Supplementary Figures & Tables

Title

Metagenomic analysis of rhizosphere microbiome provides insights into occurrence of iron deficiency chlorosis in field of Asian pears

Authors

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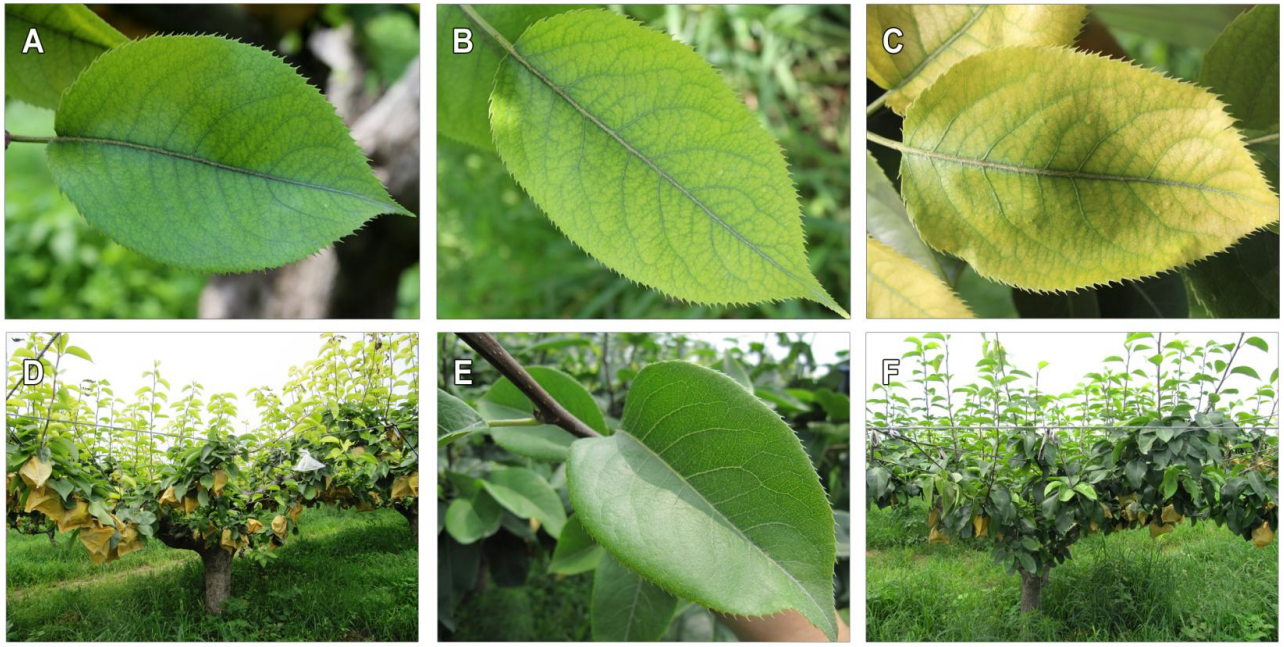

Supplementary Fig. S1 Symptoms on 'Whangkeumbea' pear leaves of Fe-deficiency chlorotic and normal plants

A. leaf with mild Fe-deficiency, lighter-green to lime-green color of interveinal tissue; B. leaf with moderate Fe-deficiency, yellow color of interveinal tissue; C. leaf with severe Fe-deficiency, leaf veins turn yellow; D. Fe-deficiency chlorotic plants; E. normal leaf; F. normal plants.

Supplementary Fig. S2 Soil layer and root growth investigations at 'Whangkeumbea' pear orchard with Fe-deficiency chlorotic and normal plants

A. Fe-deficiency chlorotic plants on the right and normal plants on the left; B. Soil layer and root growth investigations; C. A water-impermeable table formed by silt stratification, roots grow normally in the upper layer of soil, and some of the absorption roots died from anoxes condition in the middle and lower layers of soil; D. Root sampling for pathogen isolation.

Supplementary Table S1 Mineral elements in 'Whangkeumbea' pear leaves with different degrees of chlorosis

Sample types	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (mg/kg)	B (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mo (mg/kg)	Cl (mg/kg)
Severe chlorosis	1.63	0.14	2.40	2.21	0.44	0.18	91.26	27.04	50.61	12.95	27.4	1.22	578
Moderate chlorosis	1.10	0.20	2.45	1.80	0.39	0.17	125.42	29.47	47.54	16.91	30.97	1.45	549
Mild chlorosis	1.64	0.34	2.68	1.30	0.47	0.18	134.40	39.28	37.83	19.75	27.57	2.13	622
Normal leaves	2.06	0.48	3.68	1.50	0.67	0.27	328.80	52.40	60.91	32.97	41.39	41.39	773

Supplementary Table S2 Correlation analysis of mineral elements in 'Whangkeumbea' pear leaves with different degree of chlorosis

