0971-4693/94 US $ 5.00
Allelopathy Journal 27 (2): 175-184 (2011) *International Allelopathy Foundation* 2011
Tables: 4, Fig : -

Composition of root exudates from cucumber cultivars differing in resistance to Fusarium wilt

KAI PAN, FENGMING MA and FENGZHI WU*

Horticulture College
Northeast Agricultural University, Harbin, Heilongjing Province, 150030, China
E. Mail: mugonglin@yahoo.com.cn

(Received in revised form: January 12, 2011)

ABSTRACT

Seedlings of cucumber cultivars differing in their degree of resistance to *Fusarium oxysporum*. f. sp. *cucumerinum* J.H. Owen were inoculated with a fungal spore suspension (1 ×107/ml) or deionized water, thereafter the root exudates were collected. Amino acids in the root exudates were analyzed with an Automatic Amino Acid Analyzer and the other compounds in the exudates were detected by gas chromatography-mass spectrometry (GC-MS). The number of amino acid types and the overall proportion of amino acids in the resistant cultivars were higher than susceptible cultivars. Phenylalanine, valine, methionine, glycine and cystine were found in the root exudates of all cultivars in both treated and untreated seedlings. However, the total percentage of these five amino acids in root exudates from uninoculated seedlings was higher than inoculated seedlings. The total percentage of these amino acids in root exudates from susceptible cultivars was higher than resistant cultivars. Besides the soluble amino acids, 85 other compounds were detected by GC-MS in the root exudates. Regarding the uninoculated seedlings, the number and abundance of all compounds were higher in the root exudates of susceptible cultivars than in resistant cultivars. Furthermore, the number and abundance compounds were higher in the root exudates from inoculated seedlings than in uninoculated seedlings. A possible relationship between the content of organic acids (acetic acid and benzoic acid) in the root exudates and resistance to Fusarium wilt by the cucumber cultivar was indicated.

Keywords: Amino acids, cucumber cultivars, Fusarium oxysporum f.sp. cucumerinum, gas chromatography-mass spectrometry, inoculation, organic acids. root exudates

INTRODUCTION

Root exudates comprise of many substances released into the cultivation medium (such as soil or nutrient medium) through the different parts of root system during plant growth. Buxton showed that the differences in biochemical characteristics between resistant and susceptible cultivars could affect the crop's disease resistance, which may be reflected in root exudates and also showed that different crop cultivars secreted root exudates of different composition (1). Many subsequent studies have indicated that root exudates of different crops contain distinctly different types and concentrations of amino acids and organic acids (5,8,11,13,14,15).

*Correspondence Author: fzwu2006@yahoo.com.cn

Cucumber (*Cucumis sativus* L.) is a vegetable crop grown worldwide. Its Fusarium wilt (caused by *Fusarium oxysporum* f. sp. *cucumerinum* J.H. Owen) a soil disease occurring worldwide drastically reduces its crop yields (4). There is great variation in disease resistance in cucumber cultivars. Some recent studies have investigated the histopathology of cucumber cultivars differing in their resistance to Fusarium wilt, the genetic mechanism of resistance to Fusarium wilt and the relationship between resistance and other agricultural characters (2). However, there is little information about the pathogenic effects on the composition of root exudates from cucumber cultivars differing in their resistance to Fusarium wilt.

In this study, through amino acid and gas chromatography-mass spectrometry (GC-MS) analysis of the root exudates of inoculated and uninoculated cucumber seedlings, we investigated the differences in the composition of root exudates of cucumber cultivars differing in their degree of resistance to fusarium wilt and the pathogenic effects on the composition of root exudates. The research aimed to ascertain the possible mechanism by which some cucumber cultivars have increased resistance to Fusarium wilt and ultimately will lay the foundation for development and implementation of preventive measures and a curative treatment for the disease and enable biochemical screening of cucumber cultivars for resistance to fusarium wilt.

MATERIALS AND METHODS

Study material and culture media

Cucumber cultivars: The test cucumber cultivars were obtained from the Harbin Academy of Agricultural Sciences, Harbin, China and their respective disease index values are listed in Table 1.

