ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Biotechnology

ISSN 1682-296X
DOI: 10.3923/biotech.2017.85.91

Research Article
Identification of Fungal Community in Citrus Rhizosphere by ITS Gene Sequencing

[1,2]Pan Sun, [1,2]Yi-Can Zhang, [1,2]Yun-Lou Shen, [1,2]Chang-Qing Feng and [1,2]Qiang-Sheng Wu

[1]College of Horticulture and Gardening, Yangtze University, Jingzhou, 434025 Hubei, China
[2]Institute of Root Biology, Yangtze University, Jingzhou, 434025 Hubei, China

Abstract

Background and Objective: Citrus is one of the most predominant fruit trees in Southeast Asia, where soil environments are relatively bad. The soil fungal community plays a huge role in maintaining soil ecosystem balance and soil fertility. The present study was conducted to analyze the diversity of fungi community in citrus roots and soils. **Methodology:** The Internal Transcribed Spacer (ITS) fragment sequences were used to evaluate the fungal diversity in 29 years old *Citrus unshiu* Marc. trees grafted on *Poncirus trifoliata*. In the 97% similarity level, Operational Taxonomic Units (OTUs) were clustered by the UPARSE-OUT algorithm. The ITS sequences was used to analyze the alpha diversity. **Results:** There were 579 and 566 OTUs in plant roots and rhizosphere soils, respectively, whilst 462 OTUs were overlapping, confirming that many of the fungi in soils colonized plant roots. At the class, Glomeromycetes, Orbiliomycetes, Lecanoromycetes, Saccharomycetes, Incertae sedis, Leotiomycetes, Tremellomycetes, Sordariomycetes, Eurotiomycetes, Pezizomycetes, Agaricomycetes and Dothideomycetes existed. In the genus level, OTU 1, 3, 4 and 11 were in the top four of fungal community with roots and OTU 1, 5, 6 and 10 in soils. **Conclusion:** The results obtained here suggested that citrus roots exhibited greater fungal diversity than citrus soils, which provides a new highlight to enhance root growth.

Key words: Ascomycota, Basidiomycota, citrus, fungal diversity, ITS gene sequencing, operational taxonomic unit, read abundance

Received: January 02, 2017 Accepted: February 08, 2017 Published: February 15, 2017

Citation: Pan Sun, Yi-Can Zhang, Yun-Lou Shen, Chang-Qing Feng and Qiang-Sheng Wu, 2017. Identification of fungal community in citrus rhizosphere by its gene sequencing. Biotechnology, 16: 85-91.

Corresponding Author: Qiang-Sheng Wu, College of Horticulture and Gardening, Yangtze University, Jingzhou, 434025 Hubei, China Tel: 86 13986717539

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Citrus is one of the most predominant fruit trees in China[1]. Citrus trees are planted in mountain regions and hillside fields, where soils are relatively poor structure and ecological conditions. In addition, the soils of citrus orchard are coarse and soil water-stable aggregate is so bad, which is unfavorable for citrus production[2].

Citrus rhizosphere inhabit lots of microorganisms, who directly affect soil biochemical activity and nutrient composition and transformation[3,4]. Soil microorganisms are considered as the most important biological indicator involved in soil fertility. As reported by Van Heerden *et al*.[5], there were lots of fungi, including *Absidia corymbifera*, *Aspergillus fumigatus*, *Emericella nidulans*, *Penicillium diversum*, *Paecilomyces variotii*, *Rhizomucor pusillus*, *Talaromyces thermophilus* and *Thermomyces lanuginosus* in citrus rhizosphere. In addition, some Arbuscular Mycorrhizal Fungi (AMF), including *Glomus*, *Acaulospora*, *Entrophospora*, *Gigaspora* and *Scutellospora* species were frequent in occurrence in citrus orchards[6]. Such fungi populations are important for the root health, nutrient uptake and tolerance of environmental stress[7,8]. However, the information regarding the diversity of fungi community in citrus is poor known.

