Supplementary files for "Multi-omics profiling reveals comprehensive microbe-plant-metabolite regulation patterns for medicinal plant *Glycyrrhiza uralensis* Fisch"

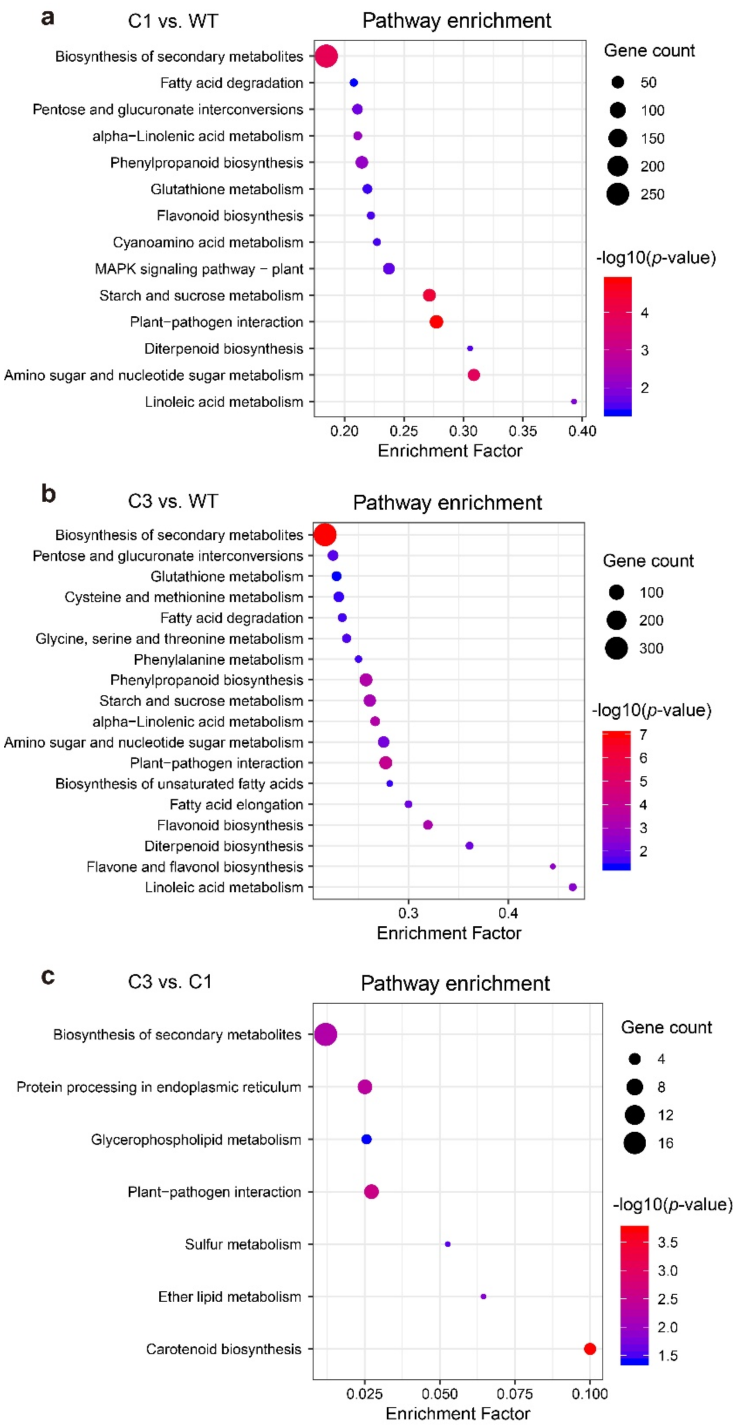
Chaofang Zhong[1,2], Chaoyun Chen[1], Xi Gao[1], Chongyang Tan[1], Hong Bai[1,*], Kang Ning[1,*]

[1]Key Laboratory of Molecular Biophysics of the Ministry of Education, Hubei Key Laboratory of Bioinformatics and Molecular-imaging, Center of AI Biology, Department of Bioinformatics and Systems Biology, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, Hubei, China

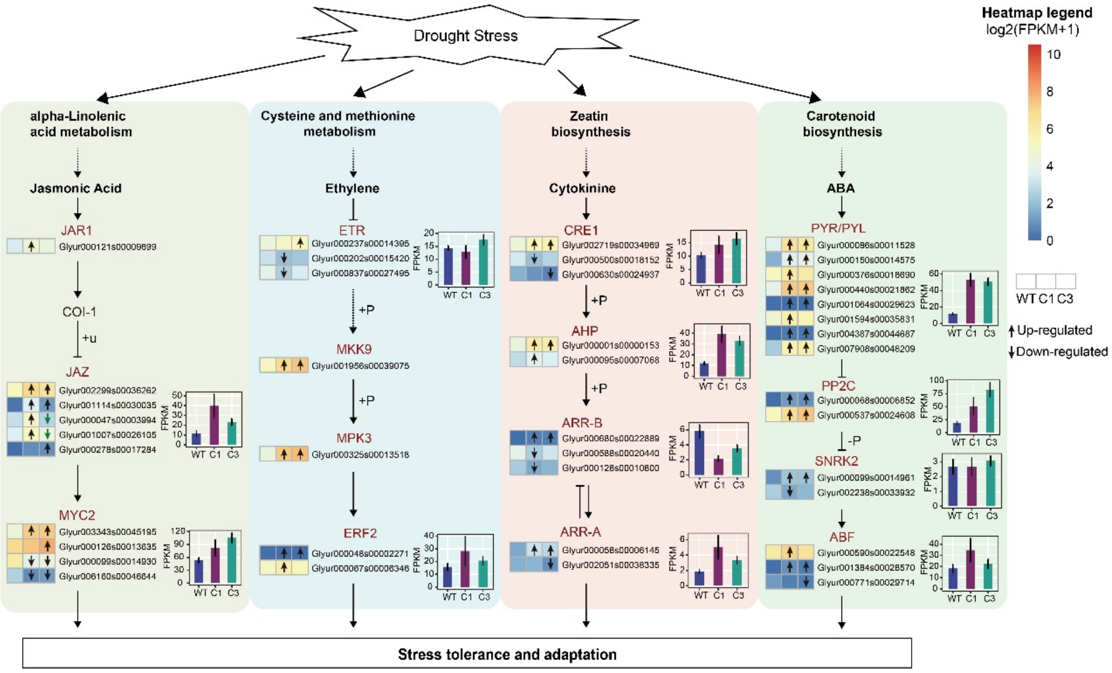
[2]Key Laboratory of Karst Biodiversity and Ecological Security, College of Environmental and Life Sciences, Nanning Normal University, Nanning 530001, China