Table 1. The cucumber cultivars used in the study and their degree of resistance to *Fusarium oxysporium* f. sp. *cucumerinum*.

Cucumber cultivar	Disease index	Disease resistance
Jinyou 3#	20.34	R
Zhongnong 13	25.33	R
Qiupeng	68.25	S
Jinyan 4#	72.67	S

R: Resistant, S: Susceptible

***Fusarium oxysporum* f. sp. *cucumerinum*:** *Fusarium oxysporum* f. sp. *cucumerinum* was isolated from the cucumber plants infected with fusarium wilt grown in our Experimental Station. It was identified as ‘race 4’ by Plant Protection Department of our University.

Abio-nutrient solution: The nutrient solution had the following composition: calcium nitrate 472 mg/L, potassium nitrate 404 mg/L, potassium dihydrogenphosphate 100 mg/L, magnesium sulphate 264mg/L, Na_2Fe-EDTA 30 mg/L, boric acid 2.86 mg/L, manganese sulphate 2.13 mg/L, zinc sulphate 2.22 mg/L, copper sulphate 0.08 mg/L and ammonium molybdate 0.02 mg/L.

Plant cultivation medium: Plants were grown in sterilized vermiculite (bulk density 0.23 g/cm^3, pH 6.7, electrical conductivity 0.8 ms/cm.

Experimental procedures

Spore suspension preparation: *Fusarium oxysporum* f. sp. *cucumerinum* was grown in potato, dextrose and agar (PDA) culture medium as per method of Ju Huiyan (11). All Petri dishes (8.5 cm dia) were cleaned, dried and autoclaved for 30 min and then dried before use. The PDA medium was autoclaved for 30 min, then 25 mL of medium was added to each Petri dish. After incubation, mycelia of *F. oxysporum* were inoculated on the medium surface in each Petri dish and incubated for 6 days at 25°C. The fungal colonies were scratched and a spore suspension containing 1×10^7 spores/mL was prepared (5).

Cucumber seedlings preparation: Seeds of cucumber cultivars were washed for several minutes with sterilized water, after which they were first soaked in water at 55°C for 15 min and then in water at 30°C for 12 h. After rinsing several times with sterilized water, the seeds were germinated in the dark at 30°C for two days. When radicles were 0.5 cm long, the seeds were sown in 50-hole agricultural seedling trays containing sterilized vermiculite. For each cultivar 24 trays of seeds were sown. The seedlings were grown at 28°C day/15°C night in a greenhouse.

Nutrients solution (either 1/4, 1/3, 1/2 or full strength) was applied once daily to the vermiculite during the experimental period. Deionized water was added daily to replace the water loss by evapotranspiration. Seedlings were inoculated by injecting 10 mL spore suspension into the root rhizosphere, when the seedlings had produced three leaves (at 21 days after germination). Deionized water was injected into the rhizosphere of the control.

Collection of root exudates: The collection of root exudates was based on the method of Wu *et al.* (15). When the fourth leaves of all seedlings had developed, 6 d after inoculation, the seedlings were removed from vermiculite, washed sequentially with water four times, with distilled water four times and finally once with deionized water. The 500 seedlings were placed into cultivation channel (150 cm × 100 cm × 12 cm) and cultured in 80L water for 2 days. Then about 22-24 L culture solution remained in each cultivation channel. The culture solutions were collected, roughly filtered through a Buchner's filter twice and then filtered through 0.45 µm film. The filtrate was collected and stored below 10°C prior to use.

Root exudate analysis

Determination of dissociative amino acids: The concentrated root exudates were analyzed with an Automatic Amino Acid Analyzer (L-8800, Hitachi, Japan).

