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RHIZOSPHERE MICROBIAL COMMUNITIES FROM RESISTANT AND SUSCEPTIBLE WATERMELON CULTIVARS SHOWED DIFFERENT RESPONSE TO *FUSARIUM OXYSPORUM* F. SP. *NIVEUM* INOCULATION

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Abstract

Fusarium oxysporum f. sp. *niveum* (FON), a soil-borne pathogen of watermelon (*Citrullus lanatus*), can cause substantial production losses worldwide. In this study, plate culture and PCR-denaturing gradient gel electrophoresis (DGGE) methods were used to evaluate the effects of inoculation of *Fusarium oxysporum* f.sp. *niveum* on rhizosphere microbial communities of different watermelon cultivars to FON. Two methods indicated that the effects of watermelon rhizosphere microbial community of different resistance cultivars to FON were much different. Populations of culturable bacteria and actinomycetes in the rhizosphere of susceptible watermelon cultivar were significantly lower than in the resistant cultivar after inoculation ($P<0.05$), but the opposite result was observed for fungi. Principal component analysis of bacterial and fungal community structure also showed that the cultivar of FON-inoculated soil treatment were separated from the non-inoculated controls after inoculation, and there was clear discrimination between the susceptible cultivars and the resistant cultivars. Sequence analysis of specific bands from DGGE profiles showed that specific rhizosphere bacterial and fungal groups differed between watermelon cultivars after inoculation . Both methods demonstrated that different resistant watermelon cultivars occupied different rhizosphere microbial communities, and and disease suppression might be correlated with high microbial diversity. *F. oxysporum* f. sp. *Niveum* alters the structure and functional diversity of microbial communities associated with watermelon rhizosphere.

Key words: *Fusarium oxysporum* f. sp. *niveum*, Fusarium wilt resistance, PCR-DGGE, Rhizosphere microbial community.

Introduction

Pathogens can have a severe impact on plant health (Roeland *et al.*, 2012). *Fusarium* wilt of watermelon (*Citrullus lanatus*), caused by *Fusarium oxysporum* f. sp. *niveum* (FON), is a serious soil-borne disease world wide and a yield-limiting factor in watermelon production (Zhou *et al.*, 2007). Continued planting would result in the build-up of soil-borne pathogens and a reduction in watermelon production. Soil-borne diseases are closely related to soil properties and microbial communities (Trivedi *et al*., 2012). The success of a pathogen is influenced by the microbial community of the soil in which the infection takes place. Every natural soil has the ability to suppress a pathogen to a certain extent. This can be deduced from the disease severity pathogen inoculation in pasteurized soils compared with non-pasteurized soils. This phenomenon is known as general disease suppression and is attributed to the total microbial activity (Roeland *et al*., 2012).

Recent advances in plant–microbe interactions research revealed that plants are able to shape their rhizosphere microbiome, that different plant species host specific microbial communities when grown on the same soil (Roeland *et al*., 2012). Current understanding of the complex plant–microbe inter-actions that take place in the rhizosphere is still in its infancy (Bisseling *et al.,* 2009). The importance of the root microbiome in plant health has been proved, that the plant is able to control the composition of its microbiome, and the number of rhizosphere microorganisms was different between resistant and susceptible plant cultivars (Liu *et al*., 2006). A significant positive correlation was also observed between microbial biomass and soil disease suppression. The population and diversity of soil microbial communities play a key role in the soil-suppressive to soil-borne pathogens (Schönfeld *et al.*, 2003; Van Elsas *et al*., 2002). Studies have demonstrated that improving soil microbial biomass can help to enhance disease suppression of *Pythium* pea root nematocysts (Leon *et al*., 2006), *Pythium* in irises (Van Os *et al*., 2001), *Rhizoctonia solani* in wheat (Pankhurst *et al*., 2002) and *F. oxysporum* in asparagus (Hamel *et al.*, 2005). Moreover, Arab El *et al.* (2001) suggested that changed rhizosphere microbial community structure, which could promote the colonization of antagonistic microorganisms, was involved in the disease suppressiveness of resistant wheat (*Triticum aestivum* L.). Significant differences were found in the number of fungi and actinomycetes in the rhizosphere of Fusarium wilt resistant and susceptible varieties of cucumber (Miao *et al.*, 2004). Further studies showed that soil suppressiveness was induced by only a limited number of watermelon cultivars that are resistant to the pathogen *F. oxysporum* f. sp. *niveum*, and the capacity of a cultivar to induce soil suppressiveness was associated with increased populations of nonpathogenic *Fusarium* spp. (Larkin *et al*., 1996; Mark, 2004), the root colonization by introduced *F. o. niveum,* indigenous *F. oxysporum,* or other microorganism groups was not consistently related to suppressiveness, suggesting that

specific antagonistic strains rather than general populations of microorganisms may be involved in suppression (Larkin *et al*., 1996). Mark (2004) demonstrated the importance of host genotype in determining the efficacy of resident and introduced microorganisms for the control of soilborne diseases, and the genetic variation within the host has the potential to enhance the interactions with plant-beneficial microorganisms. However, there is little research about the rhizosphere soil microecological response to watermelon Fusarium wilt from different resistance watermelon cultivars and the relationship between rhizosphere microorganisms and watermelon wilt disease.

In this study, the different responses of rhizosphere microbial communities of different watermelon cultivars with differing resistance to FON inoculation were compared. Viable numbers of soil bacteria, fungi and actinomycetes were measured by the plate culture method. Soil bacterial and fungal community structures were analyzed by the PCR-denaturing gradient gel electrophoresis (DGGE) method. Our hypothesis was that there would different response to FON between resistant and susceptible watermelon varieties in their rhizosphere microbial communities according to above research. The objectives of this study were to evaluate the effects of microorganisms in watermelon soil rhizosphere microecological system to wilt resistance, and reveal the relationship between the rhizosphere microbial community structure and soil-borne disease initially.

Materials and Methods

Experimental materials: Black soil (Mollisol) was collected from a 0–20 cm layer at the Northeast Agricultural University Horticulture Experiment Station (Harbin, Heilongjiang Province, China, 47° 26′N, 126° 38′E), the previous crop was tomato (continuous cropping for two years) and never previously planted with watermelon. The basic properties of the soil were: soil electrical conductivity, 1.02 $mS \cdot cm^{-1}$; pH, 7.28; organic matter, 5.27%; available nitrogen, 301.24 $mg \cdot kg^{-1}$; available phosphorus, 251.73 $mg \cdot kg^{-1}$; available potassium, 353.05 $mg \cdot kg^{-1}$.

Watermelon cv. Sweet Girl (resistant cultivar, R) and Little Angel (susceptible cultivar, S) were used as plant material. FON physiological race no. 1 was isolated from diseased watermelon plants (An *et al*., 2009).

Glasshouse experiment: FON was cultured in potato dextrose agar medium for 120 h, and then washed with sterile water and suspended to a concentration of 1×10^6 colony forming units $(CFU) \cdot mL^{-1}$. The soil was inoculated with FON suspensions and the resulting colony count was 4×10^3 $CFU \cdot g^{-1}$ soil (20) (soil) soil treated with sterile water was used as the control (soil).