The aim of this study was to analyze the fungal community diversity by ITS gene sequencing and further compared the difference of community structure in rhizosphere soils and roots of *Citrus unshiu* Marc trees grafted on trifoliate orange to provide guarantee for soil ecological management.

MATERIALS AND METHODS

Experimental set-up: The experimental site selected was 29 years old Citrus *unshiu* Marc. trees grafted on trifoliata orange (*Poncirus trifoliata* L. Raf), located in the Yangtze University Campus, Jingzhou, China (30°36' N and 112°14' E). The citrus orchard is generally kept with no-tillage soil management and also the Northern subtropical humid monsoon climate, with four distinct seasons.

The samples were collected in July 2016 (a period about the active growth of citrus trees). After cleaning the grass covered the surface soils near the perimeter of the tree, 1 kg soil samples in 5-15 cm soil depth and fibril roots were collected and stored in -80°C. Each sample replicated four times.

DNA extraction: Total genome DNA was extracted from the plant roots and soils using the DNA purification ELISA kits (ZYMO Research Company, Irvine, USA) and then total DNA was purified by means of 0.8% agarose gel.

PCR amplification: Using the special primers, ITS3_KY02 (5′-GATGAAGGCGYAGYRAA-3′), ITS4 (5′-TCCTCCGCTTATTG ATAGC-3′) and KOD-Plus-Neo DNA Polymerase, the specific ITS gene fragments were amplified through PCR reaction (The Applied Biosystems® Gene Amp® PCR System 9700 was used here)[9]. Each sample repeated three times. The PCR reaction was ended in the linear amplification stage. Then, the PCR reaction product was emerged by 1.5% agarose gel electrophoresis. Finally the aim DNA fragments were obtained by the IE buffer solution and checked by 2% agarose gel electrophoresis.

Miseq library contraction and sequencing: By means of the GE Nano system, the PCR production and quantified were detected. The TruSeq DNA PCR-Free Sample Prep Kit (FC-121-3001/3002) was used to establish the Miseq library and sequenced the samples by Miseq Reagent kit v3 (MS-102-3003).

Statistical analysis: With the Miseq sequence, we obtained the PE reads and jointed it by FLASH, controlled it from quality level and eliminated the useless sequence for the high quality sequence. In the 97% similarity level, Operational Taxonomic Units (OTUs) were clustered by the UPARSE-OUT algorithm[10]. Finally, based on the fungal community level, the Internal Transcribed Spacer (ITS) sequences provided a good classification of fungal community, which is used to analyze the alpha diversity[11,12].

RESULTS AND DISCUSSION

OTU numbers: In the proposed reading, OTUs have been widely used in distinguishing fungal and bacterial species[13]. There were 579 OTUs in plant roots and 566 OTUs in rhizosphere soils (Fig. 1). The amount of OUTs in citrus roots is relatively higher than those (91-249 OTUs) in a young transgenic poplar in France. We speculate that the citrus trees used here were 29 years old and had established better fungal communities than the 2 years old poplar[14]. The present study also showed that 462 OTUs were overlapping between citrus roots and soil samples, which confirmed that lots of fungi colonized the plant roots from the rhizosphere soils. Wu *et al*.[15] also observed that the numbers of OTUs in arbuscular mycorrhizal fungi were relatively greater in citrus roots than in citrus soils. Such results indicate that roots might be the preferential location for fungi than soils.

Fungal diversity in the phylum level: The fungal community in the phylum level showed that *Ascomycota* was the dominating fungi species in citrus roots and soils. In soils, there were Ascomycota, Basidiomycota, Glomeromycota and Zygomycota occurred in soils and Ascomycota, Basidiomycota, Glomeromycota, Zygomycota and part unidentified fungi in roots (Fig. 2). Results of the present study showed that citrus trees support a high level of fungal diversity. The result is in agreement with Sun *et al.*[16] in apple soils and Moll *et al.*[17] in maize soils. As reported by Abdelfattah *et al.*[18], many of fungi are facultative plant pathogens. It seems that roots possessed relatively higher abundance in the phylum level than soils.

