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Effects of arbuscular mycorrhizae on microbial population and enzyme activity in replant soil used for watermelon production

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Abstract

Arbuscular mycorrhizal (AM) fungi are ubiquitous fungi distributed widely in soil ecosystems. It has been showed that AM fungi play an important role in improving soil nutrition and enhancing crop disease resistance, which have great application potentials in overcoming crop replant problems. In order to evaluate the effects of AM fungi on soil microbe population and soil enzyme activities in replant soils, three replant soils respectively with 3, 7, and 12 watermelon (*Citrullus lanatus*) replanting years were employed to be investigated. Results showed that the total soil microbe, bacteria, and actinomycete population, and the activities of soil proteinase, polyphenoloxidase, urease, and saccharase in replant soils gradually declined, while the fungal population, and the fungi/total microbe ratio increased, as replanting years rose. In each replant soil, the inoculation with AM fungus *Glomus versiforme* enhanced soil bacteria and actinomycete population, and decreased the fungal numbers, and the fungi/total microbe ratio in replanting soils, and improved soil proteinase, polyphenoloxidase, urease, and saccharase activities, compared with controls. That contributed to the relative equilibrium of the three kinds of soil microorganism populations. It is concluded that the AM fungal inoculation can reduce watermelon replant problems through effectively modifying the soil microbe population and community structure, and increasing the soil enzyme activities.

Keywords: arbuscular mycorrhizae fungi; watermelon; greenhouse; soil microbial population; soil enzyme activity

1. Introduction

There are many reasons that cause crop replant problems, the main one is the non equilibrium of soil microbe community structures, which results in reducing crop yield and soil quality. Under replant conditions, crop root secretion and litter decayed materials provide rich nutrients for the pathogens, meanwhile, the long stage of suitable protective cultivation environments establish good reproductive condition to the pathogens, hence the pathogenic population continue to increase (Chin *et al.*, 1994), and soil-borne diseases are aggravated. And this change varies as the replant time rise. Meanwhile, under protective cultivation condition when pest become serious, the large amount of pesticides used result in damaging crop growth environment, disbenefiting to soil microbial population and organic matter. While the natural equilibrium of soil microbial community and inorganic components is broken by the replant cropping, then the soil-born pathogen develops and the soil-born disease overspreads. As one of the important parameters of characteristic soil biological characters, soil enzyme activities reflect soil nutrient transformation status, appear higher complexity due to be affected by many soil factors, and are directly much influenced with minor elements, organic matter and available nitrogen (Cao *et al*., 2002). It was proved that the soil microbe population and soil enzyme activities were changed in watermelon replant soil (Zhao *et al.*, 2008).

Soil fumigation is banned due to high cost and environmental concerns and long term fallow is strongly recommended before planting, but growers are not willing to wait in intensive production areas. It has been showed that arbuscular mycorrhizal (AM) fungi can change soil microbe species and ratios, and impact on the rhizosheric microbe community (Linderman *et al.*, 1996). In fact, the rhizospheric microbes could be influenced directly or indirectly by AM fungi, i.e. under the precondition of little change of the species, the microbe population was changed, the new equilibrium was established, and their activities were increased (Linderman, 1988; Kothari *et al.*, 1991). AM fungi have positive effects on replant diseases in grape or apple orchard (Camprubí *et al*., 2008; Raj and Sharma, 2009). Inoculation with vesicular-arbuscular mycorrhizal fungi *Glomus etunicatum* was successful

during the first 6 months of growth only when apple seedlings were grown for the first three weeks in a sterile substrate (sand-soil-peat). When young grafted apple trees were inoculated directly in orchard soil with Apple replant disease under non-sterile conditions, the effect of inoculation was negligible after 6 months. There were significant differences in the composition of the rhizosphere microflora after 6 months, especially between the soil with Apple replant disease and the virgin soil (Catská and Taube-Baab, 1994). Pre-inoculated peach seedlings transplanted in non-replant soils showed greater initial growth in the first year. Plant height, and lateral shoot length and number was highest in non-replant soils irrespective of mycorrhizal pre-inoculation. Similarly, biomass yield was significantly higher in seedlings in non-replant soils (Rutto and Mizutani, 2006). Inoculation with AM fungi can also change the bacteria and actinomycete numbers on the root surface and in the rhizosphere. While the benefit rhizospheric microbe which coexists with AM fungi may enhance AM fungal colonization. The author once found that AM fungi improved watermelon (*Citrullus lanatus*) seedling growth, and effectively decreased fusarium wilt diseases under replant conditions. However, we know little information on the influence of AM fungi on replant soil microbe population.

