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The effects of cotton root exudates on the growth and development of *Verticillium dahliae*

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Abstract The effects of upland cotton root exudates on the growth and development of *Verticillium dahliae* were studied, through the compared analysis of the root exudates components between the resistant and susceptible cotton materials, using a pair of resistant and susceptible isogenic lines to Verticillium wilt, Z5629 and Z421, as well as 4 other upland cotton cultivars with different resistant levels of Verticillium wilt. The results showed that the amino acids in the root exudates of the resistant cultivars were much less than that of the susceptible ones. Compared with the susceptible ones, there were a lack of aspartic acid, threonine, glutamic acid, alanine, isoleucine, leucine, phenylalanine, lysine and proline in the root exudates from the resistant cultivars. On the contrary, arginine was lacking in the susceptible cultivars. The saccharide types in the root exudates were no different between the two kinds of cultivars, but the contents of glucose, fructose and sucrose in the root exudates of the susceptible varieties were much higher than those in the resistant ones. The experiment of *Verticillium dahliae* culture showed that the cotton root exudates from resistant cultivars can effectively restrain the spore germination and mycelium growth of *Verticillium dahliae*, and the arginine was the leading amino acid in this inhibitory action, besides the nutrition of the root exudates. However, the cotton root exudates from the susceptible cotton cultivars can improve the growth and development of *Verticillium dahliae* effectively; among the amino acid in the exudates, alanine was the most active one in this stimulating function.

Keywords *Gossypium hirsutum*, root exudates, *Verticillium dahliae*

1 Introduction

To be a serious threat and a restraining factor to the development of cotton production, Verticillium wilt is one of the most serious diseases in cotton production. Since the 1990s, Verticillium wilt has occurred rampantly in most cotton growing regions and has become the main development obstacle of cotton production in China (Ma and Jian, 1997). Developing and utilizing the disease-resistant cultivars is the most economic method to control this disease. However, due to the basic research of cotton Verticillium wilt pathogen, inheritance and mechanism of host plant resistance to this disease, the cotton breeding for Verticillium wilt resistance develops slowly, and the resistance level of cotton cultivars cannot meet the needs of cotton production in China up to now (Ma et al., 2002). In addition, the lack of the resistant cotton germplasms and effective selection methods may restrict the development of the cotton breeding program for disease resistance as well (Fang et al., 2000). Therefore, it is very important to study the inheritance and mechanism of the cotton plant resistance to Verticillium wilt, and search for the germplasm with high resistant traits to the disease. Verticillium wilt is a soil-borne disease, and soil microecological environment is closely related to the growth, development and invasion of *Verticillium dahliae*. Apart from the environmental conditions of soil, first and foremost, micro-ecological environment is affected by the root exudates. A small change of root exudates will lead to great changes in the system of microorganisms. Therefore, by studying the root exudates components of cotton cultivars with different levels of disease resistance, the pathogenesis of *Verticillium dahliae* may be revealed. Furthermore, the physiological and biochemical index of cotton resistance to Verticillium wilt may be discovered, which is useful for cotton breeding and disease control.

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2 Materials and methods

2.1 Plant materials

A pair of resistant and susceptible isogenic lines to *Verticillium* wilt — Z5629 and Z421 — was used as the materials in this experiment (Zhu et al., 1999), and 2 resistant extending cultivars and 2 susceptible extending cultivars were used as the checks (Table 1).

Verticillium dahliae physiological type III, the Anyang strain and distributed widely in China, was provided by Cotton Research Institute of Chinese Academy of Agricultural Science

2.2 Inoculation of *Verticillium dahliae*

The cultured strain of *Verticillium dahliae* was washed by germfree water, filtered by a four-layer gauze, and the number of spores was counted under a microscope. The spore suspension (2.4×10^7 spores·mL⁻¹) was prepared freshly before use. Before inoculation, the bottom of the nutrition pot was ripped off in order to damage some roots of the cotton seedlings, then inoculated by adding 10 mL spore suspension to each pot. After inoculation the seedlings were cultivated in a green house at the temperature of 25–28°C/20–22°C day/night. When disease index of Jimian11 was about 50, the incidence of *Verticillium* wilt was surveyed according to the Five-grade method.