Correlation	N	P	K	Ca	Mg	S	Fe	B	Mn	Cu	Zn	Mo	Cl
N	1	0.725	0.799	-0.306	0.900	0.828	0.722	0.783	0.517	0.710	0.583	0.771	0.888
P	0.725	1	0.928	-0.796	0.888	0.842	0.903	0.993	0.368	0.958	0.782	0.845	0.922
K	0.799	0.928	1	-0.517	0.980	0.981	0.992	0.963	0.686	0.986	0.932	0.983	0.985
Ca	-0.306	-0.796	-0.517	1	-0.435	-0.345	-0.487	-0.718	0.244	-0.622	-0.327	-0.359	-0.507
Mg	0.900	0.888	0.980*	-0.435	1	0.983	0.951	0.937	0.694	0.936	0.871	0.966	0.997
S	0.828	0.842	0.981*	-0.345	0.983*	1	0.974	0.899	0.799	0.939	0.938	0.995	0.973
Fe	**0.722**	**0.903**	**0.992****	**-0.487**	**0.951***	**0.974***	**1**	0.938	0.726	0.987	0.969	0.988	0.955
B	0.783	0.993**	0.963*	-0.718	0.937	0.899	**0.938**	1	0.465	0.975	0.827	0.897	0.962
Mn	0.517	0.368	0.686	0.244	0.694	0.799	**0.726**	0.465	1	0.606	0.829	0.808	0.643
Cu	0.710	0.958*	0.986*	-0.622	0.936	0.939	**0.987***	0.975*	0.606	1	0.928	0.953	0.953
Zn	0.583	0.782	0.932	-0.327	0.871	0.938	**0.969***	0.827	0.829	0.928	1	0.967	0.865
Mo	0.771	0.845	0.983*	-0.359	0.966*	0.995**	**0.988***	0.897	0.808	0.953*	0.967*	1	0.958
Cl	0.888	0.922	0.985*	-0.507	0.997**	0.973*	**0.955***	0.962*	0.643	0.953*	0.865	0.958*	1

Note: * Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

Supplementary Table S3 Number of genes and microbiome annotated in the rhizosphere soil between Fe-deficiency chlorotic and normal plants

Sample Name	Total Gene	Annotated Gene	Annotated Kingdom	Annotated Phylum	Annotated Class	Annotated Order	Annotated Family	Annotated Genus	Annotated Species
CK1	80594	61004	5	53	93	203	401	1169	3021
CK2	55664	44323	5	54	94	205	386	1048	2525
CK3	61220	48077	5	53	96	206	392	1089	2644
Average	65826	51135	5	53	94	205	393	1102	2730
STDEV	13088	8751	0	1	2	2	8	62	259
CH1	77249	60238	5	52	94	207	406	1147	2987
CH2	62062	49617	5	51	93	201	393	1087	2687
CH3	114503	83097	5	53	96	211	414	1249	3456
Average	84605	64317	5	52	94	206	404	1161	3043
STDEV	26983	17109	0	1	2	5	11	82	388

Note: CK1-3. rhizosphere soil of chlorotic plants; CH1-3. rhizosphere soil of normal plants.

Supplementary Table S4 DEGs with pathway annotation between Fe-deficiency chlorotic and normal plants

No.	Pathway	Number of DEGs	Accounting for percentage of total DEGs	Pvalue	Pathway ID
1.	Metabolic pathways	1090	33.94	0.0102	ko01100
2.	Biosynthesis of secondary metabolites	495	15.41	0.0298	ko01110
3.	Purine metabolism	159	4.95	0.0041	ko00230
4.	Alanine, aspartate and glutamate metabolism	77	2.40	0.0067	ko00250
5.	Homologous recombination	47	1.46	0.0260	ko03440
6.	Mismatch repair	42	1.31	0.0258	ko03430
7.	PPAR signaling pathway	28	0.87	0.0431	ko03320
8.	Adipocytokine signaling pathway	15	0.47	0.0304	ko04920
9.	Toluene degradation	11	0.34	0.0416	ko00623
10.	Pathways in cancer	9	0.28	0.0351	ko05200
11.	Parkinson's disease	8	0.25	0.0141	ko05012
12.	Apoptosis	6	0.19	0.0035	ko04210
13.	Toxoplasmosis	5	0.16	0.0067	ko05145
14.	Small cell lung cancer	5	0.16	0.0067	ko05222
15.	Ethylbenzene degradation	5	0.16	0.0233	ko00642
16.	Amyotrophic lateral sclerosis (ALS)	5	0.16	0.0233	ko05014
17.	Viral myocarditis	4	0.12	0.0012	ko05416
18.	Colorectal cancer	4	0.12	0.0059	ko05210
19.	p53 signaling pathway	4	0.12	0.0070	ko04115
20.	Herpes simplex infection	4	0.12	0.0235	ko05168
21.	Biosynthesis of siderophore group nonribosomal peptides	4	0.12	0.0453	ko01053
22.	Influenza A	4	0.12	0.0491	ko05164
23.	Monoterpenoid biosynthesis	2	0.06	0.0118	ko00902

