

The effect of root exudates from two transgenic insect-resistant cotton lines on the growth of *Fusarium oxysporum*

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Abstract The attenuation of disease resistance in transgenic insect-resistant cotton has become one of the important factors restricting cotton production in China. Two transgenic insect-resistant cotton lines and their parental conventional cotton lines were used as the testing materials. The effects of root exudates of these cotton lines on the spore germination and mycelial growth of *Fusarium oxysporum* were studied and the components, contents of amino acids and sugars were determined. The results showed that the resistance of the two insect-resistant cotton lines to *F. oxysporum* was inferior to the parental lines, and that their root exudates promoted fungal spore germination and mycelial growth. Considerable differences in the components and contents of both, amino acids and sugars were found between the root exudates of

transgenic cotton lines and their parental lines, where the disease indices were highly correlated with the total amount of sugars in the root exudates.

Keywords Transgenic insect-resistant cotton · Root exudates · *Fusarium oxysporum* · Amino acids · Sugars · Disease resistance

Introduction

In 1997, commercial planting of transgenic insect-resistant cotton was officially approved in China. The planted area of transgenic insect-resistant cotton currently represents over 70% of the total cotton grown in China (Clive 2007; Stone 2008). These lines effectively control the cotton bollworm (*Helicoverpa armigera*) and other Lepidoptera, resulting in a significant reduction in the usage of chemical insecticides, thus protecting environment and human health while conferring great socioeconomic benefits. However, concerns have been raised about the environmental risks caused by transgenic plants, especially the non-target effect on soil organisms, such as soil invertebrate, soil bacteria, fungi (e.g., Saxena et al. 1999; Griffiths et al. 2000; Stotzky 2004; Turrini et al. 2004; Castaldini et al. 2005; Giovannetti et al. 2005; Liu et al. 2005a, b; Rui et al. 2005; Xue et al. 2005; Icoz and Stotzky 2008a). Further, transgenic plants also have unintentional effects (Saxena and Stotzky 2001; Chen et al. 2004;

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Flores et al. 2005), including a decrease in disease resistance in comparison with the conventional lines.

Wu (2000) studied the disease resistance of transgenic insect-resistant cotton lines in Hubei Province, China, and found that all had low resistance to disease and could be grouped among the disease-susceptible cultivars. Zhu and Feng (2005) reported that transgenic insect-resistant cotton lines performed poorly, in comparison with conventional cotton lines, with regard to resistance against *Fusarium* wilt, *Verticillium* wilt and combinations of the two diseases. Additionally, reports from the Provinces of Hebei, Shandong, Jiangxi, Anhui, Xinjiang, Shanxi, Hubei and Jiangsu in China showed that the incidence of *Fusarium* and *Verticillium* wilt was greater for transgenic insect-resistant cotton lines in comparison with the conventional ones (Fang et al. 2003; Li et al. 2005; Liu et al. 2005a, b; Zhang et al. 2006). Currently, the disease resistance levels of transgenic insect-resistant cotton lines have been decreasing and negatively impacting the cotton production in China, resulting in an increased use of chemical pesticides for disease management.

Root exudates have been proposed as one of the major biochemical factors that confer disease resistance to varieties (Buxton 1962). The objective of this study was to investigate the unintentional changes present in root exudates of transgenic insect-resistant cotton lines, as well as, their influences on the growth of *Fusarium oxysporum*.

Materials and methods

Cotton lines and the pathogen

The transgenic cotton line Zhong-41 (containing *CryIAc* gene and *CpTI* gene), was cultivated jointly by the Cotton Research Institute and the Biotechnology Research Institute of Chinese Academy of Agricultural Sciences and approved by Ministry of Agriculture of China in 2002. Zhong-23, the conventional parental cotton line of Zhong-41, was cultivated by Cotton Research Institute of Chinese Academy of Agricultural Sciences.

The transgenic *CryIAc* cotton line, GK-12 was cultivated jointly by the Biotechnology Research Institute of the Chinese Academy of Agricultural Sciences and the Jiangsu Academy of Agricultural

Sciences. SM-3, the conventional parental cotton line of GK-12, was cultivated by Siyang Cotton Seed-Breeding Farm in Jiangsu Province.

Zhong-41 and Zhong-23 were provided by the Cotton Research Institute of the Chinese Academy of Agricultural Sciences, Anyang, China. GK-12 and SM-3 were provided by the Plant Protection Research Institute of Chinese Academy of Agricultural Sciences, Beijing, China.