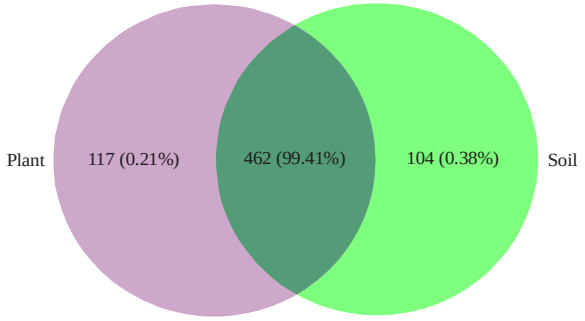

Fig. 1: Quantity of OTUs in the plant root and rhizosphere soil of *Citrus unshiu* grafted on *Poncirus trifoliata*

Fungal diversity in the class level: The fungal community at the class level indicated that Dothideomycetes, Eurotiomycetes, Agaricomycetes and Pezizomycetes were the primary in the roots and Dothideomycetes, Agaricomycetes, Pezizomycetes and Sordariomycetes in the soils (Fig. 3). Dothideomycetes, Agaricomycetes and Pezizomycetes were observed in all the roots and rhizosphere soil samples, with high relative abundance. Meanwhile, the relative abundance of Agaricomycetes was higher within rhizosphere soils than roots, showing that the fungi class is preferably inhabited in rhizosphere soils than in roots. After all, Agaricomycetes have applied as not only favorable technology for the bioremediation of contaminated soils but also as a soil environmental friend[19]. On the contrary, Eurotiomycetes and Dothideomycetes had higher relative abundance in plants in comparison with rhizosphere soils, indicating that the two classes are a stronger inhabitant within roots than within soils

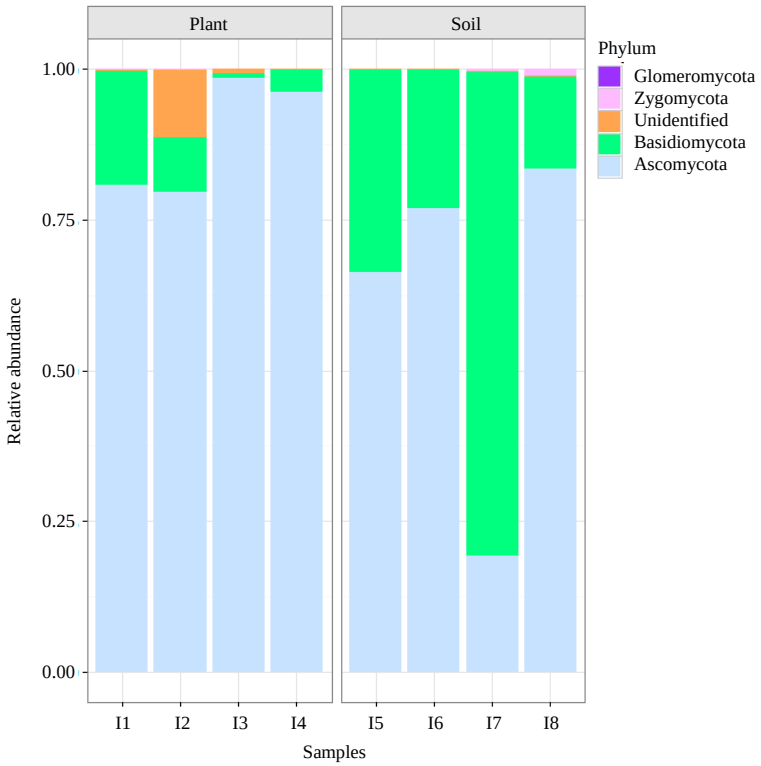

Fig. 2: Fungal relative abundance in the phylum level in plants and soils of *Citrus unshiu* grafted on *Poncirus trifoliata*
Here, I1 to I4 are the four samples from plant roots and I5 to I8 from soils

