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Root exudates of transgenic cotton and their effects on *Fusarium oxysporum*

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1. ABSTRACT

The components of the root exudates from two transgenic insect-resistant cotton lines and their parental cotton lines, and their effects on the growth of *Fusarium oxysporum* were investigated. The results demonstrated that the resistance of transgenic insect-resistant cotton to *F. oxysporum* was significantly reduced compared with their parental lines. Likewise, the root exudates from transgenic insect-resistant cotton significantly promoted the spore germination and mycelial growth of cotton *F. oxysporum*. The types of compounds found in the root exudates of transgenic insect-resistant cotton were similar to those of the parental cotton, but the composition and relative content of the compounds were different. The type and content of the fatty acids and esters were significantly reduced in the root exudates of the transgenic insect-resistant cotton, as were certain specific materials, whereas several alkanes were increased. The inhibition of the soil-borne pathogen *F. oxysporum* caused by the root exudates from the transgenic insect-resistant cotton was decreased compared with the parental cotton. This result provides a scientific basis for the decline in disease resistance in transgenic insect-resistant cotton.

2. INTRODUCTION

Cotton is an economically important crop in China. Cotton *Fusarium* wilt, a vascular disease caused by the soil-borne *F. oxysporum* f. sp. *vasinfectum*, affects the stable yield of cotton all over the world and is a major constraint on cotton production in China (1, 2). Transgenic insect-resistant cotton expressing the Cry1Ac and/or CpTI protein was first released in China in 1997 and was able to effectively control the cotton bollworm (*Helicoverpa armigera*), thus protecting the environment by reducing the application of chemical insecticides and conferring great socioeconomic benefits (3). At present, transgenic insect-resistant cotton represents more than 70% of the total cotton grown in China (4). However, transgenic proteins, such as Cry1Ab, can be released into the soil by means of root exudates and cotton residuals during the course of its growth and after harvest (5-9), which can cause environmental risks, such as adverse effects on soil microorganisms and soil invertebrates (10-15). In biosafety assessments, the ecological risk of foreign proteins expressed by transgenic plants has attracted more attention in the scientific community (16-20) than the unpredictable changes caused by the compulsory insertion of exogenous

genes and the cell and tissue cultures used during the transgenic manipulation (21-23). These associated potential risks have not received enough attention (24). A remarkable problem arising from unexpected changes in transgenic insect-resistant cotton is a clear decrease in the resistances to *Verticillium* and *Fusarium* wilts compared with those of conventional cotton (25-28).

The attenuation of the resistance of transgenic insect-resistant cotton to different diseases has been widely reported in China (23-27, 29-30). For example, the resistances to *Verticillium* wilt, *Fusarium* wilt, cotton leaf spot, and red leaf blight of transgenic insect-resistant cotton have declined to different degrees, with the most significant decline being in *Fusarium* wilt resistance (26-27). Zhu et al. (2005) evaluated the disease resistance of 35 transgenic cotton varieties and showed that transgenic insect-resistant cottons were inferior to conventional cottons against either *Fusarium* wilt or *Verticillium* wilt or against both wilts (27).

The attenuation of the disease resistance of transgenic insect-resistant cotton negatively impacted cotton production in China and resulted in an increased use of chemical pesticides for disease management. Allelochemicals, released from root exudates, play an important role in the allelopathic effect on soil-borne pathogens (31). The root exudates provide energy for the multiplication of rhizosphere microorganisms, influence these species and their quantitative distribution, and play an important role in the resistance to soil-borne disease (32). Thus, a study on the effect of the allelochemicals from root exudates is indispensable in a systematic discussion of the mechanism of the attenuated resistance of transgenic insect-resistant cotton to *Fusarium* wilt. Considering these observations, this study was performed to determine the types and amounts of the complex secondary compounds present in the root exudates of cotton and to investigate the influence of the cotton root exudates on the resistance of transgenic insect-resistant cotton varieties and their parental cotton lines to *F. oxysporum.*

2. MATERIALS AND METHODS

3.1. The cotton lines and the pathogen

The transgenic cotton line Zhong-41 (*Cry1Ac* plus *CpTI* gene) and its parental cotton line Zhong-23, along with the transgenic cotton line Zhong-30 (*Cry1Ac* gene) and its parental cotton line Zhong-16, were obtained from the Cotton Research Institute (CRI) of the Chinese Academy of Agricultural Sciences, Anyang, China.

In our study, the highly virulent strain of *F. oxysporum* f. sp. *vasinfectum* (Atk.) Snyder and Hansen was used. This fungus is a race-7 strain that causes *Fusarium* wilt disease and is responsible for significant yield losses of cotton throughout the world, including in China. This strain was also obtained from the CRI.

3.2. The collection and treatment of cotton root exudates

The cottons were cultivated under sterile hydroponic conditions as previously described by Li *et al.* (2009). Briefly, the cotton seeds were surface-disinfected with 75% ethanol followed by 0.1% HgCl2, and the seeds were transferred to 180-mm-diameter Petri dishes containing PDA medium to be pre-germinated. The seedlings with micro-roots were cultured in 50-mL beakers containing sterile Hoagland's nutrient solution. The 50-mL beakers were then placed in 2-L beakers and covered with filter paper to avoid any microbial contamination. The plants were cultured in the biochemical incubator with a regimen of 30±2 C in the light (14 h) and 20±2 C in the dark (10 h) with 50% relative humidity. The Hoagland's nutrient solution was replaced every 3 d.