2.3 Analysis of the root exudates

The fuzz-free seeds of different cultivars were sterilized by 0.1% HgCl₂ for 5 minutes, then cultured on the PDA plate at 25°C for 4 days in a growth chamber. The seeds with root length of 2 cm were selected to be planted in inverted brown bottles (no bottom), filled with sterilized sand, with 5 bottles each cultivar and 6 seedlings per bottle. Irrigation with distilled water and inorganic nutrient solution was uninterrupted. After being cultured for 30 days, the seedlings were taken out, and the cultured sand was washed by 100 mL aseptic distilled water for each bottle. The washing liquid was filtered with Nutsche filter, then freezing condensed in freeze-drying machine. Total 200 mL condensed exudates was obtained for each cultivar. One hundred mL was used for analysis of amino acids and saccharides, and the other 100 mL was used in

the growth experiment of *Verticillium dahliae*. Hitachi automatic amino acid analyzer was used for analysis of amino acids, and Shimazu high pressure liquid chromatogram was used for the measure of sugar contents.

2.4 The spore germination and mycelium growth of *Verticillium dahliae*

Determination of spore germination of *Verticillium dahliae*: Root exudates extract, amino acids, monosaccharide, etc., were put in the flute respectively and mixed with the spore suspension quantitatively. The flute (admixture) was put into the dishes, covered, and cultured at a constant temperature of 25°C. After a 12-hour culture, the spore germination was observed under a microscope. The average spore germination rate was calculated from 4 repetitions for each cultivar and 10 perspectives for each repetition, using aseptic distilled water as control.

Mycelium growth of *Verticillium dahliae*: PDA medium (1000 mL containing 25 g agar) was prepared to make PDA plate by the ratio of PDA medium and root exudates extract (amino acids and monosaccharide) filtered by sterilization filter at 3:1, respectively. Three tablets, each with a 0.5 cm diameter plate, were inoculated: 6 repetitions for each cultivar. With the check of adding 1/4 aseptic distilled water in PDA medium as control, the colony diameters were measured after culturing for 4, 7, and 11 days at 25°C.

3 Results and analysis

3.1 Identification of the *Verticillium* wilt resistance of the materials

Inoculation identification under consistent condition was carried out to understand their resistance to *Verticillium* wilt, and the results are shown in Table 2.

The results showed that the disease incidence was 17.1% and the disease index was 6.7 for Z5629. However, for the isogenic line Z421, the rate was 68.4% and 45.1, respectively. So the difference in disease resistance between Z5629 and Z421 was significant, although their genetic backgrounds were almost same. Except for the isogenic lines, disease index of two resistant cultivars, Yumian 21 and Yumian 19, were 10.5 and 13.3, respectively, and that of other two susceptible cultivars, Jimian 11 and Yumian

Table 1 Materials used in the experiment and their resistant expressions to *Verticillium* wilt

cultivar	source	resistant expression
Z5629	Zhejiang University	R
Z421	Zhejiang University	S
Yumian21	Cotton Research Institute of Chinese Academy of Agricultural Sciences	R
Yumian 19	Henan Academy of Agricultural Sciences	R
Jimian11	Cotton Research Institute of Chinese Academy of Agricultural Sciences	S
Yumian 12	Henan Academy of Agricultural Sciences	S

Table 2 Identification of the *Verticillium* wilt resistance of the experimental materials

material	disease incidence/%	disease index	resistance level
Z5629	17.7 ± 2.1	6.7 ± 0.4	R
Z421	68.4 ± 5.5	45.1 ± 5.2	S
Yumian 21	21.3 ± 1.8	10.5 ± 1.0	R
Yumian 19	23.1 ± 2.1	13.3 ± 1.0	S
Jimian11	50.5 ± 4.7	35.1 ± 3.7	S
Yumian12	56.3 ± 4.9	34.7 ± 3.1	S

12, were 35.1 and 34.7, respectively. The disease resistances among these cultivars were found significantly different (Table 2).