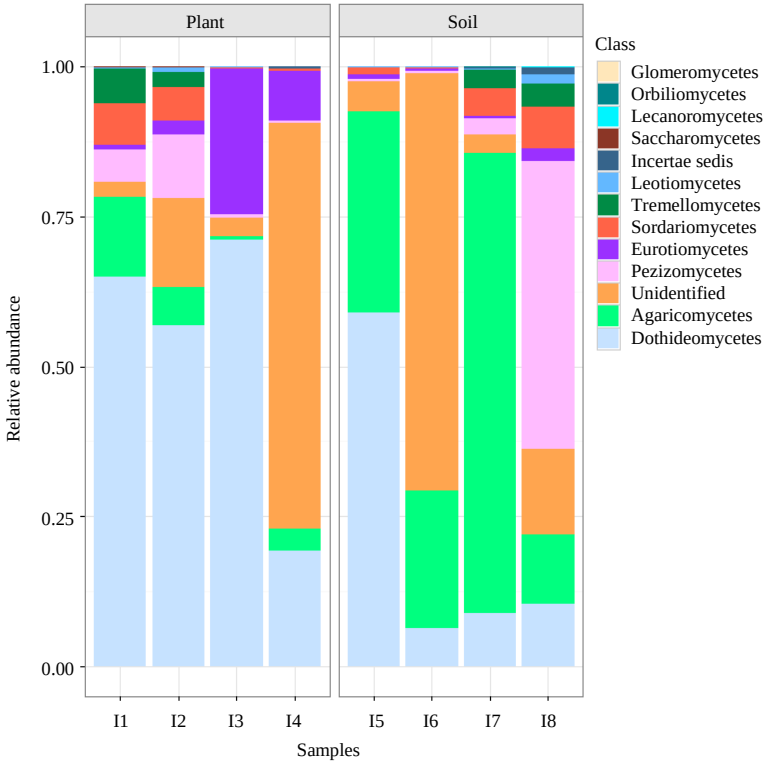

Fig. 3: Fungal relative abundance in the class level in plants and soils of *Citrus unshiu* grafted on *Poncirus trifoliata*
Here, I1 to I4 are the four samples from plant roots and I5 to I8 from soils

(Fig. 3). This is in accordance with Moll *et al.*[17], who reported that part fungal taxa were highly abundant in roots than in soils, because easily accessible substrates absorbed by fungi is originated from the roots.

Fungal diversity in the genus level: The top fifty highest read abundance of the fungal community at the genus level is shown in Fig. 4. From the diagram analysis, the top five fungi in plants were Ascomycota; c_AscomycotaOTU_1, o_Pleosporales_OTU_2, o_Pleosporales_OTU_4, o_Pleosporales_OTU_3 and f_Sporormiaceae_OTU_11 in plant and Basidiomycota.f_Ceratobasidiaceae_OTU_6, f_Ceratobasidiaceae_OTU_10, c_Agaricomycetes_OTU_7, Ascomycota. c_Ascomycota OTU_1 and o_Pleosporales_OTU_2 in soils (Fig. 4). The two fungal species, c_Ascomycota_OTU_1 and o_Pleosporales_OTU_2 were found in both plant roots and soils which were approx. 10% read abundance, suggesting that c_Ascomycota_OTU_1 and o_Pleosporales_OTU_2 were the main fungus among the citrus roots and soils. The f_Ceratobasidiaceae_OTU_6, f_Ceratobasidiaceae_OTU_10, f_Pyronemataceae_OTU_5 and c_Agaricomycetes_OTU_7 were more preferentially living in soils, as shown in Fig. 4. While o_Pleosporales_OTU_4, o_Pleosporales_OTU_3 and f_Sporomiaceae_OTU_11 were stronger inhabitant in plants than in soils (Fig. 4). It suggests that fungal species are highly varied in soils versus roots. Similar results were also found in maize soils[17]. Possibly, fungi can be influenced by abiotic and biotic factors, such as soil structure, pH, oxygen availability, resource type and availability, resulting in different fungal niche space[20, 21].

