

Effects of intercropping of wheat on specific microbial community abundances in watermelon rhizosphere

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ABSTRACT

Using the qPCR, we studied the effects of intercropping of watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) with wheat (*Triticum aestivum* L. Poaceae family) on the abundances of total bacteria, beneficial microbes (*Pseudomonas*, *Bacillus*, *Streptomyces*, *Actinomyces* and *Trichoderma* spp.) communities, total fungi, pathogenic microbes [(*Fusarium oxysporum*, *Fusarium oxysporum* f.sp. *niveum* (*FON*)] and allelochemicals 2,4-diacetylphloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA) and pyrrolnitrin (PRN) producers in watermelon rhizosphere. The intercropping decreased the abundances of *Pseudomonas* and *Actinomyces* spp., DAPG, PCA and PRN producers at 20 d, and later increased the PCA and PRN producers at 30 d and 40 d. The intercropping with wheat increased the abundances of total bacteria, *Pseudomonas* spp., *Streptomyces*, *Actinomyces* and *Trichoderma* spp., and reduced those of total fungi, *Bacillus* spp., *Fusarium oxysporum* and *FON* at 40 d. These results indicated that intercropping of wheat + watermelon affected the microbial communities and increased the abundances of allelochemicals producing genes in watermelon rhizosphere.

Key words: Bacteria, fungi, *Fusarium oxysporum*, intercropping, microbial community, *Pseudomonas* spp., rhizosphere, *Trichoderma* spp., watermelon, wheat

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai), is grown worldwide (25). *Fusarium* wilt is a major yield-limiting factor in watermelon production. It is caused by soil-borne *Fusarium oxysporum* f.sp. *niveum* (*FON*). Fungicides (such as prochloraz and hymexazol) are used to control *FON*, (12). Although these decreased the incidence of watermelon *Fusarium* wilt (12), but causes serious problems in environment and food safety. It is known that intercropping [maize/soybean (14), rice/watermelon (33) and potato/onion/tomato (11)] alleviates the soil-borne plant diseases by increasing biodiversity (34). Therefore, the adaptation of such cropping systems in commercial field not only decrease the occurrence of soil-borne diseases, but also reduced the application rate of pesticides to ensure the sustainable agriculture.

Soil microbial community is closely related to plant health, soil productivity and stability of agroecosystem (1,18,32,35,40,41). Previous studies have found that changes in soil microbial communities were different in monocropping and intercropping systems

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(24,37). These changes in the soil microbial community affects the plant growth and resistance (3,13,20,39,47). Besides in intercropping, many beneficial microorganisms (*Pseudomonas*, *Bacillus* and *Trichoderma*), can induce systemic resistance in plants. For example, specific antagonistic microbial communities (e.g., *P. fluorescens*) suppresses the soil-borne plant diseases, such as *Fusarium* wilt (2,7,28). These microbes can also produce fungicidal secondary metabolites [2,4-diacetylphloroglucinol (DPAG), phenazine-1-carboxylic acid (PCA) and pyrrolnitrin (PRN)], which suppresses the soil fungal pathogens (2,28).

Wheat (*Triticum aestivum* L. Poaceae family) (15), exhibits the allelopathy effects in intercropping system (38). Our previous study has shown that intercropping with wheat decreased the severity of *Fusarium* wilt disease and altered the microbial community structure in the rhizosphere of watermelon (43). However, changes in some special taxa in the intercropping system were unclear. Hence, this study aimed to determine the effects of wheat intercropping with watermelon on (i). the abundances of total bacteria, total fungi, *Pseudomonas* spp., *Bacillus* spp., *Actinomycetes* spp., *Streptomyces* spp., *Trichoderma* spp., *Fusarium oxysporum* and *FON* communities and (ii). producers of DPAG, PRN and PCA of watermelon using qPCR.