The purpose of the study was to investigate the effects of AM fungi on soil microbial population, the fungi/total microbe ratio, and the soil enzyme activities in watermelon replant soil, in order to provide the basis for further studying the mechanism of AM fungus overcoming replant problems.

2. Materials and methods

2.1 Materials: Seeds of watermelon (*Citrullus lanatus*) cultivar No. 1 Jingxin that was resistant to fusarium wilt disease were soaked in water for 12 hrs after being surface-sterilized with 70% ethanol, then buding under 28-30 for sowing. Fresh spores and colonized root pieces from 4-month-old pot cultures of AM fungus *Glomus versiforme* on *Trifolium repens* L. were used as inocula. Replant soil with 3, 7, and 12 replant years was collected respectively from a plastic shield field in Raogou, Changle County, Shandong Province. Clay pots (25×30 cm) and seedling trays were surface-sterilized with 40% formaldehyde solution for growing watermelon seedlings.

2.2 Experimental design: Experiments were carried in a sunlight-greenhouse. The replant soil with 3, 7, and 12 years was inoculated with or without *Glomus versiforme* (Gv) respectively, with 6 treatments. Each treatment was replicated in 30 pots, with randomly arranged.

2.3 Sowing and inoculation: Two budded seeds of watermelon were put in a tray hole filled with sterilized peat mixed with 5000 IPU of *Glomus versiforme* inocula(Liu and Luo, 1994), while the control was added the sterilized inocula with 10ml filter solution of the inocula. The temperature was 26^0C /15^0C (Day/Night). Seedlings with 3 leaves were transplanted into the pots. One seedling was maintained in each pot. Plants were watered once every 2 days, and 30% in strength of Hoagland nutrient solution without phosphorus was added once every 2 weeks.

2.4 Measuring soil microbial population: Soil samples were collected at the flowering, fruiting and harvest stages respectively, to isolate, determine the numbers of bacteria, actinomycete, and fungi. Beef extract peptone, No.1 Gao, and PDA was employed respectively to isolate bacteria, actinomycete, and fungi. Dilute plate counting method was used to measure the microbe numbers (Zhao and He, 2002). The fungi/total microbe ratio was the value of the fungal number divided by the total microbe number.

2.5 Assaying of soil enzyme activity: Soil samples were collected at the flowering, fruiting and harvest stages respectively, to determine the soil enzyme activity. Activities of proteinase, polyphenoloxidase, urease, and saccharase were determined respectively with ninhydrin colorimetry, potassium dichromate colorimetry, indophenol blue photometric method, and dinitrosalicylic acid colorimetry (Guan, 1986).

2.6 Statistical analysis: The data were subjected to Analysis of Variance (ANOVA) using the Statistical Analysis Systems (SAS 6.12) package. Comparison of multiple means was performed using the least significant difference (LSD) test at the 5% level.

3. Results and discussion

As the replanting year rose, total soil microbe population, bacterium and actinomycete numbers reduced, while the fungal population and the fungi/total microbe ratio increased in the present experiment. The results showed that AM fungal inoculation in watermelon replant soil could significantly promote the population of bacteria, actinomycete, and the total microbes, and decrease the fungal population and the fungi/total microbe ratio. In various growth and development stages of watermelon, effects of AM fungi on soil microbe population in watermelon replant soil with different replanting years showed the similar pattern. For instance, the total microbe population in the treatment with AM fungi was significantly higher than that of control at the flowering period of watermelon, indicating that AM fungi would enrich the microbe numbers in replant soil.