Alpha diversity analysis: In terms of the alpha diversity analysis, average diversity measurement value was higher in the plant samples than in the soils (Fig. 5). This suggests that the diversity and evenness of the fungal community in plants were richer than in the soils. This is in agreement with the findings of Wu *et al.*[15] in AMF communities of citrus.

Core taxa analysis: The core species can be reflected by the sequence count. The core species of plants were larger than rhizosphere soils as shown in Fig. 6. Possibly, fungal communities were selectively transferred from the soils to the plant roots for the subsistence[22]. The fungal species were preferably habited in roots, helping the host plant superior developed.

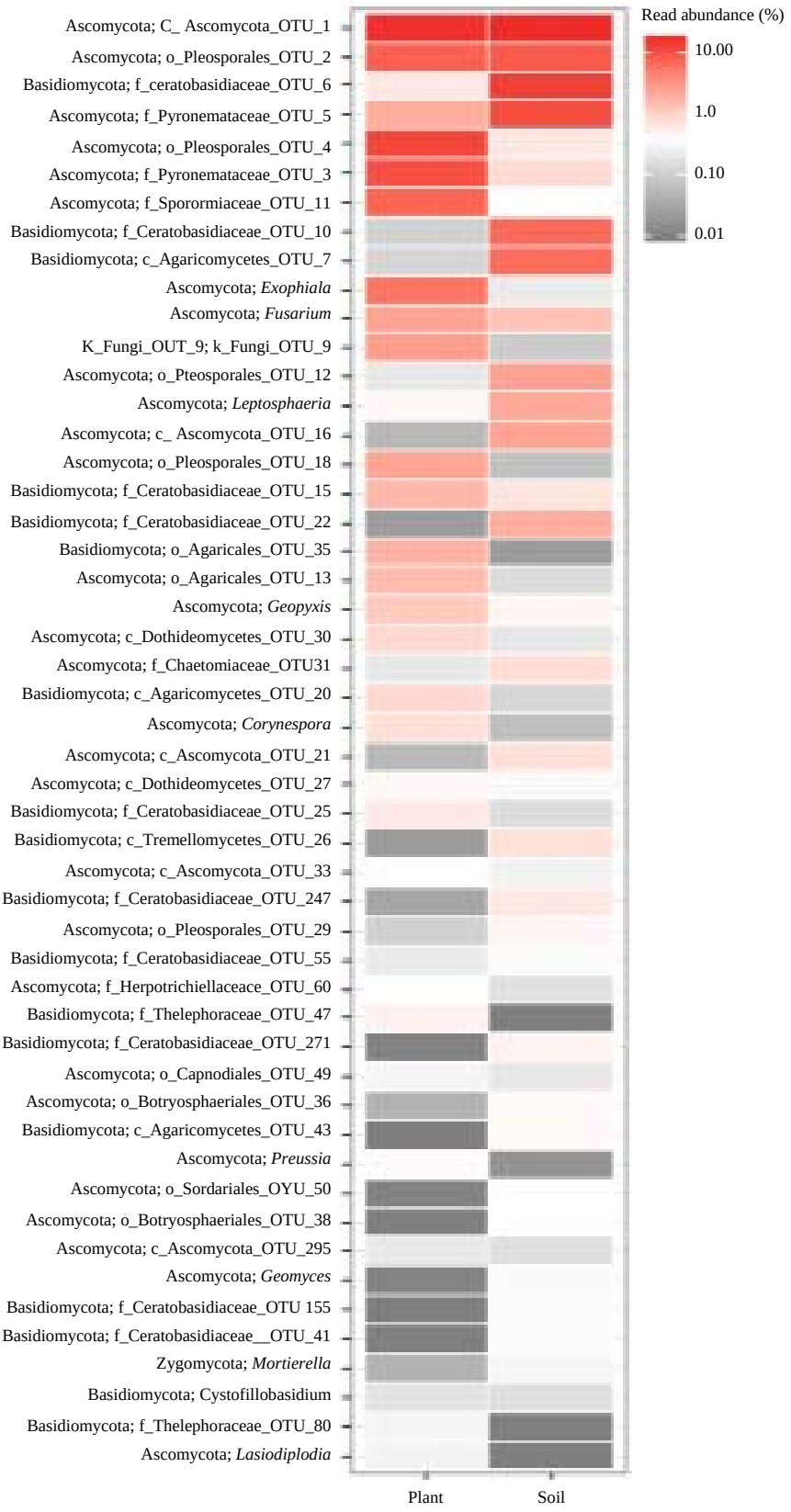

Fig. 4: Read abundance of the top fifty of fungal communities in the genus level in the plant roots and rhizosphere soil of *Citrus unshiu* grafted on *Poncirus trifoliata*