GC-MS analysis: Root exudate solution (1250 mL) was concentrated to 0.8 mL/plant by lyophilization, then filtered through 0.22 µm film and stored at 5°C before use. Two microliters of the concentrated root-exudate solutions were extracted in 20 mL butanol, shaked on an orbital shaker for 5 min and left for 10 min. The extraction solutions were dried with anhydrous calcium chloride, filtered through 0.45 µm film and analyzed by GC-MS (model GC6890-MS5973, Agilent Technologies, USA). *Detected conditions* followed the method of Han (6). Gas chromatography was performed with a HP-5MS capillary column (cross-linked 5% phenyl–methyl-siloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness). The carrier gas was helium at a flux of 1 ml/min. The temperature gradient was 40°C, retained for 2 min, raised to 250°C at 6°C/min, retained for 15 min. The injected sample volume was 1 µl. The mass spectrum conditions were: electron bombardment ion source, bombardment voltage 70 eV, electron source temperature 230°C, scan range 30–600 amu and scan speed 0.2 s in the whole course.

Compounds detected in the root exudates were identified by searching in the NIST98 mass spectral database (National Institute of Standards and Technology, USA).

Data analysis: Data were analyzed using Microsoft Office Excel 2001 and SAS 6.0.

RESULTS

Analysis of amino acids in root exudates

The amino acids detected in the different root exudates are listed in Table 2. Eleven amino acids were identified. Arginine was not detected in any exudate, whereas phenylalanine, valine, methionine, cystine and glycine were detected in all exudates. Differences between the treatments in the amino acid spectrum were evident. Many amino acid types were detected in the root exudates from the susceptible cultivars than from resistant cultivars. The number of amino acids detected were less in the inoculated seedlings.

Table 2. Amino acids detected in root exudates of inoculated and uninoculated seedlings of cucumber cultivars susceptible or resistant to *Fusarium oxysporium* f. sp. *cucumerinum*. √ indicates that the particular amino acid was detected. See Table 2 for explanation of the treatment codes

Amino acids	Susceptible cultivars				Resistant cultivars			
	Uninoculated		Inoculated		Uninoculated		Inoculated	
	Qiupeng	Jinyan 4#	Qiupeng	Jinyan 4#	Zhongnong 13	Jinyou 3#	Zhongnong 13	Jinyou 3#
Cystine	√	√	√	√	√	√	√	√
Asparine		√	√					
Threonine	√	√	√	√	√		√	√
Serine		√	√			√		√
Glycine	√	√	√	√	√	√	√	√
Alanine	√	√	√	√	√	√		√
Valine	√	√	√	√	√	√	√	√
Methionine	√	√	√	√	√	√	√	√
Phenylalanine	√	√	√	√	√	√	√	√
Lysine		√						
Histidine	√	√	√			√		

The proportion of the total amino acids for each of the five amino acids (phenylalanine, valine, methionine, cystine and glycine) detected in all treatments are listed in Table 3. In uninoculated seedlings, the total proportion of these five amino acids in susceptible cultivars (24.6%) was higher than resistant cultivars (22.1%) (Table 3). The total proportion of these five amino acids in inoculated seedlings (21.05%) was lower than uninoculated seedlings (23.36%). Except for Jinyou 3#, the proportions of each of these five amino acids in the other three cultivars showed a reduction after inoculation (i.e. Zhongnong 3#, Qiupeng, JIinyan 4#). Except for glycine, which showed a reduction in proportion, all other four amino acids increased in their proportion of the total amino acids in Jinyou 3#.

Table 3. Percentage of the total amino acids per treatment for the five amino acids detected in the root exudates of all treatments.

Cucumber culture	Proportion of the total amino acids detected (%)					
	Cystine	Glycine	Valine	Phenylalanine	Methionine	∑
Uninoculated						
Qiupeng	4.61	0.52	14.19	0.32	1.62	21.26
Jinyan 4#	5.66	1.83	17.77	0.39	2.29	27.94
Zhongnong 13#	5.54	0.48	16.32	0.56	2.15	25.05
Jinyou 3#	3.93	1.47	12.02	0.30	1.47	19.19
Inoculated						
Qiupeng	3.01	0.50	9.38	0.26	1.06	14.21
Jinyan 4#	4.59	0.16	14.79	0.33	1.78	21.56
Zhongnong 13#	3.71	0.32	11.89	0.45	1.37	17.74
Jinyou 3#	6.61	0.85	20.32	0.44	2.50	30.72

GC-MS analysis of other compounds in cucumber root exudates

Types and abundance of other compounds in cucumber root exudates

The results of two detection conditions were essentially consistent (Table 4). The number of compounds and abundance of compounds detected from root the exudates of uninoculated seedlings were greater than inoculated seedlings. Their reduced abundance in root exudates from inoculated seedlings was more pronounced in the susceptible cucumber cultivars than in resistant cultivars. Moreover in uninoculated seedlings, the number and abundance of compounds in root exudates from susceptible cucumber cultivars were higher than resistant cultivars.

