Effects of the transgenic *CrylAc* and *CpTI* insect-resistant cotton SGK321 on rhizosphere soil microorganism populations in northern China

Y.J. Zhang, M. Xie, D.L. Peng

State Key Laboratory for Biology of Plant Diseases and Insect Pests/Key Laboratory for Biological Control of Ministry of Agriculture, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China

ABSTRACT

Transgenic *CrylAc* and *CpTI* insect-resistant cotton SGK321 has been widely adopted for many years in several regions of China, however the understanding of its potential effects on soil microorganisms is limited. The impact of transgenic cotton SGK321 on microorganism populations in rhizosphere soil was investigated. The numbers of bacteria, fungi, and actinomycetes were measured by counting colony-forming units after incubation on appropriate medium in a two-year field study in the northern China. Rhizosphere soil microorganism populations between transgenic cotton SGK321 and its non-transgenic parental cotton or conventional cotton were different at some plant growth stages and/or in some years. However compared to the plant growth stage and cotton cultivar, the impacts of the transgenic trait were slight or transient. The principal component analysis also showed no significant or minor difference in the numbers of bacteria, fungi, and actinomycetes in rhizosphere soil between transgenic cotton SGK321 and its non-transgenic parental cotton. These results suggest that the transgenic cotton SGK321 has no apparent impact on microorganism populations in rhizosphere soil.

Keywords: risk assessment; toxic protein; bacteria; fungi; actinomycetes

Microorganisms are the important component of soil, tightly related to the status of soil ecosystem, and often considered as sensitive indicators reflecting the changes in soil ecosystems (Visser and Parkinson 1992). The study shows that the significant accumulation and persistence of active *Bt* toxins in rhizosphere soil were caused by the transgenic *Bt* corn, rice and potato but not of the transgenic *Bt* canola, cotton, and tobacco (Saxena et al. 2004). By contrast, the significant accumulation and persistence of active *Bt* toxins in rhizosphere soil of transgenic *Bt* cotton (Sun et al. 2007) instead of transgenic *Bt* corn and rice (Gruber et al. 2012) were observed in other studies. Ecological impacts of transgenic *Bt* crops on soil microorganisms has been studied since 1995. Most previous studies showed that the transgenic *Bt* crops could not lead to a significant difference in soil microbial ecology (Baumgarte and Tebbe 2005, Barriuso et al. 2012). However, significant effects of transgenic *Bt* crops on *Fusarium* sp were reported (Li et al. 2009). Thus the impacts of transgenic crops on soil microbial ecology are complicated, may be related with crop species, introduced gene kinds, genetic manipulation etc.

The transgenic cotton SGK321, expressing both *Cry1Ac* and *CpTI* toxic proteins, was cultivated in 1999 and was largely planted in northern China (Guo et al. 1999). The content of *Bt* toxin in rhizo-

Supported by the Project of National High-tech R&D Program of China, Project No. 2011AA10A204; by the Plant Transformation Research and Application of China, Projects No. 2009ZX08011-028B and 2008ZX08012-002/004); by the National Natural Science Foundation of China, Grant No. 31201571; by the Special Fund for Agri-scientific Research in the Public Interest of China, Grants No. 200903040 and 201003079; by the China International Cooperation Project, Projects No. 2012DFR30810 and 2011-G4, and by the Science and Technology Innovation Program of Chinese Academy of Agricultural Sciences.

sphere soil of SGK321 returned to the level of its non-transgenic parental cultivar with the senescence of cotton (Rui 2005). The cultivation of the transgenic cotton SGK321 may not affect the numbers of nitrogen-fixing, organic phosphate-dissolving, inorganic phosphate-dissolving, and potassium-dissolving bacteria (Hu et al. 2009). The understanding of impacts of the transgenic cotton SGK321 on soil microorganisms is limited. In this study, we evaluate in field the effects of the transgenic cotton SGK321 on three kinds of soil microorganisms (bacteria, fungi and actinomycetes) over a period of two years in northern China. We aimed to reveal whether or to what extent the transgenic cotton SGK321 influences the soil microbial populations, and to provide some information on the risk assessment of the transgenic cotton SGK321.