Watermelon seeds were germinated in the dark at 28°C for 5 days, and then were transplanted in pots (12×12 cm) contained 400 g soil inoculated with FON (soil) or sterile water (soil). There was one watermelon plant per pot. Each treatment was replicated three times and each replicate had 24 pots. All plants were grown in a glasshouse (average day/night temperature, 26/20°C; photoperiod, 10 h; relative humidity, 75%). The disease incidence was recorded and watermelon rhizosphere soil samples 300g(<2 mm) were collected randomly from nine plants of each replicate once every seven days until 21 days of growth, only the diseased plants were selected in the FON-inoculation treatments (soil). Part of these fresh soils was used for the viable count of soil microorganisms, and the other part was stored at -70°C for DNA extraction.

Viable counts of soil microorganisms: The following media were used to culture soil microbes: beef extract peptone agar medium (for bacteria isolation), Martin medium (for fungi isolation), Gause's No 1 synthetic medium (for actinomycetes isolation) (Zhou, 2006) and Myclobutanil agar (MBA) medium for *Fusarium* isolation (Vujanovic, 2002). Bacteria, actinomycetes, *Fusarium* and other fungi isolates, were cultured under aseptic conditions using the spread-plating method. A volume of 200 μL of 10^{-6}, 10^{-5} and 10^{-3}-fold soil dilutions were plated for each sample with three replicates. Plates were inverted and incubated at 28°C for 2-7 days, after which the number of microorganisms per gram of dry soil was calculated. The fungal genera were identified by morphological observation (Wei, 1979).

DNA extraction and PCR–DGGE: The Tianjingsha serial soil microbial DNA extraction kit (Tianze Gene Engineering Company, Sichuan, China) was used to extract and purify soil total DNA. For bacterial community, partial 16S rRNA gene was amplified with the primer set of GC-338f and 518r (Muyzer *et al*., 1993). For fungal community, the internal transcribed spacer (ITS) region of rDNA was amplified with the primer set of ITS1F and GC-ITS2 (Gardes *et al*., 1993).

DGGE was performed using an 8% (w/v) acrylamide gel with 30-60% and 30-55% denaturant gradient for bacteria and fungi, respectively, and was run in a 1×Tris-acetate-EDTA (TAE) buffer for 12 h at 60°C and 80 V with a DCode universal mutation detection system (Bio-Rad). After electrophoresis, the gel was silver stained (Bassam *et al*., 1991) and photographed.

Bands showing variations in watermelon cultivars on the DGGE gel were excised and purified with a polyacrylamide gel extraction kit (Bori Gene Engineering Company, Nanjing, China). Then, 8 μl of purified product was used as a template for PCR amplification using the bacterial primers 338f and 518r, or the fungal primers ITS1F and ITS2. After purification, the amplified product was ligated to the PucM-T vector and transformed into TG1 competent cells. Positive clones were then selected and commercially sequenced.

Statistical analyses: DGGE fingerprint analysis was carried out using Quantityone 4.3.0 software (Bio-Rad) to digitize DGGE patterns. The position and intensity of each band were determined automatically. The density value of each band was divided by the average band density of the lane in order to minimize the influence of loaded DNA concentrations among samples (Garland & Mills, 1991). Principal component analysis (PCA) of the DGGE data was also conducted using SPSS 16.0.

Population data were log-transformed prior to analysis (Loper *et al.,* 1984). The diversity of microbial community was assessed by the Shannon index calculated for each soil sample. Band positions were treated as species and the relative intensities were used to calculate the Shannon index (*H*), determined as $H = -\Sigma pi \cdot \ln pi$, where *pi* represents the abundance of the *i*th species (band) within the sample (1). Significant differences ($p<0.05$) between individual treatments were determined using Tukey's test with the SAS v.8.1 software.

The BLAST program (http://www.ncbi.nlm.nih.gov/) of the National Center for Biotechnology Information (NCBI) was used for sequence homology analysis. The nucleotide sequences were submitted to the GenBank database (Tables 2 and 3).

Results

Fusarium wilt ıncidence: As shown in Table 1, susceptible cultivars Little Angel showed 2.48% wilt at 7 days post inoculation, while resistant cultivars Sweet Girl no obvious wilt. At 14 and 21 days post inoculation, the incidence rate of Fusarium wilt resistant cultivars Sweet Girl were 2.48% and 16.67%, respectively, while susceptible cultivars Little Angel had 50% and 69.44% wilt, respectively. The incidence rate of Fusarium wilt was significantly higher in susceptible cultivars Little Angel than resistant cultivars Sweet Girl. All watermelon plants in control had not show symptoms of *Fusarium* wilt from 7 to 21 days post inoculation.

Culturable microbial populations: Differences were observed in the number of rhizosphere bacteria, fungi and actinomyces between the FON-inoculated susceptible watermelon cultivar and the resistant watermelon cultivar during different growth days (Fig. 1). Populations of culturable fungi and actinomycetes in the rhizosphere of different resistant watermelon cultivar were significantly difference at 7 days after inoculation (10 June), except the controls.

Microbial diversity in rhizosphere soil is shown in Fig. 1. The densitiy of bacteria in rhizosphere soil was markedly higher in resistant cultivars Sweet Girl (R) than susceptible cultivars Little Angel (S) at 14 and 21 days after inoculation (17 June, 24 June) ($p<0.05$), no significant difference in bacteria density was detected in rhizosphere soil of watermelon between resistant and susceptible cultivars at 7 days post FON-inoculation. In non-inoculated soil, the same trend was observed for bacteria density. The actinomycetes density in rhizosphere soil was significantly higher in resistant cultivars Sweet Girl than susceptible cultivars Little Angel at 7, 14 and 21 days after inoculation. In non-inoculated soil, differences existed between resistant and susceptible cultivars at 14 and 21 days, and actinomycetes density in rhizosphere soil was significantly higher in resistant cultivars, compared with susceptible cultivars. By contrast, fungal density in rhizosphere soil was lower in resistant cultivars than susceptible cultivars at 7 days to 21 days after inoculation. In non-inoculated soil, fungal density in rhizosphere soil was significantly lower in resistant cultivars than susceptible cultivars at 14 and 21 days, no difference in fungal density was detected in rhizosphere soils of both watermelon cultivars at 7 days.

Rhizopus, Aspergillus, Trichoderma, Penicillium and *Fusarium* were the dominant groups identified in the watermelon rhizosphere (Fig. 2). Except for *Trichoderma*, The populations of *Rhizopus, Aspergillus, Penicillium* and *Fusarium* were significantly lower in the resistant cultivar than in the susceptible cultivar at 21 days after inoculation (24 June) ($p<0.05$) for all treatments. The populations of *Aspergillus, Penicillium* and *Fusarium* were significantly lower in the resistance cultivar than in the susceptible cultivar at 14 days after inoculation (17 June) ($p<0.05$), but little difference were discovered for the controls for *Rhizopus and Trichoderma*.