Identification of compounds in cucumber root exudates

In total, 85 compounds were detected in the cucumber root exudates, including alcohols, alkyls, esters, acids, oximes, benzenes, ketones, furans, alkenes and pyrrole. Of these, 17 compounds were detected in all treatments (Table4). Nine compounds or those containing similar functional groups were identified in the eight treatments: *p*-xylene, *o*-xylene, benzene, 1,3-dimethyl, ethylbenzen, 4-heptanone, 3-methyl, 4-heptanol, 3-methyl, butanoic acid, butyl ester, 1,3-cyclopentadiene, 5-(1-methyl ethylidene), 1-hexanol, 2-ethyl. Further inspection showed that 26 compounds were only present in root exudates of resistant cultivars and inoculated seedlings. These compounds comprised of ketones, alkyls, acids, oximes, furans, alkenes, amines, esters and pyrrole. Of these, 7-organic acids (Malic acid, benzoic acid, acetic acid, maleic acid, butyric acid, acetic acid and quinoline carboxylic acid), accounted for the largest proportion (Table 4).

Table 4. Peak area (%) of compounds extracted with n-butanol from the root exudates of inoculated and uninoculated seedlings of cucumber cultivars with differing resistance to *Fusarium oxysporium* f. sp. *Cucumerinum*. See Table 2 for explanation of the treatment codes

Number	Compound`	Uninoculated				Inoculated			
		Resistant cultivars		Susceptible cultivars		Resistant cultivars		Susceptible cultivars	
		Zhongnong 13#	Jinyou 3#	Jinyan 4#	Qiupeng	Zhongnong 13#	inyou 3#	inyan 4#	Qiupeng
1	*p*-Xylene	4.28	3.99	4.17	3.91	4.23	17.79	4.43	2.01
2	*o*-Xylene	4.28	3.99	4.17	16.41	4.23	6.01	4.43	18.09
3	Benzene, 1,3-dimethyl	17.51	3.99	4.17	16.41	17.32	6.01	18.41	4.41
4	Ethylbenzen	17.51	16.56	1.00	1.05	5.83	2.55	1.99	2.01
5	4-Heptanone, 3-methyl	17.51	3.99	0.93	16.41	17.32	6.01	1.10	2.01
6	4-Heptanol, 3-methyl	0.91	16.56	1.00	16.41	2.47	2.55	1.99	2.01
7	Butanoic acid, butyl ester	0.91	0.79	1.00	1.05	17.32	6.01	1.99	2.01
8	1,3-Cyclopentadiene, 5-(1-methyl ethylidene)	17.51	16.56	1.00	1.05	5.83	6.01	1.99	2.01
9	1-Hexanol, 2-ethyl	0.91	16.56	1.00	1.05	2.47	6.01	1.99	2.01
10	Oxime-, methoxy-phenyl	0.91	16.56	0.93	1.05	5.83	6.01	1.99	2.01
11	Propanoic acid, 2-methyl-, 2-methyl propyl ester	2.43	9.98	5.56	0.81	10.39	1.08	10.85	6.08
12	Butanoic acid, 2-methylpropyl ester	1.28	9.98	9.97	0.24	1.05	1.08	35.71	10.83
13	Decane, 3,3,8-trimethyl	2.43	9.98	9.97	0.24	10.39	1.08	10.85	10.83
14	1-Penten-3-ol, 2-methyl	1.28	1.81	2.48	0.81	1.05	10.62	6.18	2.63
15	3-Hexanol, 2-methyl	1.28	1.81	32.30	9.97	1.05	0.93	10.85	0.69
16	2-Propyl-1-pentanol	1.28	9.32	3.12	9.97	0.76	7.33	10.85	10.83
17	3-Pentanol, 2,4-dimethyl	1.28	32.77	1.39	1.78	0.76	0.77	1.10	10.83
18	Pantolactone	nd	nd	9.97	nd	nd	nd	6.18	nd
19	2-Methoxycyclohexanone	nd	nd	32.30	nd	nd	nd	35.71	nd
20	2,5-Dimethylcyclohexanol	nd	nd	3.12	nd	nd	nd	1.10	nd
21	1-Bromo-2,2-dimethoxypropane	nd	1.27	nd	3.85	nd	0.77		0.61
22	1,1-Di(isobutyl)acetone	1.28	9.98	2.48	9.97	nd	nd	1.10	6.08
23	2,2,4-Trimethyl-3-pentanol	33.81	32.77	32.30	0.95	nd	nd	35.71	35.83
24	Acetaldehyde, butyl hydrazone	1.28	1.36	9.97	9.97	nd	nd	0.76	10.83
25	Piperazine, 1,4-dimethyl-	0.91	0.97	1.80	1.05	nd	nd	1.99	10.83
26	2-Furanol, tetrahydro-2-methyl	33.81	1.77	nd	nd	nd	nd	nd	nd