[*]Correspondence should be addressed to Kang Ning (Email: ningkang@hust.edu.cn) and Hong Bai (Email: baihong@hust.edu.cn)

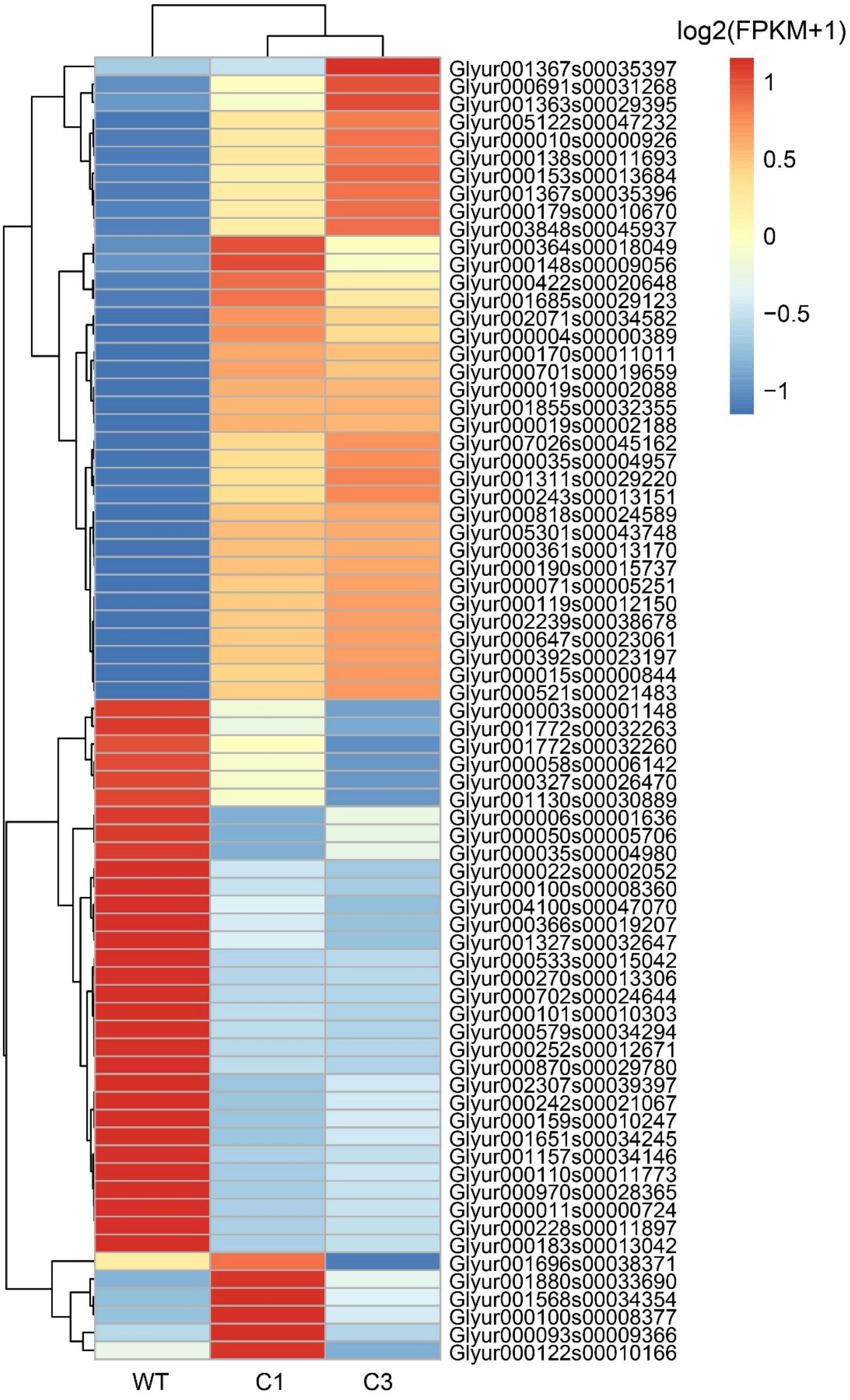
Supplementary Figures



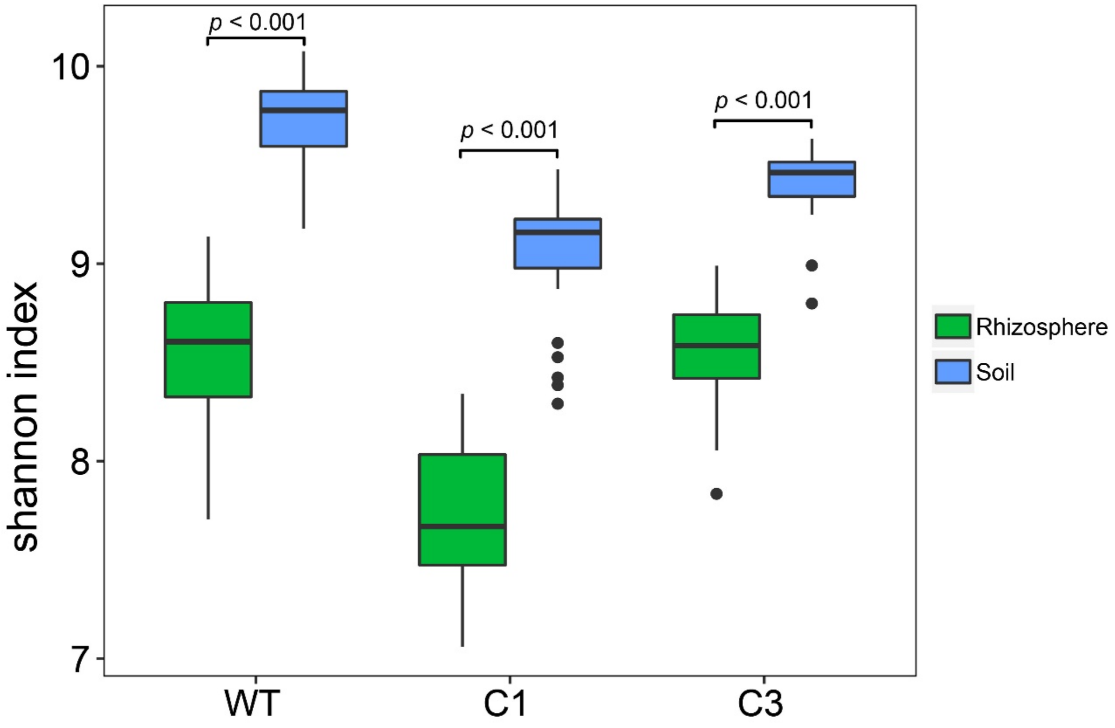
Supplementary Figure S1. KEGG pathways with the most significant enrichment of DEGs in the three groups of *G. uralensis*. (**a**) C1 vs. WT, (**b**) C3 vs. WT, (**c**) C3 vs. C1. The degree of enrichment was measured by the rich factor, FDR value and the number of genes enriched to each KEGG term. Rich factor referred to the ratio of the number of DEGs enriched in each term to the number of all DEGs annotated. The greater enrichment factor, the greater enrichment. The closer to zero, the greater enrichment. The circle size referred to the number of genes enriched to each KEGG term. WT: wild type; C1: cultivated for one year; C3: cultivated for three years.