After 40 d of cultivation, the plantlets (three per treatment) were placed into sterile 800-ml beakers containing 500 mL of sterile deionized water. The beakers were covered with aluminum foil to generate dark conditions for the roots and were maintained in a biochemical incubator at 30 C for 24 h to collect the root exudates. The collected exudates were concentrated to 100 ml using a vacuum rotary evaporator (Eppendorf AG, Hamburg, Germany) at 40 C. The concentrated root exudates were immediately stored at -20 C for analysis. The experiments described above were conducted three times under identical conditions.

3.3. The effect of the cotton root exudates on the spore germination of *F. oxysporum*

For the preparation of the conidial suspensions, a block of the stock culture was activated on PDA medium for 3 d at 28 C in the dark and then transferred to new PDA medium for 5 d at 28 C in the dark. The mycelium from 5 Petri dishes was scraped and mixed with 25 mL sterile deionized water, which was blended two times. The spore suspension was concentrated (3000×g, 10 min) in a sterile centrifuge tube and adjusted to a final concentration of 1×107conidia ml−1 in sterile distilled water using a hemocytometer. The collected exudates were filtered through 0.22-μm sterile filters (Peninsula, Millipore, China), and 50 μL of the root exudates was mixed with 50 μL of spore suspension and incubated at 25 C in the dark. After 6 h, the spore germination was determined microscopically by counting 10 fields (40× objective)/well. All of the experiments were performed in triplicate and independently replicated three times.

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

Aliquots (25 mL) of the root exudates were filtered through 0.22-μm sterile filters, mixed with 100 mL of PDA medium and poured into 90-mm diameter Petri dishes. A 0.5-cm-diameter homochronous mycelial mass of *F. oxysporum* was placed onto the center of the plate and cultured at 28 C in a thermostatic chamber (STABILITHERM^{TM}234EU2/EB2, USA). The colony diameters were measured using vernier calipers until the PDA plates were filled with mycelia. The experiments were performed in triplicate and independently replicated three times.

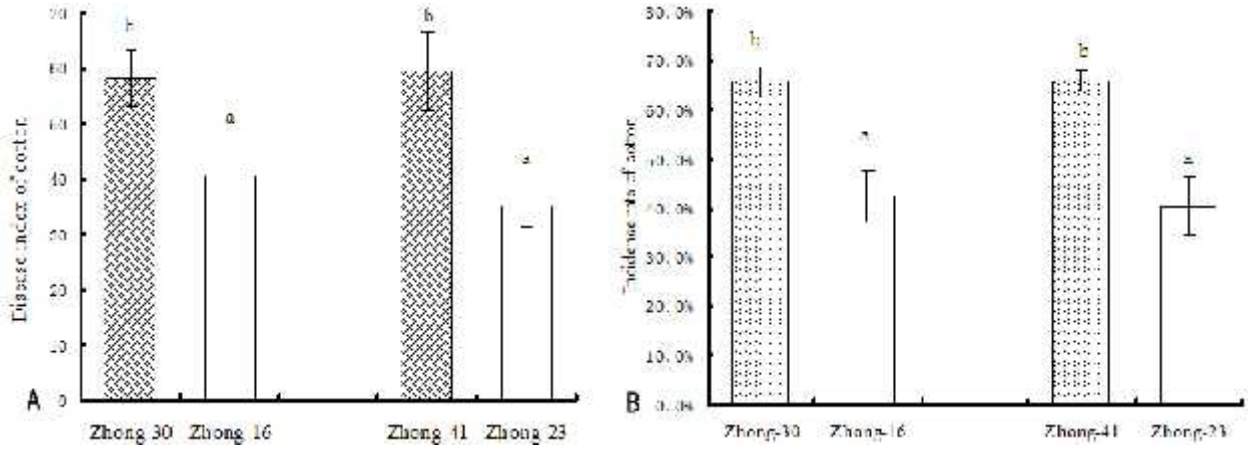

Figure 1. The disease index (A) and the incidence rate (B) of *Fusarium* wilt of cottons infected with *F. oxysporum*. The lowercase letters show the significant difference between transgenic cotton and its parent control cotton (P<0.05).

3.5. The resistance of cotton to *F. oxysporum*

This experiment used a sandy-textured soil in which cotton had never been planted. Aliquots (1 kg) of soil were uniformly mixed with 0.05 kg of organic fertilizer and moist heat sterilized twice at 121C for 3 h to obtain sterile soil for cultivation. The cotton seeds were surface-delinted in concentrated sulfuric acid and then immersed in sterile distilled water for 8 h. The cotton seeds were planted in plastic pots containing the sterile soil and then transferred to a greenhouse (day: 25-30 C, night: 20-25 C). Each cotton line was planted in triplicate, and each repetition contained fifteen cotton seedlings. At the 3-leaf stage, the roots were partially cut using a sterile knife blade and inoculated with 5 mL of a 1×10^7 conidia ml^{-1} spore suspension. After 25 d of inoculation, the degree of disease was surveyed according to the grading standards of cotton *F. oxysporum* in cotton seedlings (33).

