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Effects of transgenic watermelon with CGMMV resistance on the diversity of soil microbial communities using PLFA

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We compared the composition of phospholipid fatty acids (PLFA) to assess the microbial community structure in the soil and rhizosphere community of non-transgenic watermelons and transgenic watermelons in Miryang farmlands in Korea during the spring and summer of 2005. The PLFA data were seasonally examined for the number of PLFA to determine whether there is any difference in the microbial community in soils from two types of watermelons, non-transgenic and transgenic. We identified 78 PLFAs from the rhizosphere samples of the two types of watermelons. We found eight different PLFAs for the type of plants and sixteen PLFAs for the interaction of plant type and season. The PLFA data were analyzed by analysis of variance separated by plant type ($P < 0.0085$), season ($P < 0.0154$), and the plant type × season interaction ($P < 0.1595$). Non-parametric multidimensional scaling (NMS) showed a small apparent difference but multi-response permutation procedures (MRPP) confirmed that there was no difference in microbial community structure for soils of both plant types. Conclusively, there was no significant adverse effect of transgenic watermelon on bacterial and fungal relative abundance as measured by PLFA. We could reject our hypothesis that there might be an adverse effect from transgenic watermelon with our statistical results. Therefore, we can suggest the use of this PLFA methodology to examine the adverse effects of transgenic plants on the soil microbial community.

Keywords: NMS; PCR; transgenic plants; watermelon

Introduction

Genetic modification (GM) of plants is a powerful technology which is utilized in the process of genetic engineering that allows scientists to move genetic material between organisms with the aim of changing their characteristics for improving crop cultivars, such as resistance to certain diseases or pests (Fraley 1992; Simmonds 1999; Griffiths et al. 2000; Conner et al. 2003; Chaer et al. 2009). The scientific advances in cell and molecular biology have now culminated in genetically modified crops (Conner et al. 2003). James (2001) reported that the area of GM crops exceeded 52.6 million ha worldwide and total area of commercially grown GM crops in developing countries including China and India has increased during the last few years (Nap et al. 2003). Nowadays, Korea does not yet commercially grow any genetically modified crops and we are still in the initial stages of developing, producing, and evaluating genetically modified crops.

Even though we have developed genetically modified plants and the potential benefits from the new high technology to improve the reliability and quality of the world food supply, it is true that public and scientific concerns have been raised about the effect of genetically modified crops with transmittance of foreign genes as a human food with regard to the environment and food safety (Conner et al. 2003; Nap et al. 2003; Yi et al. 2009). Therefore, the right application of risk assessment for non-target organisms for all genetically modified crops must be the first priority for the future of human beings, and this application can resolve public and scientific concerns (Conner et al. 2003; Nap et al. 2003; Badosa et al. 2004).

Cucurbitaceae crops, such as watermelon, cucumber, squash, pumpkin, and melons, are cultivated with rootstock grafting, which involves cutting the apical stems of the underground stem (called gongdae; a wild watermelon not a breeding commercial watermelon variety) and attaching other stems on it because of the poor viability of cultivar roots. Rootstock grafting is the main cultivation method used in most of South Korea, Japan, and some European countries with their limited land resources (Park et al. 2005). However, the rootstock is very vulnerable to virus infection by cucumber green mottle mosaic virus (CGMMV) and Tobamovirus genus, and this problem causes a loss in the agro-economy of Curcurbitaceae crop farmers (Park et al. 2005). Therefore, Park et al. (2005)

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developed a partial transgenic watermelon rootstock (*Citrullus lanatus* (Twinser) cv. Gongdae) for rootstock grafting instead of genetically modifying an entire watermelon plant. The purpose of partial rootstock grafting is to satisfy simultaneously the two main demands by the public and by farmers; social concern with transmittance of foreign genes into fruit and resistance to diseases for the agro-economy.

Although CGMMV can infect many Cucurbitaceae species, causing mosaic symptoms, a yellowish leaf and finally fruit deterioration, some stable transformations are resistant to CGMMV (Lee et al. 1990; Lee 1996). CGMMV outbreaks have caused marked losses in the total yields of Cucurbitaceous crops in Korea over the past several years. Since CGMMV is easily transmitted by soil, the development of a virus-resistant rootstock holds great promise for a viable solution. However, since no genetic source is available for resistance against CGMMV infection yet, an alternative way to utilize a viral gene, such as a coat protein (CP) gene, has been found, and the use of a watermelon rootstock (gongdae) without transmittance of foreign genes into the fruit by Agrobacterium-mediated transformation with the CGMMV-CP gene has been demonstrated (Park et al. 2005).

Soils with a rhizosphere are complex and dynamic biological systems and major components in agro-ecosystems (Nannipieri et al. 2003; Li et al. 2009). Crop plants interact with soil communities and they form strong links in the rhizosphere (Brimecombe et al. 2001). The root of crop plants in the rhizosphere can affect the structures and functions of microorganisms, with high numbers of many different microbial populations found on and surrounding the crop roots with complex interactions in the agro-soil ecosystem (Atlas and Bartha 1993; Li et al. 2009). Therefore, it is rather important to examine the risk assessment of genetically modified watermelon with regard to the microbial communities in the soils and rhizosphere of the agro-ecosystem.

Furthermore, recently many transgenic crops have been introduced to agro-ecosystems all over the world (Nap et al. 2003) and it is well known that transgenic crops impact agro-ecosystems and natural ecosystems including soil and rhizosphere through direct and indirect ways, including gene flows, invasions, and community and/or food web changes (Dale et al. 2002). Although there have been many studies on microbial communities in soil ecosystems, reports related to transgenic crops and the soil microbial community have been relatively few (Lord et al. 2002). Assessing the impacts of transgenic crops on the structures and functions of agro-soil ecosystems requires an adequate determination of the soil microbial community structure (Park et al. 2006) when compared to non-transgenic watermelon. To assess the impact of transgenic crops on the soil microbial community, phospholipid fatty acid analysis (PLFA) was used. The PLFA method has been widely applied to the analysis of microbial community composition for soil communities in complex habitats (Janus et al. 2005; Muruganandam et al. 2009; Yi et al. 2009).

We hypothesized that there would be an adverse effect from TR (transgenic watermelons). Therefore, we analysed the microbial community structures with a comparison of microbial community composition between nTR (non-transgenic watermelons) and TR (transgenic watermelons) soils by using PLFA.

Materials and methods

Watermelon

The CGMMV resistant watermelon was developed by Nong Woo Bio (Park et al. 2005). The transgenic plant material used here had been developed to have resistance to infection by CGMMV. This virus causes mosaic symptoms, yellowish leaves, and fruit deterioration through overexpression of the CGMMV coat protein gene, CGMMV-CP (Park et al. 2005; Youk et al. 2009). From the 11 T_0 independent Gongdae lines created, we selected one line (12) because it had the highest degree of tolerance. This line was self-crossed to obtain the T4 generation. Seeds of both the wild-type watermelon rootstock (*Citrullus vulgaris* [Twinser] cv. gongdae) and the transgenic watermelon rootstock were surface-sterilized with 500-times concentrated Benomyl for 5 hours and the seeds were dried out in a shaded area for 2–3 hours. After hastening of germination, the seeds were planted into small pots and after 7 days they were transplanted into farm conditions with mulching by a farm vinyl cover and were watered at every day to maintain the proper wet conditions. The average temperature in the farm conditions ranged from 20°C to 38°C during the growing periods. The purposes of mulching was to maintain soil temperature, prevent loss of fertilizer, keep pests from the soil, and prevent weedy, wet soil (not over-watering; minimum waterholding capacity 2.5–2.7 potential force (pF); once a day in the morning).