MATERIAL AND METHODS

Plant materials. We used the transgenic cotton cv. SGK321 (that contains both *Cry1A* and *CpTI* gene), its non-transgenic parental cotton cv. Shiyuan 321, and the conventional cotton cv. Simian 3. All cotton seeds were provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences.

Field design and sampling. Field trials were conducted on the experimental farm of the Institute of Plant Protection of the Chinese Academy of Agricultural Sciences, located at the Cuizhuang town (39°30'N, 116°36'E), Langfang, Hebei province, China, in 2010–2011. The cotton plants were grown in a randomized block design consisting of triplicate plots (each plot 6 m × 10 m) per cultivar. Cotton was maintained in accordance with typical agronomic practices in northern China. Sampling was carried out at six developmental stages of the plants each year from 2010 to 2011, namely BBCH 31 (seedling); BBCH 55 (budding); BBCH 65 (full flowering); BBCH 75 (bolling); BBCH 85 (boll opening) and BBCH 99 (senescence), as described by Munger et al. (1998). The rhizosphere soil was defined as the soil still attached to the roots after uprooting the plant and shaking it by hand (Brusetti et al. 2005). For each sampling, rhizosphere soils from five *ad hoc* selected plants per plot were mixed and used as a composite rhizosphere soil samples. Plant and root residues were removed from soil samples with forceps, followed by sieving (2-mm mesh size). Soil samples were stored at 4°C for no longer than one week before the analysis.

Quantification of bacteria, fungi, and actinomycetes. Bacteria, fungi and actinomycetes were enumerated using a 10-fold dilution plate technique. Ten grams of homogenized soil from each sample were suspended in 100 mL sterile water, shaken for 30 min at 220 rpm, and 10-fold serially diluted. The colony forming units (CFU) of bacteria, fungi, and actinomycetes in each sample were determined by spreading 100 µL of the diluted sample on appropriate culture media in Petri plates, with five replicate plates per dilution. The following media were used to assay for different microorganism types (Shen et al. 2004): bacteria (NB agar: 10.0 g peptone, 3.0 g beef extract, 5.0 g NaCl, 15 g agar, 1000 mL distilled water, pH 7.0–7.2); fungi (Martin's Rose Bengal streptomycin agar: 10.0 g glucose, 5.0 g peptone, 1.0 g KH_2PO_4,

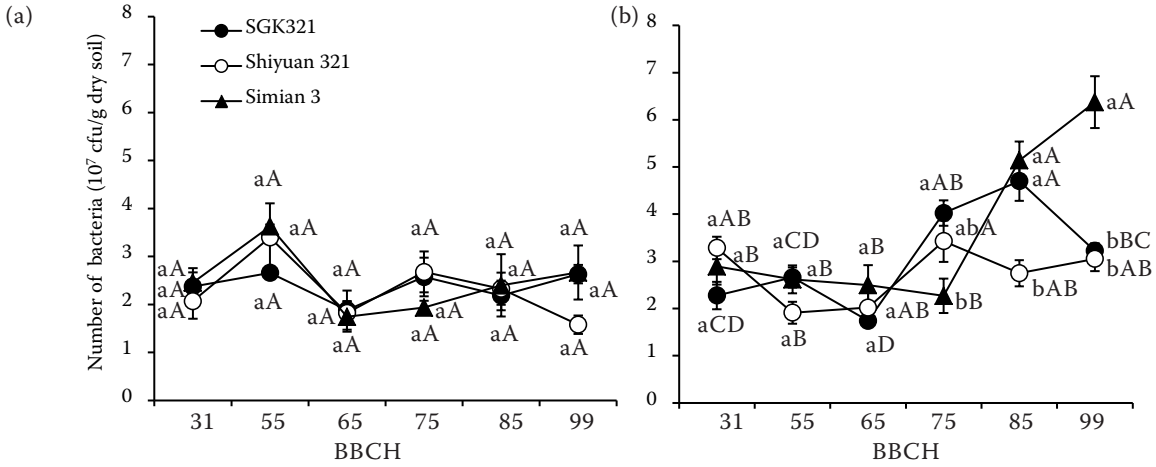

Figure 1. The number of bacteria from rhizosphere soil of transgenic cotton SGK 321, its non-transgenic parental cv. Shiyuan 321 and conventional cv. Simian 3 at different stages in 2010 (a) and 2011 (b) in the field. Significant differences between plant cultivars or plant growth stages are indicated by different lowercase or uppercase letters above bars (*LSD*, $P < 0.05$)