3.6. GC-MS analysis of root exudates

The concentrated root exudates were extracted three times with 30 mL of anhydrous ether. The ether fractions were dried over anhydrous CaSO4 and concentrated to 2 mL in a rotary evaporator at 40 C. The gas chromatographic conditions for the sample analyses were derived from Wu et al. (2000), with slight modifications (34). A DP-5 capillary column (5% phenyl-substituted methylpolysiloxane) of 30 m × 0.25 mm i.d. was used to introduce 5 µL of the concentrated root exudate with a stationary-phase thickness of 0.25 µm. The column temperature was initially held at 80 C for 1 min, then programmed to 160 C at a rate of 10 C/min, from 160 C to 235 C at a rate of 5 C/min, and from 235 C to 280 C at a rate of 50 C/min, with a final hold time of 5 min (total run time = 31 min). Helium was used as the carrier gas with a linear velocity of 34 cm/s. The injector temperature was maintained at 280 C. The ionization voltage and temperature in the electron impact (El) mode were 70 eV and 200 C, respectively. The components were identified by comparison of the retention times and mass spectral data (NIST98).

3.7. Statistical analyses

The data were verified for the homogeneity of variance using Levene's test, and an analysis of variance was subsequently performed. The mean separations were performed using Duncan's multiple range tests. Differences at P=0.05 were considered to be significant.

4.RESULTS

4.1.The resistance of different cotton lines to cotton *F. oxysporum*

The disease indices and incidences of each cotton line on day 25 post-inoculation are given in Figure 1. 25 d post-inoculation, all of the cotton lines showed noticeable disease symptoms. The transgenic insect-resistant cotton lines Zhong-41 and Zhong-30 showed significantly higher incidence rates and disease indices compared with their parents, Zhong-23 and Zhong-16, respectively (Figure 1). Therefore, the resistance of the transgenic insect-resistant cotton to cotton *F. oxysporum* decreased significantly compared with that of their parental cotton lines.

4.2. The effects of cotton root exudates on the growth of cotton *F. oxysporum*

The germination rates of *F. oxysporum* spores in the presence of the root exudates collected from each cotton line are shown in Figure 2. The root exudates collected from the transgenic lines (Zhong-41 and Zhong-30) demonstrated significantly higher spore germination of cotton *F. oxysporum* compared with the exudates from the parental lines (Zhong-23 and Zhong-16, respectively). In terms of the mycelial growth of *F. oxysporum*, the colony diameters on the PDA containing the Zhong-30 root exudates were larger than those on the PDA containing the Zhong-16 root exudates, with the differences being significant on days 5 and 6 (Table 1, P<0.05). Similarly, the colony diameters on the PDA containing the Zhong-41 root exudates were significantly larger than those on PDA containing the Zhong-23 root exudates during the entire experimental period (Table 1, P<0.05). In addition, the root exudates from all of the cotton lines significantly

Table 1. The effects of the root exudates from different cotton lines on the mycelial growth of *F. oxysporum*

Cotton lines	Average colony diameter (cm)			
	Second day	Third day	Fifth day	Sixth day
Zhong-41	3.03±0.09ac	3.95±0.07a	6.63±0.09ac	7.73±0.09ac
Zhong-23	2.57±0.05b	3.10±0.50b	6.13±0.12b	7.15±0.15b
Zhong-30	2.80±0.08a	3.70±0.08a	6.48±0.06a	7.57±0.02a
Zhong-16	2.67±0.08a	3.68±0.05a	6.22±0.10b	7.38±0.06b
Water	2.96±0.07c	4.11±0.10c	6.61±0.05c	7.72±0.07c

Within columns, means followed by different letters indicate a statistically significant difference (P<0.05) between the transgenic and parental cotton lines.

Table 2. Styles of compounds identified from root exudates of transgenic insect-resistant cotton lines and their parental lines

Cotton lines	Hydrocarbons	Alcohols	Acids	Esters	Phenols	Aldehydes and ketones	Olefins and amines	Heterocyclic
Zhong-30	69.76±21.22a	5.94±2.18a	8.44±3.55a	4.36±1.43a	5.40±2.30a	2.14±0.29a	1.92±0.62a	2.04±0.73a
Zhong-16	68.39±18.86a	6.12±1.86a	8.99±3.42a	4.92±1.27a	6.04±1.65a	2.47±0.95a	1.99±0.39a	1.08±0.28a
Zhong-41	63.01±18.65a	3.84±0.94a	13.80±2.64a	7.28±2.64	7.58±2.99a	Trace	4.49±1.97a	Trace
Zhong-23	55.95±15.78a	3.30±1.06a	22.32±4.86b	7.04±2.08	8.74±2.84a	Trace	1.12±0.74b	1.53±0.53

Within columns, means followed by different letters indicate a statistically significant difference (P<0.05) between the transgenic cotton and parental cotton lines.