The experimental design and soil sampling procedure

Non-transgenic watermelons (nTR) and the CGMMV transgenic watermelons (TR) were cultivated in isolated facilities in the greenhouse at the experimental farm at Miryang (E128° 47′, N35° 30′) in South Korea. The experimental design foreach treatment of nTR or TR consisted of a randomized block design

Table 1. PLFA profiles in the soil samples of the non-transgenic and transgenic watermelons in 2005.

	% Composition of PLFA in soil samples					
	May		June		July	
PLFA	Non-transgenic	Transgenic	Non-transgenic	Transgenic	Non-transgenic	Transgenic
9:00	0.01 (0.00)	–		–	–	–
10:0 2OH	0.03 (0.01)	–	–	0.01 (0.01)	–	–
10:0 3OH	–	–	–	0.01 (0.00)	–	–
11:0 ISO 3OH	0.05 (0.02)	0.01 (0.01)	–	0.12 (0.06)	–	0.02 (0.02)
11:0 3OH	–	–	–	0.01 (0.00)	–	–
12:00	0.30 (0.04)	0.18 (0.03)	0.06 (0.02)	0.21 (0.03)	0.09 (0.04)	0.10 (0.06)
12:0 ISO	–	–	–	0.02 (0.01)	–	–
12:0 2OH	0.01 (0.00)	0.04 (0.01)	0.04 (0.01)	0.02 (0.01)	0.02 (0.01)	0.03 (0.02)
12:0 3OH	0.04 (0.00)	0.04 (0.00)	0.02 (0.01)	0.04 (0.01)	0.03 (0.00)	0.03 (0.01)
13:00	0.07 (0.01)	0.06 (0.01)	0.01 (0.01)	0.05 (0.01)	0.03 (0.00)	0.04 (0.01)
13:0 ISO	0.11 (0.01)	0.08 (0.01)	0.01 (0.01)	0.08 (0.01)	0.03 (0.02)	0.05 (0.02)
13:0 ANTEISO	0.02 (0.01)	0.02 (0.01)	–	0.01 (0.01)	–	–
13:1 AT 12– 13	–	0.01 (0.00)	–	0.01 (0.00)	–	–
14:00	1.20 (0.03)	1.20 (0.04)	0.78 (0.05)	1.19 (0.08)	1.10 (0.08)	1.11 (0.12)
14:0 ISO	0.75 (0.03)	0.66 (0.05)	0.32 (0.04)	0.65 (0.06)	0.54 (0.07)	0.52 (0.06)
14:0 ISO 3OH	–	0.01 (0.01)	–	–	–	–
14:1 w5c	0.07 (0.01)	0.05 (0.01)	0.34 (0.04)	0.08 (0.02)	0.05 (0.02)	0.06 (0.01)
15:00	–	–	–	–	–	–
15:0 ISO	8.82 (0.20)	8.22 (0.14)	6.55 (0.37)	9.28 (0.49)	9.90 (0.66)	10.22 (0.97)
15:0 ANTEISO	4.31 (0.11)	4.29 (0.12)	3.07 (0.12)	4.10 (0.20)	3.90 (0.20)	4.20 (0.22)
15:0 ISO 3OH	0.01 (0.01)	0.05 (0.03)	0.01 (0.01)	0.06 (0.02)	–	–
15:0 2OH	0.04 (0.02)	0.05 (0.02)	0.01 (0.01)	0.06 (0.03)	–	0.04 (0.04)
15:0 3OH	–	–	–	0.06 (0.06)	–	0.46 (0.23)
ISO 15:1 AT 5	–	–	–	0.03 (0.02)	–	0.06 (0.06)
15:1 ISO G	0.59 (0.02)	0.55 (0.04)	0.48 (0.01)	0.50 (0.01)	0.43 (0.05)	0.45 (0.01)
15:1 ISO I/13:0 3OH	0.06 (0.02)	0.04 (0.02)	–	0.07 (0.04)	–	0.09 (0.09)
15:1 ANTEISO A	0.10 (0.04)	0.09 (0.04)	–	–	–	–
15:1 w8c	–	0.01 (0.01)	–	–	–	0.02 (0.02)
15:1 w6c	0.06 (0.01)	0.03 (0.01)	0.01 (0.01)	0.04 (0.01)	–	–
16:00	12.17 (0.07)	12.50 (0.21)	12.84 (0.22)	13.56 (0.19)	14.41 (0.17)	14.29 (0.55)
16:0 N alcohol	0.02 (0.02)	0.01 (0.01)	0.83 (0.12)	0.09 (0.09)	0.08 (0.04)	0.05 (0.05)
16:0 ISO	4.49 (0.10)	4.45 (0.14)	3.77 (0.09)	4.08 (0.13)	4.47 (0.17)	4.15 (0.20)
16:0 ANTEISO	0.23 (0.00)	0.23 (0.01)	0.35 (0.03)	0.24 (0.02)	0.23 (0.01)	0.24 (0.01)
16:0 10 methyl	10.84 (0.37)	10.04 (0.29)	10.86 (0.33)	10.29 (0.34)	11.41 (0.25)	11.47 (0.78)
16:0 ISO 3OH	0.02 (0.02)	–	–	–	–	–
16:0 2OH	0.10 (0.04)	0.15 (0.05)	0.13 (0.07)	0.29 (0.04)	0.08 (0.08)	0.13 (0.08)
16:1 ISO I/14:0 3OH	–	–	0.15 (0.15)	0.19 (0.19)	–	–
16:1 w7c alcohol	0.02 (0.01)	0.01 (0.01)	0.03 (0.02)	0.01 (0.01)	–	0.04 (0.02)
16:1 ISO H	1.02 (0.02)	0.87 (0.10)	0.55 (0.11)	0.67 (0.13)	0.75 (0.02)	0.77 (0.02)
16:1 w9c	0.09 (0.06)	0.37 (0.10)	0.15 (0.10)	0.16 (0.10)	–	–
16:1 w11c	0.57 (0.08)	0.26 (0.11)	0.50 (0.10)	0.57 (0.11)	0.54 (0.03)	0.67 (0.10)
16:1 w7c/15 iso 2OH	5.28 (0.13)	4.92 (0.16)	3.69 (0.08)	4.54 (0.19)	3.36 (0.07)	3.92 (0.19)
16:1 w5c	2.09 (0.08)	2.08 (0.06)	3.09 (0.19)	3.21 (0.19)	3.29 (0.26)	3.03 (0.16)
16:1 2OH	1.69 (0.05)	2.26 (0.17)	1.14 (0.29)	1.45 (0.20)	1.78 (0.15)	1.36 (0.50)
17:00	0.74 (0.02)	0.80 (0.03)	0.91 (0.03)	0.82 (0.02)	0.88 (0.03)	0.88 (0.07)
17:0 ISO	3.45 (0.03)	3.59 (0.12)	3.76 (0.06)	3.61 (0.04)	4.31 (0.06)	4.12 (0.12)
17:0 ANTEISO	3.01 (0.05)	3.05 (0.07)	3.00 (0.07)	2.70 (0.06)	2.99 (0.06)	2.94 (0.14)
17:0 3OH	–	0.03 (0.03)	–	–	–	0.11 (0.11)
17:0 CYCLO	4.04 (0.06)	3.84 (0.04)	3.69 (0.05)	3.65 (0.06)	3.75 (0.06)	3.58 (0.18)
17:0 10 methyl	1.55 (0.05)	1.60 (0.08)	1.78 (0.12)	1.37 (0.10)	1.30 (0.07)	1.16 (0.17)
17:0 ISO 3OH	0.34 (0.05)	0.25 (0.06)	1.48 (0.17)	0.35 (0.15)	0.29 (0.07)	0.21 (0.01)
17:1 ANTEISO B/i I	0.03 (0.03)	0.05 (0.05)	0.04 (0.04)	0.07 (0.05)	–	–
17:1 w8c	0.62 (0.01)	0.66 (0.02)	0.83 (0.06)	0.58 (0.04)	0.51 (0.02)	0.51 (0.03)

Table 1 (*Continued*)

