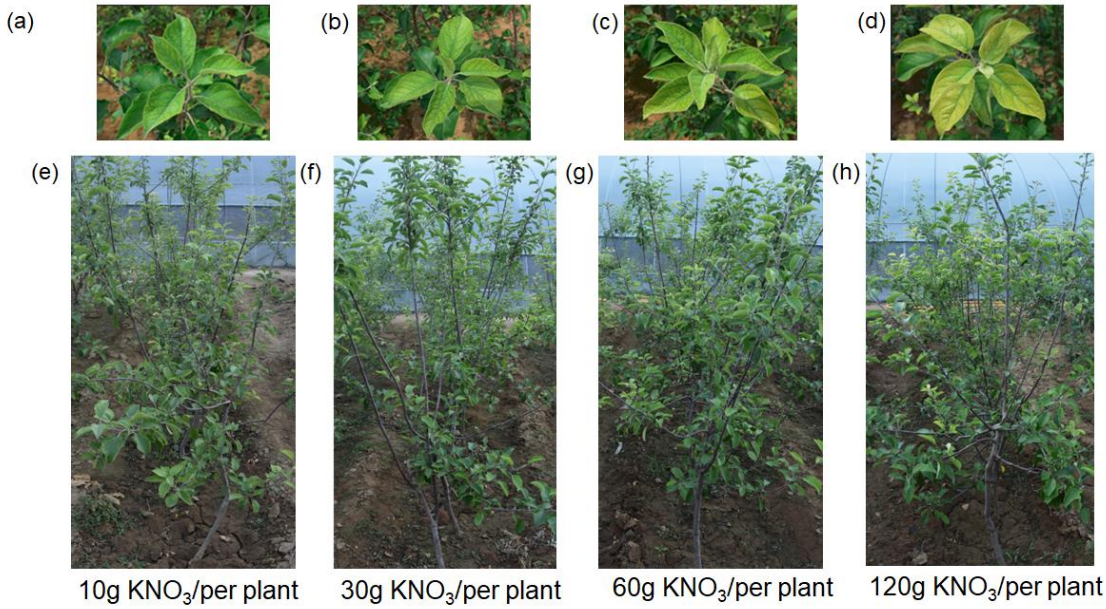

Figure S1. Effect of applicating nitrate on the Fe deficiency symptoms in the young leaves of apple. 2-year-old 'Fuji' apple trees treated with 10g KNO_3/per plant, 30g KNO_3/per plant, 60g KNO_3/per plant, 120g KNO_3/per plant for 3 months. Phenotypes of young leaves (a, b, c, d) and whole trees (e, f, g, h). Each line contains 11 trees. Each treatment contains 11 plants.

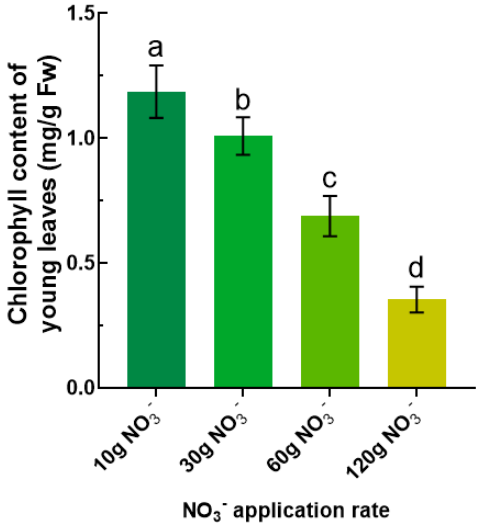

Figure S2. Chlorophyll content of young leaves of 2-year-old 'Fuji' apple trees treated with different concentration of nitrate. Error bars represent standard deviation (n=5).

Different letters represent significantly different values at *P<0.05*.