MATERIALS AND METHODS

Pot experiment

The seeds of watermelon (cv. Heimeiren) and wheat (cv. D123) were obtained from Nongyou Seed Company, Beijing, China, and Vegetable Physiological Ecology Laboratory, College of Horticulture, Northeast Agricultural University, Harbin, China, respectively. *FON* strain (37) was provided by the Vegetable Physiological Ecology Laboratory, Department of Horticulture, Northeast Agricultural University, Harbin, China.

The soil used was black soil (Mollisol) collected from a continuously monocropped watermelon (> 3 years) field in the experimental station of Northeast Agricultural University, Harbin, China (45°41'N, 126°37'E). The soil contained organic matter 30.60 g/kg; available P 246.31 mg/kg; available K 256.00 mg/kg; ammonium N 30.82 mg/kg, nitrate N 25.37 mg/kg; pH (1:2.5, w/v) 7.21 and EC (1:2.5, w/v) 1.08 mS/cm. The pot experiment was carried out in the greenhouse from April to July 2019.

Watermelon seedlings with 3-true leaves were transplanted into plastic pots (60 cm × 40 cm × 20 cm) containing the collected soils. There were 6-watermelon seedlings per pot, 20 cm apart. There were two treatments: (i) monocropped watermelon and (ii) watermelon intercropped with wheat. In intercropping system, 30 wheat seeds were sown in each pot and the distance from watermelon seedling to wheat was 5 cm. Wheat seedlings were cut, whenever they grew above 15-20 cm to allow air circulation and to prevent shading on watermelon seedlings. There were 18 pots for each treatment with a randomized arrangement resulting in a total of 36 pots (2 treatments × 3 replicates × 6 pots). No fertilizer was added and weeds were uprooted manually. In the experiment, all the plants

were kept in the greenhouse (32°C day/22°C night, relative humidity of 60-80 %, 16 h light/ 8 h dark).



Figure 1. Watermelon monoculture, wheat and watermelon intercropping

Rhizosphere soil sampling and DNA extraction

After 20, 30 and 40 days of intercropping, watermelon rhizosphere soils were collected from two pots (12 seedlings) of each replicate and were mixed to make a composite sample as previously described (45,46). Therefore, there were three composite samples for each treatment at each period. The rhizosphere soil of watermelon was sampled, gently remove the watermelon roots from the pots and carefully remove the soils loosely attached to the watermelon roots by hand shaking. Then, soil tightly adhering to the roots was removed from the root surface with a sterile brush and sieved (2 mm mesh). These fresh rhizosphere soil samples were collected and stored at -80 °C for DNA extraction. Total soil DNA was extracted from 0.25 g of each individual rhizosphere soil sample with the PowerSoil DNA Isolation Kit (QIAGEN, Venlo, the Netherlands) as per the manufacturer's instructions. The amount and purity of DNA were estimated by spectrophotometer. DNA was stored at -20 °C for further analysis.

qPCR analysis

Soil microbial abundance was estimated by qPCR assays in an IQ5 real-time PCR system (Bio-Rad Lab, LA, USA). For total bacteria, total fungi, *Pseudomonas* spp., *Bacillus* spp., *Actinomyces* spp., *Streptomyces* spp., *Trichoderma* spp., *Fusarium oxysporum* and *FON*, primer sets of F338/R518 (5), ITS1F/ITS4 (27), PSF/PSR (8),