A highly virulent strain of *F. oxysporum* f. sp. *vasinfectum* (Atk.) Snyder et Hansen, provided by the Cotton Research Institute of Chinese Academy of Agricultural Sciences, was used for all fungal experiments in this study. This fungus was a race-7 strain that causes cotton *Fusarium* wilt, which is widely distributed throughout the Yangtze River Basin and the Yellow River Basin of China.

The resistance of cotton to *F. oxysporum*

The soil used in this experiment was obtained from forested land in which cotton had never been planted. Aliquots of 1 kg of soil were uniformly mixed with, 0.05 kg of organic fertilizer and moist heat sterilized at 121°C for 3 h to obtain sterile soil for cultivation use. Cotton seeds for planting were surface-delinted in concentrated sulfuric acid for 5 min, rinsed four times in sterile distilled water, immersed in sterile distilled water for 8 h, and then planted in plastic pots containing the sterile cultivation soil. Pots were maintained in a greenhouse (day: 25–30°C, night: 20–25°C). Each cotton line was planted in triplicate and each repetition contained fifteen cotton seedlings. When seedlings reached the 3-leaf stage, the roots were partly cut using a sterile knife blade and inoculated with 5 ml of a 1×10^7 conidia·ml⁻¹ spore suspension. Plants were then cultured in the greenhouse for 20 days, and the disease incidence and index were recorded according to the grading standards for cotton *F. oxysporum* in cotton seedlings (Ma 2007).

Collection and treatment of cotton root exudates

Cotton seeds were surface-delinted by submergence in concentrated sulfuric acid for 5 min, then surface-disinfected in 75% ethanol for 10 min, followed by three washes in sterile distilled water, immersion in 0.1% HgCl₂ for 3 min and five final washes in sterile

distilled water. Seeds were transferred to PDA medium in 180-mm diameter Petri dishes, and held in a biochemical incubator at room temperature for 4 days until they pre-germinated. Contaminated seeds that had internal fungal infection were discarded and not used in the study. Seedlings of similar size (~3 cm height) were selected and transferred to micro-burettes containing 10 ml of sterile Hoagland's nutrient solution and cultured in a biochemical incubator with the following day/night cycle: 16 h $30 \pm 2^\circ\text{C}$ /8 h $25 \pm 2^\circ\text{C}$ and 75% relative humidity. After 3 days, cotton seedlings with micro-roots were transferred to 50-ml beakers containing sterile Hoagland's nutrient solution. The 50-ml beakers were then placed in 2-l beakers and covered with four layers of gauze to prevent microbial contamination. Plants were cultured under a regimen of $30 \pm 2^\circ\text{C}$ in the light (14 h) and $20 \pm 2^\circ\text{C}$ in dark (10 h) and 50% relative humidity. The Hoagland's nutrient solution was replaced every 3 days.

After 40 days of cultivation, plantlets were removed from the beakers and the roots were gently washed with sterile distilled water four times, then three times with sterile deionized water. Finally, all plantlets (three per treatment) were placed into sterile 800-ml beakers and the roots were completely submerged in 500 ml of sterile deionized water. Plantlets were maintained in a biochemical incubator at 30°C for 24 h to collect root exudates. The collected exudates were concentrated to 100 ml using a vacuum rotary evaporator (Eppendorf concentrator 5301, Germany), and 50 ml were used to determine the amino acid and sugar contents, while the other 50 ml were used to determine the effect on the growth of *F. oxysporum*.

The effect of cotton root exudates on spores germination of *F. oxysporum*

A stock culture of *F. oxysporum* was maintained on slants of PDA at 4°C . For the preparation of the conidial suspensions, a block of the stock culture was transferred to PDA medium for 4 days at 28°C in the dark and then transferred to liquid potato dextrose medium for 4 days at 28°C in the dark and rotary shaken at 150 rpm. The fungal culture was filtered under sterile conditions through five layers of gauze, and the spore-suspension was concentrated ($3,000\times$, 10 min) and adjusted to a final concentration of

1×10^7 conidia ml^{-1} in sterile distilled water using a hemocytometer. Spore germination in the presence of cotton root exudates was tested on concave glass slides (3-wells, CAT. NO.7101, China). The collected exudates were filtered through $0.22 \mu\text{m}$ sterile filters (Peninsula, Millipore, Shanghai, China) and $50 \mu\text{l}$ of the root exudates were mixed with $50 \mu\text{l}$ of spore suspension and incubated at 25°C in the dark. All experiments were performed in triplicate and independently replicated three times. After 6 h, the spore germination was determined microscopically by counting 10 fields ($40\times$ objective)/well. A spore was considered germinated if the germ tube length was, at least as long as, the length of the spore.