Rhizosphere bacterial community structure: Rhizosphere bacterial community structures was examined by PCR-DGGE. PCR-DGGE analyses showed that the compositions of rhizosphere soil bacterial communities was different between resistant and susceptible cultivars (Fig. 4). In particular, some strong and characteristic bands were observed in DGGE patterns in resistant cultivars after inoculation, which were absent in susceptible cultivars (Fig. 4b, 4c). DNA sequence data were compared to sequences in the GenBank database and homology of 90-100% was observed (Table 2). BLAST results showed that these bacteria were similar to species of Actinobacteria, Firmicutes, Proteobacteria, Sphingobacteria and uncultured bacterium (Table 2).

Band A-6, A-7, A-8, A-9, A-13, A-14, A-15 appeared only in the resistant cultivar of FON-inoculated soil treatment , band A-10 *Actinobacteria* only in the resistant cultivar of control, and band A-9, A-13, A-15 were *Actinobacteria*, A-6, A-7 were *Streptococcus*, A-8 was *alpha proteobacterium*, A-14 was *Sphingobacteria* at 21 days after inoculation. Band A-11 (*Streptomyces*) appeared in all non-inoculated controls except the FON-inoculated treatments. Actinobacteria and Firmicutes were the unique phylum in the resistant watermelon cultivar rhizosphere soil.

Table 1. Incidence of Fusarium wilt of FON inoculated and non-inoculated watermelon cultivars with differing resistance from 7 to 21 days after inoculation Shannon index calculated from the DGGE composition of soil microbial community of watermelon cultivars with differing resistance.

Treatment	Watermelon cultivar	*Fusarium* wilt ıncidence		
		10 June (7 days)	17 June (14 days)	24 June (21days)
FON-inoculation (soil)	Sweet girl (resistant cultivar)	0	2.48 ± 4.81 b	16.67 ± 4.81 b
	Little angel (susceptible cultivar)	2.48 ± 4.81 a	50.00 ± 0.00 a	69.44 ± 4.81 a
Non-inoculation (soil Π)	Sweet girl (resistant cultivar)	0	0	0
	Little angel (susceptible cultivar)	0	0	0

Mean values within the same column followed by different letters are significantly different ($p<0.05$). Each value is the mean_SE of three replicates

Table 2. Closest relatives of bacterial partial of 16S rDNA sequences derived from DGGE bands.

DGGE band	Sequence length (bp)	Accession code	Closest relatives		Similarity (%)	Alignment
			Resource	Phylogenetic affiliations		
A-1	198	FJ832140	Uncultured actinobacterium clone DOK_NOFERT_clone 463 16S ribosomal RNA gene	*Actinobacteria; Solirubrobacterales; uncultured Conexibacter* sp.	98	194/197
A-2	192	FJ832138	Uncultured bacterium clone FFCH13230 16S ribosomal RNA gene	*Actinobacteria; Propionibacterineae; Nocardioides*	96	150/156
A-3	178	FJ832146	Arthrobacter sp. VTT E-073079 16S ribosomal RNA gene	*Actinobacteria; Micrococcaceae; Arthrobacter*	99	176/177
A-4	174	FJ832142	Uncultured bacterium clone ADFI7QG3A12HL50Z/1175 16S ribosomal RNA gene	*Proteobacteria; Alphaproteobacteri*	99	167/168
A-5	173	FJ832143	Uncultured actinobacterium clone CK-11 16S ribosomal RNA gene	*Actinobacteria; Acidimicrobium*	94	162/173
A-6	191	FJ832145	Uncultured Streptococcus sp. clone BL030B74 16S ribosomal RNA gene	*Firmicutes; Bacilli; Lactobacillales; Streptococcus; Streptococcacea*	99	166/167
A-7	191	FJ832136	Uncultured Streptococcus sp. clone 2P-3-1-O06 16S ribosomal RNA gene	*Firmicutes; Bacilli; Lactobacillales; Streptococcus*	99	167/168
A-8	174	FJ832150	Uncultured bacterium clone ADFI7QG3A12HL50Z/1175 16S ribosomal RNA gene	*Proteobacteria; alpha proteobacterium*	98	165/168
A-9	178	FJ832131	Uncultured actinobacterium clone A19YF09RM small subunit ribosomal RNA gene	*Actinobacteria; Micromonosporaceae; Dactylosporangium*	99	174/176
A-10	170	FJ832134	Uncultured actinobacterium clone NPK-88 16S ribosomal RNA gene	*Actinobacteria; Actinomycetaceae; Actinomyces*	98	39/141
A-11	178	FJ832153	Uncultured Streptomyces sp. clone PS3 16S ribosomal RNA gene	*Actinobacteria; Streptomyces* sp.	97	136/141
A-12	197	FJ832135	Uncultured gamma proteobacterium clone GASP-KC1W3_B03 16S ribosomal RNA gene	*Proteobacteria; g-proteobacteria*	90	178/198
A-13	175	FJ832149	Uncultured actinobacterium clone HJH2SS58 16S ribosomal RNA gene	*Actinobacteria Acidimicrobium*	98	170/174
A-14	173	FJ832139	Uncultured Sphingomonas sp. clone QuLP2F33 16S ribosomal RNA gene, partial sequence	*Sphingobacteria; Sphingomonas* sp.	100	135/135
A-15	239	FJ832151	Unidentified bacterium clone 67_H_RHIZO_H1_T7s 16S ribosomal RNA gene, partial sequence	*Actinobacteria; Streptophyta*	93	144/155
A-16	191	FJ832144	Uncultured bacterium clone sE10-21 16S ribosomal RNA gene	*Firmicutes; Bacilli; Lactobacillales; Streptococcus*	99	166/167
A-17	173	FJ832147	Uncultured bacterium clone FFCH8323 16S ribosomal RNA gene	*Actinobacteria; Acidimicrobium*	97	168/173
A-18	181	FJ832133	Nocardioides sp. MI-59a 16S ribosomal RNA gene, partial sequence	*Actinobacteria; Nocardioidaceae; Nocardioides*	95	170/179
A-19	198	FJ832141	Uncultured Nocardioides sp. clone R2C-6 16S ribosomal RNA gene, partial sequence	*Actinobacteria Actinomycetales;Nocardioidaceae; Nocardioides*	98	175/178
A-20	98	FJ832148	Uncultured actinobacterium clone HG-B02196 16S ribosomal RNA gene, partial sequence	*Actinobacteria*	97	74/76
A-21	191	FJ832137	Uncultured bacterium clone GP28373cO3 16S ribosomal RNA gene, partial sequence	Uncultured bacterium	98	147/150

