Front. Agric. China 2008, 2(1): 61–65
DOI 10.1007/s11703-008-0005-7

RESEARCH ARTICLE

Benli HUANG, Yundong XU, Ye WU, Shunqi ZHANG, Xuehao CHEN

Effect of root exudates of different resistant varieties of cucumber on fusarium wilt and preliminary studies on their resistance mechanism

Abstract This study investigated the effect of root exudates of cucumber varieties, Jinyan 4 (susceptible variety), Jinchun 4 (resistant variety) and Yinnan Black seed squash on fusarium wilt. The results showed that fusarium wilt occurrence of plants treated with the root exudate of Jinyan 4 was earlier. The infection rate was significantly higher 15 days after inoculation, but similar to the control 20 days after inoculation. In contrast, the infection rate of plants treated with the root exudate of Jinchun 4 was significantly lower than that of the control. The plant height and fresh weight of Jinyan 4 treated with its own root exudate were lower than those of the control, and the root vigor decreased but the conductance increased. There was no significant effect of the root exudates from Jinchun 4 and Black seed squash on plant height and fresh mass of Jinyan 4. We found that the root exudate of susceptible cucumber variety stimulated the growth of *Fusarium oxysporum* pathogen, in contrast, that of resistance variety and Black seed squash suppressed the growth.

Keywords cucumber varieties, root exudate, fusarium wilt, mechanism

1 Introduction

Soil-borne diseases seem to be more serious because of specialized production of vegetables and continuously cropping (Huang et al., 2004a; Huang et al., 2004b). It is commonly considered that the aggravation of soil-borne diseases is due to the accumulation of pathogens in soil. Recently, plant allelopathy has been attracted attention. It has been reported that there is a significant effect of root exudate, plant residue extract and decomposed liquid on plant growth (Yu and Matsui, 1994, 1997; Zhou et al., 1997; Wang et al., 2001; Zhen et al., 2004). Some researchers found that root exudate could stimulate the growth of soil-borne pathogens (Jia et al., 1997; Ju et al., 2002; Wu et al., 2002). The results showed that root exudate from resistant and susceptible cotton varieties to verticillium wilt showed an opposite effect on the growth of *Verticillium dahliae* (Yuan et al., 2002). The soil after successive plantation of resistant varieties for many years in cotton fields where serious *Fusarium oxysporum* occurs has antimicrobial against the pathogen (Ma et al., 1992). We considered that the impacting factors of soil-borne disease occurrence were complex in soil ecosystem.

Cucumber fusarium wilt is a typical disease caused by continuously cropping. Periodical progress from light disease to serious occurrence usually needs five to six years for the open field and less than five years for the protected land. Although common methods are difficult to be used to control the disease, some researchers have obtained some good controlling efficacy by grafting the method with Yunnan Black seed squash as stock (Chen and Xu, 2001). Therefore, this study examined the effect of root exudates of different resistant cucumber varieties and Yunnan Black seed squash variety on the occurrence of fusarium wilt in order to provide a theoretical basis for the rational utilization of resistant and susceptible variety with high yield in agricultural production, as well as to explore the possibility of controlling or abating the continuous cropping diseases by root exudates of resistant varieties.

2 Materials and methods

2.1 Materials

Pathogen materials were *Fusarium oxysporum* Schlecht. Plant materials were Jinchun 4 (resistant variety) and Jinyan 4 (susceptible variety) (Lü et al., 1994), provided by Agricultural College of Yangzhou University. Black seed squash

Translated from *Chinese Journal of Applied Ecology*, 2007, 18(3): 559–563 [译自: 应用生态学报]

Benli HUANG (✉), Yundong XU, Ye WU, Shunqi ZHANG, Xuehao CHEN
Agricultural College of Yangzhou University, Yangzhou 225009, China
E-mail: yzhbl2003@yzcn.net; yzhbl2003@163.com

variety was purchased from Yunnan Institute of Agricultural Crop Resources.