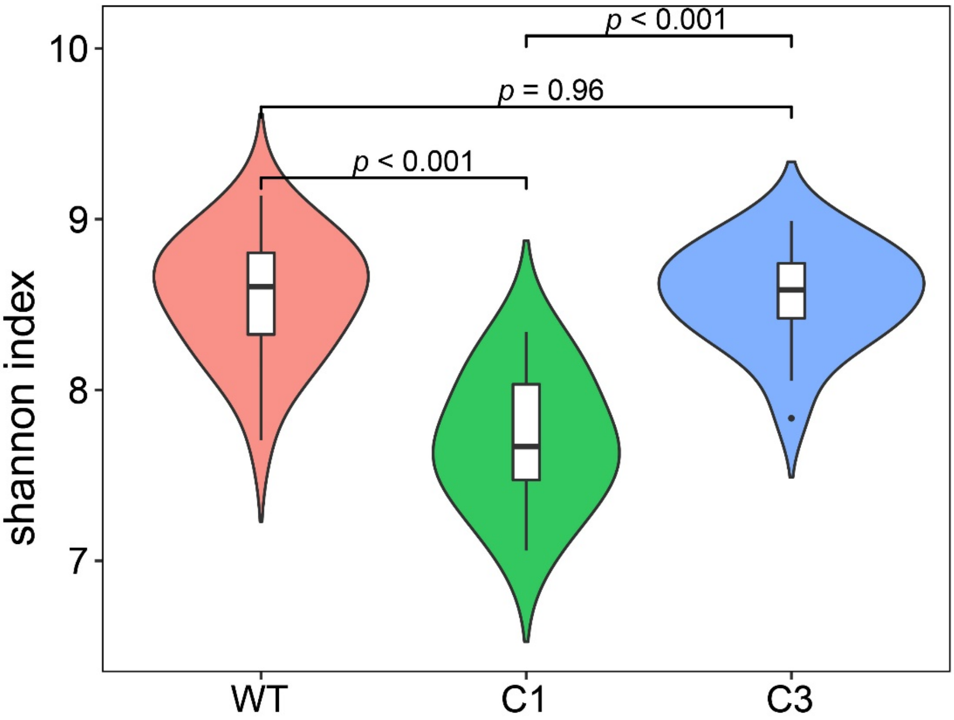
Supplementary Figure S2. Expression patterns of genes involved in plant hormone signal transduction pathways. Enzymes encoded by the differentially expressed genes were marked in red and the heatmap shows log2 (FPKM+1) values of each DEGs. Each row of heatmap represented one gene and each column represents one a group (from left to right, WT, C1, C3). The black arrow in the heatmap indicated that up (arrow up) or down (arrow down) regulation of C1 and C3 compared to wild, respectively. The bar box showed the average FPKM of genes encoding the same enzymes. WT: wild type; C1: cultivated for one year; C3: cultivated for three years.



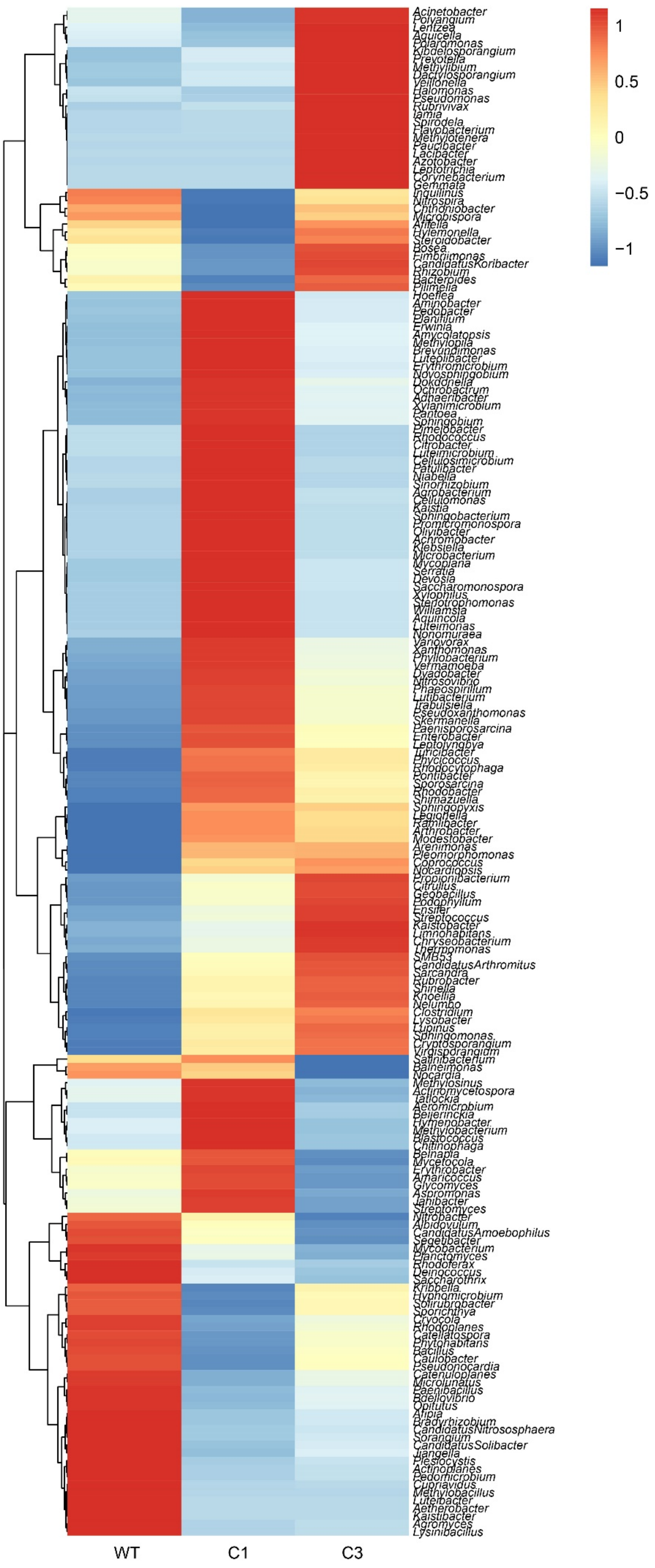
Supplementary Figure S3. Expression patterns of genes involved in plant-pathogen interaction. Each row of heatmap represented one gene and each column represented one a group (from left to right, WT, C1, C3). The heatmap was scaled in the row direction based on the FPKM of genes. WT: wild type; C1: cultivated for one year; C3: cultivated for three years.