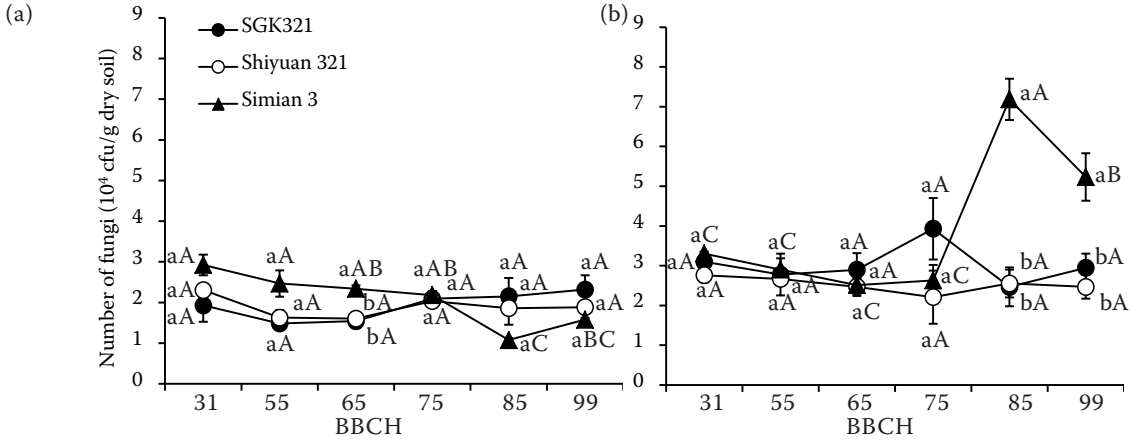

Figure 2. The number of fungi from rhizosphere soil of transgenic cotton SGK321, its non-transgenic parental cv. Shiyuan 321 and conventional cv. Simian 3 at different stages in 2010 (a) and 2011 (b) in the field. For explanations see Figure 1

0.5 g $MgSO_4 \cdot 7\ H_2O$, 33 mg Rose Bengal, 1000 mL distilled water, and 3.0 mL 1% streptomycin added immediately before use); actinomycetes (Gauze No. 1 agar: 20.0 g soluble starch, 1.0 g KNO_3, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 0.5 g NaCl, 0.01 g $FeSO_4 \cdot 7\ H_2O$, 1000 mL distilled water, and 3.3 mL 3% potassium dichromate added immediately before use). Plates were incubated at 30°C for 3 days for the assay of bacteria, 25°C for 5 days for fungi, and 30°C for 7 days for actinomycetes. Colonies were counted visually and expressed as CFU/g dry soil. All the results are expressed on the basis of oven-dried soil.

Data analysis. The number of bacteria, fungi and actinomycetes were expressed as the means ± standard deviation. The statistical differences among data were determined by the Fisher's least significant difference (*LSD*) test at the 5% significance level. The principal component analysis (PCA) for effects of the cultivar or growth stage on microbial populations were conducted. All statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

The numbers of bacteria, fungi and actinomycetes in rhizosphere soil from the same cotton were significantly different among different growth stages in both 2010 and 2011 (Figures 1–3). PCA analyses revealed that the plant growth stage was the strongest explanatory factor for differences in the numbers of microorganisms in rhizosphere soil (Figure 4). Cotton cultivar also had significant contribution to explanation of differences in the numbers of microorganisms in rhizosphere soil (Figure 4). However there were no significant differences in the numbers of microorganisms in rhizosphere soil between the transgenic cotton SGK321, with two exceptions (bacteria at BBCH 85 in 2011 and actinomycetes at BBCH 31 in 2010) (Figures 1–3). Data analyses of ANOVAs showed that both PC1 and PC2 score of the transgenic cotton SGK321 were significantly different from its non-transgenic parental cotton at some growth stage and/or in some year (Figure 4).