2.2 Effects of root exudate on growth of plants

2.2.1 Extraction of root exudate

The seeds of Jinchun 4 (resistant variety), Jinyan 4 and Black seed squash variety were soaked at 55°C for 10 min and 30°C for 3–4 h, and then germinated on moist filter paper at 25°C–23°C. Seeds were sown in pots filled with BW-22 stroma. After washing the stroma off the roots with tap water, the seedlings at the one-leaf-stage were immediately transplanted to plastic vessels containing 20-Litre Yamasaki culture solution per vessel (Wang, 2000). The hydroponic nutrient solution was prepared using deionized water and replaced every week.

The seedlings at the 2 to 3-leaf-stage were carefully moved and grown for 4–5 h in 4 L hydroponic sterile deionized water to release the root exudate into water, which was collected as the source of root exudate (Han et al., 2000).

2.2.2 Seedling treatment

As described above, the seeds of Jinyan 4 were sown in pots, and then were watered daily with 10–12 mL of fresh root exudate while the rest were watered with water only as controls at the cotyledon expanding stage. The plant height, fresh mass, root vigor (Zou, 2000) as well as the electrical conductance of roots were measured 12 days after the treatment (Zou, 2000).

2.3 Effects of root exudate on the growth of *Fusarium oxysporum* pathogen

2.3.1 Preparation of medium containing root exudate

2.3.1.1 Extraction from rhizosphere soil

The seedlings of Jinchun 4 (resistant variety), Jinyan 4 and Black seed squash variety were transplanted to the Experimental Base of Yangzhou University. At the flowing stage, the rhizosphere soil around Jinchun 4, Jinyan 4 and Black seed squash were sampled, respectively, socked for 2 h with distilled water (soil/water was 1 : 1), filtered with filter paper. One half of the extraction solution was processed to potato dextrose agar medium and the rest was processed to water agar culture medium. The soil from a non-cropped site nearby was used as the control.

2.3.1.2 Collection from deionized water

After washing the soil off the roots with running tap water, the seedlings at the initial bloom stage were initially washed with distilled water and deionized water three times, then immersed in 1-Litre deionized water, and kept at 25°C for 5 h. At the end of the exudation period, the solution containing root exudate was divided into two halves, one of which was processed into PDA culture medium and the other was processed into water agar culture medium. The control culture medium was prepared with deionized water.

2.3.1.3 Cultivation in nutrient solution

The seedlings at the 2 to 3-leaf-stage were grown in a 1500 mL nutrient solution at 25°C–30°C for 5 days. The solution containing root exudate was processed into the PDA culture medium and water agar culture medium when the solution without seedlings cropping was served as the control.

2.3.2 Occurrence of fusarium wilt

Fifteen days after the root exudate treatment, the plants from each treatment were inoculated with *Fusarium oxysporum* pathogen by watering 10 mL conidial suspension, prepared by culturing the pathogen in the PDA medium and diluted with the distilled water to a given concentration of 10^3 conidia/mL. The occurrence of fusarium wilt was measured two days after inoculation.

The culture medium described above was dispensed aseptically into 15-cm-culture dishes. Mycelial plugs of single-spore isolates of *Fusarium oxysporum* pathogen were then transferred to the centre of media. The dishes were incubated at 25°C and colony diameter was measured. Each treatment was replicated ten times.

2.4 Statistical analysis

All experimental data were analyzed with DPS software. The analysis of variance (ANOVA) was conduced (SAS Institute, 1989) thereafter.

3 Results

3.1 Effect of root exudate on physiological characteristic of cucumber

3.1.1 Plant height and fresh mass

The plant height (8.25 cm) and fresh mass (1.40 g) in control group were higher than those of other treatments (Fig. 1). The plant height (7.56 cm) and fresh mass (1.15 g) of Jinyan 4 treated with its own root exudate were the lowest. There was no significant effect of the root exudate of Jinchun 4 and Black seed squash on the plant height and fresh mass of Jinyan 4. Statistical analyses showed that the root exudate of susceptible variety (Jinyan 4) has a significant inhibitory effect on the plant height and fresh mass ($P < 0.01$).