Table 3. Closest relatives of fungal ITS sequences derived from DGGE bands

DGGE band	Sequence length (bp)	Accession code	Closest relatives		Similarity (%)	Alignment
			Resource	Phylogenetic affiliations		
F-1	316	GQ225116	Uncultured Ascomycota clone NPL1-18085 18S ribosomal RNA gene, partial sequence	*Ascomycota*	99	282/283
F-2	347	GQ225117	Fungal sp. AB58 18S ribosomal RNA gene	Fungi	100	260/260
F-3	249	GQ225118	Uncultured Cladosporium clone ME4_9 18S ribosomal RNA gene, partial sequence	*Ascomycota; Dothideomycetes; Capnodiales*	100	248/248
F-4	287	GQ225119	Hypocreales sp. LM566 18S ribosomal RNA gene, partial sequence	*Ascomycota; Sordariomycetes; Hypocreales*	100	247/247
F-5	306	GQ225120	Fusarium oxysporum strain CR10 18S ribosomal RNA gene, partial sequence	*Ascomycota; Fusarium; Fusarium oxysporum* species complex	100	265/265
F-6	322	GQ225121	Uncultured Alternaria clone d344_6_45 18S ribosomal RNA gene, partial sequence	*Ascomycota; Pezizomycotina; Dothideomycetes; Pleosporaceae*	99	282/283
F-7	275	GQ225122	Uncultured Cladosporium clone EF13-8405 18S ribosomal RNA gene, partial sequence	*Ascomycota; Dothideomycetidae; Capnodiales*	99	272/273
F-8	358	GQ225123	Uncultured Ascomycota clone 1149 18S ribosomal RNA gene, partial sequence	*Ascomycota*	100	265/265
F-9	279	GQ225124	Uncultured ascomycete ITS region including 18S rRNA gene	*Ascomycota*	99	255/256
F-10	248	GQ225125	Uncultured fungus clone Contig177-117-1219_0676 18S ribosomal RNA gene, partial sequence	uncultured fungus	97	240/248
F-11	173	GQ225158	Fusarium sp. w33 18S ribosomal RNA gene, partial sequence	*Ascomycota*; *Fusarium*.	99	160/161
F-12	321	GQ225126	Uncultured basidiomycete 246 18S ribosomal RNA gene, partial sequence	*Basidiomycota*	99	281/282
F-13	269	GQ225160	Uncultured soil fungus clone D148 18S ribosomal RNA gene, partial sequence	Fungi	100	263/263
F-15	371	GQ225128	Uncultured fungus clone 7-Z-M13F(-47) 18S ribosomal RNA gene, partial sequence	Fungi	100	371/371
F-16	305	GQ225129	Fusarium oxysporum strain CR10 18S ribosomal RNA gene, partial sequence	*Ascomycota; Fusarium; Fusarium oxysporum* species complex	100	265/265
F-17	278	GQ225130	Fusarium oxysporum strain CR10 18S ribosomal RNA gene, partial sequence	*Ascomycota; Fusarium; Fusarium oxysporum* species complex	100	265/265
F-18	322	GQ225131	Myrmecridium schulzeri strain CBS 642.76 18S small subunit ribosomal RNA gene	*Ascomycota; Myrmecridium*	100	260/260
F-19	343	GQ225132	Uncultured fungus clone 19-M13F(-47) 18S ribosomal RNA gene, partial sequence	Fungi	100	343/342

Table 3. (Cont'd.).

DGGE band	Sequence length (bp)	Accession code	Closest relatives		Similarity (%)	Alignment
			Resource	Phylogenetic affiliations		
F-20	253	GQ225133	Podospora communis 18S ribosomal RNA gene, partial sequence	*Ascomycota; Lasiosphaeriaceae; Podospora.*	99	216/217
F-21	337	GQ225134	Acremonium sp. YT03 18S ribosomal RNA gene, partial sequence	*Ascomycota; Acremonium*	99	296/298
F-22	278	GQ225135	Uncultured fungus clone 5-M13F(-47) 18S ribosomal RNA gene, partial sequence	Fungi	100	259/259
F-24	295	GQ225137	Uncultured fungus clone f3Fc50 18S ribosomal RNA gene, partial sequence	Fungi	9	268/269
F-25	304	GQ225138	Uncultured ascomycete clone Mesq_E07 18S ribosomal RNA gene	*Ascomycota*	98	258/264
F-26	293	GQ225139	Cryptococcus sp. QMW-2009a strain KTAPG1-11.63 18S ribosomal RNA gene	*Basidiomycota*	*99*	249 /250
F-27	174	GQ225141	*Fusarium* sp. JD-109.2 18S ribosomal RNA gene	*Ascomycota; Hypocreales; Fusarium* sp.	99	264/266
F-29	286	GQ225140	Uncultured fungus clone Singleton_ (120-1057_3487) 18S ribosomal RNA gene	Fungi	91	253/276
F-30	174	GQ225156	Coccinia adoensis 18S ribosomal RNA gene	*Ascomycota; Streptophyta; Cucurbitaceae; Benincaseae; Coccinia*	98	157/160
F-31	172	GQ225142	Uncultured ascomycete ITS region including 18S rRNA gene	*Ascomycota; Alternaria* sp.	99	230/232
F-32	456	GQ225143	Uncultured fungus clone ontig438-108-1211_1493 18S ribosomal RNA gene, partial sequence	Fungi	99	248/258
F-33	275	GQ225161	*Verticillium dahliae* 18S ribosomal RNA gene	*Ascomycota; Verticillium dahliae*	98	210/214
F-34	314	GQ225144	Uncultured soil fungus clone A34 18S ribosomal RNA gene	Fung*i*	99	272/273
F-35	306	GQ225145	*Fusarium oxysporum* strain BD 18S ribosomal RNA gene	*Ascomycota; Fusarium oxysporum f.* sp.	100	265/265
F-36	314	GQ225146	Uncultured soil fungus clone A29 18S ribosomal RNA gene	Fungi	99	272/274
F-37	304	GQ225147	*Fusarium oxysporum* strain BD 18S ribosomal RNA gene	*Ascomycota; Fusarium oxysporum*	100	265/265
F-38	306	GQ225148	*Fusarium sp.* 19010 18S ribosomal RNA gene	*Ascomycota; Fusarium oxysporum*	100	263/263
F-39	260	GQ225149	Uncultured ascomycete clone MO1_A02 18S ribosomal RNA gene	*Ascomycota; Alternaria* sp.	99	117/122
F-40	302	GQ225150	Fusarium oxysporum strain CR10 18S ribosomal RNA gene, partial sequence	*Ascomycota; Fusarium; Fusarium oxysporum* species complex	100	265/265
F-41	295	GQ225151	Plectosphaerella cucumerina genes for 18S rRNA gene	*Ascomycota; Plectosphaerella cucumerina*	100	242/242

