文章编号:1001 - 7380(2015)01 - 0016 - 07

转基因 *Bt* 棉对根际土壤营养元素及酶活性的不同影响

刘根林

(江苏省林业科学研究院,江苏 南京 211153)

摘要:在根际箱中种植2个转基因 *Bt* 棉品系99BC-4,99BC-8及其非 *Bt* 受体泗棉3(SM3)后,于花期采集其根际土壤,进行化学分析、微生物生物量测量、Bt毒蛋白检测和酶活性测定。结果显示,2品系的根际土壤内能检测出Bt毒蛋白。相比于受体,99BC-4或99BC-8根际土壤中N, Ca, Zn, Co和Cu元素含量高,而K, Mg元素的含量低,C/N, P和Mn的含量无显著差异;Fe元素,99BC-4根际土壤内含量低,99BC-8根际土壤内含量高。2个转基因 *Bt* 棉品系根际土壤内微生物生物量碳显著高于其受体,但微生物生物量碳与总碳量的比值,99BC-4与受体相比,有显著差异。不同根际土壤内磷酸酶活性无显著差异,但相比于受体,FDA水解酶活性99BC-4根际土壤内显著升高,而99BC-8显著降低。推断Bt毒蛋白的存在可能通过改变某些微生物的代谢模式来刺激它们的生长,而已变的代谢模式似乎主宰着土壤中微生物的反应,虽然其反应在2转基因 *Bt* 棉品系的根际土壤内可能会不一致。

关键词:转基因 *Bt* 棉;毒蛋白;土壤;酶活性

中图分类号:S562;S154.3 **文献标识码**:A **doi**:10.3969/j.issn.1001 - 7380.2015.01.004

Different effects of transgenic *Bt* cotton on rhizospheric soil nutrition and soil enzyme activities

LIU Gen-lin

(Jiangsu Academy of Forestry, Nanjing 211153, China)

Abstract: After cultivation of 2 transgenic *Bt* cotton lines, 99BC-4 and 99BC-8, and their non-*Bt* recipient Simian3 (SM3) in rhizoboxes, the rhizospheric soils during blooming period were sampled. By the chemical analysis, microbial biomass measurement, Bt toxic protein detection, enzyme activity determinations, this study revealed that the rhizospheric soils of 99BC-4 and 99BC-8 were positive for Bt endotoxin content. N, Ca, Zn, Co and Cu contents had higher values in the rhizospheric soils of 99BC-4/99BC-8 than in that of SM3 while lower values found with 2 *Bt* cotton lines for K and Mg contents. No significant differences ($p > 0.05$) for C/N, P and Mn contents. For Fe content, a lower value was observed in 99BC-4 rhizospheric soil whereas a higher value in that of 99BC-8. C_{mic} (microbial biomass C) with 2 transgenic *Bt* cotton lines showed higher values with significant differences ($p < 0.05$). But for C_{mic}/C_{total}, a significant difference ($p < 0.05$) found between those of SM3 and 99BC-4 only. Compared with SM3, 2 *Bt* cotton lines performed more or less inconsistently as no significant difference emerged for phosphatase, a higher value in 99BC-4 rhizospheric soils but a lower value in that of 99BC-8 for FDA hydrolysis. It can be deduced that the presence of Bt endotoxin likely stimulated the growth of some microbes by altering their metabolic patterns, which seemed to dominate the microbial responses in the soil although the responses were inconsistent in 2 transgenic *Bt* lines.

Key words: Transgenic *Bt* cotton; Toxic protein; Soil; Enzyme activity

---

收稿日期:2014-10-22;**修回日期**:2014-12-28

作者简介:刘根林(1963 -),男,江苏姜堰人,高级工程师,博士,主要从事农林土壤微生物群落的分子生物学研究等工作。

1 Introduction

In recent years, a striking achievement has been made in the research of transgenic crops and their commercialization. According to a new report from International Service for the Acquisition of Agri-biotech Applications (ISAAA)[1], in 2009 global dimensions of transgenic crops in 25 countries reached 134 million hectares, among which the area of transgenic cotton soared to 16.2 million hectares, accounting up 12.1% of transgenic crops grown globally. China is the largest producer of cotton in the world, with 68% of its 5.4 million hectares successfully planted with transgenic *Bt* (*Bacillus thuringiensis*) cottons in 2009. Transgenic *Bt* cottons, into which the *Cry* gene encoding insecticidal Cry1Ac *Bt* toxic protein was introduced, have been grown worldwide for their resistance to cotton bollworm (e. g. *Helicoverpa armigera*)[2-3]. However, along with the increasing potential for widespread commercial use and the potential benefits of transgenic crops, considerable concerns have been raised about their potentially environmental risks[4-8]. Although risk assessments have been extensively conducted, the issue of transgenic *Bt* cottons on soil environmental risk still remains unclear or controversial[4-7]. Here the effects of transgenic *Bt* cotton lines on rhizospheric soil nutrition and soil enzyme activities are reported after their cultivation in China.