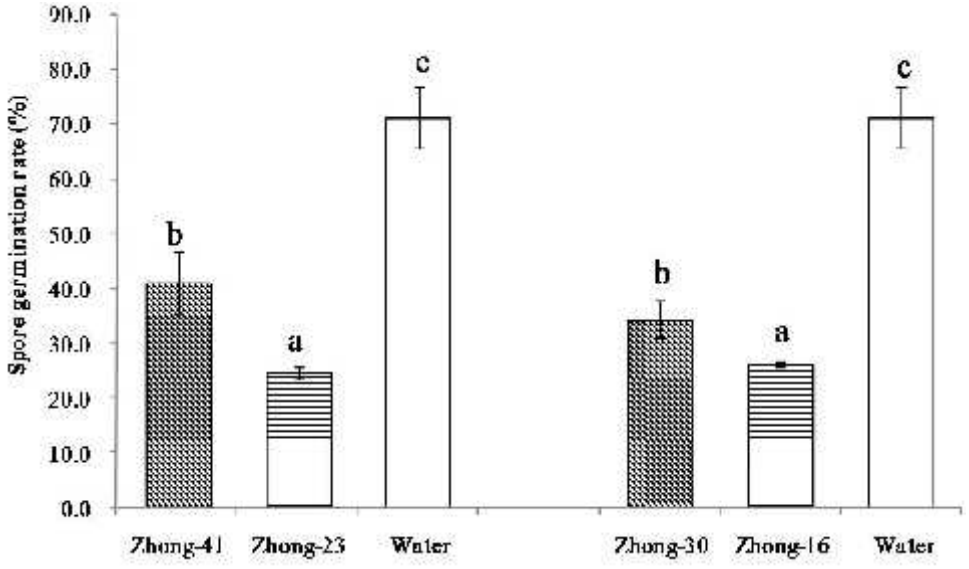

Figure 2. The effects of root exudates from different cottons on spore germination of *F. oxysporum*. The lowercase letters show the significant difference between transgenic cotton, its parent control cotton and control (P<0.05).

suppressed the growth of *F. oxysporum* compared with the control treatment (water).

4.3. The chemical composition of the root exudates from transgenic insect-resistant cotton and their parental cotton lines

The main components of the root exudates in the transgenic (Zhong-30 and Zhong-41) and parental (Zhong-16 and Zhong-23) cotton lines were basically similar. Eight substance types were detected in the root exudates of the transgenic cotton line Zhong-30 and its parental cotton line Zhong-16. Six and seven substances were detected in the root exudates of the transgenic cotton line Zhong-41 and its parental cotton line Zhong-23, respectively (Table 2). Hydrocarbons and acids were the main compounds detected in the root exudates. There were no significant differences for the eight substance types detected in the root exudates of the transgenic Zhong-30 and its parental Zhong-16 cotton lines. The content of acids was significantly higher in the root exudates of the parental cotton line Zhong-23 than in the transgenic cotton line Zhong-41. The content of amines was significantly lower in the root exudates of Zhong-23 than in Zhong-41. For the other detected substances, there were no significant differences between Zhong-41 and Zhong-23 (Table 2).

4.4. Comparison of the chemical composition of the root exudates in the transgenic insect-resistant cotton and their parental cotton lines

The compositions of the root exudates in the transgenic (Zhong-30 and Zhong-41) and parental (Zhong-16 and Zhong-23) cotton lines determined by GC-MS analysis are shown in Figure 3. The content of 2,6,10-trimethyl tetradecane in the transgenic Zhong-30 line was significantly higher than that in Zhong-16, but no significant differences were observed for the other detected compounds. In the root exudates of the transgenic Zhong-41 line, the contents of 2,6,10-trimethyl pentadecane,