3.2 The component and content of amino acids in cotton root exudates

The components and contents of amino acids in cotton root exudates among the different cotton cultivars were analyzed by an automatic amino-acid analyzer, and the results are shown in Table 3.

Table 3 shows that there was a great difference in the root exudates among the different cotton cultivars. The amino acids in the root exudates from susceptible cultivars were much more than those from resistant cultivars, as well as the content of amino acids in which most of them were much higher from susceptible cultivars than that from resistant cultivars. There were only 6–7 amino acids in the root exudates of resistant cultivars, while that of susceptible cultivars were more than 13, two times more than that of resistant cultivars. Compared with susceptible ones, the amino acid from the resistant cultivars lack aspartic acid, threonine, glutamic acid, alanine, isoleucine, leucine, phenylalanine, lysine, and proline; but on the contrary, arginine was lacking in the susceptible cultivars. As far as total amino acid content was concerned, the total content of

amino acid was 3.05–4.80 $\mu\text{g}\cdot\text{plant}^{-1}$ in the root exudates of resistant cultivars, which was significantly lower than that of susceptible ones (9.64–12.07 $\mu\text{g}\cdot\text{plant}^{-1}$). The correlation between total contents of amino acid in root exudates of various materials and disease index of *Verticillium* wilt was positively significant ($r = 0.99^{**}$).

Among the resistant and susceptible isogenic lines, there were 6 types of amino acids in root exudates of resistant line (Z5629), the total content of amino acid was 3.05 $\mu\text{g}\cdot\text{plant}^{-1}$, while there were 15 amino acids in susceptible line (Z421) and the total content of amino acid was 12.07 $\mu\text{g}\cdot\text{plant}^{-1}$, so their difference was obvious. It was shown that the component and content of amino acid in cotton root exudates might closely relate to their resistance to *Verticillium* wilt, due to their similar genetic backgrounds.

3.3 Content of saccharides in the root exudates

The results of the saccharides analysis of the root exudates from the different cotton cultivars (Table 4) show that there was no significant difference in the types of saccharides in the root exudates among the tested materials, mainly including glucose, fructose, sucrose, and three unknown saccharides with a very small proportion. However, the concentration of saccharides in the root exudates from resistant cultivars was about 2 to 3 times higher than that of susceptible cultivars. The correlation between total saccharide content in cotton root exudates and disease index of *Verticillium* wilt was also positively significant ($r = 0.81^*$).

3.4 Effects of cotton root exudates on the growth and development of *Verticillium dahliae*

Results of the spore germination and growth of *Verticillium dahliae* affected by root exudates are shown in Table 5.

Table 3 The contents of amino acids in the root exudates of different cotton cultivars ($\mu\text{g}\cdot\text{plant}^{-1}$)

amino acid	isogenic line		resistant cultivar		susceptible cultivar	
	Z5629	Z421	Yumian 21	Yumian 19	Yumian 12	Jimian11
ASP	< 0.01	0.51	< 0.01	< 0.01	0.49	0.41
THR	< 0.01	0.91	< 0.01	< 0.01	0.66	0.66
SER	0.31	1.54	0.44	0.33	1.31	1.21
GLU	< 0.01	0.59	< 0.01	< 0.01	0.61	0.56
GLY	< 0.01	0.36	0.22	< 0.01	0.18	0.15
RLA	< 0.01	0.67	< 0.01	< 0.01	0.34	< 0.01
CYS	0.56	0.98	0.77	0.64	0.66	0.98
UAL	0.78	1.65	0.91	0.93	1.46	1.01
MET	0.18	0.45	0.29	0.17	0.23	0.28
ILE	< 0.01	1.09	< 0.01	< 0.01	1.19	0.89
LEV	< 0.01	0.18	< 0.01	< 0.01	0.13	< 0.01
TYR	0.88	0.88	1.78	1.89	0.89	0.89
PHE	< 0.01	0.72	< 0.01	< 0.01	0.66	0.67
LYS	< 0.01	0.45	< 0.01	< 0.01	0.65	0.62
ARG	0.34	< 0.01	0.39	0.41	< 0.01	< 0.01
PRO	< 0.01	1.09	< 0.01	< 0.01	1.32	1.31
total	3.05	12.07	4.80	4.37	10.78	9.64