The effect of root exudates on the mycelial growth of *F. oxysporum*

Aliquots of 25 ml of root exudates were mixed with 100 ml of the PDA medium and poured into 180-mm-diameter Petri dishes. A 0.5 cm-diam homochronous mycelial mass of *F. oxysporum* was placed onto the center of the plate and cultured at 28°C in a thermostatic chamber (STABILITHERM™ 234EU2/EB2, USA). The colony diameters were measured using vernier calipers on days 2, 3, 4, 5 and 6, or until the PDA plates were filled with mycelia. The experiments were performed in triplicate and independently replicated three times.

Determination of amino acid and sugars in cotton root exudates

Cotton root exudates were filtered through a $0.22 \mu\text{m}$ sterile filter prior to, the determination of the contents of amino acids and sugars. The components and contents of the amino acids were determined using high performance liquid chromatography (HPLC) for amino acids (HP1100, USA). The analytical conditions were as follows: chromatographic column: $4.0 \times 125 \text{ mm C}_{18}$, temperature of column: 40°C , velocity of flow: 1.0 ml/min, wavelength: 338, 262 nm (Pro), mobile phase: A: 20 mmol sodium acetate solution, B: 20 mmol sodium acetate solution: methanol: acetonitrile = 1:2:2 (v/v/v).

The components and contents of sugars in cotton root exudates were determined using analysis/semi-preparation HPLC (Waters 600/650E, USA). The

analytical conditions were as follows: chromatographic column: Sugarpak1, 6.5 mm i.d. \times 300 mm, mobile phase: ddH₂O, velocity of flow: 0.4 ml·min⁻¹, temperature of column: 85°C. Each cotton line was tested in triplicate.

Statistical analyses

Data were verified for homogeneity of variance using Levene's test and subsequently, an analysis of variance was performed. Mean values were compared using Fisher's least significant difference. All statistical analyses were performed using Statgraphics Plus 5.0.

Results

Resistance of different cotton lines to cotton *F. oxysporum*

Different cotton lines were inoculated with cotton *F. oxysporum* under identical conditions, to determine their resistance level to cotton wilt disease. The disease index and incidence of each cotton line on days 20, 29 and 38 post-inoculation are given in Table 1.

Twenty days post-inoculation, all cotton lines showed obvious disease symptoms. The edges of infected leaves were wilted, appearing chlorotic and water stained at first, whereby the symptoms then gradually extended to the entire leaf. Both the incidence rate and the disease index of Zhong-23 maintained a stable but slow increase, while SM-3 had a more rapid increase. According to grading

standards of resistance types to cotton *F. oxysporum* (Ma 2007), Zhong-23 is a disease-resistant cultivar with good resistance, while SM-3 is a susceptible cultivar with poor resistance. Overall, both of the transgenic lines exhibited symptoms of infection three to 5 days, earlier than the conventional ones. The results obtained on day 20 indicated that, Zhong-41 had a higher incidence rate and disease index than its parent Zhong-23. Over the next two investigation days, Zhong-41 was significantly higher ($P < 0.05$) than Zhong-23 in both incidence rate and disease index. In comparison, when the susceptible cultivar and its transgenic line were investigated, GK-12 was significantly higher ($P < 0.05$) than SM-3 on day 20 in terms of both incidence rate and disease index. Over the subsequent two investigation days, however, the differences between them were not significant ($P > 0.05$), although, GK-12 was higher than SM-3 for both measures. These results indicate that, the disease resistance of Zhong-41, whose parent was a disease-resistant cultivar, significantly decreased due to the introduction and expression of the transgenes (*Cry1Ac* gene and *CpTI* gene). While for GK-12, whose parent was a susceptible cultivar, the disease resistance also decreased but not to a significant level. In comparison with their respective parental lines, the resistance of the two transgenic insect-resistant cotton lines to cotton *F. oxysporum* decreased.