In replant soils with various replanting years, the AM fungal inoculation treatments showed less fungal numbers, more bacteria and actinomycete, and lower the fungi/total microbe ratio (from 1/2 to 1/4) compared with control (Table 1). It was suggested that AM fungi can be beneficial to keeping a suitable proportion of microbes in replant soil on some degree.

At the fruit setting stage of watermelon plants, soil bacterium, actinomycete, fungal, and the total microbe population significantly increased (Table 2). Compared with the fruit setting stage, the soil bacterium, actinomycete, and the total microbe population significantly reduced, while the fungal number and the fungi/total microbe ratio increased at harvest stage (Table 3). And the AM fungal treatment gave the same effects as above mentioned. These results supported the findings by Secilia and

Bagyaraj (1988). As we know that the mycorrhiza formation impacts greatly the rhizospheric microbe community and population, second to the influence by plant species (Linderman *et al.*, 1996). AM fungi may directly or indirectly influence the rhizospheric microbe, i.e. under the condition of no great changes of microbe species, alter their population, let forming a new equilibrium, and enhance their activities (Kothari *et al*., 1991). This was very important in stabilization of the microbe equilibrium during the whole development of crops.

Table 1 Effects of AM fungi on soil microbe population in watermelon replant soil at the flowering period of watermelon plants

Treatments	Total microbe numbers ($\times10^4$CFU·g^{-1} dry soil)	Number of fungi ($\times10^4$ CFU·g^{-1} dry soil)	Number of bacteria ($\times10^4$ CFU·g^{-1} dry soil)	Number of actinomycete ($\times10^4$ CFU·g^{-1} dry soil)	Fungi / Total Microbe ratio
3-CK	1705.6 c	3.1 c	1107.5 c	595.0 c	0.002 cd
3-AM	2769.2 a	1.7 f	1705.0 a	1062.5 a	0.001 e
7-CK	1417.4 d	4.9 b	1080.0 d	342.5 d	0.004 b
7-AM	2039.9 b	2.4 e	1332.5 b	705.0 b	0.001 d
12-CK	976.6 f	6.6 a	707.5 f	262.5 f	0.007 a
12-AM	1117.7 e	2.7 d	822.5 e	292.5 e	0.002 c

* Means in each column followed by different letters are significantly different based on LSD test(P<0.05).
3-CK, replant soil with 3 replanting years; 3-AM, inoculating AM fungi in replant soil with 3 replanting years;
7-CK, replant soil with 7 replanting years; 7-AM, inoculating AM fungi in replant soil with 7 replanting years;
12-CK, replant soil with 12 replanting years; 12-AM, inoculating AM fungi in replant soil with 12 replanting years.

Table 2 Effects of AM fungi on soil microbe population in watermelon replant soil at the fruit setting stage of watermelon plants

Treatments	Total microbe numbers ($\times10^4$CFU·g^{-1} dry soil)	Number of fungi ($\times10^4$ CFU·g^{-1} dry soil)	Number of bacteria ($\times10^4$ CFU·g^{-1} dry soil)	Number of actinomycete ($\times10^4$ CFU·g^{-1} dry soil)	Fungi / Total microbe ratio
3-CK	4371.7 c	6.2 c	3523 d	642.5 b	0.0014 b
3-AM	6612.3 a	3.8 d	5801 a	807.5 a	0.0006 d
7-CK	4227.2 d	7.2 b	3625 d	595.0 c	0.0017 ab
7-AM	5361.1 e	5.6 cd	4613 b	742.5 a	0.0010 c
12-CK	3666.9 f	8.4 a	3416 e	242.5 e	0.0023 a
12-AM	4539.6 b	6.6 c	3998 c	535.0 d	0.0015 b

* Means in each column followed by different letters are significantly different based on LSD test(P<0.05).
3-CK, replant soil with 3 replanting years; 3-AM, inoculating AM fungi in replant soil with 3 replanting years;
7-CK, replant soil with 7 replanting years; 7-AM, inoculating AM fungi in replant soil with 7 replanting years;
12-CK, replant soil with 12 replanting years; 12-AM, inoculating AM fungi in replant soil with 12 replanting years.