2 Materials and Methods

2.1 Rhizobox design and soil sampling and processing

The experiments were performed at the Institute of Genetics & Physiology, Academy of Agriculture Sciences of Jiangsu Province, Nanjing, China. One cotton variety (genotype) was used, including 2 transgenic *Bt* cotton lines 99BC-4 and 99BC-8 (into which GFMCry1A *Bt* gene was introduced in 1999), and their non-*Bt* recipient variety, i. e., Simian3 (SM3)[9]. The rhizobox used for their cultivation was designed as 200 mm in length, 150 mm in width, and 200 mm in depth, divided into 5 compartments with 4 nylon nets (30 μm mesh size) that were impermeable to roots but allowed exchange of water or nutrients. There were 3 large compartments separated by two 10-mm compartments. Cottons grew in the middle compartment with the same cultivation practices, such as irrigation and fertilization, no pesticide used, and the rhizospheric soils during blooming period were collected for 6 times randomly in July, 2010 from the two adjacent 10-mm compartments. Each sample was immediately put in a sterile plastic bag and transported to the laboratory in a cooled box, then sieved (2mm) and homogenized in a rotary cylinder, and finally, stored at 4 ℃ before used for chemical analysis, microbial biomass measurement, Bt toxic protein detection and enzyme activity determinations.

2.2 Soil chemical analysis and microbial biomass measurement

The soil elements analysis was carried out at the Center of Modern Analysis of Nanjing University. Soil samples were dried at 105 ℃ for 24 h and subsequently weighed for dry mass determination. The inductively coupling plasma emission spectroscopy (ICP Analyzer, Jarrell-Ash Co., Ltd.) was used for soil inorganic elements and total P analysis. Perkin-Elmer 240 was used for soil total C analysis. Foss Heraeus CHN-O-Rapid was used for determination of soil total N.

Microbial biomass C (C_{mic}) was measured by the fumigation extraction (FE) procedure[10]. Shimadzu TOC 500 was used to measure total organic C of the filtrate. The difference between total organic C in the fumigated and non-fumigated samples divided by conversion factor (Kc) is C_{mic}, where Kc is soil-specific, estimated to be 0.45.

2.3 Determination of Bt endotoxin in samples

Buffer Solution (EnviroLogix, Portaland, ME, USA) (1 mL) was added to 0.7 g soil sample, then the soil mixture was mixed and centrifuged (13,000 × g) at 4 ℃ for 10 min; the Bt toxin concentration in the supernatant was determined by ELISA, using the EnviroLogix Cry 1Ab/Cry 1Ac plate Kit (detection limit < 0.25 parts per million)[11].

2.4 Analysis of soil enzyme activities

Acid phosphatase activity was determined spectrophotometrically by the disodium phenol phosphate

method, as described by Tabatabai[12]. The analysis method of alkaline phosphatase activity was similar to that of acid phosphatase activity, but with a different level (pH 11.0) of the phosphate buffer. Activity (vs phenol) was expressed in mg/(h · kg fresh soil).

Fluorescein diacetate (FDA) method was used to analyze the soil FDA hydrolase activity. Each fresh sample (0.5 g) was put in a 50 mL measuring flask, followed by the addition of 5 mL of phosphate buffer (pH 7.6) and 50 μL of 4.8 mmol/L FDA. The solution obtained was incubated at 37 ℃ for 2 h, next, added 5 mL acetone to terminate the reaction. After the solution centrifuged for 5 min at 5 000 rpm, the supernatant was taken and the OD at 490 nm was measured to collect the data. FDA hydrolase activity was expressed as mg luciferin produced per gram fresh soil per hour.

2.5 Statistical analysis

All the experiments were performed at least 3 repetitive independent treatments. An analysis of variance was carried out by using the SPSS13 software. The values were expressed as means ± SD. The significant differences among the means were calculated by using LSD-test. Statistical significance threshold was set to less than 0.05 for p value.