Supplementary Table S5 Phyla having a relative abundance greater than 0.5% in any one sample from chlorotic and normal plants

Phyla	CK1	CK2	CK3	Mean of CK	CH1	CH2	CH3	Mean of CH	P value
Proteobacteria	48.408	49.271	44.868	47.515	48.426	44.211	35.427	42.688	0.330
Unclassfied	29.992	28.519	31.261	29.924	27.199	27.803	36.803	30.601	0.859
Thaumarchaeota	3.513	4.707	5.010	4.410	13.630	18.593	5.120	12.448	0.128
Gemmatimonadetes	3.907	3.611	3.783	3.767	0.463	0.534	2.893	1.297	0.037
Nitrospirae	0.932	2.822	3.778	2.511	1.049	0.940	0.634	0.874	0.057
Actinobacteria	2.745	2.240	2.217	2.401	2.058	1.626	2.474	2.053	0.312
Firmicutes	1.858	2.033	2.188	2.026	1.560	1.470	2.250	1.760	0.402
Acidobacteria	2.176	1.259	1.347	1.594	1.558	1.099	6.812	3.156	0.491
Others	1.580	1.450	1.550	1.526	1.239	1.312	1.560	1.370	0.229
Cyanobacteria	1.383	1.232	1.250	1.289	1.094	0.973	1.937	1.335	0.900
Bacteroidetes	1.290	0.908	0.952	1.050	0.669	0.434	1.388	0.830	0.549
Planctomycetes	0.563	0.437	0.463	0.487	0.376	0.320	1.118	0.605	0.708
Crenarchaeota	0.202	0.750	0.469	0.474	0.067	0.121	0.042	0.077	0.024
Chloroflexi	0.488	0.363	0.372	0.408	0.272	0.265	0.847	0.461	0.819
Verrucomicrobia	0.375	0.371	0.383	0.376	0.278	0.262	0.561	0.367	0.938
Ignavibacteriae	0.589	0.029	0.109	0.242	0.063	0.037	0.133	0.078	0.305

Note: Note: CK1-3. rhizosphere soil of chlorotic plants; CH1-3. rhizosphere soil of normal plants. There was a significant difference of the average relative abundance between CH and CK, when the P value was less than 0.05.

Supplementary Table S6 Species with a higher relative abundance and the increment of CK vs. CH

Species	CK1	CK2	CK3	Mean	CH1	CH2	CH3	Mean	Increase of CK vs CH	P value
Unclassfied	69.75	67.92	67.89	68.52	72.63	72.89	64.97	70.16	2.3	0.7111
Others	21.31	19.97	19.18	20.16	18.53	17.89	21.48	19.30	-4.24	0.7075
Uncultured bacterium	1.75	1.76	1.82	1.78	1.41	1.31	4.70	2.47	28.0	0.6944
Candidatus Nitrosoarchaeum koreensis	0.86	0.06	0.53	0.48	3.03	3.14	1.03	2.40	80.0	0.0328
Gemmatirosa kalamazoonesis	2.34	2.05	2.09	2.16	0.29	0.32	1.93	0.85	-60.83	0.0424
Candidatus Nitrosoarchaeum limnia	0.23	0.10	0.22	0.18	0.97	1.18	0.37	0.84	78.0	0.0288
Nitrospira defluvii	0.80	2.68	3.63	2.37	0.91	0.80	0.50	0.74	-68.96	0.2269
Candidatus Solibacter usitatus	0.51	0.28	0.32	0.37	0.26	0.16	1.59	0.67	44.5	0.6878
Candidatus Nitrosopumilus salaria	0.12	0.03	0.11	0.09	0.63	0.89	0.22	0.58	84.5	0.0380
Chloracidobacterium thermophilum	0.25	0.12	0.20	0.19	0.22	0.11	1.20	0.51	62.2	0.4887
Candidatus Koribacter versatilis	0.23	0.16	0.15	0.18	0.31	0.28	0.69	0.42	57.9	0.1117
Nitrososphaera viennensis	0.86	3.73	2.74	2.44	0.29	0.50	0.16	0.32	-87.07	0.1147
Sulfuricella denitrificans	0.27	0.57	0.50	0.45	0.34	0.36	0.18	0.29	-34.66	0.3643
Uncultured *Acidobacteria bacterium*	0.16	0.09	0.11	0.12	0.12	0.07	0.58	0.26	53.9	0.5443
Gemmatimonas aurantiaca	0.55	0.49	0.51	0.51	0.07	0.10	0.42	0.20	-61.43	0.0227

Note: Note: CK1-3. rhizosphere soil of chlorotic plants; CH1-3. rhizosphere soil of normal plants. There was a significant difference of the average relative abundance between CH and CK, when the P value was less than 0.05.