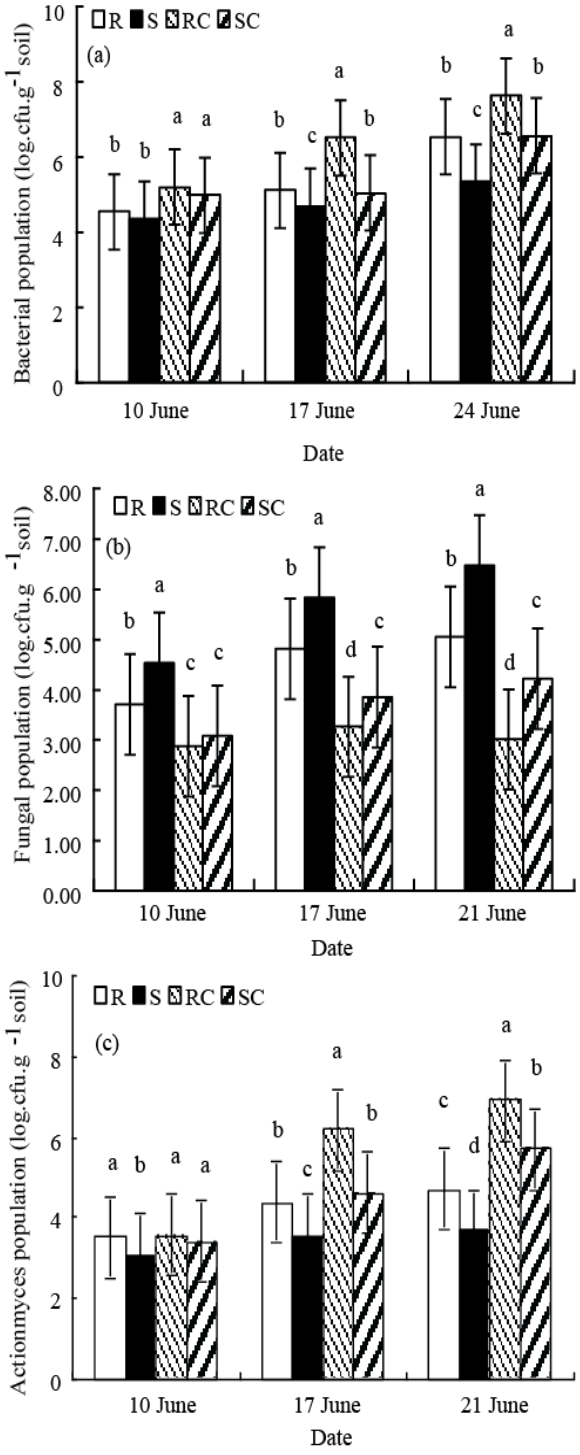

Fig. 1. Populations of culturable bacteria (a), fungi (b) and actinomycetes (c) in watermelon rhizosphere estimated by plate culture method.. R, FON-inoculated soil resistant cultivar treatment; S, FON-inoculated soil susceptible cultivar treatment; RC, non-inoculated soil resistant cultivar treatment; SC, non-inoculated soil susceptible cultivar. Samples for each cultivar were collected at 7, 14, and 21 days after inoculation on 10 June, 17 June, and 24 June, respectively. Each value was the mean ± SE of nine replicates. Values with different letters in the group were significantly different between treatments at the 0.05 level.

The Shannon index (*H*) of the bacterial community structure in the rhizosphere of the susceptible cultivar (S)were significantly lower than in the resistant cultivar (R) ($p<0.05$) from 7 days (10 June) after inoculation, and similarly, non-inoculation evidently decreased the Shannon index (*H*) of the bacterial community structure ($p<0.05$) on 24 June (Fig. 3). Principal component analysis (PCA) of soil bacterial community clearly separated resistant cultivars and susceptible cultivars after inoculation (Fig. 5). Figure 5 showed that resistant cultivars far from susceptible cultivars at three sampling time points after inoculation. In non-inoculating treatment, PCA analysis of soil bacterial community separated resistant cultivars and susceptible cultivars on 24 June.

Rhizosphere fungal community structure: DGGE profiles revealed that soil fungal community was affected by different cultivars and inoculating FON (Fig. 6). Differences in fungal community structures were observed between the different treatments. With the exception of the common bands, thirty eight characteristic bands (indicated by arrow in (Fig. 6-a,b,c) were cloned and sequenced (Table 3). Most of the Fungal bands from the DGGE profiles were affiliated to *Ascomycota* and fungi, Bands F-12 and F-26 were affiliated to *Basidiomyc*ota, F-10 to uncultured fungi. Sequencing results showed that band F-40 was the pathogenic fungi of watermelon Fusarium wilt (FON).

Band F-10, F-18 showed only in the resistant cultivar of FON-inoculated treatment and band F-16, F-20 showed in the susceptible cultivar of FON -inoculated treatment at 14 days (17 June) post inoculation, but F-19 existed in the susceptible cultivar of control.F-20 was observed in all susceptible cultivar treatments. Band F-30 were in the resistant cultivar of FON-inoculated treatment and band F-27 in the susceptible cultivar of FON -inoculated treatment at 21 days after inoculation, band F-24, F-33, F-41 were observed in the resistant cultivar of control, band F-25, F-30, F-32, F-35 were detected in the susceptible cultivar of control, and band F-26, F-34, F-38 were showed in all controls. Band F-36 appeared in the resistant cultivar of FON -inoculated treatment and the susceptible cultivar of non-inoculated control at 21 days (24 June) after inoculation, F-37 existed in all treatments except the susceptible cultivar of inoculation treatment. The Shannon index (*H*) of the fungal community structure in the rhizosphere of the susceptible cultivar (S) were significantly lower than in the resistant cultivar (R) ($p<0.05$) from 7 to 21 days after inoculation, and the controls tend to the same conclusion ($p<0.05$) (Fig. 3) after 17 June. PCA analysis clearly separated the fungal community between resistant cultivars and susceptible cultivars at three sampling points after inoculation. (Fig. 7-A, B, C)., the controls showed same trend after 17 June (Fig. 7-B, C).

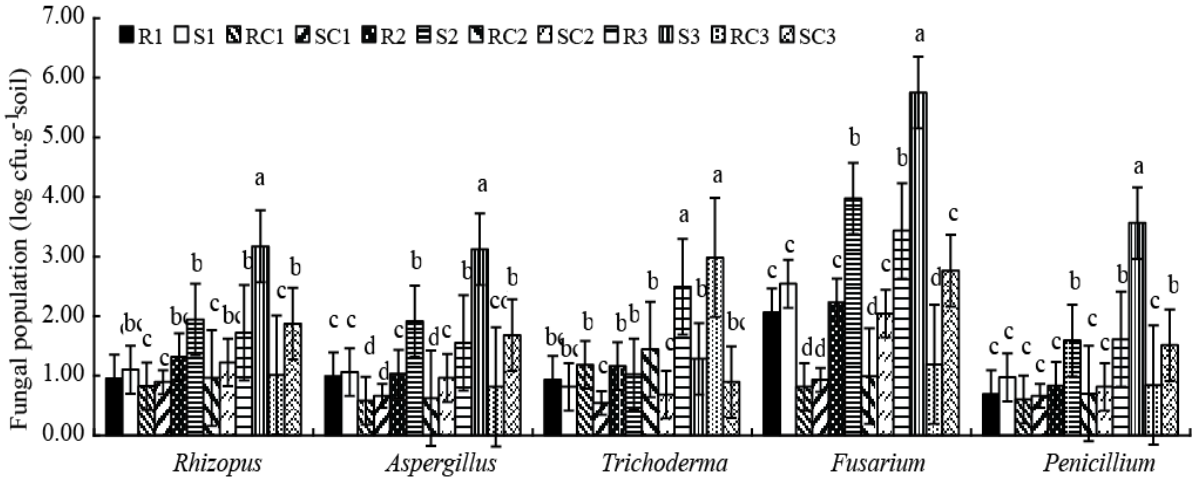

Fig. 2. Populations of the main fungal groups in the watermelon rhizosphere. R, FON-inoculated soil□ resistant cultivar treatment; S, FON-inoculated soil□ susceptible cultivar treatment; RC, non-inoculated soil□ resistant cultivar treatment; SC, non-inoculated soil□ susceptible cultivar. Samples for each cultivar collected at 7, 14, and 21 days after inoculation are denoted by R1 and S1, R2 and S2, R3 and S3, the same days are denoted by RC1 and SC1, RC2 and SC2, and RC3 and SC3, respectively. Each value was the mean ± SE of nine replicates. Values with different letters in a group were significantly different between treatments at the 0.05 level.