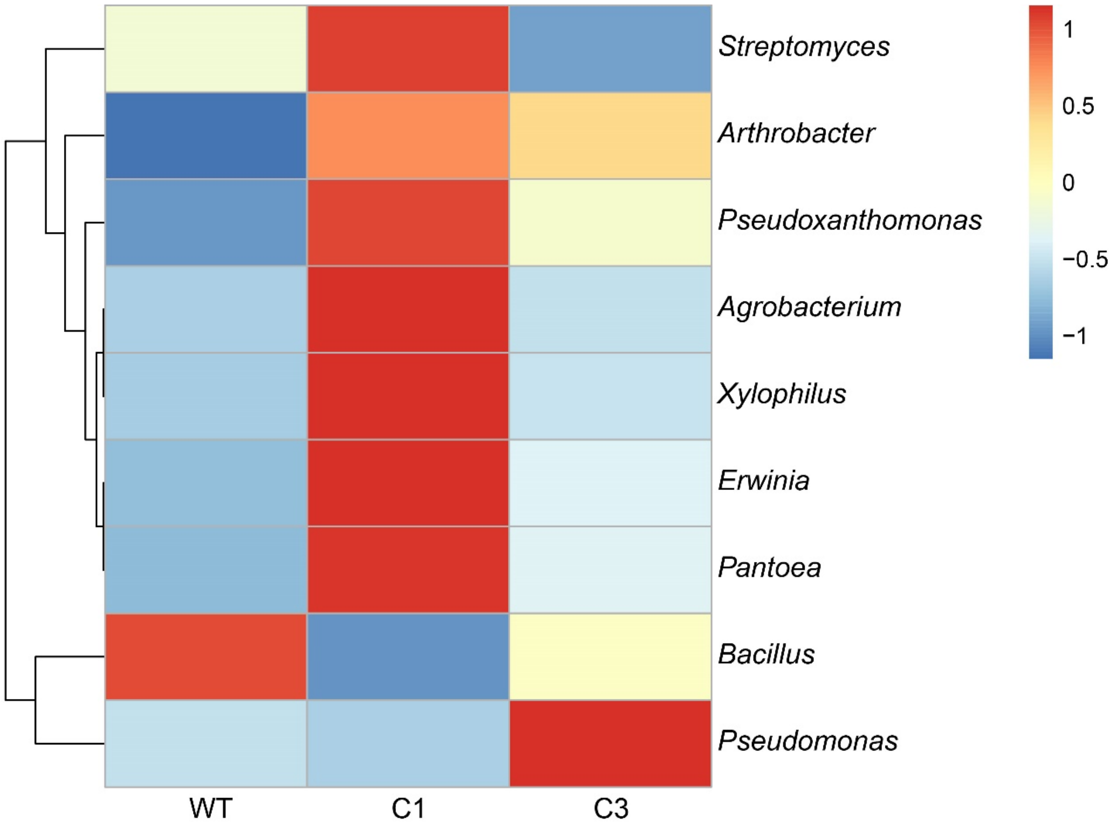
Supplementary Figure S4. The comparison of microbial community diversities between the rhizosphere and soil. WT: wild type; C1: cultivated for one year; C3: cultivated for three years.



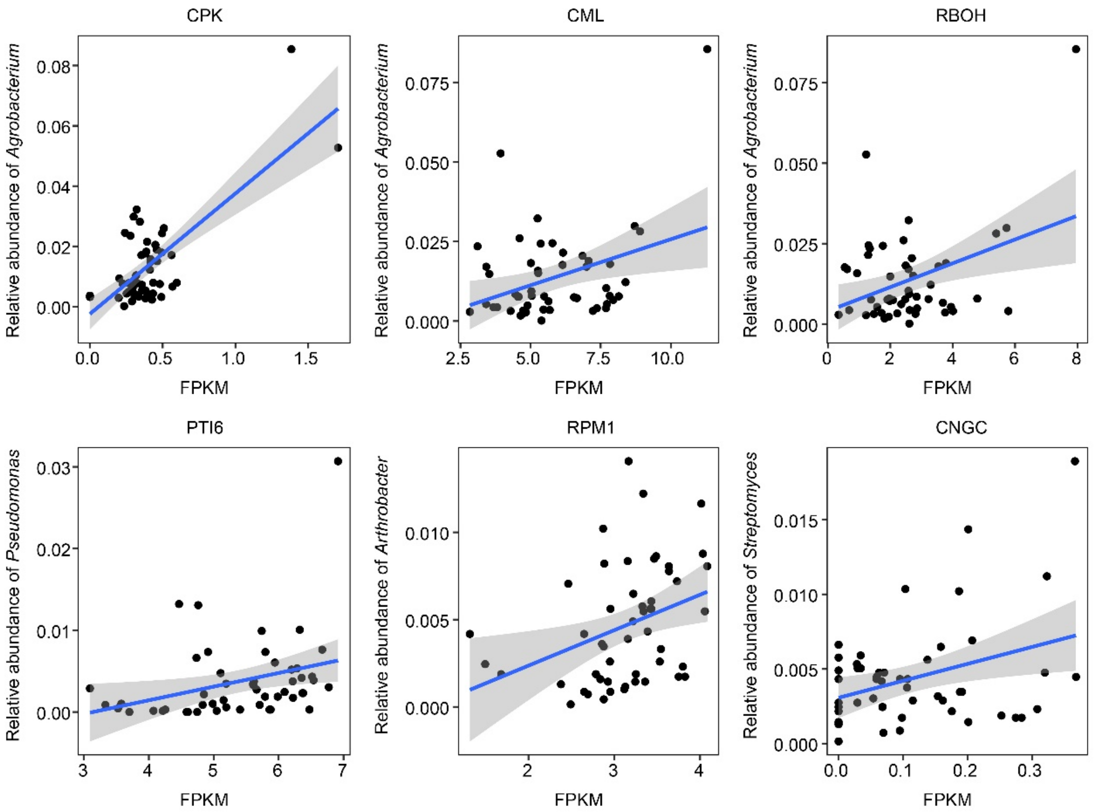
Supplementary Figure S5. The comparison of rhizosphere microbial community diversity between wild, C1 and C3 *G. uralensis*. WT: wild type; C1: cultivated for one year; C3: cultivated for three years.