	% Composition of PLFA in soil samples					
	May		June		July	
PLFA	Non-transgenic	Transgenic	Non-transgenic	Transgenic	Non-transgenic	Transgenic
ANTEISO 17:1 w9c	0.75 (0.01)	0.81 (0.03)	0.81 (0.11)	0.54 (0.12)	0.52 (0.26)	0.23 (0.23)
18:00	3.90 (0.09)	4.23 (0.19)	5.46 (0.19)	4.55 (0.17)	5.39 (0.23)	5.02 (0.62)
18:0 ISO	0.31 (0.07)	0.38 (0.05)	0.45 (0.03)	0.34 (0.03)	0.32 (0.04)	0.27 (0.03)
TBSA 10Me18:0	3.53 (0.06)	3.46 (0.09)	3.31 (0.07)	3.08 (0.11)	3.21 (0.17)	3.26 (0.15)
18:0 3OH	0.02 (0.01)	0.22 (0.09)	–	0.01 (0.01)	–	–
18:0 2OH	0.23 (0.05)	0.37 (0.06)	0.04 (0.04)	0.23 (0.07)	0.28 (0.10)	0.22 (0.04)
18:1 w9c	3.78 (0.04)	3.87 (0.10)	4.10 (0.11)	4.15 (0.10)	3.29 (0.04)	3.59 (0.14)
18:1 w7c	5.90 (0.12)	5.76 (0.14)	4.53 (0.14)	4.70 (0.06)	3.47 (0.06)	3.66 (0.06)
11 methyl 18:1 w7c	0.92 (0.04)	0.93 (0.05)	1.18 (0.07)	0.88 (0.08)	0.70 (0.06)	0.65 (0.01)
18:1 2OH	0.04 (0.02)	0.14 (0.08)	–	0.09 (0.06)	–	–
18:2 w6,9c/18:0 ANTE	1.13 (0.07)	1.38 (0.11)	1.78 (0.12)	1.52 (0.10)	0.96 (0.07)	1.05 (0.12)
18:3 w6c(6,9,12)	0.06 (0.04)	0.19 (0.06)	1.19 (0.14)	0.37 (0.12)	0.31 (0.04)	0.27 (0.02)
19:00	1.06 (0.12)	0.98 (0.18)	0.24 (0.16)	0.60 (0.15)	1.11 (0.24)	1.01 (0.14)
19:0 ISO	0.73 (0.06)	0.62 (0.13)	1.13 (0.04)	0.43 (0.13)	0.66 (0.12)	0.48 (0.05)
19:0 CYCLO w10c/19w6	–	–	1.38 (0.74)	–	–	1.71 (1.71)
19:0 ANTEISO	0.29 (0.15)	0.41 (0.21)	–	0.10 (0.10)	–	–
19:0 CYCLO w8c	6.21 (0.14)	6.43 (0.18)	5.04 (0.97)	6.26 (0.20)	6.18 (0.45)	4.55 (2.29)
19:0 10 methyl	0.09 (0.06)	0.12 (0.08)	0.28 (0.12)	0.23 (0.11)	–	–
19:1 w11c/19:1 w9c	–	–	–	0.06 (0.06)	–	–
19:1 w6c/.846/19cy	–	0.09 (0.09)	0.58 (0.19)	0.11 (0.11)	0.17 (0.17)	0.23 (0.23)
19:1 ISO I	–	0.05 (0.05)	0.48 (0.06)	0.06 (0.06)	–	–
20:00	0.80 (0.19)	0.68 (0.23)	0.93 (0.38)	0.78 (0.20)	1.65 (0.40)	1.33 (0.06)
20:0 ISO	0.05 (0.03)	–	0.03 (0.03)	0.07 (0.05)	0.04 (0.04)	0.06 (0.03)
20:1 w9c	0.29 (0.09)	0.26 (0.09)	0.17 (0.12)	0.40 (0.11)	0.36 (0.18)	0.53 (0.02)
20:4 w6,9,12,15c	0.08 (0.06)	0.10 (0.07)	0.27 (0.11)	0.30 (0.13)	–	–
Species Richness	49.92 (0.80)	50.90 (1.21)	46.00 (0.73)	50.56 (0.82)	45.67 (1.45)	48.33 (1.45)
Evenness	0.83 (0.00)	0.83 (0.01)	0.86 (0.01)	0.82 (0.01)	0.82 (0.01)	0.80 (0.00)
Shannon-Wiener Diversity	3.23 (0.02)	3.27 (0.03)	3.31 (0.02)	3.22 (0.03)	3.14 (0.02)	3.11 (0.02)

–, not detected.

with three replication plots (3 m × 5 m each) for the two plant types in which ten watermelon plugs were planted per plot under restricted regulation. The photo and experimental scheme of watermelon plots were as in Yi et al.'s (2006) report and we modified the experiment plots to six plots (three replicates of nTR and TR). Their growth rate in 2005 was 1–2 m per month. Soil samples per plot were taken from the top 5 cm of soil by using 2.5-cm-diameter cores and the samples consisted of four replicates ($n = 24$) of three subplots of nTR and TR on 12 May, three replicates ($n = 18$) of three subplots of nTR and TR on 16 June, and then four subplots were harvested and we left one subplot each of nTR and TR. We sampled three replicates ($n = 6$) of soil from each subplot on 25 July in 2005. All soil samples which were taken from the experimental plots were immediately placed on dry ice. Upon returning to our laboratory, the samples were frozen at -80°C before PLFA analyses (McKinley et al. 2005).

Microbial communities by PLFA analysis

The phospholipid fatty acid (PLFA) profiles were analyzed to determine the microbial community composition (Janus et al. 2005; McKinley et al. 2005). Fatty acids with carbon chain lengths between 9 and 20 carbons were extracted and identified from all soil samples using White et al.'s (1979) and Kelly et al.'s (1999) modified methods. To extract PLFA, the frozen soils were lyophilized by a freeze dryer (Labconco, USA). Then, 8.0 g of the dried sample was extracted using a single-phase chloroform–methanol–aqueous buffer system (Bligh and Dyer 1959). The total lipid extract was fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid chromatography (Sep-pak® cartridge, Waters, Ireland) and the polar lipid

Table 2. The significance levels for testing effects of two plant types, non-transgenic and transgenic watermelons, season, and their interaction, in the Miryang greenhouse facility during 2005. Each PLFA was tested by ANOVA ($P < 0.05$).