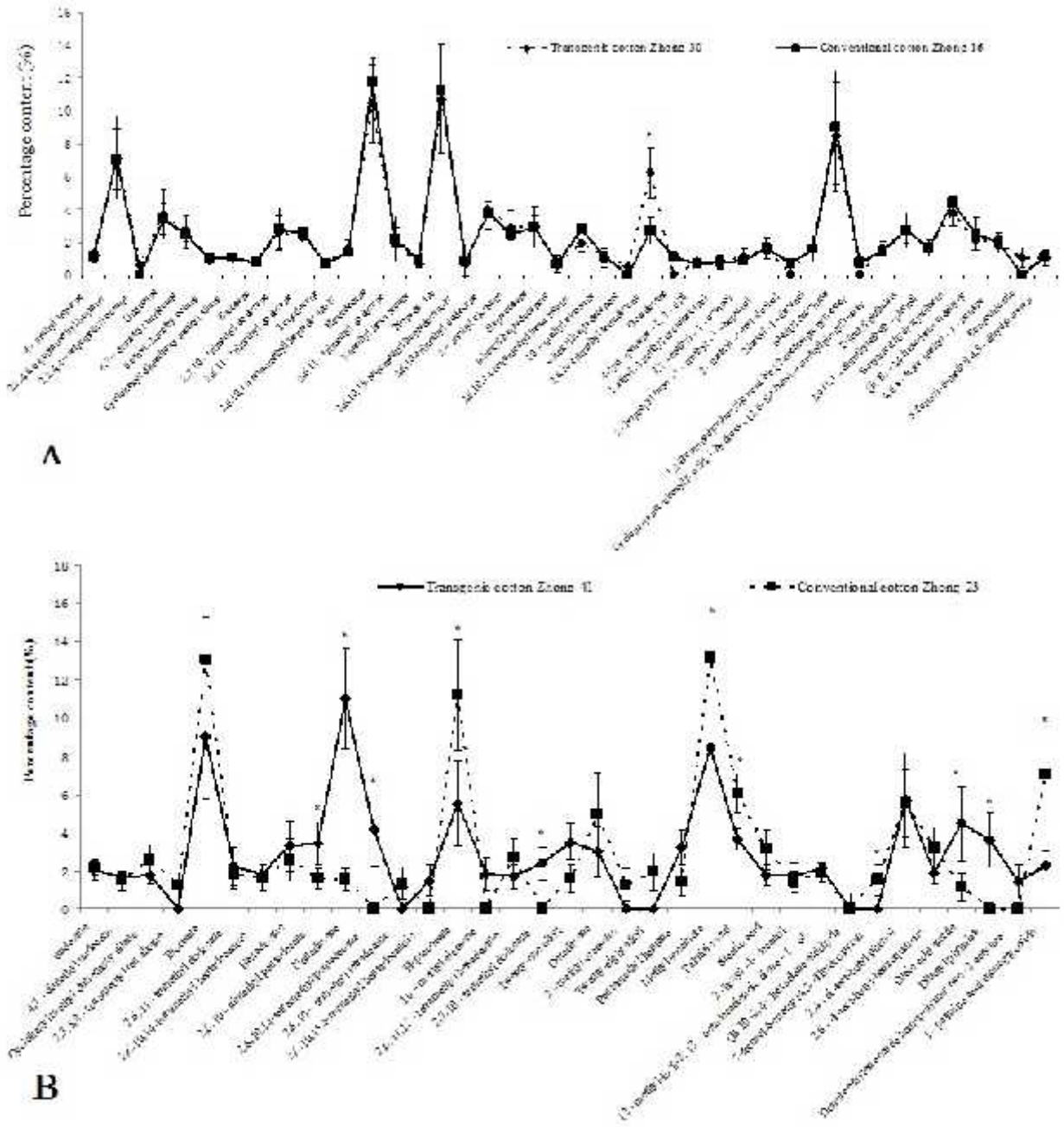

Figure 3. Relative contents of compounds identified from the root exudates of transgenic insect-resistant cotton lines Zhong-30 (A), Zhong-41 (B) and their parental lines Zhong-16, Zhong-23 respectively. "*" indicate a statistically significant difference between transgenic cotton and parental cotton where $P<0.05$.

nonadecane, 2,6,10,14-tetramethyl pentadecane, 2,7,10-trimethyl dodecane, oleic acid amide, and dibutyl phthalate were significantly higher, and the contents of heptacosane, methyl succinic acid, palmitic acid, 5-formyl-6-methyl-4,5-dihydropyran and 1-palmitic acid monoglyceride were significantly lower compared with its parental cotton line Zhong-23. Overall, compared with their respective parental cotton lines, the compounds in the root exudates from the transgenic *Cry1Ac* cotton changed slightly, whereas the compounds changed to a greater extent in the root exudates from the transgenic *Cry1Ac* and *CpTI* cotton.

5. DISCUSSION

In recent years, the attenuation of disease resistance to the *Fusarium* and *Verticillium* wilts in transgenic insect-resistant cotton due to unintentional changes has become an interesting research topic for the

scientific community. The results of this study showed that the resistance of Zhong-30 and Zhong-41 to cotton *F. oxysporum* declined significantly compared with that of their parental cotton lines. These findings were largely consistent with the results obtained from previous field experiments and investigations (27-28, 35). Cotton *F. oxysporum* is a soil-borne pathogen that invades the cotton seedling from the root, and thus, studying the effect on cotton *F. oxysporum* of allelochemicals derived from the root exudates of transgenic insect-resistant cotton is necessary. In addition, the mechanism underlying the attenuated resistance of transgenic insect-resistant cotton to *Fusarium* wilt should be explored.

Root exudates from disease-resistant varieties are known to supply fewer nutrients to *F. oxysporum* or to contain substances that are inhibitory to pathogens (36-37). Yuan et al. (2002) and Han et al. (2006) investigated the effects of the root exudates from cucumber and cotton on *Fusarium* and *Verticillium dahliae* and demonstrated that the root exudates from resistant varieties inhibited spore germination and mycelial growth whereas the root exudates of the susceptible varieties promoted the growth of the pathogens (38-39). In the present study, the root exudates of cottons were collected under sterile hydroponic conditions, and their effects on the spore germination and mycelial growth of *F. oxysporum* were studied. The results showed that the root exudates of the transgenic lines significantly promoted the spore germination and mycelial growth of cotton *F. oxysporum* compared with the root exudates from their parental lines. However, the root exudates of both the transgenic insect-resistant and parental cotton lines significantly inhibited the germination rate and mycelial growth of *F. oxysporum* compared with the control treatment (water). These results indicated that the inhibitory capability of the root exudates from the transgenic cottons on the growth of *F. oxysporum* was decreased after the compulsory insertion of exogenous genes, which consequently changed the disease resistance of the transgenic cotton lines.