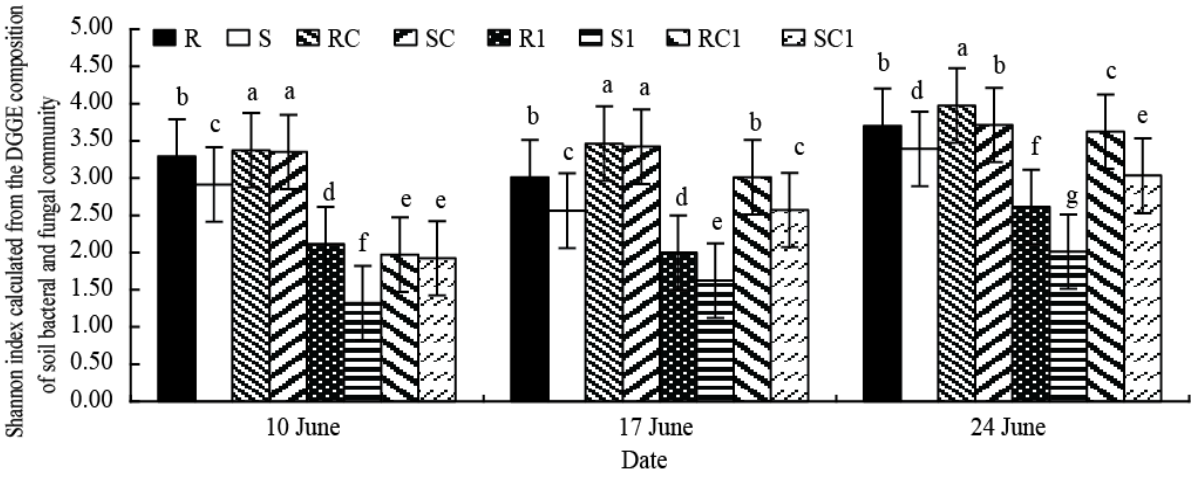

Fig. 3. Shannon index of DGGE profiles of bacterial and fungal community structure in watermelon rhizosphere. M, DNA marker 2000. BA, before transplanting. For bacteria: R, FON-inoculated soil□ resistant cultivar treatment; S, FON-inoculated soil□ susceptible cultivar treatment; RC, non-inoculated soil□ resistant cultivar treatment; SC, non-inoculated soil□ susceptible cultivar. For fungi: R1, FON-inoculated soil□ resistant cultivar treatment; S1, FON-inoculated soil□ susceptible cultivar treatment; RC1, non-inoculated soil□ resistant cultivar treatment; SC1, non-inoculated soil□ susceptible cultivar.

Discussion

DGGE analysis, PCA analysis and a culturable microbial population were used to assess the microbial community characteristics in the rhizosphere of the watermelon cultivars. Although our experiment included only two watermelon cultivars grown in two different soils and this is a limitation to confirm the effect of plant genotype on soil microbial community, all the methods suggested that the effects of watermelon rhizosphere microbial community of different resistance cultivars to *Fusarium Oxysporum* f. sp. *niveum* were different.

The plant genotype can affect the accumulation of microorganisms that help the plant to defend itself against pathogen attack (Roeland *et al*., 2012). The study revealed that FON-inoculated soil treatments significantly reduced the rhizosphere culturable bacteria and actinomycetes population, and increased fungi, but the same genotype treatments had difference significantly. These might be owing to that soil-borne fungal pathogens were lower in resistant cultivars compared to susceptible cultivars, and the resistant cultivars had relatively high numbers of actinomycetes, which might induce the decrease of soil-borne pathogens (Yao *et al*., 2010). The interactions between a plant and its root microbiome might change when the plant is attacked (Trivedi *et al*., 2012). Thus, FON invade watermelon rhizosphere just as Huanglongbing alters the structure and functional diversity of microbial communities associated with citrus rhizosphere (Trivedi *et al*., 2012).

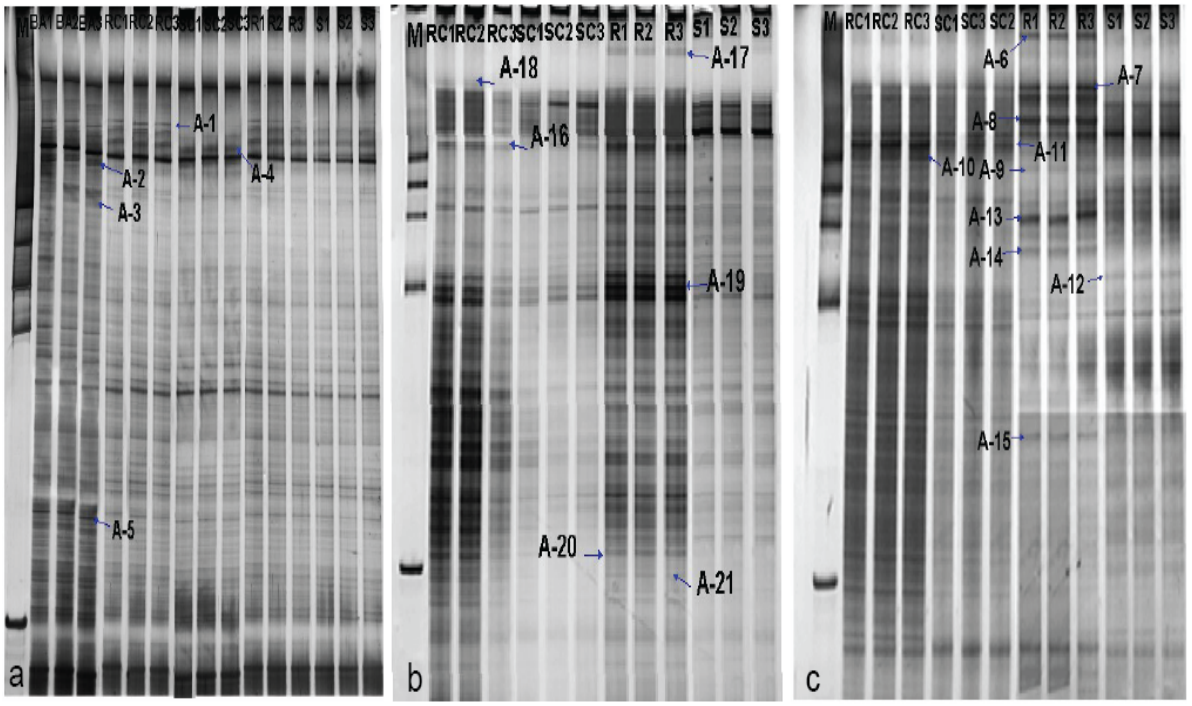

Fig. 4. DGGE banding pattern of DGGE profiles of bacterial community structure in watermelon rhizosphere. M, DNA marker 2000. BA, before transplanting. R, FON-inoculated soil resistant cultivar treatment; S, FON-inoculated soil susceptible cultivar treatment; RC, non-inoculated soil resistant cultivar treatment; SC, non-inoculated soil susceptible cultivar. Samples for each cultivar collected at 7, 14, and 21 days after inoculation are denoted by R1 and S1, R2 and S2, R3 and S3, the same days are denoted by RC1 and SC1, RC2 and SC2, and RC3 and SC3, respectively. Arrows indicate bands excised from the gel for sequencing.