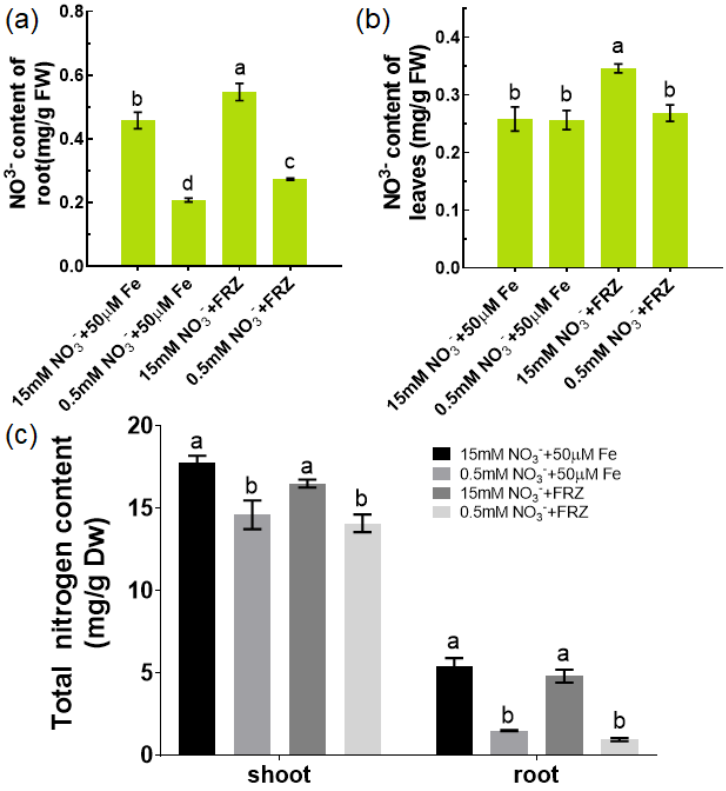

Figure S3. Nitrogen content of differently treated seedlings. NO_3^- content of roots (a), leaves (b) and total nitrogen content of leaves and roots (c) of 6-week-old seedlings treated with 15 mM KNO_3 + 50 μM Fe, 0.5 mM KNO_3 + 50 μM Fe, 15 mM KNO_3 + 200 μM FRZ, 0.5 mM KNO_3 + 200 μM FRZ for two weeks. Error bars represent standard deviation (n=3). Different letters represent significantly different values at *P<0.05*.

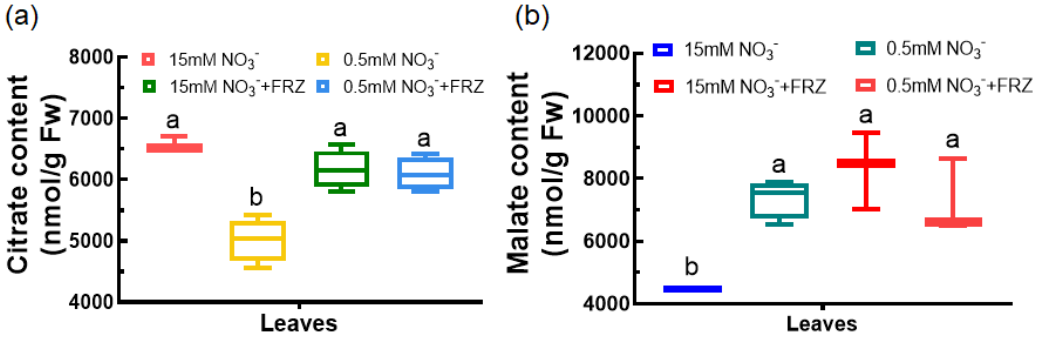

Figure S4. Effects of different nitrate treatment on citrate content (a) and malate content (b) in leaves. 6-week-old seedlings were treated with 15 mM KNO_3 + 50 μM Fe, 0.5 mM KNO_3 + 50 μM Fe, 15 mM KNO_3 +200 μM FRZ, 0.5 mM KNO_3 + 200 μM FRZ for two weeks. Error bars represent standard deviation (n≥3). Different letters represent significantly different values at *P<0.05*.