Table 4 The content of saccharides in the root exudates of different cotton cultivars ($\mu\text{g}\cdot\text{plant}^{-1}$)

carbohydrate	isogenic line		resistant cultivar		susceptive cultivar	
	Z5629	Z421	Yumian 21	Yumian 19	Yumian 12	Jimian11
GLU	78.5	143.1	88.3	85.1	250.6	138.5
FRU	11.3	41.1	13.1	8.7	43.4	41.3
SUC	12.1	33.2	16.3	9.5	30.2	34.9
others	2.1	1.2	3.1	0.9	0.8	2.0
total	104.0	218.6	120.9	104.3	325.0	216.7

Table 5 The effects of cotton root exudates on the growth and development of *Verticillium dahliae*

treatment	spore germination/%	colony diameter/mm		
		4 days after inoculation	7 days after inoculation	11 days after inoculation
Z5629	25.31 \pm 3.12	11.45 \pm 1.10	16.11 \pm 1.23	24.21 \pm 2.01
Z421	56.73 \pm 5.77	15.11 \pm 1.21	22.35 \pm 1.89	30.77 \pm 2.67
Yumian21	25.60 \pm 3.10	10.37 \pm 1.00	15.90 \pm 1.25	25.70 \pm 3.32
Yumian 19	25.80 \pm 3.30	12.00 \pm 1.00	17.40 \pm 1.45	28.40 \pm 3.47
Jimian11	58.24 \pm 6.12	14.75 \pm 1.21	21.80 \pm 2.00	31.10 \pm 3.51
Yumian 12	47.70 \pm 5.31	14.60 \pm 1.31	21.00 \pm 2.21	29.80 \pm 3.43
CK	36.60 \pm 2.78	13.00 \pm 1.26	18.00 \pm 1.45	29.00 \pm 3.34

The spore germination results of *Verticillium dahliae* showed that the germination rate of *Verticillium dahliae* on the medium with the root exudates from resistant cultivars was significantly lower than that of water control, while that from susceptible cultivars was higher than that of water control. It is suggested that the root exudates of resistant cotton cultivars had a restraining effect on spore germination of *Verticillium dahliae*, while that of susceptible cotton cultivars may have some effects on spore germination of *Verticillium dahliae*.

The mycelium growth rate of *Verticillium dahliae* on the PDA medium with the root exudates from different cultivars are shown in Table 5. It shows that the mycelium growth of *Verticillium dahliae* on the medium with resistant cultivars' root exudates obviously restrained and the average colony diameter was smaller than that of control, although the difference did not reach a statistically significant level. However, the mycelium growth of *Verticillium dahliae* was stimulated by root exudates of susceptible cultivars and colony diameter was bigger than that of control, although the difference did not reach the statistical significant level as well. Between the resistant line and susceptible line of the isogenic lines, the difference of colony size was significant after being cultured for 4 days and 7 days, but was not significant after being cultured for 11 days and later. In other words, the restraint effect of the resistant cultivars root exudates and stimulation function of susceptible cultivars root exudates declined with the extension of culture time.

3.5 Effects of amino acid on the growth and development of *Verticillium dahliae*

In order to explore ingredients of the root exudates influencing the growth and development of *Verticillium dah-*

liae, based on the results of amino acid analysis in root exudates, the spore germination and mycelium growth experiments were carried out using the specific amino acid, exudates from resistant cultivars or susceptible cultivars, and the results are shown in Table 6.

Table 6 shows that the spores germination rate of *Verticillium dahliae* ranged from 20.31% to 45.80% for a single amino acid treatment, and that on the medium with arginine treatment was the lowest (20.31%), significantly lower than that of water control, while that with alanine treatment was the highest (45.80%), significantly higher than that of water control. Other treatments had some variations compared with water control, but the difference did not reach a statistically significant level.