1
0.5
0
−0.5
−1
WT
C1
C3
Acinetobacter
Polyangium
Lentzea
Aquicella
Polaromonas
Kibdelosporangium
Prevotella
Methylibium
Dactylosporangium
Veillonella
Halomonas
Pseudomonas
Rubrivivax
Iamia
Spirodela
Flavobacterium
Methylotenera
Paucibacter
Lacibacter
Azotobacter
Leptotrichia
Corynebacterium
Gemmata
Inquilinus
Nitrospira
Chthoniobacter
Microbispora
Afifella
Hylemonella
Steroidobacter
Bosea
Fimbriimonas
CandidatusKoribacter
Rhizobium
Bacteroides
Pilimelia
Hoeflea
Aminobacter
Pedobacter
Planifilum
Erwinia
Amycolatopsis
Methylopila
Brevundimonas
Luteolibacter
Erythromicrobium
Novosphingobium
Dokdonella
Ochrobactrum
Adhaeribacter
Xylanimicrobium
Pantoea
Sphingobium
Pimelobacter
Rhodococcus
Citrobacter
Luteimicrobium
Cellulosimicrobium
Patulibacter
Niabella
Sinorhizobium
Agrobacterium
Cellulomonas
Kaistia
Sphingobacterium
Promicromonospora
Olivibacter
Achromobacter
Klebsiella
Microbacterium
Mycoplana
Serratia
Devosia
Saccharomonospora
Xylophilus
Stenotrophomonas
Williamsia
Aquincola
Luteimonas
Nonomuraea
Variovorax
Xanthomonas
Phyllobacterium
Vermamoeba
Dyadobacter
Nitrosovibrio
Phaeospirillum
Lutibacterium
Trabulsiella
Pseudoxanthomonas
Skermanella
Paenisporosarcina
Enterobacter
Leptolyngbya
Turicibacter
Phycicoccus
Rhodocytophaga
Pontibacter
Sporosarcina
Rhodobacter
Shimazuella
Sphingopyxis
Legionella
Ramlibacter
Arthrobacter
Modestobacter
Arenimonas
Pleomorphomonas
Coprococcus
Nocardiopsis
Propionibacterium
Citrullus
Geobacillus
Podophyllum
Ensifer
Streptococcus
Kaistobacter
Limnohabitans
Chryseobacterium
Thermomonas
SMB53
CandidatusArthromitus
Sarcandra
Rubrobacter
Shinella
Knoellia
Nelumbo
Clostridium
Lysobacter
Lupinus
Sphingomonas
Cryptosporangium
Virgisporangium
Salinibacterium
Balneimonas
Nocardia
Methylosinus
Actinomycetospora
Tatlockia
Aeromicrobium
Beijerinckia
Hymenobacter
Methylobacterium
Blastococcus
Chitinophaga
Belnapia
Mycetocola
Erythrobacter
Amaricoccus
Glycomyces
Aspromonas
Janibacter
Streptomyces
Nitrobacter
Albidovulum
CandidatusAmoebophilus
Segetibacter
Mycobacterium
Planctomyces
Rhodoferax
Deinococcus
Saccharothrix
Kribbella
Hyphomicrobium
Solirubrobacter
Sporichthya
Cryocola
Rhodoplanes
Catellatospora
Phytohabitans
Bacillus
Caulobacter
Pseudonocardia
Catenuloplanes
Microlunatus
Paenibacillus
Bdellovibrio
Opitutus
Afipia
Bradyrhizobium
CandidatusNitrososphaera
Sorangium
CandidatusSolibacter
Jiangella
Plesiocystis
Actinoplanes
Pedomicrobium
Cupriavidus
Methylobacillus
Luteibacter
Aetherobacter
Kaistibacter
Agromyces
Lysinibacillus