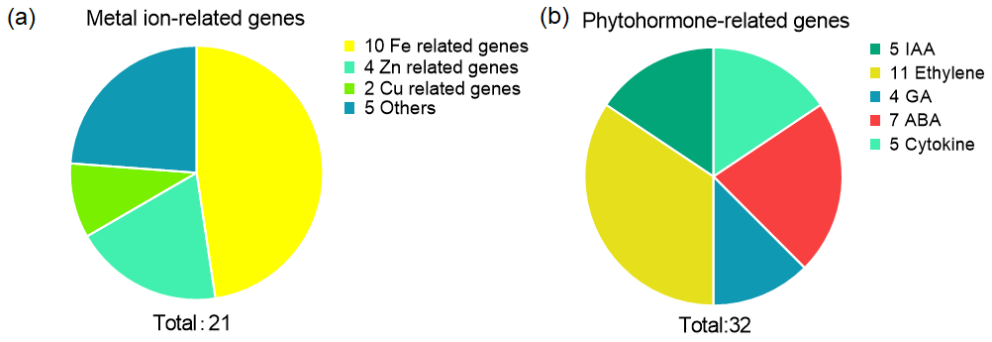

Figure S5. Nitrate regulates metal ion and phytohormone related genes expression. Number of metal ion-related genes regulated by nitrate (a) and number of phytohormone related genes regulated by nitrate (b) in roots. The data were filtered at |Foldchange|≥1.5, *pval<0.05*.

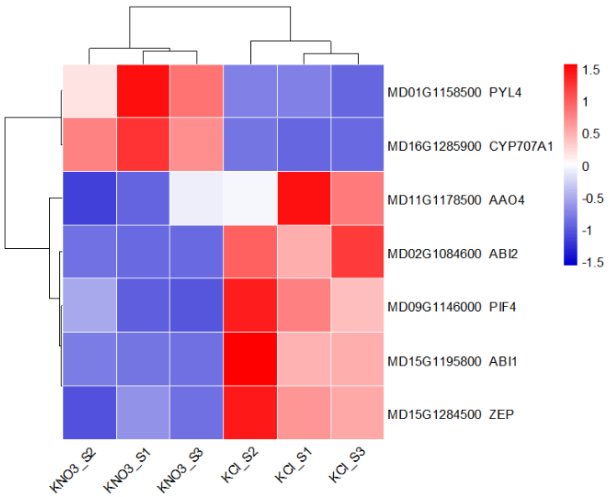

Fig S6. ABA biosynthesis and signal pathway genes which are regulated by nitrate. S1, S2, S3 represent 3 biological repeats. The data were filtered at |Foldchange|≥1.5, *pval<0.05*.

Table 1 qRT-PCR Primers used in this study

Primer name	Primer sequence_F	Primer sequence_R
MdActin	GGACAGCGAGGACATTCAGC	CTGACCCATTCCAACCATAACA
MdFRD3	TACAAGCGTGTTTTCAAATGGGATA	CTTTCGCAGATTCCACGTTCATTT
MdMATE43	AAGTGGAAGATGCCTGTTGGTGTT	CTTTTCCCGCTTCTCACCTTTCGC
MdNAS1	CCCTCCCAAGATGTCAACATGCTC	ACAAAGGCAATTTTGCTAGGCACA
MdFIT	GTCAAGCTGGTTCTACTACTCCA	TGTTAGGAACTAAAGACCGCAAT
MdFER1	CGGGACAATGGTGGTGCTGTTAG	TTATGGCGGCTTCGGACTCTTTT
MdYSL1	ATACAATAAACGGCTAGGATGTG	CAAGTAACTAAAGAAGGCAAGGA
MdYSL3	ATCATCCCGCTTATGTTTCCTCA	CAGACCACAGCCTACAAGTCCAG
MdCYP82C4	TCTGCCTTATGCAAACTCTATCA	CACTGCTGCTTATCACCAACGAC
MdNRAMP1	GACTATTTGGTAGCGAAGATGTG	AAACAAGTGATAGACACGGACAA
MdAHA2	AGCCTGGAGGAGATCAAGAACGAG	AAAATCTTGCCAATCTGGCGGCTT
MdFRO2	AATCAGACGAAAGGCTAACTCAA	GAAGATAGATACAACCCAACACC