The effects of cotton root exudates on the spore germination and mycelial growth of cotton *F. oxysporum*

The germination of *F. oxysporum* spores in the presence of root exudates collected from each cotton

Table 1 Virulence of *F. oxysporum* to different cotton lines (mean \pm SD)

Cotton lines	August 2		August 10		August 19	
	Disease index	Incidence rate (%)	Disease index	Incidence rate (%)	Disease index	Incidence rate (%)
Zhong-23	39.4 \pm 8.1a*	46.7 \pm 12.7a	41.1 \pm 5.1a	47.4 \pm 8.0a	41.6 \pm 0.8a	47.4 \pm 8.1a
Zhong-41	52.8 \pm 8.4a	65.1 \pm 3.9a	53.3 \pm 0.6b	64.3 \pm 1.6b	76.0 \pm 6.1b	79.6 \pm 5.3b
SM-3	38.5 \pm 6.2A*	45.3 \pm 6.4a	53.7 \pm 12.7a	61.8 \pm 11.6a	63.7 \pm 2.7a	72.1 \pm 6.8a
GK-12	61.4 \pm 4.1B	66.3 \pm 8.3b	67.5 \pm 12.9a	72.1 \pm 8.0a	74.8 \pm 6.8a	83.7 \pm 5.3a

The table shows the disease index and incidence of transgenic (Zhong-41 and GK-12) and parental (Zhong-23 and SM-3) cotton lines on days 20, 29 and 38 after incubation with the spore suspension of cotton *F. oxysporum*. The resistance to cotton *F. oxysporum* of transgenic insect-resistance cotton lines decreases as compared to their respective parental lines

Asterisks * indicates that the small and capital letters in the same column showed significant difference between transgenic cotton and its parental cotton at $P < 0.05$ and $P < 0.01$, respectively

line was tested on concave glass slides; all rates are shown in Table 2. Spore germination rate was significantly higher ($P = 0.001$) in the presence of root exudates from Zhong-41 than in root exudates from the parent Zhong-23. Similarly, the spore germination rate in the presence of root exudates from GK-12 was higher, than in the presence of root exudates from the conventional parental cotton line, however, this difference was not statistically significant ($P = 0.059$). These results indicated that the root exudates from the two transgenic lines promoted the spore germination of cotton *F. oxysporum* in comparison with their parental lines, and that the root exudates from Zhong-41 had the most significant promoting effect on spore germination.

The effects of the root exudates from the two transgenic lines and their respective parental lines on the mycelial growth of *F. oxysporum* were tested through plate culture (Table 2). Overall, the colony diameters on PDA containing root exudates from Zhong-41 were significantly larger ($P < 0.05$) than colonies grown on PDA containing exudates from Zhong-23. Likewise, the colony diameters on PDA containing root exudates from GK-12 were larger than colonies grown on PDA containing exudates from SM-3, however, the colony diameters were not significantly different ($P > 0.05$). This was consistent with the results obtained from the spore germination trials. Compared with the root exudates from the parental lines, the root exudates of the two transgenic lines promoted the mycelial growth of *F. oxysporum*

and the root exudates of Zhong-41 had a significant growth-promoting effect.

Contents of amino acid and sugars in cotton root exudates

Sixteen amino acids were detected in root exudates of the four lines (Fig. 1), including: Threonine (Thr), Alanine (Ala), Valine (Val), Isoleucine (Ile), Aspartic acid (Asp), Leucine (Leu), Phenylalanine (Phe), Glycine (Gly), Methionine (Met), Histidine (His), Glutamic acid (Glu), Tyrosine (Tyr), Lysine (Lys), Serine (Ser), Arginine (Arg) and Proline (Pro). The most abundant amino acids were Gly, Glu, Ser, Ile, Asp and Thr. The root exudates of Zhong-41 contained two additional amino acids, Met and Lys, than Zhong-23, and the contents of some, including Asp, Glu, Ser, Ala, Val, Leu and Tyr were higher. No significant differences were found in the other amino acids contents, between the root exudates of Zhong-41 and Zhong-23. The amino acid components were the same in the root exudates of SM-3 and GK-12, with the latter having significantly lower levels ($P < 0.05$) of Tyr, Val and Leu.