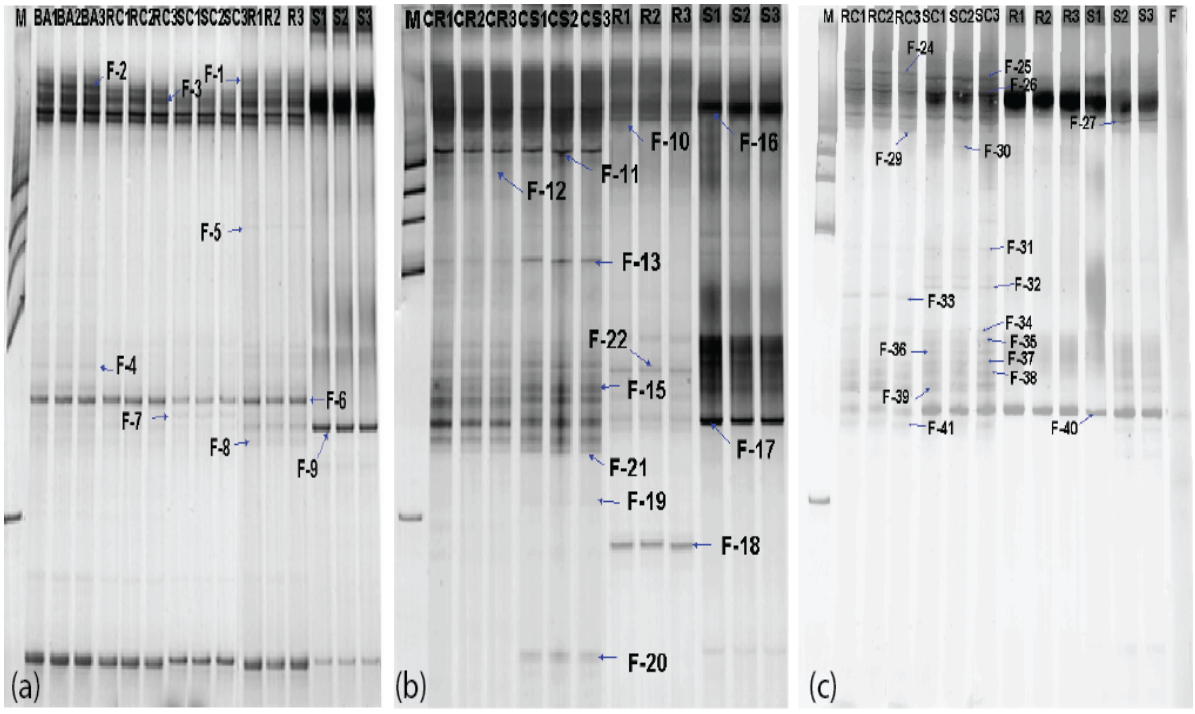

Fig. 6. DGGE banding pattern of DGGE profiles of fungal community structure in watermelon rhizosphere. M, DNA marker 2000. BA, before transplanting. R, FON-inoculated soil resistant cultivar treatment; S, FON-inoculated soil susceptible cultivar treatment; RC, non-inoculated soil resistant cultivar treatment; SC, non-inoculated soil susceptible cultivar. Samples for each cultivar collected at 7, 14, and 21 days after inoculation are denoted by R1 and S1, R2 and S2, R3 and S3, the same days are denoted by RC1 and SC1, RC2 and SC2, and RC3 and SC3, respectively. Arrows indicate bands excised from the gel for sequencing.

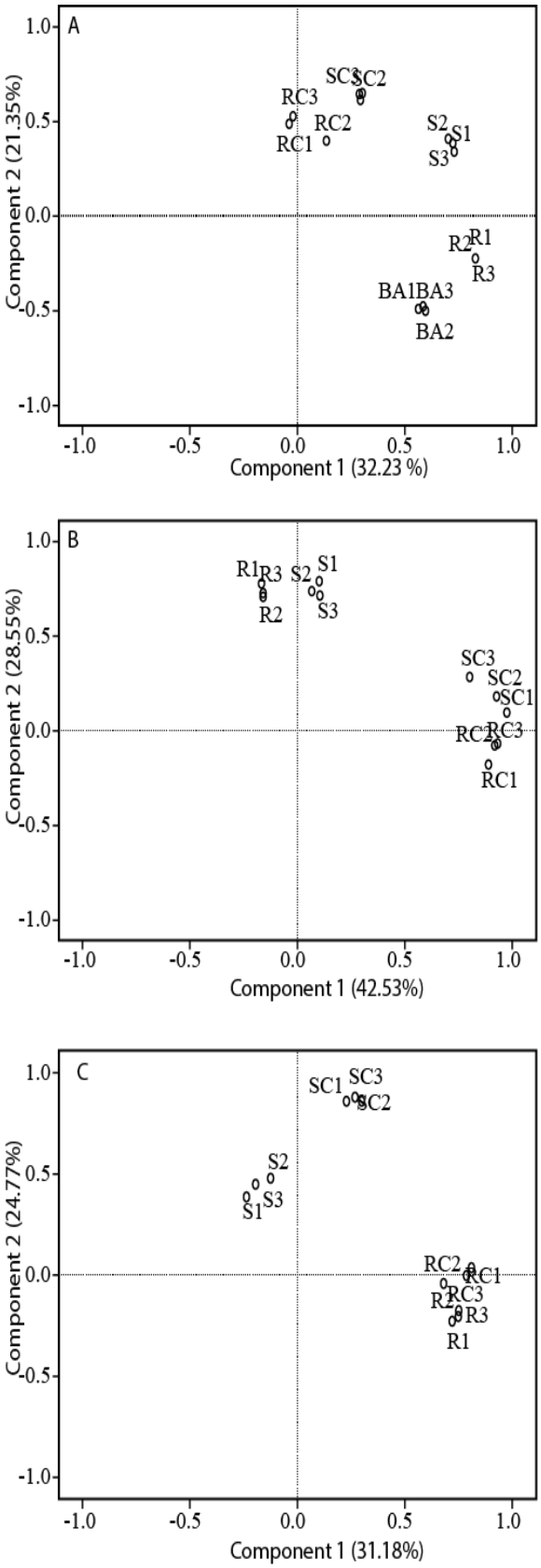

Fig. 5. Principal component analysis of DGGE profiles of bacterial community structure in watermelon rhizosphere.