3.1.2 Root vigor

The accumulation of the root exudate of susceptible variety (Jinyan 4) in soil resulted in harmful effects on the root vigor

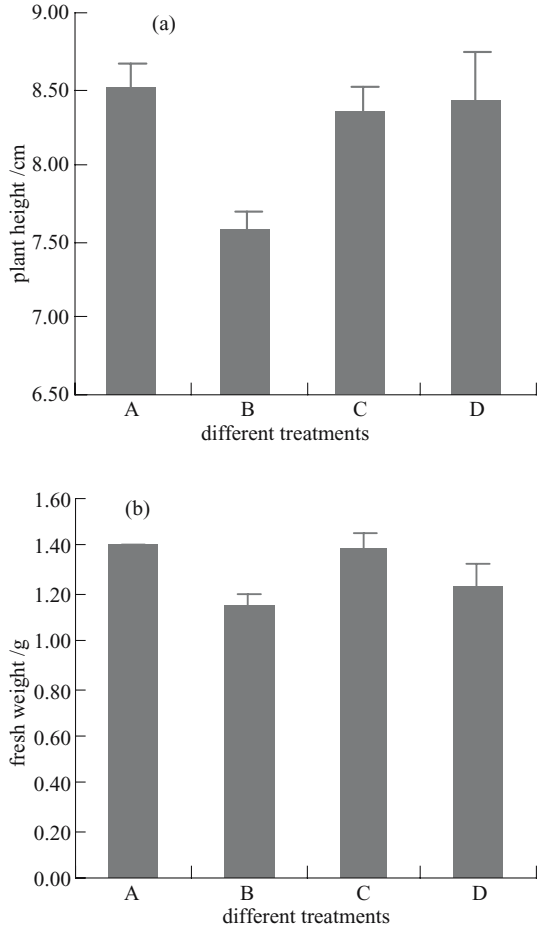

Fig. 1 Effect of different root exudate treatments on plant height (a) and fresh weight (b) of cucumber seedlings. Note: A, B, C and D represent CK, Jinyan 4 root exudate, Jinchun 4 root exudate and Black seed squash root exudate, repectively. The same below.

(Fig. 2). The active absorption area of roots treated with the root exudate of Jinyan 4 was the lowest, and there was a significant difference compared with the control ($P<0.01$), except on the early stage of treatment. In addition, the active absorption area of roots treated with the root exudate of Jinchun 4 increased by 39.81% and 44.31% compared with that in other treatments during the early stage of measurements (0 and 4 days), but at the later stages of measurements, the active absorption area of roots in the control plants was higher, being 48.41%, which was significantly higher than that of the treatment with the root exudate of Jinyan 4 ($P<0.01$).

3.1.3 Conductance of roots

The results of the root conductance are shown in Fig. 3. The damage degree of roots treated with the root exudates of Jinchun 4 and Black seed squash did not change significantly during the measurements. The damage degree of roots treated with the root exudate of resistant variety (Jinchun 4) was significantly lower than that of other treatments ($P<0.01$),

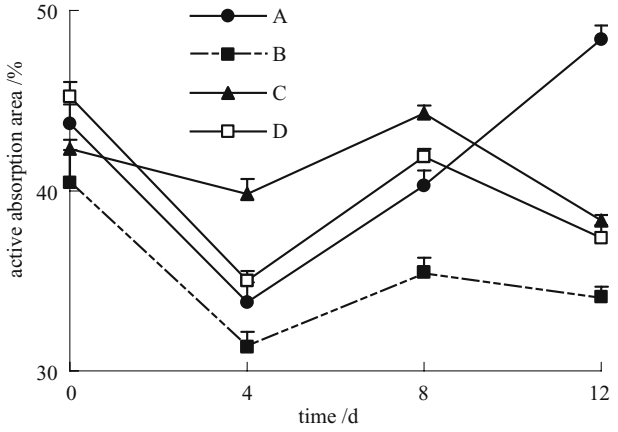

Fig. 2 Changes of root active absorption area of cucumber plants after being treated with different root exudates

especially on the early stage of treatment. On the contrary, the damage degree of roots treated with the root exudate of susceptible variety (Jinyan 4) was the highest with a rate of 12.7%, and that of 20 days after treatment was significantly higher than those in treatments with the root exudates of Jinchun 4 and Black seed squash ($P<0.01$), which were 0.61% and 4.98%, respectively.