In terms of the mycelium growth rate of *Verticillium dahliae* on PDA medium with single amino acid, the colony diameters varied to some extent, but the difference was not significant except for the colony of arginine and alanine treatments. The colony size was significantly smaller on the medium with arginine than that of water control, while the colony on the medium with alanine treatment was significantly larger than that of water control. Thus it can be seen that arginine can inhibit the spore germination and mycelium growth of *Verticillium dahliae*, and the alanine, on other hand, can stimulate the spore germination and mycelium growth of *Verticillium dahliae*. Other amino acids were found to have no significant effect on the growth and development of *Verticillium dahliae*. When 25 $\mu\text{g}\cdot\text{L}^{-1}$ of alanine was added in the root exudates extract of resistant cultivars, the spore germination and mycelium growth of *Verticillium dahliae* were close to those of susceptible control, and 25 $\mu\text{g}\cdot\text{L}^{-1}$ arginine in the root exudates extract of susceptible cultivars led to almost the same result of resistant cultivar control

Table 6 The effects of amino acid on the growth and development of *Verticillium dahliae*

amino acid	concentration/ $\mu\text{g}\cdot\text{L}^{-1}$	spore germination/%	colony diameter/mm		
			4 days after inoculation	7 days after inoculation	11 days after inoculation
ASP	25.0	35.45 \pm 3.32	13.23 \pm 1.21	16.88 \pm 1.32	32.22 \pm 2.98
THR	25.0	36.43 \pm 4.11	15.23 \pm 1.42	19.55 \pm 2.09	31.47 \pm 3.12
GLU	25.0	35.60 \pm 4.10	12.43 \pm 1.21	20.10 \pm 2.05	29.40 \pm 3.12
RLA	25.0	45.80 \pm 4.30	16.01 \pm 1.45	25.41 \pm 2.43	38.44 \pm 3.77
ILE	25.0	38.24 \pm 3.22	12.76 \pm 1.32	21.32 \pm 2.11	31.08 \pm 3.02
LEV	25.0	37.36 \pm 3.33	12.61 \pm 1.28	21.10 \pm 2.01	29.77 \pm 3.01
PHE	25.0	36.60 \pm 3.18	14.11 \pm 1.41	19.01 \pm 1.32	31.01 \pm 3.01
LYS	25.0	33.50 \pm 2.88	13.21 \pm 1.32	17.90 \pm 1.75	30.04 \pm 3.04
ARG	25.0	20.31 \pm 1.78	8.43 \pm 0.88	12.89 \pm 1.02	20.34 \pm 2.11
PRO	25.0	34.40 \pm 3.04	13.45 \pm 1.23	20.12 \pm 1.91	28.33 \pm 3.33
CK1+ARG	25.0	28.41 \pm 2.45	11.99 \pm 1.23	17.14 \pm 1.34	25.26 \pm 2.41
CK2+RLA	25.0	50.44 \pm 5.01	14.12 \pm 1.32	21.30 \pm 1.96	29.76 \pm 2.73
CK1	–	56.73 \pm 5.77	15.11 \pm 1.21	22.35 \pm 1.89	30.77 \pm 2.67
CK2	–	25.31 \pm 3.12	11.45 \pm 1.10	16.11 \pm 1.23	24.21 \pm 2.01
CK3	–	36.60 \pm 2.78	13.00 \pm 1.26	18.00 \pm 1.45	29.00 \pm 3.34

Note: CK1 = theroot exudates from resistant cultivars; CK2 = theroot exudates from susceptible cultivars; CK3 = water.

(Table 6). According to the detection results of amino acid in the root exudates from different cotton cultivars, arginine was a specific amino acid in the root exudates of resistant cultivars. Apart from the types and content of amino acids in the root exudates of resistant cultivars, arginine may be one of the important factors to inhibit the growth, development and invasion of *Verticillium dahliae*. For susceptible cultivars, more amino acids and lack of arginine in the root exudates can provide nutrition for the growth and development of pathogens, and stimulate its growth and development. Among the amino acids, the role of alanine was found to be particularly important.