BacF/BacR (10), F243/R518 (44), Sm5R/Sm6F (29), UtF/UtR (19), FnSc-1/FnSc-2 and Fon-1/Fon-2 (26) were used, respectively. Antibiotic producer genes *phlD*, *prnD*, and *phzC* responsible for the production of DAPG, PRN and PCA were amplified with primer sets of B2BF/B2BR3 (2), PRND1/PRND2 (21) and PHZJR1/PHZJR2 (28), respectively. The amplicon sizes for total bacteria, fungi, *Pseudomonas* spp., *Bacillus* spp., *Actinomyces* spp., *Streptomyces* spp., *Trichoderma* spp., *Fusarium oxysporum*, *FON*, and *phlD*, *prnD* and *phzC* producers were 230, 750, 960, 995, 303, 620, 540, 327, 174, 319, 786 and 522 bp, respectively. The annealing temperatures for total bacteria, total fungi, *Pseudomonas* spp., *Bacillus* spp., *Actinomyces* spp., *Streptomyces* spp., *Trichoderma* spp., *Fusarium oxysporum* and *FON*, and *phlD*, *prnD*, and *phzC* producers were 58, 60, 63, 53, 67.5, 62.5, 55.2, 63, 58, 67, 69, 56.5 °C, respectively. Each composite soil sample was accompanied by three amplifications. Negative control was sterile water. The specificity of the product was determined by melting curve analysis and agarose gel electrophoresis. All standard curves were constructed from a 10-fold dilution series of plasmids containing the target genes. The threshold circulation (Ct) values obtained for each sample were compared with the standard curve to determine the initial copy number of the target genes.

Statistical analysis : All data were analyzed by Student's *t*-tests using the SAS 9.2 software. Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Total fungi and bacteria abundances

After 40 days of intercropping, the abundance of total bacteria ($P < 0.05$) in intercropped watermelon rhizosphere was higher, but that of total fungi ($P < 0.05$) was lower than monocropped watermelon (Figure 2). The results showed that on the 20th and 30th days after intercropping, the abundance of total bacteria and fungi in watermelon rhizosphere soil were not different between intercropping and monoculture system.

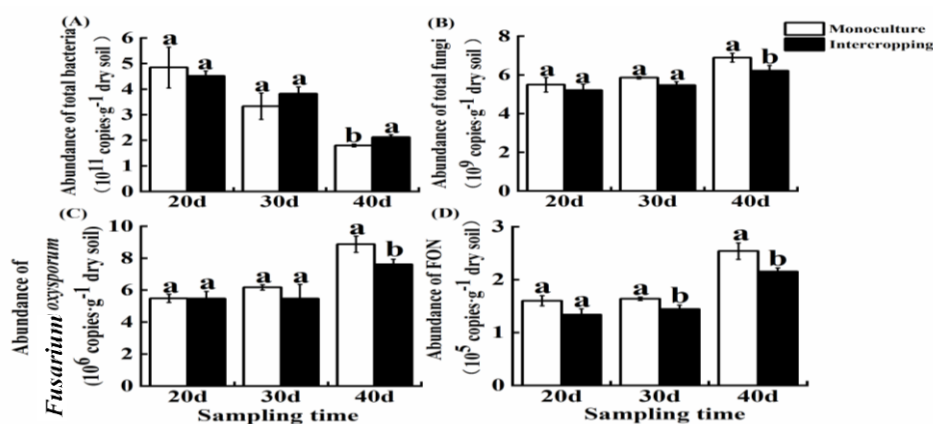


Figure 2. Effects of intercropping with wheat on the abundances of total bacteria (A), total fungi (B), *Fusarium oxysporum* (C) and *FON* (D) in watermelon rhizosphere soil. Different letters at each sampling time indicate a significant difference based on *t*-tests method ($P < 0.05$).

Fusarium oxysporum and *FON* abundances

After 40 days of intercropping, the abundance of *Fusarium oxysporum* rhizosphere soil was lower in watermelon intercropped with wheat than in watermelon monoculture ($P < 0.05$) (Figure 2C). After 30 days and 40 days of intercropping, the abundance of *FON* was lower in rhizosphere soil in watermelon intercropped with wheat than in watermelon monoculture treatment ($P < 0.05$) (Figure 2D).