Significant differences were found in the kinds and contents of sugars identified in the root exudates of the transgenic lines with respect to their parental lines (Fig. 2). Four sugars were detected in the root exudates of Zhong-41, glucose, fructose, maltose and an unknown sugar, while only glucose was detected in the root exudates of Zhong-23. In the root exudates

Table 2 Effects of the root exudates from transgenic (Zhong-41 and GK-12) and parental (Zhong-23 and SM-3) cotton lines on cotton *F. oxysporum* (mean \pm SD)

Cotton lines	Spore germination rate (%)	Average colony diameters (cm)				
		2nd day	3rd day	4th day	5th day	6th day
Zhong-41	78.53 \pm 3.68A*	2.40 \pm 0.21a	3.99 \pm 0.07A	5.48 \pm 0.13A	6.90 \pm 0.10a	7.84 \pm 0.08A
Zhong-23	48.41 \pm 4.58B	2.03 \pm 0.33a	3.58 \pm 0.08B	5.00 \pm 0.09B	6.46 \pm 0.25b	7.49 \pm 0.05B
SM-3	85.25 \pm 1.91a*	2.28 \pm 0.18a	3.88 \pm 0.18a	5.28 \pm 0.07a	6.79 \pm 0.16a	7.78 \pm 0.05a
GK-12	89.77 \pm 2.28a	2.37 \pm 0.23a	4.02 \pm 0.11a	5.40 \pm 0.09a	6.89 \pm 0.21a	7.86 \pm 0.12a

Column 2 shows the germination rate of *F. oxysporum* spores in the presence of root exudates collected from transgenic (Zhong-41 and GK-12) and parental (Zhong-23 and SM-3) cotton lines. Columns 3–7 shows the average colony diameters of *F. oxysporum* in the presence of root exudates collected from two transgenic lines and their respective parental lines at each stage of development from 2nd to 6th day. The root exudates of the two transgenic cotton lines promote the spore germination and mycelial growth of *F. oxysporum* as compared to their respective parental lines, and the root exudates of transgenic *Bt* and *CpTI* cotton line, Zhong-41 have the most significant promoting effect

Asterisks * indicates that the small and capital letters in the same column showed significant difference between transgenic cotton and its parental cotton at $P < 0.05$ and $P < 0.01$, respectively

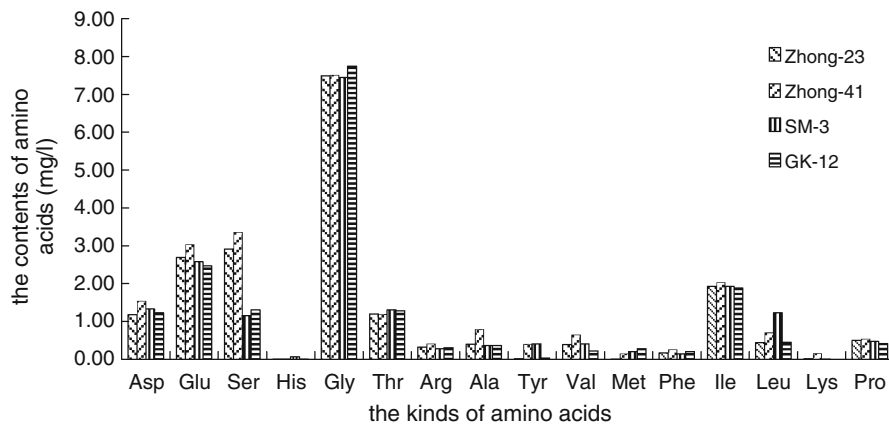


Fig. 1 The components and contents of amino acid in the root exudates from transgenic (Zhong-41 and GK-12) and parental (Zhong-23 and SM-3) cotton lines. The components and contents of the amino acids were determined using high performance liquid chromatography (HPLC) for amino acids. The root exudates of Zhong-41 contain two additional amino

acids (Met and Lys), and the contents of Asp, Glu, Ser, Ala, Val, Leu and Tyr are higher compared to its parental Zhong-23. The amino acid components are the same in the root exudates of SM-3 and GK-12, with the latter having significantly lower levels ($P < 0.05$) of Tyr, Val and Leu