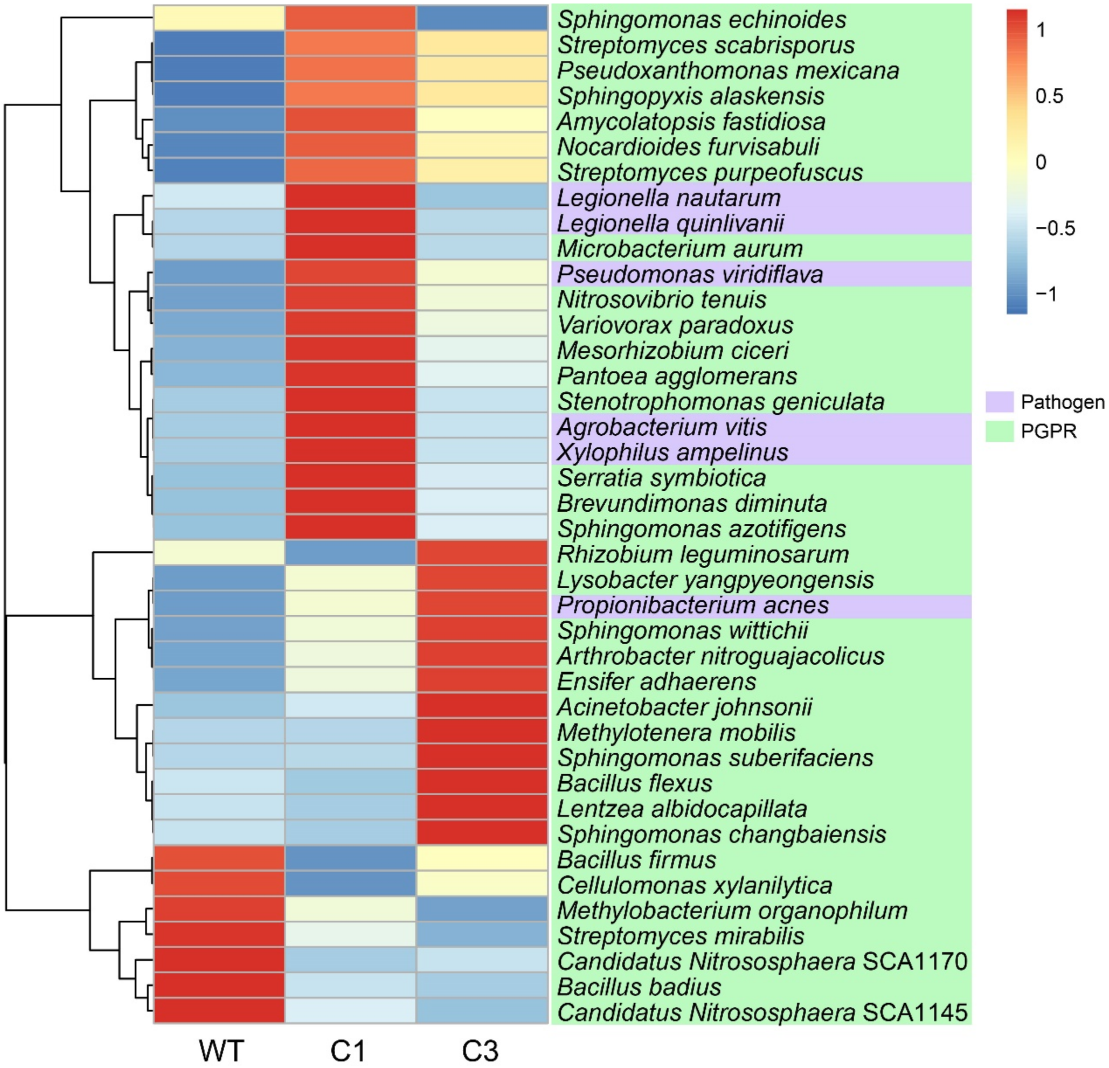
Supplementary Figure S6. Marker genera in rhizosphere samples of wild, C1 and C3 *G. uralensis*. The heatmap was scaled in the row direction based on the microbial relative abundance. WT: wild type; C1: cultivated for one year; C3: cultivated for three years.



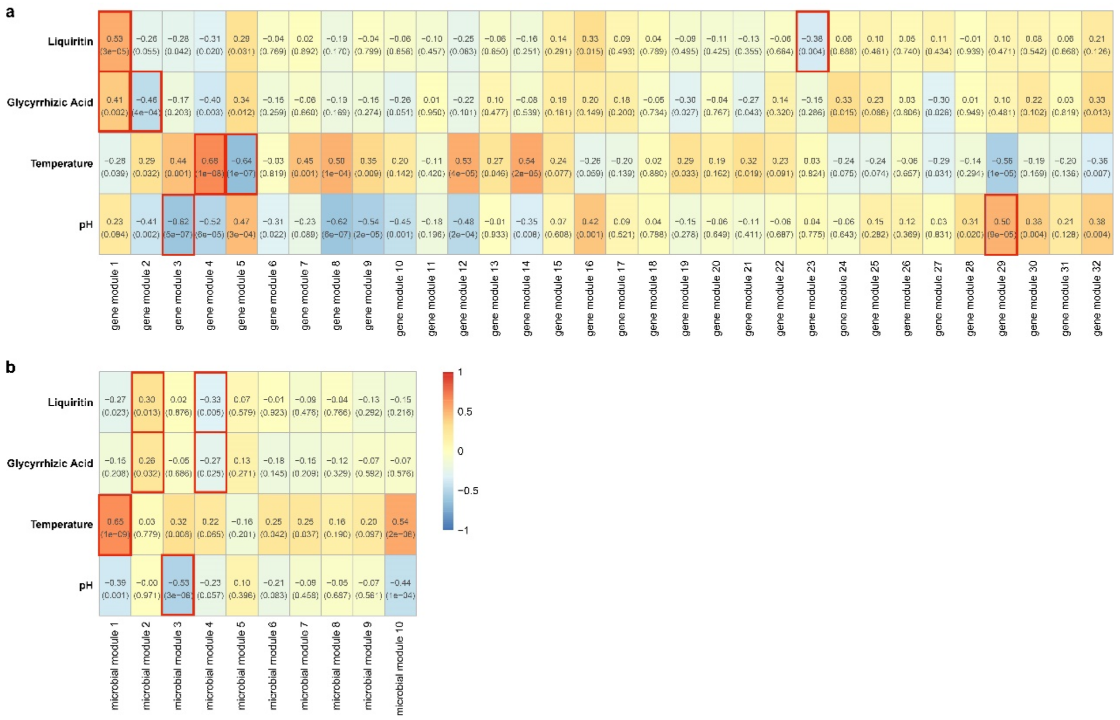
Supplementary Figure S7. Marker pathogen genera in rhizosphere samples of wild, C1 and C3 *G. uralensis*. The heatmap was scaled in the row direction based on the microbial relative abundance. WT: wild type; C1: cultivated for one year; C3: cultivated for three years.