Beneficial and antibiotics-producing microbial communities

After 20 days of intercropping, the abundance of *Pseudomonas* spp. (Figure 3A), *Actinomycetes* spp. (Figure 3C), DAPG-producing (Figure 4A), PRN- (Figure 4B) and PCA-producing (Figure 4C) were lower in rhizosphere soil in watermelon intercropped with wheat than in watermelon monoculture treatment ($P < 0.05$). After 40 days of intercropping, the abundance of *Pseudomonas* spp. (Figure 3A), *Actinomycetes* spp. (Figure 3C), *Streptomyces* spp. (Figure 3D) and *Trichoderma* spp. (Figure 3E), DAPG-producing (Figure 4A), PRN- (Figure 4B) and PCA-producing (Figure 4C) were higher in rhizosphere soil in wheat/watermelon companion system compared with monoculture system ($P < 0.05$).

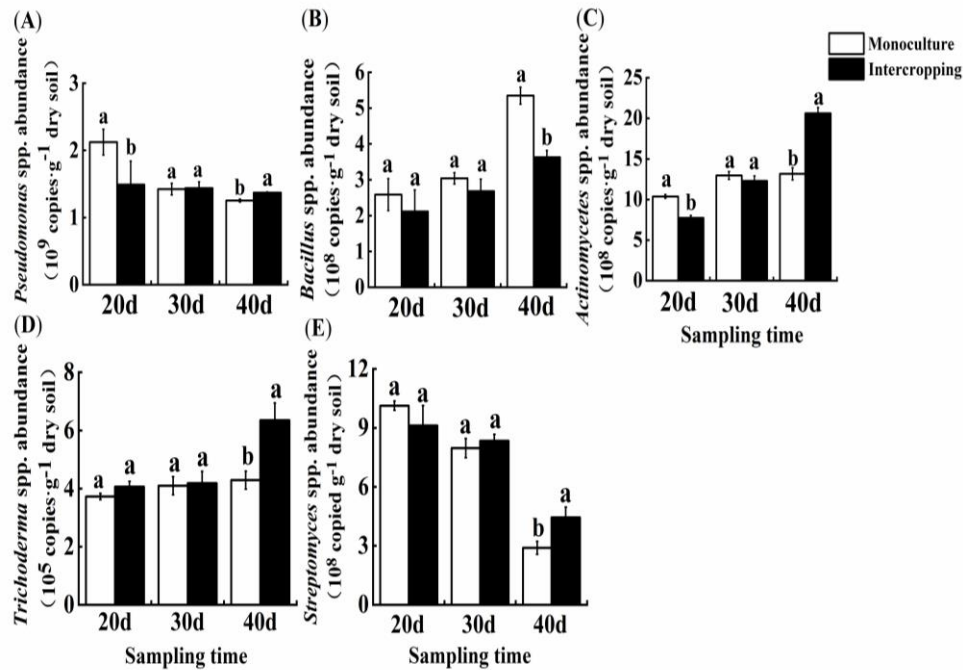


Figure 3. Effects of intercropping with wheat on the abundances of *Pseudomonas* spp. (A), *Bacillus* spp. (B), *Actinomycetes* spp. (C), *Streptomyces* spp. (D) and *Trichoderma* spp. (E) in watermelon rhizosphere soil. Different letters at each sampling time indicate a significant difference based on *t*-tests method ($P < 0.05$).

Watermelon wilt is a soil-borne disease caused by *FON*. In this study, the abundances of *Fusarium oxysporum* and *FON* in the rhizosphere soil of wheat/watermelon intercropping were lower than in pure watermelon on the 30th and 40th day. Our results are similar to previous studies that, 40 days after transplanting, the number of *FON* in the rhizosphere soil of watermelon under the condition of dry rice and watermelon intercropping was significantly higher than in watermelon monocropping (34). These results indicated that the effects of intercropping wheat on pathogenic microorganisms in watermelon rhizosphere soil was affected by the intercropping period (34). Previous research results show that wheat/watermelon intercropping system decreased the watermelon wilt disease incidence (23). Thus, the decrease in pathogens abundance may be one of the reasons for the decreased incidence of watermelon *Fusarium* wilt.