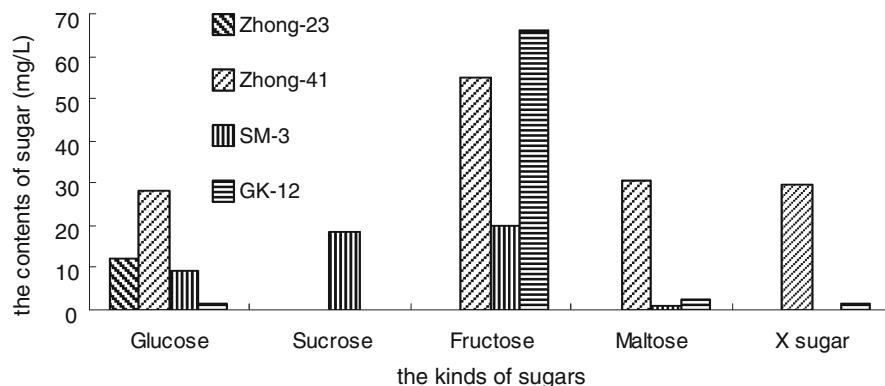


Fig. 2 The components and contents of sugar in the root exudates from transgenic (Zhong-41 and GK-12) and parental (Zhong-23 and SM-3) cotton lines. The components and contents of sugars in cotton root exudates were determined using analysis/semi-preparation HPLC. Glucose, fructose, maltose and an unknown sugar were detected in the root

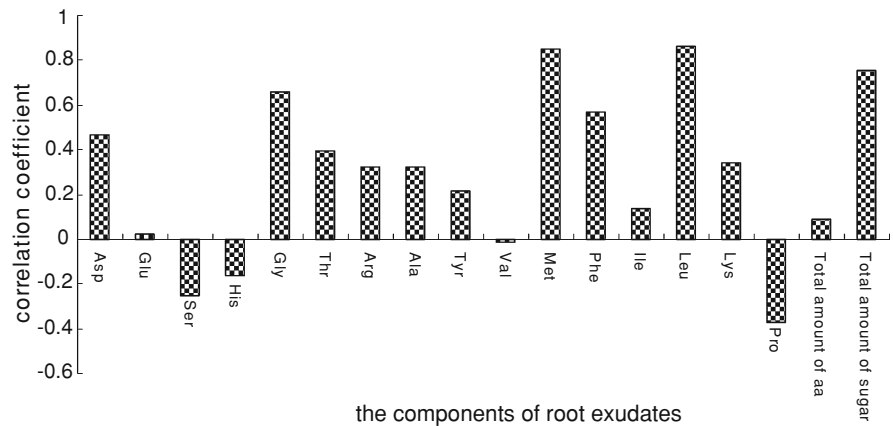
exudates of Zhong-41, while only glucose was detected in the root exudates of Zhong-23. Glucose, fructose and maltose were detected in the root exudates of GK-12 and SM-3. In addition, the root exudates of SM-3 also contained sucrose, while the exudates of GK-12 contained an unknown sugar

of GK-12 and SM-3, glucose, fructose and maltose, were detected in both lines. The root exudates of SM-3 also contained sucrose, while the exudates of GK-12 contained an unknown sugar. The total amount of the sugars in the root exudates of Zhong-41 and GK-12 (143.25 mg/l and 71.49 mg/l, respectively) were significantly higher ($P < 0.05$) than that, in their respective parental lines Zhong-23 and SM-3 (12.30 mg/l and 48.59 mg/l, respectively).

Analysis of the correlation between the components and contents of amino acids and sugars in the root exudates and the disease indices of different cotton lines

Many studies have shown that there is a correlation between the composition of a plant's root exudates and the plant's resistance to pathogens (Claudius and Mehrotra 1972; Shen and Zhang 1999; Bais et al.

Fig. 3 Correlation between the cotton root exudates and the disease indices of the cotton lines to *F. oxysporum*. The figure shows the correlation coefficients for the contents of amino acids and sugar in root exudates from transgenic (Zhong-41 and GK-12) and parental (Zhong-23 and SM-3) cotton lines and their disease indices to cotton *Fusarium oxysporum*



2006; Han et al. 2006b). Due to the lower wilt resistance of the two transgenic lines compared to the parental lines, we analyzed the correlation between the contents of amino acids and sugars in the root exudates and the disease resistance indices of the cotton lines to *F. oxysporum* (Fig. 3). The results showed that there was a negative correlation between the contents of four amino acids (Ser, His, Pro and Val) in the root exudates and the disease indices for *F. oxysporum*. The content of Pro had the highest correlation, but this was not found to be significant ($r = -0.375$, $P > 0.05$). However, the contents of the other 12 amino acids were positively correlated with disease index for *F. oxysporum*, and the contents of Leu, Met and Phe had a significantly higher correlation ($P < 0.05$), where $r = 0.861$, 0.851 and 0.567 . Overall, the total amount of the amino acids in the root exudates had a low correlation with disease index ($r = 0.092$), and this relationship was not statistically significant ($P > 0.05$). Conversely, the correlation between the total amount of sugars in the root exudates and the disease index of *F. oxysporum* was significantly high ($r = 0.752$, $P < 0.05$).