Contd.

Table 4. Contd.

27	dl-Malic disodium salt	0.89	0.97	nd	nd	1.05	1.08	1.95	1.12
28	1,2,4-Benzene tricarboxylic acid, 1,2-dimethyl ester	nd	nd	nd	nd	0.76	nd	nd	nd
29	Dipropyl oxy di acetate	nd	nd	nd	nd	34.32	nd	nd	nd
30	2-Ethylhexyl hydrogen maleate	nd	nd	nd	nd	0.76	nd	nd	nd
31	7-Chlorocinchoninic acid	nd	nd	nd	nd	nd	35.00	nd	nd
32	Vinyl butyrate	nd	nd	nd	nd	nd	nd	nd	0.61
33	Acetic acid, 2,2'-oxybis-, dibutyl ester	nd	9.98	nd	nd	nd	nd	nd	nd
34	Furan, tetrahydro-2-methyl-	nd	nd	nd	nd	nd	nd	0.63	35.83
35	2,2'-Bifuran, octahydro-	nd	9.32	nd	nd	nd	nd	nd	nd
36	*N*-Hydroxy methyl acetamide	nd	nd	nd	nd	nd	1.08	nd	nd
37	2-(*p*-Tolyl)ethylamine	nd	nd	nd	nd	nd	10.62	10.85	2.63
38	1*H*-Pyrrole-2,5-dione, 1-(4-chlorophenyl)	nd	nd	nd	nd	nd	35.00	nd	nd
39	2-Ethyl acridine	nd	nd	nd	nd	nd	nd	1.99	nd
40	2-Methyl valeroyl chloride	nd	nd	nd	nd	nd	nd	0.63	nd
41	Heptane, 3-ethyl-	nd	nd	nd	nd	nd	nd	35.71	nd
42	Germacyclopent-3-ene, 1,1,3,4-tetramethyl-	nd	nd	nd	nd	7.44	nd	nd	nd
43	Ethanone, 1,1',1''-(1,3,5-benzene triyl)tris-	nd	nd	nd	nd	nd	nd	nd	6.50
44	Tripropyl orthoformate	nd	nd	nd	nd	nd	nd	nd	1.49
45	1,5-Heptadiene-3,4-diol	nd	nd	nd	nd	nd	nd	35.71	n
46	4-Hexen-3-ol	nd	nd	nd	nd	nd	nd	1.10	10.83
47	2-Decene, 5-methyl-, (Z)	33.81	nd	nd	nd	nd	nd	nd	nd
48	Oxirane, 3-hydroxy propyl	0.89	nd	nd	nd	nd	nd	nd	nd
49	1-Hepten-3-ol, 3-methyl	nd	1.27	nd	nd	nd	nd	nd	nd
50	2'-Hydroxy decanophenone oxime	nd	9.98	nd	nd	1.05	7.33	nd	10.83
51	1-Propanol, 2-methyl-2-nitro-	1.83	1.27	nd	nd	nd	35.00	nd	nd
52	Malic Acid	1.18	0.97	nd	9.97	6.21	1.08	1.95	nd
53	Benzoic acid, 2-amino-4-methyl	1.18	0.97	1.00	nd	10.39	nd	0.76	nd
54	Butane, 2,2-dimethyl-	nd	nd	nd	9.97	nd	nd	0.63	10.83
55	Chloroacetic acid, 2-ethylhexyl ester	0.89	nd	3.12	nd	1.87	7.33	0.63	6.50
56	Ethyl indole-4-carboxylate	nd	0.97	32.30	nd	nd	35.00	nd	1.96
57	Heptane, 1,1'-oxybis-	0.89	1.36	4.03	nd	34.32	nd	35.71	1.49
58	Octane, 2,3-dimethyl-	1.18	9.32	nd	0.44	nd	1.08	0.15	1.49