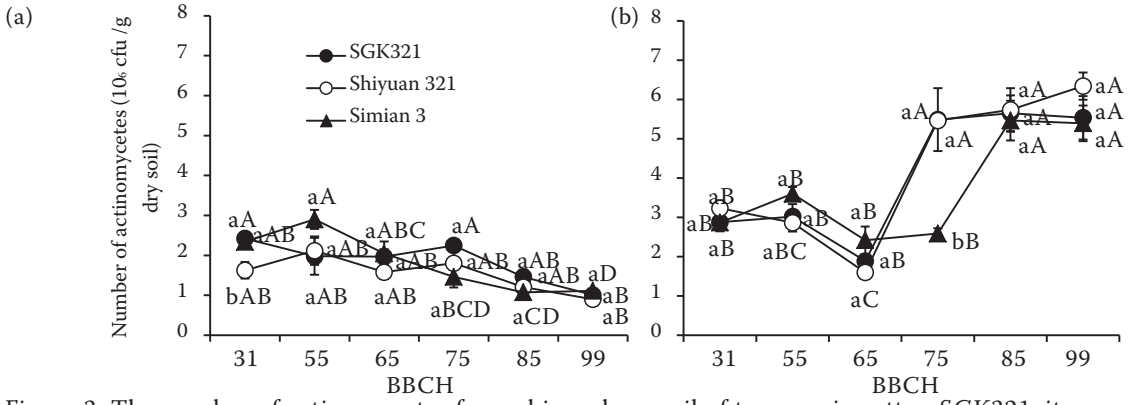

Figure 3. The number of actinomycetes from rhizosphere soil of transgenic cotton SGK321, its non-transgenic parental cv. Shiyuan 321 and conventional cv. Simian 3 at different stages in 2010 (a) and 2011 (b) in the field. For explanations see Figure 1

Most studies proved that transgenic *Bt* crops had no significant effects on soil microbial populations, or only had the transient effects (Donegan et al. 1995, Saxena et al. 2002, Li et al. 2011). Monogene transgenic cotton cultivars were used in previous researches, while our tested transgenic cotton cv. SGK321 carries double toxic genes. The present study showed significant seasonal and developmental variations in rhizosphere soil microorganism populations. The significant differences of microorganism populations in rhizosphere soil between SGK321 and its parent were transient. Those were in accordance with not only previous studies showing that the transgenic trait had no significant or minor effects on microorganism populations in rhizosphere soil (Saxena et al. 2002, Li et al. 2011), but also a recent report that the tissues of transgenic cotton SGK321 have no apparent impact on soil microorganisms associated with residue decomposition (Hu et al. 2013). These results indicated that the species and number of introduced genes may not always lead to a significant change of microbial populations in rhizosphere soil of transgenic crops.

Although the experimental methods or technologies were discriminated, most of researches had revealed no or minor effect of *Bt* traits on soil microbial communities (Zhang et al. 2013). The *Bt* cotton had no significant effect on the richness and diversity of soil microbial community compared to near-isogenic non-*Bt* cotton using the biolog system (Shen et al. 2006). The variations detected in the rhizobacterial community structure were possibly due to climatic factors rather than to the presence of the *Bt* gene and no variation was observed in the diversity between non-*Bt* and *Bt* maize utilizing the next generation sequence (Barriuso et al. 2012). No significant difference between *Bt* cottons and their non-transgenic parents in rhizosphere soil bacterial communities was detected as determined by DGGE at the same growth stage in the same province (Na et al. 2011).

In conclusion, the differences of bacterial, fungal and actinomycete populations in rhizosphere