Discussion

The attenuation of disease resistance in transgenic insect-resistant cotton to pathogens such as cotton *F. oxysporum* has become one of the important factors restricting cotton production in China. The results from this study showed that the resistance of GK-12 and Zhong-41 to cotton *F. oxysporum* declined in

comparison with their parental lines. These findings were consistent with the results obtained from previous field experiments and investigations (Wu et al. 1998; Wu 2000; Zhu and Feng 2005). Because cotton *F. oxysporum* invades the cotton seedling from the root, it is necessary to explore the reasons for the attenuation of disease resistance in transgenic cotton, taking into account the components and composition of the root exudates.

The relation between the root exudates and the plant's resistance to *Fusarium* wilt, is connected with the components from the root exudates and the microorganisms present in the rhizosphere (including cotton *F. oxysporum*) (Liu et al. 1998; Steinberg et al. 1999; Bertin et al. 2003; Steinkellner et al. 2005; Bais et al. 2006). Root exudates from disease-resistant lines are known to supply fewer nutrients to *F. oxysporum* (Claudius and Mehrotra 1972; Naqvi and Chauhan 1980; Steinkellner et al. 2008), or to contain inhibitory substances to pathogens (Nóbrega et al. 2005). Yuan et al. (2002) and Han et al. (2006a) studied the effects of the root exudates from cucumber and cotton on *Fusarium* and *Verticillium dahliae*, and found that the root exudates from resistant lines inhibited spore germination and mycelial growth, while the root exudates of the susceptible lines promoted growth of the pathogen. The results from our study showed that the root exudates of the transgenic lines promoted spore germination and mycelial growth of cotton *F. oxysporum* in comparison with the root exudates from their respective parental lines, in which the root exudates from Zhong-41 significantly promoted both the spore germination and mycelial growth of cotton

F. oxysporum. This is possibly one of the main reasons for the observed attenuation of resistance to *F. oxysporum*.

Feng et al. (1991) found that the root exudates from susceptible lines contained higher levels of Ala, Asp and Glu than the resistant lines. They also identified that some amino acids, including, Ala, Ser, Glu, Asp, Phe, Tyr and Val, significantly promoted the spore germination of *F. oxysporum*. Booth (1969) compared the amino acid contents of the root exudates of cotton lines that were either resistant or susceptible to Verticillium wilt. Among the eight amino acids tested in his study, the greatest difference in the amino acid content was found for Ala, and no major differences were determined in the contents of the other seven amino acids. Wu et al. (2007) also observed that Ala promoted spore germination of *V. dahliae*. The results of our study indicated that Zhong-41 had two additional amino acids (Met and Lys) than Zhong-23 had. Moreover, the contents of Asp, Glu, Ser, Ala, Val, Leu and Tyr in the root exudates of Zhong-41 were significantly higher ($P < 0.05$) than in Zhong-23, with Ala being the most abundant. The correlation analysis also showed that these amino acids were highly positively correlated with disease index. These results were consistent with Feng et al. (1991) and Booth (1969), indicating that the higher amino acid content in the root exudates stimulated spore germination in *F. oxysporum*. This was one of the possible reasons for the observed decline in resistance to cotton Fusarium wilt in the transgenic lines. In cotton lines SM-3 and GK-12, the same amino acid components were present in the root exudates. In terms of abundance, there was a great difference in the contents of Tyr, Val and Leu. However, the contents of the other amino acids (including the ones known to stimulate spore germination of *F. oxysporum*) had no notable differences. The results were consistent with the conclusion of Feng et al. (1991) and Booth (1969), and also explained, why there was no significant difference in the germination rates and mycelial growth of *F. oxysporum*, in the presence of the root exudates from any of the cotton lines tested. Our results indicated that the levels of the amino acids capable of stimulating growth of *F. oxysporum* were higher after the introduction of transgenes into the genomes of the parental cotton lines. This is possibly one of the reasons for the observed attenuation of disease resistance in the transgenic lines.