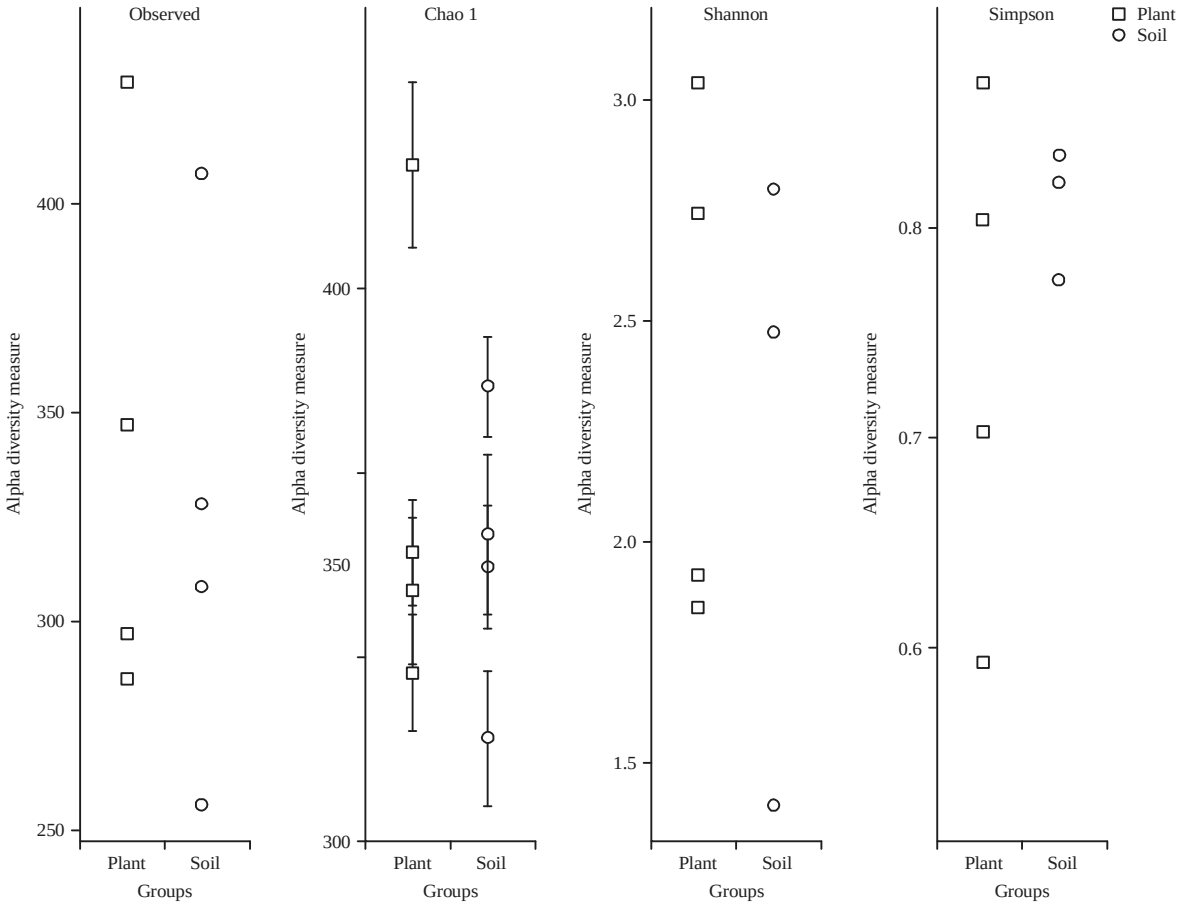

Fig. 5: Species richness plot in plant roots and soils of *Citrus unshiu* grafted on *Poncirus trifoliata*

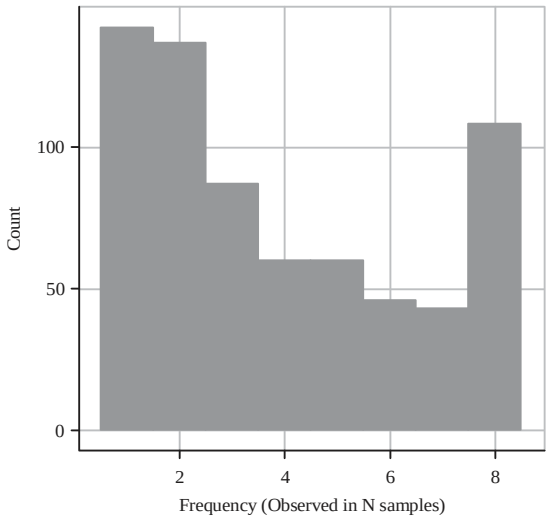

Fig. 6: Sequence count in the plant roots and rhizosphere soil of *Citrus unshiu* grafted on *Poncirus trifoliata*

CONCLUSION

In short, the numbers of OTUs were dramatically greater in citrus roots than in citrus soils. Citrus roots exhibited relatively higher Ascomycota and lower Basidiomycota in the phylum than citrus soils. Dothideomycetes, Agaricomycetes and Pezizomycetes were observed in all the roots and rhizosphere soil samples, with high relative abundance. Citrus roots showed higher species richness than soils.

SIGNIFICANCE STATEMENTS

This study analyzed the diversity of fungi community in citrus roots and soils. In this work, citrus roots exhibited relatively higher Ascomycota and lower Basidiomycota in the phylum than citrus soils. Dothideomycetes, Agaricomycetes and Pezizomycetes were observed in all the roots and rhizosphere soil samples, with high relative abundance. This

study will provide the highlight that management of root fungal community in citrus orchard is more important than soil for greater soil ecosystem balance and fertility.

ACKNOWLEDGMENTS

This study was supported by the Plan in Scientific and Technological Innovation Team of Outstanding Young, Hubei Provincial Department of Education (T201604) and the Creative Experimental Project of National Undergraduate Students in Yangtze University (2016139).