Contd.

Table 4. Contd.

59	4-Ethylbenzoic acid, cyclohexyl ester	1.18	9.32	nd	1.38	7.44	nd	0.15	6.50
60	2-Furanmethanol, tetrahydro-	1.28	1.27	9.97	nd	nd	nd	0.63	10.83
61	Benzene ethanol, .alpha.,.beta.-dimethyl	1.28	9.98	nd	0.81	1.48	10.62	1.99	2.01
62	2(3*H*)-Furanone, dihydro- 3-hydroxy-4, 4-dimethyl	nd	nd	9.97	nd	nd	nd	1.99	1.12
63	2-Ethyl-1-dodecanol	nd	nd	9.97	nd	nd	nd	nd	nd
64	1-Pentanol, 2-ethyl-4-methyl-	nd	1.27	nd	0.95	nd	nd	nd	nd
65	2,4-Dimethyl-1-penten-3-ol	0.89	nd	nd	0.95	nd	nd	nd	nd
67	3-Heptanone, 2,4-dimethyl	1.18	nd	nd	32.54	nd	nd	nd	nd
68	Ethane-1,1-diol dibutanoate	nd	9.32	1.39	nd	nd	nd	nd	nd
69	1-Decene, 2,4-dimethyl-	0.89	nd	9.97	0.95	0.76	7.33	1.10	0.61
70	4-Heptanone	0.91	0.97	0.93	16.41	nd	nd	1.10	nd
71	1-Methyl-2-phenylpiperidin-4-one	nd	nd	nd	0.95	34.32	35.00	nd	nd
72	3-Methoxy-1-pentene	nd	nd	32.30	9.97	34.32	nd	nd	nd
73	1-Benzylamino-3-benzyloxy propane	1.83	32.77	3.12	nd	34.32	nd	nd	nd
74	2,2,3-Tri ethyl oxirane	1.41	nd	1.00	nd	nd	10.62	6.18	35.83
75	2-Hexen-1-ol, 3-methyl-, (E)	0.91	nd	nd	5.53	nd	nd	nd	10.83
76	3-Pentanone, 2,4-dimethyl-	0.91	nd	nd	0.24	nd	nd	2.62	10.83
77	5-Hydroxy-4-octanone	nd	nd	nd	9.97	nd	nd	1.10	nd
78	Cis-3-methylpent-3-ene-5-ol	nd	nd	9.97	9.97	1.88	nd	nd	1.12
79	Cyclopropane, 1-(2-methylbutyl)-1-(1-methylpropyl)-	33.81	nd	nd	3.85	0.76	7.33	nd	6.50
80	Pentane, 3,3-dimethyl-	nd	1.27	1.80	0.95	7.44	1.08	1.95	1.12
81	Thiazole, 2-ethyl-4-phenyl-	nd	1.36	32.30	0.62	34.32	35.00	nd	1.12
82	2-Phenyl-hex-5-en-3-ol	1.02	1.81	2.77	5.53	10.39	10.62	2.62	nd
83	2-Amino-6-methylbenzoic acid	0.14	0.97	1.00	5.53	10.39	10.62	2.62	nd
84	Thiophene-2-carboxaldehyde, 5-(2-pyridyl)-	nd	32.77	nd	0.95	nd	nd	nd	2.25
85	Undecane, 3,3-dimethyl-	1.28	nd	nd	0.24	nd	nd	nd	0.69
Total no. of compounds detected		19	20	30	26	19	19	19	20
Total peak area		4.295E+08	4.448E+08	4.986+08	4.689E+08	4.268E+08	4.192E+08	4.304E+08	4.148E+08