PLFAs	Plant type (T) (df = 1)	Season (S) (df = 2)	T × S (df = 2)
9:00	–	–	–
10:0 2OH	–	*	**
10:0 3OH	–	–	–
11:0 ISO 3OH	–	–	*
11:0 3OH	–	–	–
12:00	–	*	*
12:0 ISO	–	–	–
12:0 2OH	–	–	*
12:0 3OH	–	*	–
13:00	–	–	–
13:0 ISO	–	**	**
13:0 ANTEISO	–	–	–
13:1 AT 12– 13	–	*	–
14:00	*	–	*
14:0 ISO	–	*	*
14:0 ISO 3OH	–	–	–
14:1 w5c	**	**	**
15:00	–	–	–
15:0 ISO	–	–	*
15:0 ANTEISO	–	–	–
15:0 ISO 3OH	–	–	–
15:0 2OH	–	–	–
15:0 3OH	–	–	–
ISO 15:1 AT 5	–	–	–
15:1 ISO G	–	–	–
15:1 ISO I/13:0 3OH	–	–	–
15:1 ANTEISO A	–	*	–
15:1 w8c	–	–	–
15:1 w6c	–	–	–
16:00	–	–	–
16:0 N alcohol	–	–	–
16:0 ISO	–	–	–
16:0 ANTEISO	*	*	*
16:0 10 methyl	–	–	–
16:0 ISO 3OH	–	–	–
16:0 2OH	–	–	–
16:1 ISO I/14:0 3OH	–	–	–
16:1 w7c alcohol	–	–	–
16:1 ISO H	–	*	–
16:1 w9c	–	–	–
16:1 w11c	–	–	–
16:1 w7c/15 iso 2OH	–	**	–
16:1 w5c	–	*	–
16:1 2OH	–	*	–
17:00	–	–	–
17:0 ISO	–	–	–
17:0 ANTEISO	–	–	–
17:0 3OH	–	*	–
17:0 CYCLO	–	–	–
17:0 10 methyl	–	–	–
17:0 ISO 3OH	*	**	*
17:1 ANTEISO B/i I	**	*	*
17:1 w8c	–	–	–
ANTEISO 17:1 w9c	–	–	–
18:00	–	–	–
18:0 ISO	–	–	–
TBSA 10Me18:0	–	–	–
18:0 3OH	–	–	–
18:0 2OH	–	–	–
18:1 w9c	–	*	–
18:1 w7c	–	**	–
11 methyl 18:1 w7c	–	*	–
18:1 2OH	*	*	–
18:2 w6,9c/18:0 ANTE	–	*	–
18:3 w6c (6,9,12)	**	**	**
19:00	–	–	–
19:0 ISO	–	–	–
19:0 CYCLO w10c/19w6	–	–	–
19:0 ANTEISO	–	–	–
19:0 CYCLO w8c	–	–	–
19:0 10 methyl	–	–	–
19:1 w11c/19:1 w9c	–	–	–
19:1 w6c/.846/ 19cy	–	–	*
19:1 ISO I	*	**	*
20:00	–	–	*
20:0 ISO	–	*	–
20:1 w9c	–	*	–
20:4 w6,9,12,15c	–	–	–
Total abundance	–	–	–
Species richness	*	*	–
Evenness	*	*	**
Shannon-Wiener diversity	–	**	*

* $P < 0.05$; ** $P < 0.001$; – , not significant.

fraction containing the phospholipids was isolated and transesterified into fatty acid methyl esters using a mild alkaline methanolysis reaction (Guckert et al. 1985). Fatty acid methyl esters (FAMEs) were analyzed by capillary gas chromatography with flame ionization detection on an HP 6890N (Hewlett-Packard Inc., USA) chromatograph using a 25 m non-polar column

Table 3. Phospholipid fatty acid composition (PLFA) of soil from non-transgenic (nTR) and transgenic (TR) watermelons. Unidentified PLFA are not included. Identified PLFA are grouped into four categories: saturated, branched (i, iso; a, anteiso), monounsaturated (MUFA) and polyunsaturated (PUFA) PLFA.

	% Composition of PLFA in sample from plant type and season						
	May		June		July		
PLFA	nTR	TR	nTR	TR	nTR	TR	Marker
Normal saturated	20.239	20.631	21.223	21.747	24.667	23.777	
Mid-chain branched saturated	**16.004**	**15.216***	**16.221**	**14.973***	15.920	15.893	Actinomycetes
10me 16:0	10.841	10.043	10.856	10.292	11.410	11.47	Gram(+), actinomycetes
10me 18:0	3.528	3.463	3.307	3.079	3.213	3.263	Actinomycetes
Terminally branched saturated	26.569	25.994	**22.438**	**25.725****	27.393	27.237	
i14:0	0.750	0.657	0.320	0.648	0.540	0.517	Gram (+)
i15:0	8.821	8.222	**6.551**	**9.283****	**9.897**	**10.217***	Gram (+)
a15:0	4.313	4.289	**3.066**	**4.099***	**3.900**	**4.197***	Gram (+)
i16:0	4.493	4.453	3.773	4.083	4.470	4.153	Gram (+)
i17:0	3.451	3.587	3.763	3.614	4.313	4.123	Gram (+)
a17:0	3.008	3.052	2.997	2.696	2.993	2.94	Gram (+)
Branched monounsaturated	7.826	7.384	6.199	6.634	5.057	5.460	
Monounsaturated	23.031	22.843	23.56	23.76	21.493	22.407	
16:1w9c	**0.093**	**0.372****	0.148	0.156	0.000	0.000	Gram (−)
16:1w7c	**5.278**	**4.923***	**3.691**	**4.541***	3.360	3.920	Gram (-)
16:1w5c	2.086	2.081	3.093	3.208	3.290	3.030	Gram (−), fungi
cy17:0	**4.044**	**3.841***	3.69	3.648	3.753	3.583	Gram (−)
18:1w9c	3.778	3.865	4.097	4.149	3.287	3.587	Fungi, plant
18:1w7c	5.9	5.757	4.533	4.702	3.470	3.660	Gram (−)
cy19:0	6.209	6.429	**5.036**	**6.261***	**6.177**	**4.553****	Gram (−)
20:1w9c	0.286	0.258	0.173	0.396	0.363	0.533	Fungi
Polyunsaturated	**1.273**	**1.673***	**3.238**	**2.193**	1.270	1.320	
18:2w6	1.128	1.383	1.777	1.518	0.963	1.047	Fungi, plant
18:3w3	**0.064**	**0.189****	**1.193**	**0.374****	0.307	0.273	Fungi, plant
20:4w6	**0.082**	**0.101****	0.268	0.301	0.000	0.000	Protozoa

Bold numbers indicate that the *P*-value between nTR and TR was less than 0.05. * $P < 0.05$, ** $P < 0.01$ on **bold** numbers of TR.

(0.2 mm i.d., 0.33 μm film thickness) with the injector and detector maintained at 250 and 300°C, respectively. The column temperature was programmed to start at 170°C, and then ramp up at a rate of 5°C min^{-1} to 260°C, followed by a ramp of 40°C min^{-1} to 310°C (MIDI Inc. 2002). PLFAs were analyzed by using the MIDI Sherlock Microbial Identification System (MIDI Inc. 2002). Individual PLFA values were expressed as a percentage of the total PLFAs in the sample (Janus et al. 2005).

Statistical analyses

For this aspect of the experiment, we initially compared the responses of the microbial communities between nTR and TR and treatments using the split plot in time approach by analyses of variance (ANOVA), 1 df for treatment and 2 df of time (seasons) (Sokal and Rohlf 1995). ANOVAs were used to test the differences in mean abundance, mean species richness, and mean species diversity for the microbial community with PLFA (SAS Institute 2001). *F*-statistics were calculated for season, treatment, and interactions. The Tukey-Kramer procedure was used to compare treatment means. In all analyses, the level of significance was at least $P \leq 0.05$ (SAS Institute 2001). Ordination analyses were done using PC-ORD version 4.28 (McCune and Grace 2002). The pooled main matrices for each data set had high beta diversity, moderate to extreme row and column skewness, and a high coefficient of variation (CV) among the sums of the columns (species) in the matrix. Thus, rare species that occurred in less than 5% of the samples were deleted, and the data were then transformed by taking logarithms. Relativization by column (species) maxima was then