REFERENCES

1. Liu, K.H., Y. Zhou and Z.A. Li, 2016. Technical progress on shoot-tip grafting of citrus. Acta Hortic. Sin., 43: 1665-1674.
2. Srivastava, A.K. and S.K. Malhotra, 2014. Nutrient management in fruit crop: Issues and strategies. Ind. J. Ophthalmol., 10: 72-88.
3. Srivastava, A.K. and S. Singh, 2002. Soil analysis based diagnostic norms for Indian citrus cultivar. Commun. Soil Sci. Plant Anal., 33: 1689-1706.
4. Ortas, I., 2012. Mycorrhiza in Citrus: Growth and Nutrition. In: Advances in Citrus Nutrition, Srivastava, A.K. (Ed). Springer, Dordrecht, pp: 333-351.
5. Van Heerden, I., C. Cronje and J.M. Kotze, 2002. Microbial, chemical and physical aspects of citrus waste composting. Bioresour. Technol., 81: 71-76.
6. Wu, Q.S and A.K. Srivastava, 2012. Rhizosphere Microbial Communities: Isolation, Characterization And Value Addition For Substrate Development. In: Advances In Citrus Nutrition. Srivastava, A.K (Ed.). Springer, Netherlands., pp: 169-194.
7. Wu, Q.S., A.K. Srivastava and Y.N. Zou, 2013. AMF-induced tolerance to drought stress in citrus: A review. Scient. Hortic., 164: 77-87.
8. Srivastava, A.K. and S. Singh, 2008. Citrus nutrition research in India: Problems and prospects. Indian J. Agric. Sci., 78: 3-16.
9. Toju, H., A.S. Tanabe, S. Yamamoto and H. Sato, 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PloS One, Vol. 7.
10. Koljalg, U., R.H. Nilsson, K. Abarenkov, L. Tedersoo and A.F. Taylor *et al*., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol., 22: 5271-5277.
11. Gentleman, R. and R. Ihaka, 2011. R: A language and environment for statistical computing. Computing, 14: 12-21.
12. Edgar, R.C., 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nature Meth., 10: 996-998.
13. Koeppel, A.F. and M. Wu, 2013. Surprisingly extensive mixed phylogenetic and ecological signals among bacterial operational taxonomic units. Nucleic Acids Res., 41: 5175-5188.
14. Danielsen, L., A. Thurmer, P. Meinicke, M. Buee and E. Morin *et al*., 2012. Fungal soil communities in a young transgenic poplar plantation form a rich reservoir for fungal root communities. Ecol. Evol., 2: 1935-1948.
15. Wu, Q.S., P. Sun. and A.K. Srivastava, 2017. AMF diversity in citrus rhizosphere. Ind. J. Agric. Sci., 87: 653-656.
16. Sun, J., Q. Zhang, X. Li, B. Zhou and Q. Wei, 2017. Apple replant disorder of pingyitiancha rootstock is closely associated with rhizosphere fungal community development. J. Phytopathol., 165: 162-173.
17. Moll, J., B. Hoppe, S. Konig, T. Wubet, F. Buscot and D. Kruger, 2016. Spatial distribution of fungal communities in an arable soil. PloS One, Vol. 11.
18. Abdelfattah, A., S.O. Cacciola, S. Mosca, R. Zappia and L. Schena, 2017. Analysis of the fungal diversity in citrus leaves with greasy spot disease symptoms. Microbial. Ecol., 73: 739-749.
19. Mohammadi-Sichani, M.M., M.M. Assadi, A. Farazmand, M. Kianirad, A.M. Ahadi and H.H. Ghahderijani, 2017. Bioremediation of soil contaminated crude oil by *Agaricomycetes*. J. Environ. Health Sci. Eng., Vol. 15.
20. Moll, J., K. Goldmann, S. Kramer, S. Hempel and E. Kandeler *et al*., 2015. Resource type and availability regulate fungal communities along arable soil profiles. Microbial Ecol., 70: 390-399.
21. Lauber, C.L., M.S. Strickland, M.A. Bradford and N. Fierer, 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem., 40: 2407-2415.
22. Zuo, X., S. Wang, P. Lv, X. Zhou, X. Zhao, T. Zhang and J. Zhang, 2016. Plant functional diversity enhances associations of soil fungal diversity with vegetation and soil in the restoration of semiarid sandy grassland. Ecol. Evol., 6: 318-328.