The relationship between the root exudates and the plant's resistance to *Fusarium* wilt is connected to the components of the root exudates (40-41). The root exudates produced by plant secondary metabolism include various allelopathic inhibitory substances, e.g., autotoxins, as well as allelopathic acceleratory substances (42-44). Thus far, several low-molecular-weight organic compounds, such as long-chain fatty acids, benzoic acid and its derivatives, straight-chain alcohols, aliphatic aldehydes, ketones, phenols, and alkanes and their derivatives, are considered to be allelochemicals (43-44). Ju et al. (2002) reported that high-concentration phthalic acid and malonic acid have allelopathic inhibitory effects on the growth of the pathogenic fungi that cause soybean root rot. They inferred that the low pH value caused by the high-concentration organic acids was unsuitable for the growth of the pathogenic fungi (45). Chai et al. (2007) found phthalic acid, an allelochemical, in the root exudates of maize with strong autotoxicity. Esters, amines ketones, and acids in the root exudates of cotton were reported to be botanical antimicrobial substances that could act as growth inhibitors (43, 46). In the present study, we determined by GC-MS the composition of cotton root exudates with respect to different types of components that are thought to be allelochemicals. Several acids (such as 2-methylbutanedioic acid, palmitic acid and stearic acid) and esters (such as dibutyl phthalate, methylation ester, 1-palmitic acid monoglyceride) were decreased, with relatively low contents, in the root exudates of the transgenic insect-resistant cotton. Certain alkanes (such as 9-hexyl-17-alkyl, 2,6,10-trimethyl-tetradecane, 2,3,5,8-tetramethyl dodecane, 2,6,10-trimethyl tetradecane, 2-methyleicosane, octacosane and nonadecane) were increased in the root exudates of transgenic insect-resistance cotton lines, with relatively higher contents. Additionally, certain aldehydes, ketones, and phenols were significantly reduced. In summary, the types and contents of the acids and esters were significantly reduced, certain alkanes were significantly induced, and certain other chemicals were reduced in the transgenic cotton compared with the conventional parental cotton. Therefore, we speculated that the changing pattern of the chemical composition of the root exudates of the transgenic insect-resistant cotton might have led to the change in the disease resistance compared with their parental cotton lines.

The compulsory insertion of exogenous genes and the cell and tissue cultures used during the transgenic manipulation can cause unexpected changes in the growth and physiological characteristics of transgenic plants (21, 47-49). For example, compared with conventional cotton, transgenic insect-resistant cotton in China appeared to have thriving vegetative and reproductive growth and significantly more active carbon and nitrogen metabolism (50). Our early studies also found that the contents of glucide and amino acids/proteins in the leaf and root exudates of transgenic insect-resistant cotton were significantly higher than in the parental lines (28, 51). These unexpected changes, similarly to the attenuation of disease resistance in transgenic insect-resistant cotton demonstrated in this study, may be caused by the insertion of foreign genes in transgenic plants, and particular attention should be paid to the stress resistance of these plants in transgenic biosafety research. The root exudates obviously play a vital role in the decline of the disease resistance observed in the transgenic insect-resistance cotton lines, but a comprehensive study on this issue must be performed because root exudates are complex and vary greatly in composition. In addition, only a subset of the allelochemical components can be extracted by organic solvents, and many of these compounds have biological activities, which can have cumulative effects. Therefore, the comprehensive effect of the root exudates needs to be determined to obtain a better understanding of the mechanism responsible for the decline in the disease resistance observed in transgenic insect-resistant cottons.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

1. JK Kochman: *Fusarium* wilt in cotton - a new record in Australia. *Aust Plant Pathol* 24, 74 (1995)

2. C Ma, GL Jian, CL Zheng: The advances in cotton breeding resistance to *Fusarium* and *Verticillium* wilts in china during past fifty years. *Scientia Agr Sinica* 35, 508-513 (2002)

3. KM Wu, YH Lu, HQ Feng, YY Jiang, JZ Zhao: Suppression of cotton bollworm in multiple crops in China in areas with *Bt* toxin-containing cotton. *Science* 321, 1676-1678 (2008)

4. R Stone: China plans $3.5 billion GM crops initiative. *Science* 321, 1279 (2008)

5. KK Donegan, CJ Palm, VJ Fieland, LA Porteous, LM Ganio, DL Schaller, LQ Bucao, RJ Seidler: Changes in levels, species, and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin. *Appl Soil Ecol* 2, 111–124 (1995)