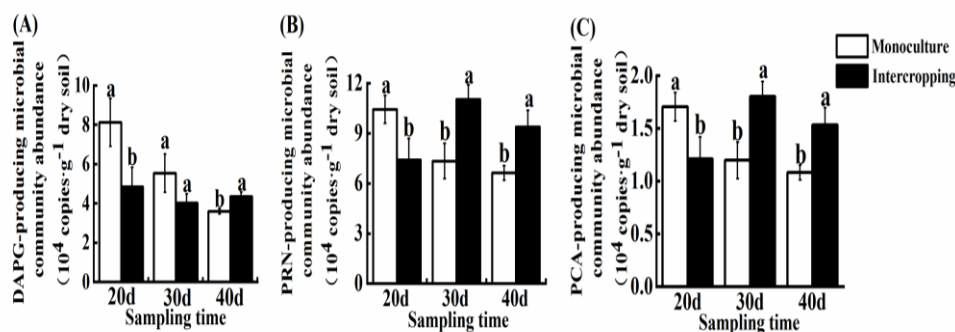


Figure 4. Effects of intercropping with wheat on the abundances of antibiotics-producing microbial communities in watermelon rhizosphere soil. Different letters at each sampling time indicate a significant difference based on *t*-tests method ($P < 0.05$).

Many beneficial microorganisms inhibits the pathogenic microorganisms (6,30). In the wheat/watermelon intercropping system, the decrease in the abundance of pathogenic microorganisms may be due to the increase in the abundance of some beneficial microorganisms (*Pseudomonas*, *Bacillus* spp.) (30). There is growing evidence that some species of *Pseudomonas*, *Bacillus*, *Trichoderma*, *Actinomycetes* and *Streptomyces* play important role in plant health by inhibiting the pathogens and inducing plant resistance (4,9,31,36,42). Plant diversity increases the abundances of some soil beneficial microorganisms (22,33). Our results showed that the abundances of *Pseudomonas* spp. and *Actinomycetes* spp. in watermelon rhizosphere soil of intercropping system were significantly lower than in monoculture system on the 20th day of intercropping of wheat and watermelon. Moreover, on the 40th day of wheat/watermelon intercropping, the abundances of *Pseudomonas* spp., *Actinomycetes* spp., *Streptomyces* spp. and *Trichoderma* spp. in the monoculture system were significantly lower than in intercropping system. These results indicated that the effects of intercropping wheat on certain microorganisms in watermelon rhizosphere soil may be affected by intercropping period.

At the same time, we also detected the abundances of antibiotics (DAPG, PCA and PRN) produced by specific antagonistic microorganisms (e.g., *Pseudomonas fluorescens*) in watermelon rhizosphere soil. DAPG, PCA and PRN are well-known antifungal compounds suppressing a wide range of plant pathogens (16,17). Our results showed that on the 20th day of wheat and watermelon intercropping, the abundances of DAPG, PRN and PCA in the intercropping system were significantly lower than in the watermelon monoculture system. However, on the 30th and 40th day of intercropping, a reverse trend was found in the abundances of DAPG, PRN and PCA between the two cropping systems. These results indicated that the increased abundances of antibiotics producing genes in rhizosphere soil of wheat + watermelon intercropping might be another reason for the decrease in watermelon wilt disease incidence.

CONCLUSIONS

This study was done to find the effects of intercropping with wheat on the abundances of total fungi, bacteria, selected microbial communities and antibiotics-producing genes in watermelon rhizosphere soil. Our results showed that, 40 days after watermelon + wheat intercropping differently affected the microbial communities' abundances in watermelon rhizosphere. Besides, the abundances of antibiotics producing genes increased in intercropped watermelon rhizosphere. Thus, we speculated that wheat/watermelon intercropping system could decrease the abundance of *FON* through regulating the beneficial or antagonistic microorganisms in watermelon rhizosphere soil.

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CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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