In uninoculated seedlings, 11 organic acids were present in the root exudates of resistant cultivars, whereas only 5-compounds were present in susceptible cultivars (Table 4). Following inoculation, organic acids (acetic acid, benzoic acid and maleic acid) were increased in the root exudates of resistant cultivars, whereas ketones, alcohols and alkyls were reduced. After inoculation of susceptible cultivars, organic acids (acetic acid, benzoic acid, butyric acid and phosphate acid) increased in the root exudates and new ketone, alcohol, alkyl compounds were detected (Table 4).

DISCUSSION

The main ecological effect of amino acids in root exudates is their nutritional effect in the rhizosphere and root exudates from disease-susceptible cultivars tend to contains more number of amino acids and in higher concentrations. Liu *et al.* (9) analyzed amino acids present in the root exudates of cotton cultivars differing in their resistance to wilt and found that root exudates of susceptible cultivars contained more amino acids than resistant cultivars. The results of Yuan (17) and our present study are consistent with the findings of Liu *et al.* (9). Pan and Wu (10) suggested that arginine only occurred in root exudates of cucumber cultivars with intermediate resistance to fusarium wilt and found that five amino acids (cystine, glycine, valine, phenylalanine, methionine) and disease resistance were negatively correlated (10). In this study we found that after inoculation of Jinyou 3#, the changes in the 4 amino acids (cystine, valine, phenylalanine and methionine) were different than in other cultivars (Zhongnong 3#, Qiupeng and JIinyan 4#), which indicats that there may be different resistance mechanisms in various cucumber cultivars. This hypothesis should be studied further.

These results showed that there were differences in the types and abundance of compounds in root exudates of cucumber cultivars, which is consistent with the findings of previous studies (1,13,17). The change in composition of cucumber root exudates following inoculation is similar to the results of Han (7). Elroy and Truelove (3) considered that after infection of plant by pathogen, carbohydrates, amino acids, proteins, lipids and nucleic acid metabolism changed and the root exudation accelerated. However, in the present study, the abundance of compounds detected in root exudates from uninfected seedlings was higher than inoculated seedlings. The reason for this might be the short time period after inoculation over which the root exudates were collected. The seedlings were probably still at an early stage of disease development. A longer experimental period might result in different conclusions.

We found a possible relationship between the organic acids (especially acetic acid and benzoic acid) in root exudates and resistance to Fusarium wilt. These results are similar to previous studies. Some plants excrete citric acid, guavas acid, malic acid, benzoic acid and cinnamic acids. Of these compounds, benzoic acid, cinnamic acid and some hydroxybenzene compounds could have allelopathic effects in the rhizosphere (12,19). However, in the present study, cinnamic acid and *p*-hydroxybenzoic acid, which are reported to be allelochemicals, were not detected in cucumber root exudates. This finding might be a result of the use of different cultivars and treatment methods. The reason for the absence of these compounds should be studied further. In addition, we found that there was a consistent change in the contents of ketones, alcohols, alkyls in the root exudates. The biological characteristics of these organic substances and their relationship with disease resistance should also be further investigated.

ACKNOWLEDGEMENTS

This research was funded by the Doctor Foundation of Northeast Agricultural University, the National Natural Science Foundation of China (30771252), Outstanding Youth Foundtion of Heilongjing Province(JC200803) and the National Industrial Technology System of Staple Vegetables.