6. CJ Palm, DL Schaller, KK Donegan, RJ Seidler: Persistence in soil of transgenic plant-produced *Bacillus thuringiensis* var. *kurstaki* d-endotoxin. *Can J Microbiol* 42, 1258–1262 (1996)

7. VVSR Gupta, S Watson: Ecological impacts of GM cotton on soil biodiversity: Below-ground production of *Bt* by GM cotton and *Bt* cotton impacts on soil biological processes. Australian Government Department of the Environment and Heritage, CSIRO Land and Water, pp 1–72 (2004)

8. YK Rui, GX Yi, J Zhao, BM Wang, ZH Li, ZX Zhai, ZP He, Q Li: Changes of *Bt* toxin in the rhizosphere of transgenic *Bt* cotton and its influence on soil functional bacteria. *World J Microbiol Biotech* 21, 1279–1284 (2005)

9. OGG Knox, VVSR Gupta, DB Nehl, WN Stiller: Constitutive expression of Cry proteins in roots and border cells of transgenic cotton. *Euphytica* 154, 83–90 (2007)

10. M Giovannetti, C Sbrana, A Turrini: The impact of genetically modified crops on soil microbial communities. *Biology Forum/Riv Biol* 98, 393–417 (2005)

11. BS Griffiths, S Caul, J Thompson, ANE Birch, C Scrimgeour, J Cortet, A Foggo, CA Hackett, PH Krogh: A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* Cry1Ab toxin. *Plant Soil* 275, 135–146 (2005)

12. BS Griffiths, S Caul, J Thompson, ANE Birch, C Scrimgeour, MN Andersen, J Cortet, A Messean, C Sausse, B Lacroix, PH Krogh: Soil microbial and faunal community responses to *Bt* maize and insecticide in two soils. *J Environ Qual* 35, 734–741 (2006)

13. I Icoz, D Saxena, D Andow, C Zwahlen, G Stotzky: Microbial populations and enzyme activities in soil in situ under transgenic corn expressing Cry proteins from *Bacillus thuringiensis*. *J Environ Qual* 37, 647–662 (2008)

14. L Hönemann, C Zurbrügg, W Nentwig: Effects of *Bt*-corn decomposition on the composition of the soil meso- and macroinvertebrate. *Appl Soil Ecol* 40, 203–209 (2008)

15. XG Li, B Liu, JJ Cui, DD Liu, S Ding, B Gilna, JY Luo, ZX Fang, W Cao, ZM Han: No evidence of persistent effects of continuously planted transgenic insect-resistant cotton on soil microorganisms. *Plant soil* 339, 247–257 (2011)

16. PJ Dale, B Clarke, EMG Fontes: Potential for the environmental impact of transgenic crops. *Nature Biotech* 20, 567–574 (2002)

17. MH Devare, CM Jones, JE Thies: Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community. *J Environ Qual* 33, 837–843 (2004)

18. B Liu, Q Zeng, FM Yan, HG Xu, CR Xu: Effects of transgenic plants on soil microorganisms. *Plant Soil* 271, 1–13 (2005)

19. A Lang, M Arndt, R Beck, J Bauchhenss, G Pommer: Monitoring of the environmental effects of the *Bt* gene. Bavarian State Research Center for Agriculture Vöttinger Straße 38, 85354 Freising-Weihenstephan Internet. http://www.lfl-neu.bayern.de/publikationen/daten/schriftenreihe_url_1_43.pdf . Accessed 26 June 2011 (2006)

20. I Icoz, G Stotzky: Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biol Biochem 40, 559-586 (2008)

21. D Saxena, G Stotzky: Bt corn has a higher lignin content than non-Bt corn. Am J Bot 88, 1704-1706 (2001)

22. CX Sun, JQ Sun, L Miao: Photosynthetic characteristics of Bt or CpTI-Bt transgenic cotton at seedling stage. Acta Agronomica Sinica 33, 469-475 (2007)

23. YS Zhang, SX Yu, XM Guo, ZW Wang, QL Wang, L Chu: Dissection of genetic effects of quantitative trait loci (QTL) in transgenic cotton. Euphytica 159, 93-102 (2008)

24. S Flores, D Saxena, G Stotzky: Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biol Biochem 37, 1073–1082 (2005)

25. HY Fang, GY Zhang, CY Ma: Disease dynamic and resistance identification to Verticillium wilt of transgenic cotton. Cott Science 15, 210-214 (2003)

26. XL Liu, ZB Zhang, GQ Duan, XM Wang, HJ Zhang: Influence of cotton species and soil nourishment on red leaf blight disease. Plant Prot 31, 69-71 (2005)

27. KQ Zhu, ZL Feng: Analysis on disease resistance in anti-insect cotton cultivars (lines) in China. *Chinese Cotton* 32, 23 (2005)

28. XG Li, B Liu, S Heia, DD Liu, ZM Han, KX Zhou, JJ Cui, JY Luo, YP Zheng: The effect of root exudates from two transgenic insect-resistant cotton lines on the growth of *Fusarium oxysporum. Transgenic Res* 18, 757–767 (2009)

29. AL Xu, GQ Duan, XM Wang, ZF Ji: The response of transgenic *Bt* cotton to *Verticillium* wilt. *China Cott* 32, 17 (2005)