Yuan et al. (2002) and Wu et al. (2007) found that the contents and kinds of sugars in the root exudates of disease-susceptible cotton lines were more abundant, than in disease-resistant cotton. Our study indicated that the root exudates from Zhong-41 were more abundant and more diverse with regard to sugars, than those from Zhong-23. Moreover, there were large differences in the sugars identified in the root exudates of GK-12 and SM-3, and the correlation analysis showed that there was a significantly positive correlation between the contents of the total sugars in the root exudates and the disease index ($P < 0.05$). The increase in the kinds and contents of sugars in the root exudates, which provide a carbon source for the growth of *F. oxysporum*, might be another reason for the decreased disease resistance in the transgenic cotton lines.

Some research has indicated that the introduction of transgenes into plants results in unintentional changes in the phenotype, physiology and biochemistry of transgenic plants, and could effect the relationship between the transgenic plants and other organisms (Wolfenbarger and Phifer 2000; Yan et al. 2002; Chen et al. 2004; Ammann 2005). For instance, the density of trichomes covering the leaves of transgenic *Bt* cotton was significantly lower than in the parental cotton line. This difference facilitates aphid feeding on the transgenic leaves, causing cotton aphid breakouts to be heavier in transgenic *Bt* cotton fields, than in conventional ones (Xue et al. 2008). In this study, two transgenic cotton lines were found to have lower resistance to cotton *F. oxysporum* than their respective parental lines, and also to differ significantly in the kinds and contents of amino acids and sugars in their root exudates. This is another example of the unintentional changes that may have occurred in transgenic plants. In addition, we also found the resistance to Verticillium wilt was lower in the two transgenic cotton lines, and the root exudates promoted growth of the Verticillium wilt pathogen (unpublished data). Currently, we are investigating whether the resistance of other transgenic insect-resistant cotton lines to cotton *F. oxysporum* and other cotton pathogens, is universally lower than their respective parental lines.

Plant diseases caused by soil-borne pathogens result from multiple and complex interactions between plants and both abiotic soil compartments and biotic ones (e.g., microbial biomass and activity,

diversity and structure of microbial communities, and the induced respiration of the soil microflora after glucose supply) (Stotzky and Martin 1963; Alabouvette et al. 1986; Höper et al. 1995; Janvier et al. 2007). For example, soils with the addition of either montmorillonite or illite significantly increased the degree of soil suppressiveness to *Fusarium* wilts (Stotzky and Martin 1963; Höper et al. 1995). The suppressive effect of soil disappeared when soil organisms were destroyed by steam, but, can be restored by blending a mixture of microorganisms into the previously disinfected soil (Alabouvette et al. 1986). The soil used in the present study was treated through moist heat sterilization at 121°C for 3 h to eliminate the influence of soil organisms. Therefore, the result of resistance of the two transgenic lines to *F. oxysporum* is supposed to reflect the direct interaction between transgenic cotton and *F. oxysporum*. In addition, many lines of transgenic cotton have been planted commercially in Yangtze and Yellow River Basins of China, where the types of soil mainly include cinnamon soil, brown soil, paddy soil, and skeleton soil. However, the resistance of transgenic cotton lines to *F. oxysporum* was found to decrease in all these soil types. This study concentrated on the resistance of transgenic cottons to *F. oxysporum* in only one type of soil. Therefore, further investigations on the disease resistance of transgenic cotton lines to *F. oxysporum* in other soil types are expected.

Cry proteins have been detected in root exudates of transgenic *Bt* corn, rice, potato and cotton (Saxena and Stotzky 2000; Saxena et al. 1999, 2004; Gupta and Watson 2004; Rui et al. 2005; Knox et al. 2007; Icoz and Stotzky 2008b). Fungi appear to be the organisms most affected by Cry proteins released to soil, in root exudates (Turrini et al. 2004; Castaldini et al. 2005; Villányi et al. 2006; Icoz and Stotzky 2008a). Therefore, it is necessary to detect the transgenic proteins (*CryIAc* toxin and *CpTI* protein) in root exudates of transgenic cotton lines, as well as, their influences on *F. oxysporum* and other soil-borne pathogens.

The mechanisms for plant pathogen resistance include: the organizational structure mechanism, the physiological and biochemical mechanisms and the micro-ecological mechanisms. The differences in the components and contents of amino acids and sugars in the root exudates of transgenic and their respective parental cotton lines were possibly one of the key reason for the attenuation of resistance in

transgenic cotton to cotton *F. oxysporum*. However, besides amino acids and sugars, the cotton root exudates are also composed of other substances, such as organic acids, phenols and growth regulators. It is important to study the changes of the other components in root exudates of transgenic insect-resistant cotton, as well as, their effects on cotton pathogen resistance.

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