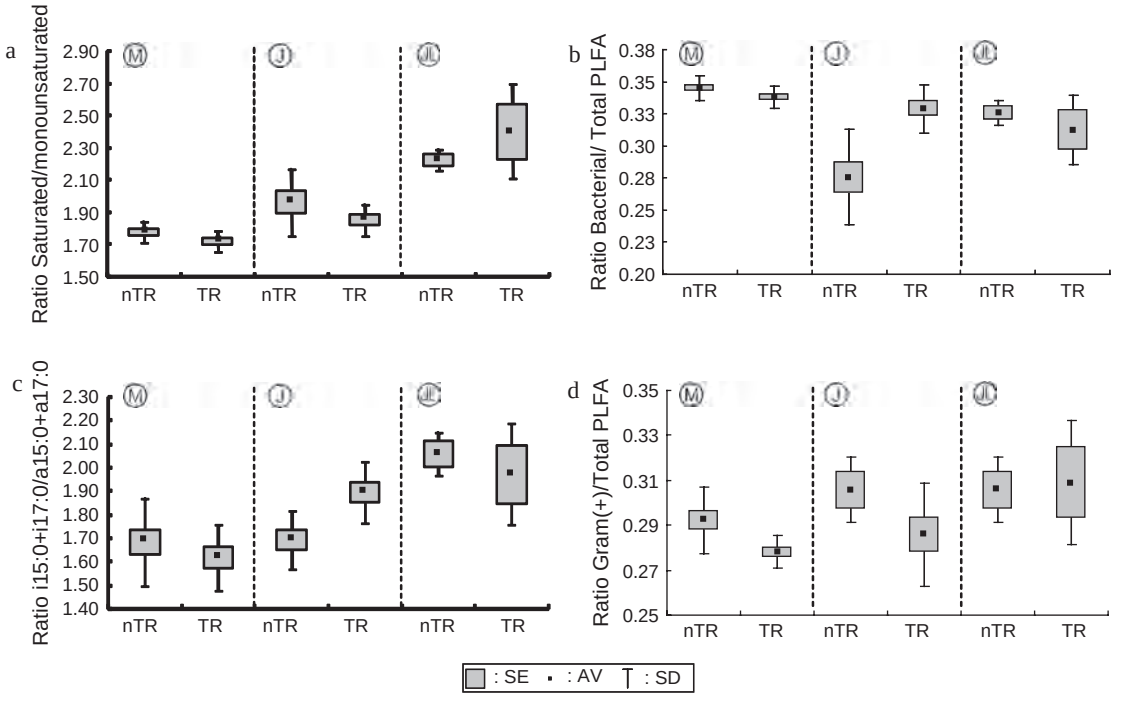

Figure 1. Amount of (a) normal saturated to monosaturated PLFA, (b) bacterial PLFA markers to total PLFA, (c) iso to anteiso branched PLFA (i15:0 + i17:0/a15:0 + a17:0), and (d) Gram(+) PLFA markers to total PLFA found in the soil of the nTR and TR watermelons farm in Miryang in May (M), June (J), July (JL) in 2005. SE: standard errors, AV: average, SD: standard deviation.

done to equalize the weights between abundant and less abundant species. The Sørensen distance measure was used for all analyses. The transformed data were used for ordination analysis at this point. The suite of multivariate analyses gave us rapid insight into the variability in microbial populations within and between different treatments. One of the ordinations, non-metric multidimensional scaling (NMS), is an iterative method based on rank distances between sample units. It is conceptually simple (Rees et al. 2004) and useful for ecological gradient studies because of its general robustness and lack of assumptions about the distribution or type of data (Mather 1976). Therefore, NMS was used to determine the number of factors affecting the complex community structures and to qualitatively summarize the overall distribution of species assemblages across the gradients of different treatments. NMS was used in lieu of other ordination methods because it avoids the 'zero-truncation problems' of Beals (1984). Although its usefulness is great on the ecological data sets including the microbial community ecology (van Hannen et al. 1999; Díez et al. 2001), its use is still rare as an ordination technique in microbial ecology (Rees et al. 2004). Species richness, species diversity indices, and species evenness were calculated with the PC-Ord. Our simple repeated-measure designs, which are common in ecology, required multi-response permutation procedures (MRPP) (McCune and Grace 2002). This MRPP procedure is useful for analyzing ecological data because it does not require assumptions of normality and constant variance (Biondini et al. 1988; McCune and Mefford 1999). Since our research fitted well to the analysis, we used MRPP. The analysis is a nonparametric procedure for testing a hypothesis of no difference between two or more groups of entities and it randomly reassigns the observed values to different treatments.

Results

We determined the PLFA profiles from the nTR and TR soil samples and we seasonally examined the PLFA profiles and analyzed each PLFA with plant type, season, and their interaction by using ANOVA to compare the microbial community structures between nTR and TR.

Microbial community structure by PLFA

We profiled the PLFA composition from the nTR and TR soil samples as shown in Table 1. In total 78 PLFAs were identified in the nTR and TR soils and the PLFA compounds ranged from C_9 to C_{20}. The number of PLFA detected and relative mean proportion were

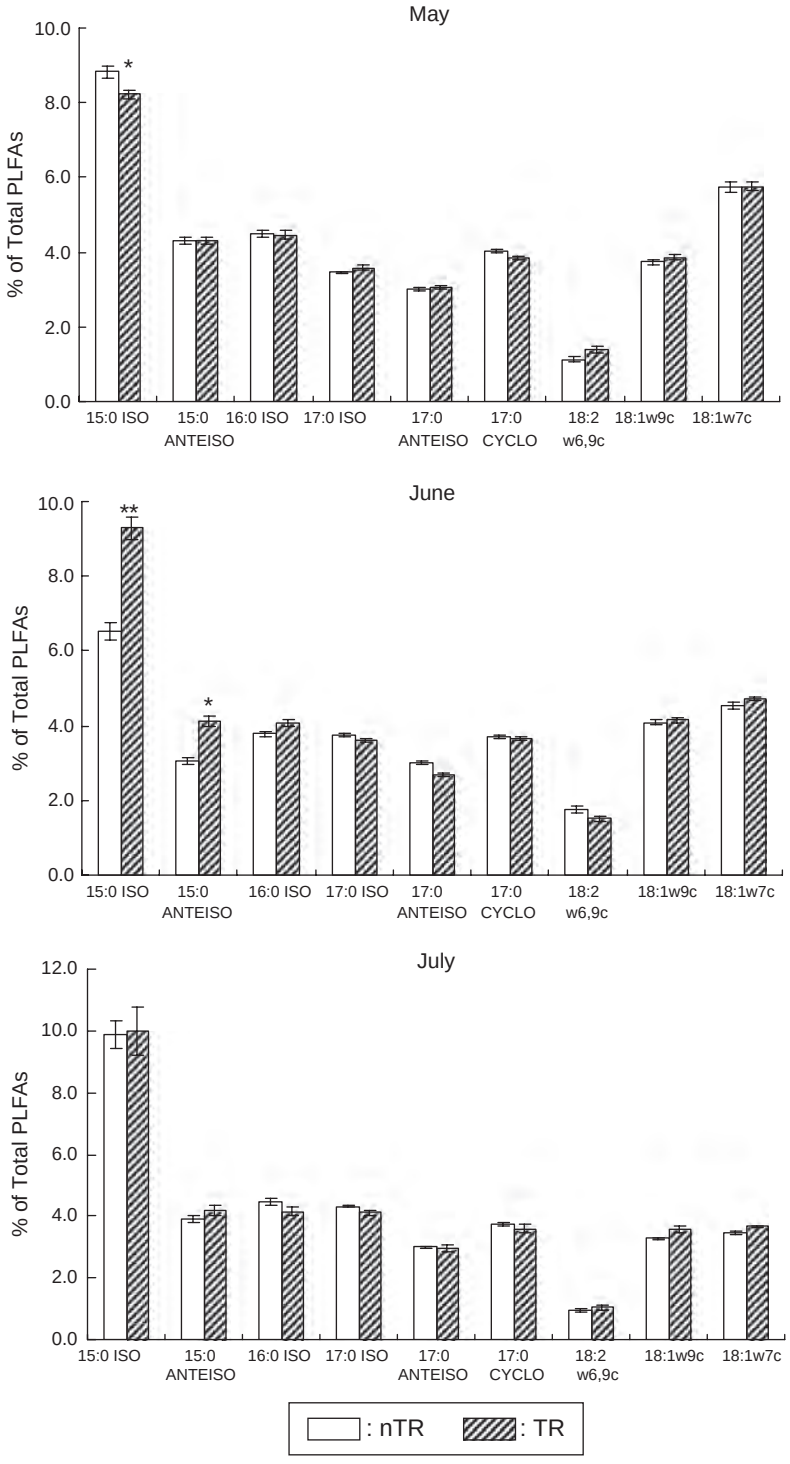

Figure 2. The percentile of total PLFA in soil for three sampling seasons (May, June, and July) with comparison of nTR and TR in 2005. (*$P < 0.05$, **$P < 0.001$)

varied seasonally. We identified 49.92 PLFAs for nTR and 50.90 PLFAs for TR in May, 46.00 PLFAs for nTR and 50.56 PLFAs for TR in June, and 45.67 PLFAs for nTR and 48.33 PLFAs for TR and we used the number of PLFA as the measure of species richness in Table 1. When we examined the evenness, the evenness in May was 0.83 between nTR and TR ($P > 0.05$), the evenness in June was 0.86 for nTR and 0.82 for TR ($P < 0.05$), and the evenness in July was 0.82 for nTR and 0.80 for TR ($P < 0.05$). The evenness was statistically