30. HQ Zhu, ZL Feng, XY Liu, YQ Shi, LH Zhao: Occurrence and control technology of transgenic cotton Verticillium wilt. *China Cott* 36, 20-21 (2009)

31. L Lioussanne, M Jolicoeur, M St-Arnaud: Mycorrhizal colonization with *Glomus intraradices* and development stage of transformed tomato roots significantly modify the chemotactic response of zoospores of the pathogen *Phytophthora nicotianae*. *Soil Biol Biochem* 40, 2217–2224 (2008)

32. HL Li, HX Yuan, Y Wang, JX Cai, L Huang, SZ Wang: Study on the relationship between diversity of microbes in rhizosphere and resistance of cotton cultivars to *Verticillum dahliae*- Ⅰ.the population of microbes in cotton rhizosphere and cultivars resistant to *V. dahliae. Acta phytopathologica sinica* 28, 341-345 (1998)

33. C Ma: Study on *Fusarium oxysporum* f.sp.*vasinfectum* and *Verticillium dahliae* Kleb. China Agriculture Publishing House, Beijing, China (2007)

34. H Wu, T Haig, J Pratley, D Lemerle, M An: Distribution and exudation of allelochemicals in wheat (Triticum aestivum) *J Chem Ecol* 26, 2141–2154 (2000)

35. ZB Wu: Analysis on resistance of anti-insect and hybrid cotton cultivars (lines) at test area in Hubei Province. *Hubei Agr Sci* **5**, 36–38 (2000)

36. S Steinkellner, R Mammerler, H Vierheilig: Germination of *Fusarium oxysporum* in root exudates from tomato plants challenged with different *Fusarium oxysporum. Eur J Plant Pathol* 122, 395–401 (2008)

37. FM Nóbrega, IS Santos, MD Cunha, AO Carvalho, VM Gomes: Antimicrobial proteins from cowpea root exudates: inhibitory activity against *Fusarium oxysporum* and purification of a chitinase-like protein. *Plant Soil* 272, 223-232 (2005)

38. HY Yuan, HL Li, Y Wang, WP Fang, ZY Wang: The root exudates of cotton cultivars with the different resistance and their effects on *Verticillium dahliae. Acta Phytopathologica Sinica* 3**2**, 127–131 (2002)

39. Y Han, K Pan, FZ Wu: Effect of root exudates from cucumber cultivars on pathogen of *Fusarlum* wilt. *China Vegtable*, 13–15(2006)

40. S Steinkellner, R Mammerler, H Vierheilig: Microconidia germination of the tomato pathogen *Fusarium oxysporum* in the presence of root exudates. *J Plant Interact* 1, 23–30 (2005)

41. HP Bais, TL Weir, LG Perry, S Gilroy, JM Vivanco: The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol* 57, 233–266 (2006)

42. LM Han, SQ Wang, HY Ju, F Yan, GQ Li, JP Liu, JC Yan: Identifition and study on allelopathy of soybean root exudates. *Soybean Sci* 19, 119-125 (2000)

43. Q Chai, FX Feng: Identification of root exudation of *Zea mays* L. and allelopathy of 1,2-benzenedicarboxylic acid. *J Gansu Agri Univers* 42, 43-48 (2007)

44. N Liu, BL Zhou, YX Li, J Hao, YW Fu: Allelopathy of the eggplant/tomato grafted eggplants root exudates to *Verticillium* wilt (*Verticillium dahllae*). *Acta Horticulturae Sinica* 35, 1297-1304 (2008)

45. HY Ju, LM Han: Influence of root exudations on pathogenic fungi produced by root rot and seed germination in continuous cropping soybean. *J Jilin Agri Univers* 24, 45-49 (2002)

46. CW Wu, XF Du, JM Xu, WZ Wang: Research advances in natural antibacterial constituents from plant origin. *Acta Agri Boreali-occidentalis Sinica* 13, 81-88 (2004)

47. A Wilson, J Latham, R Steinbrecher: Genome scrambling – myth or reality? Transformation-induced mutations in transgenic crop plants. EcoNexus, UK (2004)

48. ANE Birch, RE Wheatley: GM pest-resistant crops: assessing environmental impacts on non-target organisms. *Issues Sci Technol* 21, 31–54 (2005)

49. AA Snow, DA Andow, P Gepts, EM Hallerman, A Power, JM Tiedje, LL Wolfenbarger: Genetically engineered organisms and the environment: Current status and recommendations. *Ecol Appl* 15, 377–404 (2005)

50. X Zhang, DH Chen, JY Wang, WS Xia, Y Chen, WY Gu, YK Wu: Growth and development of transplanted plants and their metabolism of carbohydrate and nitrogen in *Bacillus thuringiensis* transgenic cotton. *Cotton Sci* 1, 37–42 (2006)

51. B Liu, J J Cui, J Meng, W J Hu, JY Luo, YP Zheng: Effects of transgenic *Bt*+CpTI cotton on the growth and reproduction of earthworm *Eisenia foetida*. *Front Biosci* 14, 4008–4014 (2009)

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