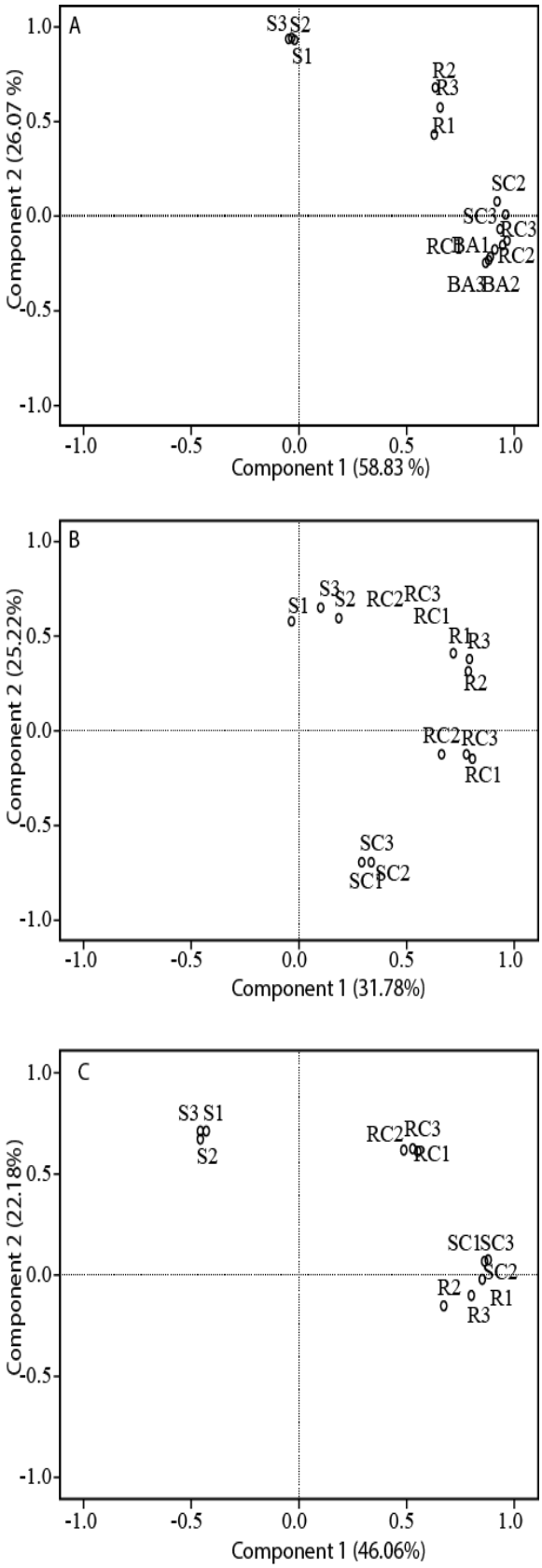

Fig. 7. Principal component analysis of DGGE profiles of fungal community structure in watermelon rhizosphere.

The resistant difference of variety was reflected through microbial species, quantity, and diversity difference. While the rhizosphere microbial community structure of high disease-resistant varieties were more rich, species were more homogeneous, and diversity was higher, that indicated the soil microbial ecological system was more stable and balanced, which would be conducive to crop growth and disease resistance (Prosser, 2002; Wang *et al*., 2012). In this study, the PCR-DGGE analysis showed that the rhizosphere bacterial and fungal community structure differed between FON-inoculated and non-inoculated cultivars, and there were more different bands of fungal communities were observed in the DGGE profile of non-inoculated samples than inoculated samples. PCA analysis also indicated the shifts of watermelon rhizosphere bacterial and fungal communities with growth days, and the community structures among two watermelon genotypes were different, supporting that the microbial communities in individual sample from one watermelon genotype are identical. In addition, the Shannon index (*H*) of the bacterial and fungal community structure in the rhizosphere of the susceptible cultivar were significantly decreased than that in the resistant cultivar ($p<0.05$) after inoculation. Although the relationship between microbial diversity and disease suppression is complex, it was assumed that high overall microbial diversity will lead to the suppressiveness to soil-borne diseases, because in some cases, disease suppression is correlated with high microbial diversity (Postma *et al.,* 2005). Furthermore, it proved that a negative correlation between the diversity of the soil microbiota and survival of the invader and the relationship which could be explained by a decrease in the competitive ability of the invader in species-rich vs. species-poor bacterial communities (Jan Dirk van Elsas *et al*., 2012). Therefore, the disease suppressiveness might not only dependent on the rhizosphere microorganism abundance, but also on the rhizosphere microorganism community structure.

The abundance and structure of rhizosphere soil microbial communities differed among plant cultivars and genotype, and that resistance of plants to soil-borne diseases related to the presence of antagonistic microorganisms (Hardoim *et al*., 2011; Kavino *et al*., 2007). Sequence analysis of different bands derived from fungal DGGE profile showed that *Verticillium* sp. were found in the rhizosphere soil of resistant non-inoculated watermelon cultivar on 24 June. The results of plate culturing also showed that the number of populations of *Rhizopus, Penicillium*、*Aspergillu,* and *Fusarium* were significantly lower in the resistant cultivar than in the susceptible cultivar of all treatments, but the *Trichoderma* populations showed the opposite trend. It was ascribable to that *Trichoderma*, *Chaetomium*, *Verticillium*, mycorrhizal fungi, *Saccharomyces* and *Paecilomyces lilacinus* can produce antibiotics (Li *et al*., 2000). Sequence analysis of some bands below derived from fungal DGGE profile, belong to *F. oxysporum*. F-11, F-38 were in all controls and F-37 was in all treatments except the FON-inoculated susceptible cultivar on 17 June. Perhaps *F. oxysporum* was the primary antagonist responsible for suppression in this suppressive soil, although other organisms may contribute to suppressiveness, and several isolates of *F. Oxysporum* from this suppressive soil have potential for development as biocontrol agents (Larkin *et al.*, 1996). As a result, the resistance of the resistant watermelon cultivar might attribute to the presence of antagonistic microorganisms in its rhizosphere.

Differences were found in the microbial community composition between rhizosphere microbiology with FON inoculated and non-inoculated treatments in the experiment. The rhizosphere soil difference between the health watermelon and infected watermelon might be contributing factor in the susceptible watermelon cultivar sufferring and the resistance in watermelon could be overcome by high inoculum levels of *F. oxysporum* f. sp. *Niveum* (Martyn *et al.*, 1991). So, *F. oxysporum* f. sp. *Niveum* alters the structure and functional diversity of microbial communities associated with watermelon rhizosphere.

In conclusion, the study indicated that the effects of watermelon rhizosphere microbial community of different resistance cultivars to FON were different. Different resistant watermelon cultivars occupied different rhizosphere microbial communities, and disease suppression might be correlated with high microbial diversity. Although specific microbial populations may be responsible for *Fusarium oxysporum* f.sp. *niveum* suppression in the soil samples examined, it was possible to correlate the presence of distinct bacteria or fungal species with the inhibition of *Fusarium oxysporum* f.sp. *niveum* growth through soil or suppression of disease development in watermelon.

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