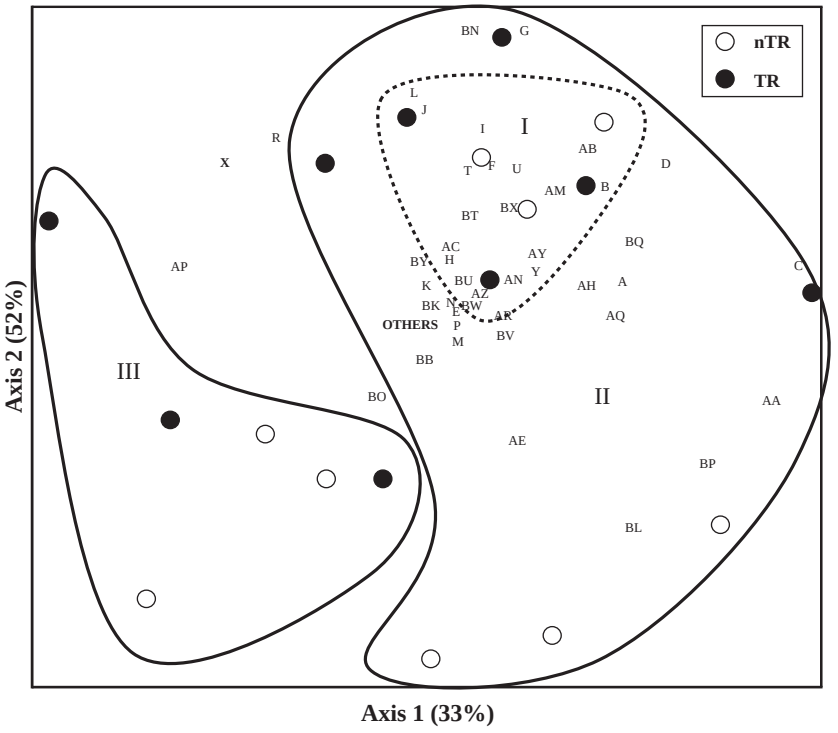

Figure 3. Non-metric multidimensional scaling ordination for evaluation of soil collected from both plant types, nTR and TR (78 PLFAs) during 12 May (I), 16 June (II), and 25 July (III) in 2005 (Final Instability; 0.0000, Final Stress; 13%, Iteration; 57). A, 9:00; B, 10:0 2OH; C, 10:0 3OH; D, 12:0 ISO; E, 12:00; F, 11:0 ISO 3OH; G, 11:0 3OH; H, 13:0 ISO; I, 13:0 ANTEISO; J, 13:00; K, 13:1AT 12–13; L, 12:0 2OH; M, 12:0 3OH; N, 14:0 ISO; P, 14:00; R, 15:1 ISO G; T, 15:1 ANTEISO A; U, 15:0 ISO; X, 15:1 w6c; Y, 15:00; AA, 14:0 ISO 3OH; AB, 16:1 w7c alcohol; AC, 16:1 ISO H; AE, 16:0 ISO; AH, 16:1 w11c; AM, 15:0 2OH; AN, 16:0 10 methyl; AP, 17:1 ANTEISO B/i I; AQ, ANTEISO 17:1 w9c; AR, 17:0 ISO; AY, 16:0 2OH; AZ, 17:0 10 methyl; BB, 18:0 ISO; BK, 19:1 ISO I; BL, 19:0 ISO; BN, 19:1 w6c/.846/19cy; BO, 19:0 CYCLO w10c/19w6; BP, 19:0 ANTEISO; BQ, 19:0 CYCLO w8c; BT, 18:0 2OH; BU, 19:0 10 methyl; BV, 20:4 w6,9,12,15c; BW, 18:0 3OH; BX, 20:0 ISO; BY, 20:1 w9c; OTHERS, 14:1 w5c, ISO 15:1 AT 5, 15:1 ISO I/13:0 3OH, 15:0 ANTEISO, 15:1 w8c, 16:1 ISO I/14:0 3OH, 16:0 ANTEISO, 16:1 w9c, 16:0 N alcohol, 16:1 w7c/15 iso 2OH, 16:1 w5c, 16:00, 15:0 ISO 3OH, 15:0 3OH, 17:0 ANTEISO, 17:1 w8c, 17:0 CYCLO, 17:00, 16:1 2OH, 16:0 ISO 3OH, 18:3 w6c (6,9,12), 18:2 w6,9c/18:0 ANTE, 18:1 w9c, 18:1 w7c, 18:00, 11 methyl 18:1 w7c, 17:0 ISO 3OH, TBSA 10Me18:0, 17:0 3OH, 19:1 w11c/19:1 w9c, 19:00, 18:1 2OH, 20:00.

significantly different in June and July but not different in May between the two types of plant. When we also compared the Shannon-Wiener diversity among the seasons, the diversity in June was highest, at 3.31 for nTR and 3.22 for TR, and relatively the diversity in July was lowest, at 3.14 for nTR and 3.11 for TR.

We examined the significance levels from all PLFAs for plant type, season, and their interaction (Table 2). The PLFA data were examined to determine whether there was any difference in the microbial community in soils from the two types of watermelons, nTR and TR. The PLFA data were analyzed by analysis of variance (ANOVA) separated by plant type ($P < 0.0085$), season ($P < 0.0154$), and the type × season interaction ($P < 0.1595$). Each PLFA was examined to determine any statistical difference with regard to the type of plants and season using ANOVA. We found that eight PLFAs (**14:1 w5c**, 14:00, 16:0 ANTEISO, **17:1 ANTEISO B/i l**, **18:3 w6c (6,9,12)**, 17:0 ISO 3OH, 19:1 ISO l, 18:1 2OH) for the plant type showed a statistical difference, 25 PLFAs (10:0 2OH, 12:00, **13:0 ISO**, 13:1 AT 12–13, 12:0 3OH, 14:0 ISO, **14:1 w5c**, 15:1 ANTEISO A, 16:1 ISO H, 16:0 ANTEISO, **16:1 w7c/15 iso 2OH**, 16:1 w5c, 17:1 ANTEISO B/i l, 16:1 2OH, **18:3 w6c (6,9,12)**, 18:2 w6,9c/18:0 ANTE, 18:1 w9c, **18:1 w7c**, 11 methyl 18:1 w7c, **17:0 ISO 3OH**, 17:0 3OH, **19:1 ISO l**, 18:1 2OH, 20:0 ISO, 20:1 w9c) for season showed a statistical difference, and 16 PLFAs (**10:0 2OH**, 12:00, 11:0 ISO 3OH, **13:0 ISO**, 12:0 OH, 14:0 ISO, **14:1 w5c**, 14:00, 15:0 ISO, 16:0 ANTEISO, 17:1 ANTEISO B/i l, **18:3 w6c (6,9,12)**, 17:0 ISO 3OH, 19:1 ISO l, 19:1 w6c/.846/19cy, 20:00) for the interaction between plant types and season showed a statistical difference ($P < 0.05$). Only six PLFAs (14:1

w5c, 16:0 ANTEISO, 17:1 ANTEISO B/i l, 18:3 w6c (6,9,12), 17:0 ISO 3OH, 19:1 ISO l) among the above statistically different PLFAs showed statistical difference for the plant types, season, and their interactions, together. (**Bold** PLFA means that P values are less than 0.001.)