4 Discussion

Verticillium wilt is one of the most important and serious diseases in cotton production. Since the 1990s, the incidence rate of *Verticillium* wilt has been an increasing trend (Ma and Jian, 1997; Ma et al., 2002). *Verticillium* wilt is a soil-borne disease, and the ecological factors, such as rhizosphere microorganisms, rhizosphere secretions, et al., have a close relationship with the growth of soil-borne pathogens and disease resistance of plants. Apart from the environmental conditions of soil, rhizosphere microorganisms are mainly affected by the root exudates. Singh (1971) considered that the variation of amino acids and other components in the root exudates of different cotton cultivars affected the rhizosphere bacterial population. Booth (1969, 1974) compared the amino acid content in the root exudates of cotton between resistant and susceptible cultivars, and the results showed that the most obvious difference was alanine among 8 kinds of amino acids, and the difference among 7 other amino acids was not obvious. Using a pair of resistant and susceptible isogenic lines, Z5629 and Z421, as the materials and 2 resistant extending

cultivars and 2 susceptible extending cultivars as checks, the differences in the root exudates of different cotton cultivars were studied in the present paper. It was found that the total content of amino acids and saccharides in the root exudates of the cotton cultivars was significantly related to their resistance to *Verticillium* wilt. There were only 6–7 amino acids in the root exudates of resistant cultivars, compared with the susceptible ones. There was a lack of aspartic acid, threonine, glutamic acid, alanine, isoleucine, leucine, phenylalanine, lysine and proline; but arginine that was a specific amino acid in the root exudates of resistant cultivars. The content of saccharides in the root exudates of resistant cultivars was also significantly lower than that of the susceptible genotypes. More amino acids and soluble saccharides in the root exudates of susceptible cultivars can provide abundant nutritional substances for the growth, development and invasion of *Verticillium dahliae*. Less amino acids and soluble saccharides in the root exudates of resistant cultivars may inhibit the growth and development of *Verticillium dahliae* to a certain extent. The results also showed that, besides the component and content of amino acids and soluble saccharides which provided nutritional substances for *Verticillium dahliae*, the arginine (a specific amino acid in the root exudates of resistant cultivars) may be one of the important factors to inhibit the growth, development and invasion of *Verticillium dahliae*, and the alanine in the root exudates of susceptible cultivars may be one of the important factors to stimulate the growth and development of *Verticillium dahliae*. However, except for amino acids and saccharides, there are vitamins, organic acids, growth regulators, growth factors and inhibiting factors in the cotton root exudates. Therefore, it is necessary to study the effect of various components on the growth, development and invasion of *Verticillium dahliae*, in order to reveal the mechanism of cotton resistance to *Verticillium* wilt.

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References

- Booth J A (1969). *Gossypium hirsutum* tolerance to *Verticillium ablatrum* infection I. Amino acids exudation from aseptic roots of tolerant and susceptible cotton. *Phytopathology*, 59: 43–46
- Booth J A (1974). Effect of cotton root exudation constituents on growth and pectolyticenzyme production to *Verticillium ablatrum*. *Canadian Journal of Botany*, 52: 22–27
- Fang W P, Ji D F, Zhu S J (2000). Advance of research on the resistant mechanism of cotton to *Verticillium* wilt. *Cotton Science*, 12(5): 277–280 (in Chinese)
- Ma C, Jian G L (1997). Current status, problem and countermeasure on resistance breeding to *Verticillium* wilt of cotton in China. *Scientia Agricultura Sinica*, 30(2): 58–64 (in Chinese)
- Ma C, Jian G L, Zheng C L (2002). The advances in cotton breeding resistance to *Fusarium* and *Verticillium* wilts in China during past fifty years. *Scientia Agricultura Sinica*, 35(5): 508–513 (in Chinese)
- Singh D (1971). Effect of alanine on development of *Verticillium* wilt in cotton cultivars with different level of resistance. *Phytopathology*, 61: 880–884
- Zhu S J, Wang R H, Wang H M (1999). Breeding research of resistant near-isogenic lines to *Verticillium* wilt of upland cotton. *China Cotton*, 26(3): 22–23 (in Chinese)