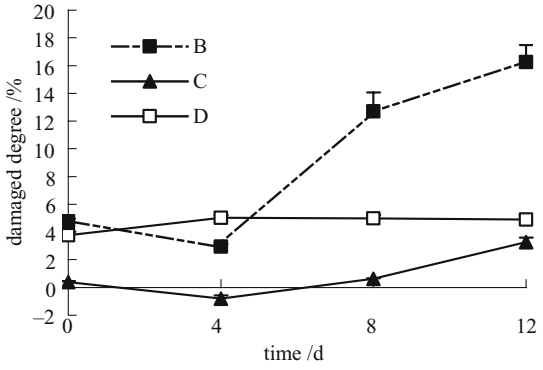

Fig. 3 Changes of severity damage of cucumber plants after being treated with different root exudates

3.2 Effect of root exudate on cucumber fusarium wilt occurrence

3.2.1 Incidence of cucumber fusarium wilt

The results indicated that the plants treated with the root exudate of Jinyan 4 showed disease symptom 4 days after inoculation. The disease incidence of the plants treated with the root exudates of Jinyan 4 and Black seed squash were 18.5% and 10.0% compared with 2.5% of the root exudate of Jinchun 4 and 1.0% of CK 10 days after inoculation. Until the 15th day, the incidence of plants treated with the root exudate of Jinyan 4 (34.5%) was significantly higher but that treated with the root exudate of Jinchun 4 (14.5%) was lower than

that of CK ($P < 0.01$). On the 20th day after inoculation, the incidences with treatment of the root exudates of Jinyan 4 and CK were nearly the same (41.0%), compared with 24.0% of the root exudate of Jinchun 4. All results indicated that root exudates from resistant varieties inhibited the occurrence of fusarium wilt. In contrast, the root exudates from susceptible varieties promoted the occurrence (Fig. 4).

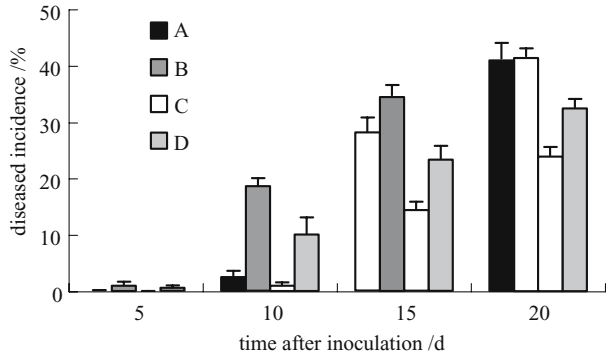

Fig. 4 Infection rate of fusarium wilt in cucumber seedlings treated with different root exudates

3.2.2 Growth of *Fusarium oxysporum* colony

The results showed that the root exudates from resistant varieties and Black seed squash inhibited the growth of *Fusarium oxysporum* colony, which could be promoted by the root exudates from susceptible varieties (Fig. 3). Statistical analyses revealed that there was no significant difference among the effects of different root exudates on the growth of this pathogen grown on the medium made by remnants of nutrient solution; on water agar culture medium, growth rate of pathogen in control group was the greatest, followed by the Jinyan 4 treatment, while the growth rate in Jinchun 4 and Black seed squash was minimum although the content of root exudate extracted with deionized water was small. The root exudate from Jinchun 4 inhibited the growth of the pathogen (Table 1).