The proportion of normal saturated PLFA was lowest in May and highest in July, but there was no statistical difference between the two types of plants in each season ($P > 0.05$, Table 3). Mid-chain branched saturated PLFA were similar in May and July but we found a statistical difference between nTR and TR in the soil samples from June ($P < 0.05$), the month between May and July, with nTR being higher than TR. The proportion of terminally branched saturated PLFAs, largely contributed by Gram(+) organisms, was not statistically different between the two types in May ($P > 0.05$) and July, but nTR PLFA (22.44) of June was lower than TR PLFA (25.73) of June ($P < 0.05$). TR PLFA (9.28) of i15:0 was higher than nTR PLFA (6.55) of i15:0. For other PLFAs, mono-unsaturated PLFAs, mainly from Gram(−) organisms and polyunsaturated PLFAs, mainly from fungi or plant, there was not much significantly different between plant types ($P > 0.05$) but sampling times did show a difference ($P < 0.05$).

We examined the PLFA results to see whether there are any changes by season or any effects of plant types. We found the a significant difference in season but there was no difference between the soil microbial communities from the plant types in each season in the proportion of Gram-positive PLFA to Gram-negative PLFA (Figure 1a) and in the ratio of iso to anteiso PLFA (Figure 1c). Both ratios showed increasing trends with time. The ratio of bacterial PLFA to total PLFA was similar in each season but nTR PLFA from June was relatively lower than at other times and the variance of PLFA ranged widely (Figure 1b). Figure 1d shows the ratio of Gram-positive to total PLFA, and the ratio for TR was a little lower than that for nTR in May and June but it was similar in July between nTR and TR.

Figure 2 shows percentile of total PLFA at each sampling time. We compared PLFA community composition for Gram-positive, Gram-negative, and fungi biomarkers between nTR and TR. The proportion of 15:0 ISO, a Gram-positive biomarker, was much higher than other PLFAs, but that of 18:2 w6,9c, a fungi biomarker, was lower than others at each sampling time. We also found that 15:0 ISO (Gram-positive), 17:0 CYCLO, and 18:2 w6,9c (fungi) biomarkers in May were the statistically significantly different ($P < 0.05$). 15:0 ISO ($P < 0.001$), 15:0 ANTEISO (Gram-positive, $P < 0.001$), and 17:0 ANTEISO ($P < 0.05$) in June were the statistically significantly different but there was no statistical difference in July between nTR and TR.

Multivariate analyses

Based on 78 PLFAs identified, non-parametric multi-dimensional scaling (NMS) was done as multivariate analysis. The NMS showed that the difference attributable to plant types, nTR and TR, was less than that for sampling times, May, June and July (Figure 3). Although there was a clear distinction for the sampling times, the difference for the plant types was not clearly distinguished. Axis 1 of the NMS explained 33% of the variation and axis 2 explained 52% of variation, and in total 85% of the variation is explained in Figure 3. To confirm whether there is any difference attributable to plant types or sampling times, we used MRPP analysis and it verified that there was no difference between the plant types ($T = -0.508$, $A = 0.017$, $P = 0.252$) but there was difference among the sampling times ($T = -6.536$, $A = -0.003$, $P = 0.0001$).

Discussion

Our study has illustrated the sensitivity of microbial communities associated with TR and nTR in soil ecosystems. Analysis of PLFA has already been applied in various study fields and the method is one of the most useful tools as a chemical method to determine the relative differences in soil microbial community structures (Ritchie et al. 2000; Wilkinson et al. 2002). According to the number of PLFA forms, we could measure the microbial diversity to compare the different soil communities (Rajendran et al. 1994). Variations in the microbial community structure have already been reported in the different study fields and most of the fatty acids identified have been proposed as bacterial signature fatty acids. Ritchie et al. (2000) suggested that PLFA methods are a better tool for the assessment of microbial communities than accurate and time-consuming techniques such as cloning and sequencing. Therefore, we determined the soil microbial community composition from the rhizosphere of watermelon root stock by using PLFA.

We identified 78 PLFAs from our results and statistically examined all PLFAs to compare the difference in microbial soil community structure among the plant types, seasons, and their interactions by using ANOVA. Generally, the number of PLFA forms, indicated as species richness, was higher in the TR than nTR soil and we found a statistical difference in the plant type ($P < 0.05$), the season ($P < 0.05$), and their interaction ($P < 0.001$) on the significance level with the species richness. For the significant difference with regard to plant type, it is the main reason for the

relatively big difference between nTR (46.00 ± 0.73) and TR (50.56 ± 0.82) for June soil samples. This denotes that the microbial activity is very high in June and the time is in the middle of the growing season for watermelons in Korea. Our results on community structures were influenced by seasonal variation, as indicated by significant changes in the fatty acid composition of the microbial community associated with the sampling time (Table 1). The soil microbial communities associated with the rhizosphere of the transgenic plants were significantly different depending on the time of sampling, but the differences during the sampling times were not consistent throughout the entire field season. Previous studies have also shown that there were differences in the composition of the rhizosphere microbial communities with the other varieties tested. The microbial communities examined throughout a field season have depended on the seasonal activity of microbes and it has often been indicated that there is seasonal variability in the microbial communities (Siciliano et al. 1998; Siciliano and Germida 1999; Griffiths et al. 2000; Heuer et al. 2002; Dunfield and Germida 2003). Generally, seasonal variation in microbial communities is common and complicating (di Cello et al. 1997; Dunfield and Germida 2003; Grayston et al. 2001; Griffiths et al. 2000; Heuer et al. 2002; Lottmann et al. 1999; Lottmann et al. 2000; Smalla et al. 2001).

In conclusion, the composition of microbial communities using the PLFA method was largely determined by seasonal changes. The PLFA profiling method provided a sensitive and meaningful measure of microbial community compositions in the soil ecosystem. It was also a good tool to compare the effect of transgenic plants on the soil conditions. The effects of transgenic plants on the soil ecosystem were examined throughout our experiments. Although NMS results also demonstrated the sensitivity with which the microbial communities respond to changes in local conditions, we could reject our hypothesis that there might be an adverse effect from TR with our statistical results. Our current results did not show any adverse effect on microbial community structures from the comparison between nTR and TR soils.

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References

Atlas RM, Bartha R. 1993. Microbial ecology: fundamentals and applications. 4th edition. Benjamin Cummings. 640 pp.

Badosa E, Moreno C, Montesinos E. 2004. Lack of detection of ampicillin resistance gene transfer from Bt176 transgenic corn to culturable bacteria under field conditions. FEMS Microb Ecol. 48:169–178.

Beals EW. 1984. Bray-Curtis ordination: an effective strategy for analysis of multivariate ecological data. Adv Ecol Res 14:1–55.

Biondini ME, Mielke PW Jr, Berry KJ. 1988. Data-dependent permutation techniques for the analysis of ecological data. Vegetatio 75:161–168.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 37:911–917.

Brimecombe MJ, De Leij FA, Lynch JM. 2001. The effect of root exudates on rhizosphere microbial populations. In: Willig S, Uaranini Z, Nanniperi P, editors. The rhizosphere: biochemistry and organic substances at the soil-plant interface. New York: Marcel-Dekker. p. 95–140.

Chaer GM, Fernandes MF, Myrold DD, Bottomley PJ. 2009. Shifts in microbial community composition and physiological profiles across a gradient of induced soil degradation. Soil Sci Soc Am J. 73:1327–1334.

Conner AJ, Glare TR, Nap JP. 2003. The release of genetically modified crops into the environment, Part II. Overview of ecological risk assessment. Plant J. 33:19–46.