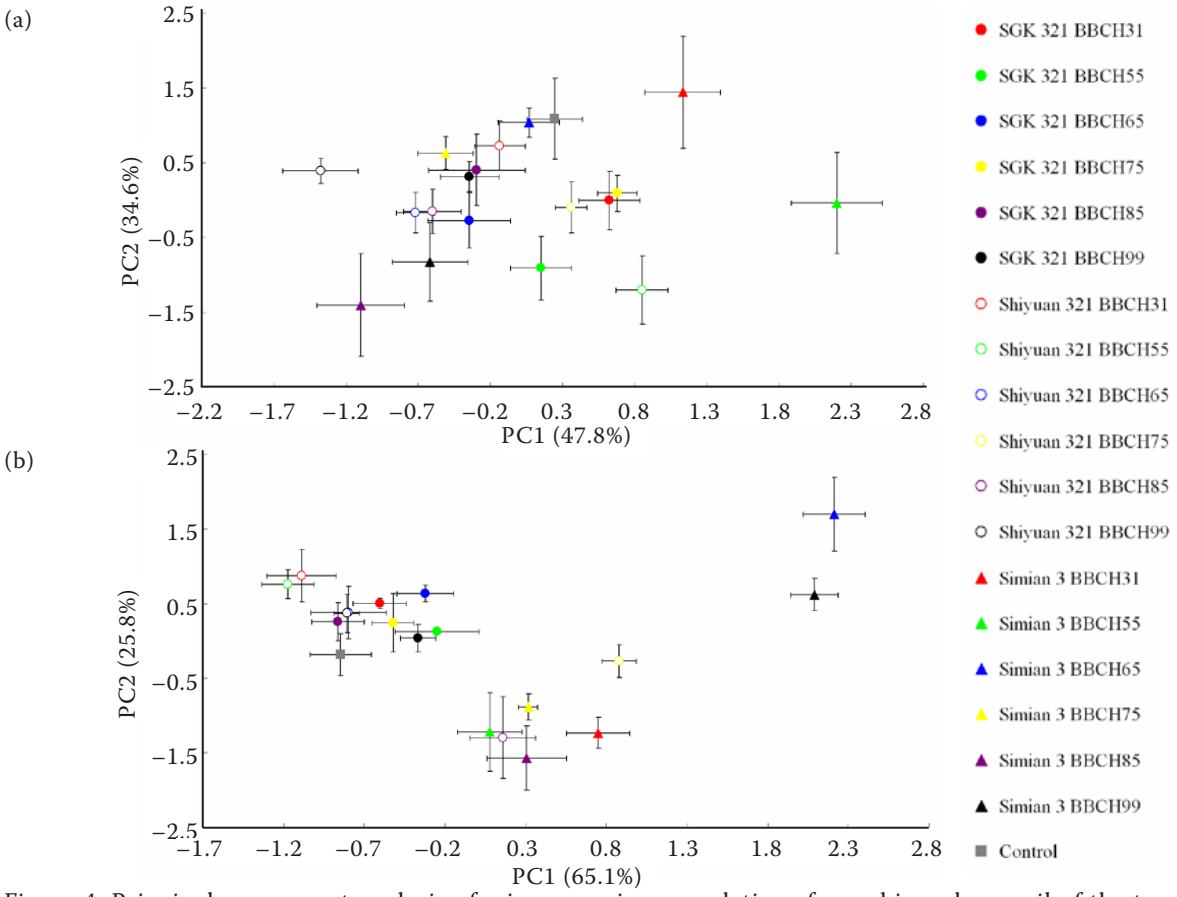

Figure 4. Principal component analysis of microorganism populations from rhizosphere soil of the transgenic cotton SGK321, its non-transgenic parental cotton cv. Shiyuan 321 and conventional cotton cv. Simian 3 at different stages in 2010 (a) and 2011 (b) in the field. Pre-cropping samples are used as control. The level of variation explained by each principal component is indicated in parentheses

soil between transgenic cotton SGK321 and non-transgenic parental cotton were either transient or absent. In the overall level, the transgenic cotton SGK321 had no significant effects on microbial populations in rhizosphere soil. However, the results presented here should be considered preliminary because a culture-based technique (which detects only a small portion of the microbial community) was used. Therefore, more work has to be done to further evaluate the effects of the transgenic cotton SGK321 on soil microorganisms, especially the soil microbial community structure.

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Received on March 6, 2014
Accepted on April 22, 2014

Corresponding author:
Dr. Ming Xie, Chinese Academy of Agricultural Sciences, Institute of Plant Protection (South Campus), No. 12, Zhong-Guan-Cun Nan-Da-Jie, Haidian, 100081 Beijing, P.R. China
phone/fax: + 86 10 8210 9568, e-mail: xiem406@126.com