4 Discussion

The root exudate collected from the cucumber root systems was easily polluted by soil organic matters, organism and its metabolites, while the root exudate collected from the nutrient solution with seedlings cropping was easily affected by nutrient elements. Moreover, it is difficult to collect with other methods during a short time. Thus the seedlings in our test were grown in the nutrient solution and transplanted into sterile water everyday in order to avoid the disadvantages described above. In addition, the same variety of cucumber excluded the effects of different genetic backgrounds.

The present results showed that the root exudate of the susceptible cucumber variety could stimulate the occurrence of fusarium wilt, while that of the resistance variety was in adverse. The cause may be the harmful effects of the root exudate of the susceptible cucumber variety on the plants, especially the roots. The plant height and fresh weight of Jinyan 4 (susceptible variety) treated with its own root exudate were lower than those of the control, and the root vigor decreased which affected the water and nutrient absorption of seedlings. The increase of conductance would provide nutrients for the pathogen growth and stimulate the spore germination. In addition, the root exudate of cucumber in continuous cropping soil would promote the growth of *Fusarium oxysporum* pathogen according to the present findings. Our results showed that the root exudate of the susceptible cucumber variety could stimulate the growth of its pathogen while that of resistance variety was in adverse.

Some studies on the allelopathic effect of root exudate of the grafted eggplant have demonstrated that the grafting technique was a good method to reduce the continuous cropping barrier begotten by autointoxication (Zhang et al., 2005). The root exudate was collected from Black seed squash in this experiment. The results showed that the incidence was

Table 1 Effect of different root exudate treatments on growth of *Fusarium oxysporum* colony

origin of root exudate	treatment	colony diameter on potato medium /cm				colony diameter on water agar medium /cm			
		three days		five days		three days		five days	
remnants of nutrient solution	Jinchun 4	4.35	aA	7.16	aA	3.26	cC	5.13	cC
	Jinyan 4	4.41	aA	7.28	aA	3.50	bB	5.43	bcBC
	Black seed squash	4.30	aA	7.06	aA	3.30	cC	5.29	cC
	CK	4.38	aA	7.24	aA	3.81	aA	6.06	aA
non-ion water	Jinchun 4	3.81	bA	5.79	bA	2.78	aA	4.59	aA
	Jinyan 4	4.09	aA	6.12	aA	2.84	aA	4.69	aA
	Black seed squash	3.88	AbA	5.84	bA	2.76	aA	4.55	aA
	CK	3.95	aA	5.93	abA	2.81	aA	4.64	aA
water extract of rhizosphere soil	Jinchun 4	3.45	cC	5.10	cC	2.71	bA	4.34	cB
	Jinyan 4	4.08	aA	6.09	aA	2.94	aA	4.79	aA
	Black seed squash	3.73	bB	5.63	bB	2.73	bA	4.44	bcB
	CK	3.90	aA	5.76	bAB	2.81	abA	4.58	bAB

Note: Means within a column followed by the different small letters and capital letters showed different significances.

lower than that treated with the root exudates of susceptible varieties and CK, but the inhibitory effect was still less than that treated with the root exudate from resistance variety.

Currently, some researchers are testing the added autointoxication (e.g., cinnamic acid) into soil in order to study the effect on the soil-borne diseases (Wang and Li, 2003). However, these studies have not explained the roles of root exudates in occurrence of diseases. Our experiments proved that the effects of root exudates on the occurrence of the soil-borne diseases varied with cucumber varieties. In addition, some findings demonstrated that the formation of pathogen suppressive soil after planting the resistance variety of *Citrullus lanatus* (Thunb) Matsum. and Nakai for many years could be caused by the decrease of pathogen density which was related to the root exudates (Larkin et al., 1993; Yang et al., 1995; Yuan et al., 2002). So it has a significant implication that the effects of root exudates on the diseases occurrence need to be studied to find out the causes of the soil-borne diseases after continuous vegetable cropping and to reduce the disease through reasonable utilization of resistance varieties.

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