Dale PJ, Clarke B, Fontes EMG. 2002. Potential for the environmental impact of transgenic crops. Nat Biotechnol 20:567–574.

Di Cello F, Bevivino A, Chiarini L, Fani R, Paffeti D, Tavacchioni S, Dalmastri C. 1997. Biodiversity of a *Burkholderia cepacia* population isolated from the maize rhizosphere at different plant growth stages. Appl Environ Microbiol. 63:4485–4493.

Díez B, Pedrós-Alió C, Marsh TL, Massana R. 2001. Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. Appl Environ Microbiol. 67:2942–2951.

Dunfield KE, Germida JJ. 2003. Seasonal changes in the rhizosphere microbial communities associated with field-grown genetically modified Canola (*Brassica napus*). Appl Environ Microbiolm. 69:7310–7318.

Fraley R. 1992. Sustaining the Food Supply. Bio/Technology. 10:40–43.

Grayston SJ, Griffith GS, Mawdsley JL, Campbell CD, Bardgett RD. 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biol Biochem. 33:533–551.

Griffiths BS, Geoghegan IE, Robertson WM. 2000. Testing genetically engineered potato, producing the lectins GNA and Con A, on non-target soil organisms and processes. J Appl Ecol. 37:159–170.

Guckert JB, Antworth CP, Nichols PD, Whithe DC. 1985. Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microb Ecol. 31:147–158.

Heuer J, Kroppenstedt RM, Lottmann J, Berg G, Smalla K. 2002. Effects of T4 lysozyme release from transgenic potato roots on bacterial rhizosphere communities are

negligible relative to natural factors. Appl Environ Microbiol. 68:1325–1335.
James C 2001. Global review of commercialized transgenic crops. ISSA Briefs 24.
Janus LR, Angeloni NL, McCormack J, Rier ST, Tuchman NC, Kelly JJ. 2005. Elevated atmospheric CO2 alters soil microbial communities associated with trembling aspen (*Populus tremuloides*) roots. Microb Ecol. 50:102–109.
Kelly JJ, Haggblom M, Tate RL. 1999. Changes in soil microbial communities over time resulting from one time application of zinc: a laboratory microcosm study. Soil Biol Biochem. 31:1455–1465.
Lee KY. 1996. Current occurrence and control of CGMMV'Konjak' disease. Plant Disease Agri. 2:38–39.
Lee KY, Lee BC, Park HC. 1990. Occurrence of cucumber green mottle mosaic virus disease of watermelon in Korea. Korean J Plant Path. 6:250–255.
Li XG, Liu B, Heia S, Liu DD, Han ZM, Zhou KX, Cui JJ, Luo JY, Zheng YP. 2009. The effect of root exudates from two transgenic insect-resistant cotton lines on the growth of *Fusarium oxysporum*. Transgenic Res. 18:757–767.
Lord NS, Kaplan CW, Shank P, Kitts CL, Elrod SL. 2002. Assessment of fungal diversity using terminal restriction fragment (TRF) pattern analysis: comparison of 18S and ITS ribosomal regions. FEMS Microb Ecol. 42:327–337.
Lottmann J, Heuer H, Smalla K, Berg G. 1999. Influence of transgenic T4 lysozyme-producing potato plants on potentially beneficial plant associated bacterial. FEMS Microbiol Ecol. 29:365–377.
Lottmann J, Heuer H, de Vries J, Mahn A, During K, Wackernagel W, Smalla K, Berg G. 2000. Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect of the bacterial community. FEMS Microbiol Ecol. 33:41–49.
Mather PM. 1976. Computational methods of multivariate analysis in physical geography. London: Wiley and Sons.
McCune B, Grace JB. 2002. Analysis of ecological communities. Oregon, USA: MjM Software Design, Gleneden Beach.
McKinley VL, Peacock AD, White DC. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biol Biochem. 37:1946–1958.
MIDI Inc. 2002. Microbial identification system: MIS operating manual, Version 4.5. Newark (DE).
Muruganandam S, Israel DW, Robarge WP. 2009. Nitrogen transformations and microbial communities in soil aggregates from three tillage systems. Soil Sci Soc Am J. 74:120–129.
Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G. 2003. Microbial diversity and soil functions. Europ J Soil Sci. 54:655–670.
Nap JP, Metz PLJ, Escaler M, Conner AJ. 2003. The release of genetically modified crops into the environment, Part I. Overview of current status and regulations. Plant J. 33:1–18.
Park S, Ku YK, Seo MJ, Kim DY, Yeon JE, Jeong S-C, Yoon WK, Kim HM. 2006. The characterization of bacterial community structure in the rhizosphere of watermelon (*Citrullus vulgaris* SCHARD.) using culture-based approaches and terminal fragment length polymorphism (T-RFLP). Agric Ecosyst Environ. Appl Soil Ecol. 33:79–86.
Park SM, Lee JS, Jegal S, Jeon BY, Jung M, Park YS, Han SL, Shin YS, Her NH, Lee JH, et al. 2005. Transgenic watermelon rootstock resistant to CGMMV (cucumber green mottle mosaic virus) infection. Plant Cell Rep. 24:350–356.
Rajendran N, Matsuda O, Urushigawa Y, Simidu U. 1994. Characterization of microbial community structure in the surface sediment of Osaka bay, Japan, by phospholipid fatty acid analysis. Appl Environ Microbiol. 60:248–257.
Rees GN, Baldwin DS, Watson GO, Perryman S, Nielsen DL. 2004. Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics. Antonie van Leeuwenhoek Inter J Gen Mol Microbiol. 86:339–347.
Ritchie NJ, Schutter ME, Dick RP, Myrold RD. 2000. Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterized microbial communities in soil. Appl Environ Microbiol. 66:1668–1675.
SAS Institute. 2001. PROC user's manual. 6th ed. Cary, North Carolina: SAS Institute.
Siciliano SD, Theoret CM, de Freitas JR, Hucl PJ, Germida JJ. 1998. Differences in the microbial communities associated with the roots of different cultivars of canola and wheat. Can J Microbiol. 44:844–851.
Siciliano SD, Germida JJ. 1999. Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest, compared to the non-transgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. FEMS Microbiol Ecol. 29:263–272.
Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G. 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol. 67:4742–4751.
Sokal RR, Rohlf FJ. 1994. Biometry. 3rd ed. New York, NY: W.H. Freeman.
Van Hannen EJ, Zwart GJM, van Agterveld MP, Gons HJ, Ebert J, Laanbroek HJ. 1999. Changes in bacterial and eukaryotic community structure after mass lysis of filamentous canobacteria associated with viruses. Appl Environ Microbiol. 65:795–801.
White DC, Davis WM, Nickels JS, King JD, Bobbie RJ. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia. 40:51–62.
Wilkinson S, Anderson J, Scardelis S, Tisiafouli M, Taylor A, Wolters V. 2002. PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biol Biochem. 34:189–200.
Yi HB, Park JE, Kwon MC, Park SK, Kim CG, Jeong SC, Yoon WK, Park SM, Han SL, Harn CH, Kim HM. 2006. Environmental risk assessment of watermelon grafted onto transgenic rootstock resistant to cucumber green mottle mosaic virus (CGMMV) on non-target insects in conventional agro-ecosystem. J Ecol Field Biol. 29:323–330.
Yi HB, Kim HJ, Kim CG, Harn CH, Kim HM, Park SK. 2009. Using T-RFLP to assess the impact on soil microbial communities by transgenic lines of watermelon rootstock resistant to cucumber green mottle mosaic virus (CGMMV). J Plant Biol. 52:577–584.
Youk ES, Pack IS, Kim Y-J, Yoon WK, Kim C-G, Ryu SB, Harn CH, Jeong S-C, Kim HM. 2009. A framework for molecular genetic assessment of a transgenic watermelon rootstock line. Plant Sci. 176:805–811.