Table 3 Effects of AM fungi on soil microbe population in watermelon replant soil at the harvesting time of watermelon

Treatments	Total microbe numbers ($\times10^4$CFU·g^{-1} dry soil)	Number of fungi ($\times10^4$ CFU·g^{-1} dry soil)	Number of bacteria ($\times10^4$ CFU·g^{-1} dry soil)	Number of actinomycete ($\times10^4$ CFU·g^{-1} dry soil)	Fungi / Total microbe ratio
3-CK	3116.6 d	9.1 d	2545.0 b	562.5 b	0.003 c
3-AM	4259.5 c	4.5 f	3425.0 a	830.0 a	0.001 e
7-CK	1772.3 b	9.8 b	1367.5 d	395.0 d	0.006 b
7-AM	2458.7 a	6.2 e	1970.0 c	482.5 c	0.003 c
12-CK	1294.7 f	12.2 a	1150.0 e	132.5 f	0.010 a
12-AM	1522.1 e	9.6 c	1307.5 d	205.0 e	0.006 b

* Means in each column followed by different letters are significantly different based on LSD test(P<0.05).
3-CK, replant soil with 3 replanting years; 3-AM, inoculating AM fungi in replant soil with 3 replanting years;
7-CK, replant soil with 7 replanting years; 7-AM, inoculating AM fungi in replant soil with 7 replanting years;
12-CK, replant soil with 12 replanting years; 12-AM, inoculating AM fungi in replant soil with 12 replanting years.

There are many mechanisms of inhibiting plant pathogens by AM fungi; one of them is that AM fungi change the rhizospheric microbe population and community structure. After colonization on plant roots with AM fungi, the quantity of rhizospheric microbes significantly increased (John, 2001). The number of both rhizospheric bacteria and actinomycete enhanced when plant formed mycorrhizas, while the dominant species composition also changed (Secilia and Bagyaraj, 1987). There may be two

pathways for AM fungi to change microbe community structure, the first one is that the AM fungal hypha secretion directly impacts microbe community structures; the another one is that both AM fungi in roots and on the roots alter plant physiological and biochemical processes, then directly or indirectly change the plant root secretion (Bansal and Mukerji, 1994; Andrade *et al*., 1998; Bais *et al.*, 2008; Badri and Vivanco, 2009), thus alter those structures (Zhu *et al.*, 2005). In present study, AM fungi significantly decreased the fungal number and the fungi/total microbe ratio, increased the amount of bacteria and actinomycete, which may effectively alleviate the replant problem due to the microbe nonequlibrium.

In our experiment we further observed that activities of soil proteinase, polyphenoloxidase, urease, and saccharase in watermelon replant soils gradually reduced as the replanting year increased, while the inoculation with AM fungi could enhance the soil enzyme activities in different degree. For instance, at the flowering period of watermelon plants, soil proteinase and polyphenoloxidase activities were improved with AM fungal inoculation, the activity of soil proteinase was significantly enhanced from 1.13 NH_2-N mg·g^{-1} to 1.36 NH_2-N mg·g^{-1} in replant soil with 3 replanting years. AM fungi promoted the activity of soil polyphenoloxidase in every replant soils compared with controls, soil urease and saccharase activities were also increased in some degree (Table 4). The soil proteinase, polyphenoloxidase, urease, and saccharase activities at the fruit setting stage of watermelon plants were higher than that at the flowering period. In each replant soil, AM fungal inoculation showed the greatest effects on increasing the soil polyphenoloxidase, and saccharase activities (Table 5). At the harvesting time of watermelon, activities of soil proteinase, polyphenoloxidase, urease, and saccharase were low, while the one with AM fungal inoculation was still higher than that of controls (Table 6).