REFERENCES

1. Buxton, E.W. (1962). Root exudates from banana and their relationship to strains of the Fusarium causing Panama wilt. *Annals Applied Biology* **50**: 269-282.
2. Chen, M., Wang, G.P. and Wu, D.H. (2003). Histopathological differences between cucumber cultivars with different resistances to Fuasrium Wil. *Journal of South China Agricultural University* **24**: 110-112. (In Chinese).
3. Elroy, A. C. and Truelove, B. (1986). *The Rhizosphere*. Pp. 510-523. Springer-Verlag, New York.
4. Feng, D.T. and Li, B.D. (1994). The development of resistance breeding of cucumber and the *Fusarium oxysporum* f.sp. *Cucumerinum*. *China Vegetables* : 56-58. (In Chinese).
5. Han, X., Wu, F.Z. and Pan, K. (2006). Effect of root exudates from cucumber cultivars on pathogens of fusarium wilt. *China vegetables* **5**: 13-15. (In Chinese)
6. Han, L.M., Shen, Q.R. and Wang, S.Q. (2002). Identification on decomposing products of soybean stubs by *Trichoderma r koningii* and their allelopathy. *Chinese Journal of Applied Ecology* **13** : 1295-1299. (In Chinese).
7. Han, L.M., Ju, H.M. and Yang, Z.M. (2005) Allelopathy of root exudates from two genotypes soybeans on root rot pathogenic fungi. *Chinese Journal of Applied Ecology* **16**□137-141. (In Chinese).
8. Kraffizyk, I. (1984). Soluble root exudates of maize: Influence of potassium supply and rhizosphere microoganisms. *Soil Biology and Biochemistry* **16** : 315 - 322.
9. Liu, S.P., Wang, R.X. and Zang, R. (1998). Effects of sugar and amino acid in root exudation of different resistant cotton cultivars on cotton fusarium wilt pathogen. *Journal of Northwest A & F University*(*Natural Science*) **26**: 30-35. (In Chinese).
10. Pan, K. and Wu, F.Z. (2007). Correlation analysis of amino acids components in cucumber root exudates and fusarium wilt resistance. *Acta Ecologica Sinica* **27**: 1946-1950. (In Chinese).
11. Perez, F.J. and Ormeno-Nunez, J. (1991). Root exudates of wild oats: Allelopathic effect on spring wheat. *Phytochemistry* **30** : 2199 - 2201.
12. Shi, R.G. (2004). Ecological effects of plant root exudates. *Chinese Journal of Ecology* **23**: 97-101.(In Chinese)
13. Wang, F. (2003). *The Mechanism of Eggplant (Solanum melongena L.) Replanting Problem.* Dissertation Ph. D. degree. China Agricultural University (Beijing) Pp. 46-51. (In Chinese).
14. Wu, F.Z., Meng, L.J. and Wen, J.Z. (2002). Effect of cucumber root exudates on mycelial growth of Fusarium wilt. *China vegetables* **5**: 26-27. (In Chinese).
15. Wu, F.Z., Xue, H. and Wang, X.Z. (2006). Allelopathic effect of root exudates of cucumber cultivars on fusarium oxysporum. *Allelopathy Journal* **18**: 163-172.
16. Yuan, H.X., Li, H.L., Wang, Y., Fang, W.P. and Wang, Z.Y. (2002). Analysis of root exudates from different resistance cultivars of cotton and effect Verticillium dahliae in cotton. *Acta Phytopathologica Sinica* **32**: 127-131. (In Chinese).
17. Yuan, H.X., Li, H.L. and Wang, Y. (2002). The root exudates of cotton cultivars with the different resisitance and their effects on verticillium dahliae. *Acta Phytopathologica Sinica* **32**: 127-131.
18. Zhao, J.X., Gu, X.H. and Liu, Y. (2006). Study on the volatile flavor compounds of the traditional Chinese soy bean paste. *Food Science* **27**: 684-687. (In Chinese).
19. Zhu, L.X., Zhang, J.E. and Liu, W.G.. (2003). Review of studies on interactions between root exudates and rhizopheric microorganisms. *Ecology and Environment* **12**: 102-105. (In Chinese).