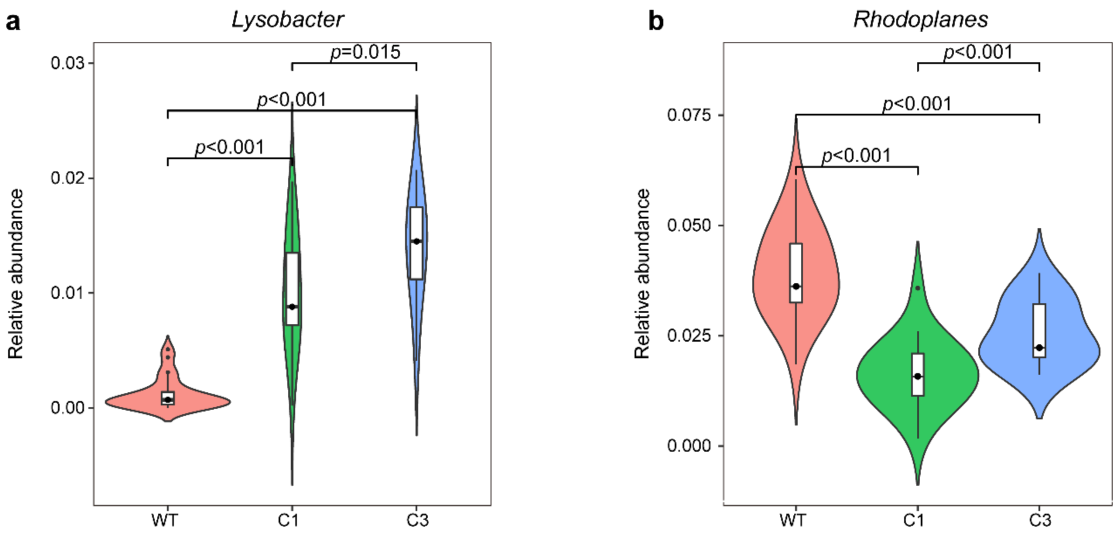
Supplementary Figure S8. The correlation between pathogen abundance and the expression of genes related to plant-pathogen interaction in *G. uralensis*. Significance of the correlation using *t*-test, p<0.05



Supplementary Figure S9. Marker PGPRs and pathogens at the species level in rhizosphere samples of wild, C1 and C3 *G. uralensis*. The heatmap was scaled in the row direction based on the microbial relative abundance. WT: wild type; C1: cultivated for one year; C3: cultivated for three years.



Supplementary Figure S10. Matrix of Module-Trait Relationships (MTRs) and *p*-values for selected traits. **a**. Correlation distribution of traits and gene modules. **b**. Correlation distribution of traits and microbial modules. Each row corresponded to a gene or microbial module, and each column corresponded to a histopathological trait. Each cell contained a corresponding correlation and *p*-value. The table was color-coded by correlation according to the color legend.



Supplementary Figure S11. Relative abundance comparison of *Lysobacter* and *Rhodoplanes* in three types of *G. uralensis*. a. Relative abundance comparison of *Lysobacter* in three types of *G. uralensis*: wild (WT), cultivated *G. uralensis* that were grown for one year (C1) and three years (C3). **b.** Relative abundance comparison of *Rhodoplanes* in wild, C1 and C3 *G. uralensis*.

Supplementary Tables

Supplementary Table S1. The information about collected samples in this study

Sample ID	Group	Transcriptome	Metabolome	Rhizosphere microbiome	Soil microbiome	Temperature	pH	Village
WT1	Wild	√	√	√	√	22.47	6.87	Shiji Quan
WT2	Wild	√	√	√	√	19.70	6.70	Shiji Quan
WT3	Wild	√	√	√	√	19.30	6.80	Shiji Quan
WT4	Wild	√	√	√	√	19.43	6.83	Shiji Quan
WT5	Wild	√	√	√	√	19.53	6.87	Shiji Quan
WT6	Wild	√	√	√	√	19.53	6.87	Shiji Quan
WT7	Wild	√	√	√	√	19.83	6.87	Shiji Quan
WT8	Wild	√	√	√	√	18.87	6.90	Shiji Quan
WT9	Wild	√	√	√	√	19.40	6.93	Shiji Quan
WT10	Wild	√	√	√	√	19.37	6.87	Shiji Quan
WT11	Wild	√	√	√	√	19.27	6.90	Shiji Quan
WT12	Wild	√	√	√	√	20.50	6.93	Shiji Quan
WT13	Wild	√	√	√	√	20.90	6.87	Shiji Quan
WT14	Wild	√	√	√	√	19.23	6.87	Shiji Quan
WT15	Wild	√	√	√	√	19.50	6.87	Shiji Quan
WT16	Wild	√	√	√	√	19.77	6.87	Shiji Quan
WT17	Wild	√	√	√	√	21.33	6.90	Shiji Quan
WT18	Wild	√	√	√	√	21.60	6.93	Shiji Quan
WT19	Wild	√	√	√	√	20.30	6.87	Shiji Quan
WT20	Wild	×	√	√	√	20.37	6.90	Shiji Quan
WT21	Wild	×	√	√	√	21.63	6.93	Shiji Quan

WT22	Wild	×	√	√	√	21.57	6.80	Shiji Quan
WT23	Wild	×	√	√	√	20.43	6.90	Shiji Quan
WT24	Wild	×	√	√	√	20.13	6.93	Shiji Quan
WT25	Wild	√	√	√	√	19.50	6.93	Shiji Quan
C1-1	Cultivated for one year	×	√	√	√	27.77	6.63	TianJi Zhang
C1-2	Cultivated for one year	√	√	√	√	23.47	6.90	TianJi Zhang
C1-3	Cultivated for one year	×	√	√	√	22.50	6.57	TianJi Zhang
C1-4	Cultivated for one year	×	√	×	√	22.47	6.87	TianJi Zhang
C1-5	Cultivated for one year	×	√	√	√	19.70	6.77	TianJi Zhang
C1-6	Cultivated for one year	√	√	×	√	23.83	6.73	TianJi Zhang
C1-7	Cultivated for one year	×	√	√	√	20.67	6.80	TianJi Zhang
C1-8	Cultivated for one year	√	√	√	√	22.10	6.77	TianJi Zhang
C1-9	Cultivated for one year	×	√	√	√	18.17	6.80	TianJi Zhang
C1-10	Cultivated for one year	√	√	√	×	19.83	6.80	TianJi Zhang
C1-11	Cultivated for one year	×	√	√	√	21.23	6.80	TianJi Zhang

C1-12	Cultivated for one year	√	√	√	√	22.13	6.90	TianJi Zhang
C1-13	Cultivated for one year	×	√	√	√	20.63	6.87	TianJi Zhang
C1-14	Cultivated for one year	×	√	√	√	20.77	6.83	TianJi Zhang
C1-15	Cultivated for one year	×	√	√	√	21.83	6.83	TianJi Zhang
C1-16	Cultivated for one year	√	√	√	√	22.47	6.77	TianJi Zhang
C1-17	Cultivated for one year	×	√	×	√	24.93	6.67	TianJi Zhang
C1-18	Cultivated for one year	×	√	√	√	23.10	6.80	TianJi Zhang
C1-19	Cultivated for one year	×	√	√	√	21.03	6.77	TianJi Zhang
C1-20	Cultivated for one year	√	√	√	√	20.57	6.70	TianJi Zhang
C1-21	Cultivated for one year	√	√	√	√	23.17	6.83	TianJi Zhang
C1-22	Cultivated for one year	×	√	√	√	21.03	6.70	TianJi Zhang
C1-23	Cultivated for one year	√	√	×	√	22.13	6.70	TianJi Zhang
C1-24	Cultivated for one year	×	√	√	√	23.93	6.80	TianJi Zhang