Table 4 Effects of AM fungi on soil enzyme activities of watermelon replanting at the flowering period of watermelon plants

Treatments	Proteases (NH_2-N mg·g^{-1})	Polyphenoloxidase (Gallicin mg·g^{-1})	Urease (NH_3-N mg·g^{-1})	Saccharase (Glucose mg·g^{-1})
3-CK	1.13 b	0.47 b	0.32 ab	0.35 a
3-AM	1.36 a	0.80 a	0.35 ab	0.37 a
7-CK	1.03 cd	0.43 b	0.33 ab	0.20 b
7-AM	1.08 bc	0.77 a	0.38 a	0.21 b
12-CK	0.99 d	0.30 c	0.29 b	0.12 cd
12-AM	1.07 bc	0.41 b	0.32 b	0.19 bc

* Means in each column followed by different letters are significantly different based on LSD test(P<0.05).
3-CK, replant soil with 3 replanting years; 3-AM, inoculating AM fungi in replant soil with 3 replanting years;
7-CK, replant soil with 7 replanting years; 7-AM, inoculating AM fungi in replant soil with 7 replanting years;
12-CK, replant soil with 12 replanting years; 12-AM, inoculating AM fungi in replant soil with 12 replanting years.

Table 5 Effects of AM fungi on soil enzyme activities of watermelon replanting at the fruit setting stage of watermelon plants

Treatments	Proteases (NH_2-N mg·g^{-1})	Polyphenoloxidase (mg·g^{-1})	Urease (NH_3-N mg·g^{-1})	Saccharase (Glucose mg·g^{-1})
3-CK	1.35 b	2.01 b	0.37 ab	0.40 b
3-AM	1.47 a	2.61 a	0.44 a	0.50 a
7-CK	1.32 bc	1.22 c	0.37 ab	0.35 bc
7-AM	1.40 ab	1.82 b	0.40 ab	0.40 b
12-CK	1.20 c	1.02 d	0.32 b	0.21 d
12-AM	1.34 b	1.49 c	0.42 ab	0.30 c

* Means in each column followed by different letters are significantly different based on LSD test (P<0.05).
3-CK, replant soil with 3 replanting years; 3-AM, inoculating AM fungi in replant soil with 3 replanting years;
7-CK, replant soil with 7 replanting years; 7-AM, inoculating AM fungi in replant soil with 7 replanting years;
12-CK, replant soil with 12 replanting years; 12-AM, inoculating AM fungi in replant soil with 12 replanting years.

There are positive correlations between soil enzyme activity and soil microbe quantity, microbe diversity, microbe biomass, and soil animal numbers (Groffman *et al.*, 2001; Taylor *et al.*, 2002; Bandick and Dick, 1999). Inoculation with AM fungi enrich soil microbe quantities, equilibrate proportion of various microbes, maintain a stabilization of proper proportion of the microbes, enhance soil carbon, nitrogen, and phosphorous cycling power, thus improve the soil enzyme activity.

Table 6 Effects of AM fungi on soil enzyme activities of watermelon replanting at the harvesting time of watermelon

Treatments	Proteases (NH_2-N mg·g^{-1})	Polyphenoloxidase (mg·g^{-1})	Urease (NH_3-N mg·g^{-1})	Saccharase (Glucose mg·g^{-1})
3-CK	1.17 b	1.52 ab	0.35 a	0.30 ab
3-AM	1.34 a	1.64 a	0.39 a	0.36 a
7-CK	1.06 c	1.39 bc	0.25 ab	0.22 bc
7-AM	1.24 ab	1.52 ab	0.27 ab	0.30 ab
12-CK	1.00 c	0.84 d	0.24 b	0.14 c
12-AM	1.19 b	1.18 c	0.33 a	0.26 b

* Means in each column followed by different letters are significantly different based on LSD test (P<0.05).
3-CK, replant soil with 3 replanting years; 3-AM, inoculating AM fungi in replant soil with 3 replanting years;
7-CK, replant soil with 7 replanting years; 7-AM, inoculating AM fungi in replant soil with 7 replanting years;
12-CK, replant soil with 12 replanting years; 12-AM, inoculating AM fungi in replant soil with 12 replanting years.

4. Conclusion

The inoculation with AM fungus *Glomus versiforme* enhanced soil bacteria and actinomycete population, and decreased the fungal numbers, and the fungi/total microbe ratio in replanting soils, and improved soil proteinase, polyphenoloxidase, urease, and saccharase activities, compared with controls. That contributed to the relative equilibrium of the three kinds of soil microorganism populations. So, the AM fungal inoculation can reduce watermelon replant problems through effectively modifying the soil microbe population and community structure, and increasing the soil enzyme activities.

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