C1-25	Cultivated for one year	√	√	√	√	22.17	6.73	TianJi Zhang
C3-1	Cultivated for three years	√	√	√	√	20.13	5.93	TianJi Zhang
C3-2	Cultivated for three years	√	√	√	√	20.37	6.37	TianJi Zhang
C3-3	Cultivated for three years	√	√	√	√	21.63	6.17	TianJi Zhang
C3-4	Cultivated for three years	√	√	√	√	20.10	6.30	TianJi Zhang
C3-5	Cultivated for three years	√	√	√	√	21.87	6.20	TianJi Zhang
C3-6	Cultivated for three years	√	√	√	√	20.73	6.67	TianJi Zhang
C3-7	Cultivated for three years	√	√	×	√	22.90	6.87	TianJi Zhang
C3-8	Cultivated for three years	√	√	√	√	21.87	6.53	TianJi Zhang
C3-9	Cultivated for three years	√	√	√	√	23.27	6.63	TianJi Zhang
C3-10	Cultivated for three years	√	√	√	×	23.43	6.77	TianJi Zhang
C3-11	Cultivated for three years	√	√	√	√	22.60	6.90	TianJi Zhang
C3-12	Cultivated for three years	√	√	√	√	23.17	6.90	TianJi Zhang

C3-13	Cultivated for three years	√	√	√	√	23.40	6.67	TianJi Zhang
C3-14	Cultivated for three years	√	√	√	√	22.67	6.67	TianJi Zhang
C3-15	Cultivated for three years	√	√	×	√	22.07	6.70	TianJi Zhang
C3-16	Cultivated for three years	√	√	√	√	22.27	6.60	TianJi Zhang
C3-17	Cultivated for three years	√	√	√	√	23.37	6.80	TianJi Zhang
C3-18	Cultivated for three years	√	√	√	√	23.70	6.80	TianJi Zhang
C3-19	Cultivated for three years	×	√	√	√	20.80	6.37	TianJi Zhang
C3-20	Cultivated for three years	√	√	√	√	23.43	6.50	TianJi Zhang
C3-21	Cultivated for three years	√	√	√	√	24.57	6.60	TianJi Zhang
C3-22	Cultivated for three years	√	√	√	√	22.40	6.77	TianJi Zhang
C3-23	Cultivated for three years	√	√	√	√	24.20	6.70	TianJi Zhang
C3-24	Cultivated for three years	√	√	√	√	24.37	6.70	TianJi Zhang
C3-25	Cultivated for three years	√	√	√	√	24.57	6.83	TianJi Zhang

Supplementary Table S2. The sequencing information about collected samples in this study

Sample ID	Transcriptome reads	Rhizosphere mircrobial reads	Soil mircrobial reads
WT1	47,799,762	400,528	176,054
WT2	45,806,484	189,932	167,000
WT3	49,128,144	382,816	234,160
WT4	49,736,312	157,834	168,762
WT5	49,562,546	138,304	199,314
WT6	50,328,210	149,168	168,806
WT7	48,621,854	215,694	152,826
WT8	47,933,578	136,396	191,286
WT9	49,289,478	195,346	179,722
WT10	48,650,092	144,366	179,848
WT11	52,775,672	146,752	185,208
WT12	49,808,910	159,218	161,952
WT13	50,809,828	190,604	177,764
WT14	49,552,490	174,858	171,240
WT15	53,064,638	219,576	163,848
WT16	53,606,680	205,562	160,432
WT17	46,267,576	204,158	203,290
WT18	52,714,148	167,140	177,852
WT19	46,935,588	171,038	157,722
WT20	48,314,440	180,042	171,066
WT21	NA	181,796	164,166
WT22	NA	241,556	169,176
WT23	NA	227,396	150,436
WT24	NA	180,296	150,346
WT25	48,585,478	227,990	190,586
C1-1	NA	253,700	607,660
C1-2	53,158,416	218,252	487,962
C1-3	NA	176,908	152,608
C1-4	NA	NA	457,266
C1-5	NA	161,536	410,804
C1-6	47,593,790	NA	397,380
C1-7	NA	197,306	219,880
C1-8	47,005,566	273,836	450,092
C1-9	NA	257,980	166,014
C1-10	46,437,566	258,426	NA
C1-11	NA	239,264	189,280
C1-12	51,321,014	215,728	138,882
C1-13	NA	179,416	184,200
C1-14	NA	270,336	138,306
C1-15	NA	221,146	164,180

C1-16	47,401,372	230,258	143,486
C1-17	NA	NA	207,412
C1-18	NA	194,856	191,304
C1-19	NA	206,756	174,420
C1-20	52,750,846	213,478	195,206
C1-21	41,451,592	175,506	168,234
C1-22	NA	193,622	209,870
C1-23	50,120,412	NA	184,580
C1-24	NA	255,850	152,680
C1-25	53,908,780	265,948	262,156
C3-1	52,668,672	197,414	176,842
C3-2	55,034,984	219,394	189,492
C3-3	51,528,026	187,290	194,388
C3-4	50,487,742	224,396	154,718
C3-5	55,257,210	169,948	200,256
C3-6	50,548,492	236,964	237,324
C3-7	53,428,780	NA	213,306
C3-8	51,302,848	203,874	190,526
C3-9	55,931,934	204,620	201,700
C3-10	52,775,118	217,712	NA
C3-11	67,279,696	184,602	167,376
C3-12	47,713,652	211,490	155,358
C3-13	54,059,372	220,440	160,420
C3-14	44,550,252	153,562	153,240
C3-15	57,015,350	NA	235,204
C3-16	43,860,278	144,962	172,462
C3-17	43,846,454	153,058	183,532
C3-18	44,476,450	135,682	177,118
C3-19	NA	152,994	165,606
C3-20	47,960,128	214,072	178,848
C3-21	52,199,638	442,414	186,660
C3-22	45,853,510	396,372	183,778
C3-23	52,590,556	208,094	227,364
C3-24	49,218,040	254,240	200,952
C3-25	48,620,476	358,548	188,470
Total	2,758,648,920	14,740,616	14,921,664