3 Results

3.1 Effects of 99BC-4, 99BC-8 and SM3 on the rhizosphere soil chemical properties

Table 1 showed the values of the rhizospheric soil chemical indicators of 2 transgenic *Bt* cotton lines (99BC-4 and 99BC-8), and their recipient (SM3). In the rhizospheric soils of 99BC-4, 99BC-8 and SM3, there occurred changes in content of some organic, inorganic nutritional elements at different levels. In detail, N, Ca, Zn, Co and Cu contents had higher values in the rhizospheric soils of 99BC-4/99BC-8 than in that of SM3, increased by 54.2%/31.8%, 3.0%/26.2%, 12.3%/57.9%, 11.6%/2.0% and 23.8%/28.6% respectively, while lower values found in those of the 2 *Bt* cotton lines for K and Mg contents, decreased by 13.0%/7.5%, 7.9%/10% respectively. There were no significant differences for C/N, P and Mn contents, but a slightly decreased value with the recipient. Exceptionally, 2 *Bt* cotton lines expressed incoherently in that for Fe content, a lower value was observed in 99BC-4 rhizospheric soil compared with the recipient, decreased by 4.8% whereas a higher value in 99BC-8 rhizospheric soil, increased by 0.3%.

Table 1 Value of chemical indicators in the rhizospheric soils

	99BC-4	99BC-8	SM3
C/%	5.499 ± 0.633	5.672 ± 0.133	4.636 ± 0.413
N/%	0.723 ± 0.051^{a}	0.618 ± 0.062^{ab}	0.469 ± 0.017^{b}
C/N	7.753 ± 0.254	9.337 ± 0.848	9.892 ± 0.879
P/%	0.141 ± 0.009	0.135 ± 0.000	0.122 ± 0.013
K/%	1.399 ± 0.025^{b}	1.487 ± 0.019^{ab}	1.608 ± 0.068^{a}
Ca/%	0.821 ± 0.028^{b}	1.006 ± 0.012^{a}	0.797 ± 0.019^{b}
Mg/%	0.574 ± 0.007^{b}	0.560 ± 0.006^{b}	0.623 ± 0.003^{a}
Fe/%	3.432 ± 0.034^{b}	3.617 ± 0.019^{a}	3.605 ± 0.037^{a}
Mn/%	0.066 ± 0.002	0.062 ± 0.001	0.065 ± 0.002
Zn/(mg/kg)	125.806 ± 0.610^{b}	176.886 ± 5.690^{a}	112.051 ± 1.255^{c}
Cu/(mg/kg)	22.272 ± 1.197^{a}	23.135 ± 1.000^{a}	17.991 ± 0.998^{b}
Co/(mg/kg)	15.259 ± 0.093^{a}	13.954 ± 0.090^{b}	13.676 ± 0.594^{b}

Different letters in apex within row indicated significant differences ($p < 0.05$).

3.2 Effects of 99BC-4, 99BC-8 and SM3 on the rhizospheric soil microbial biomass C

In comparison with SM3, C_{mic} in their rhizospheric soils was largely affected by 2 transgenic *Bt* cotton lines (99BC-4 and 99BC-8), showing higher values with significant differences (Fig. 1-a), which accorded well with another study[13]. But for such an indicator as C_{mic}/C_{total}, there was a significant difference between the rhizospheric soils of SM3 and 99BC-4 only (Fig. 1-b). It was in disagreement with the finding of Fig. 1-a,

since C_{total}, specially for the rhizospheric soil of 99BC-8, not only included C_{mic} and simple organic C compounds, but also significant amounts of inorganic C and relatively recalcitrant organic C, though it was regarded that soil C_{mic} was positively related with soil C[14].

3.3 ELISA of Cry1Ab/Cry1Ac endotoxin in the rhizospheric soils of 99BC-4, 99BC-8 and SM3

In our Cry1Ab/Cry1Ac endotoxin detection experiment, the OD of the rhizospheric soil sample extract of SM3 was close to that of the Blank (CK) and less than that of PC whilst the OD's of the rhizospheric soil sample extracts of 99BC-4 and 99BC-8 were significantly higher than those of CK and PC, illustrating that SM3 rhizospheric soil was presumed to be free of Bt endotoxin, yet the rhizospheric soils of 99BC-4 and 99BC-8 were positive for Bt endotoxin content (Fig. 2). Owing to Bt endotoxin as a root exudate existing in 2 transgenic *Bt* cotton lines (99BC-4 and 99BC-8) rhizospheric soils, which likely resulted in more microbes occurring, there might be a significant positive correlation between C_{mic} and Bt endotoxin content.

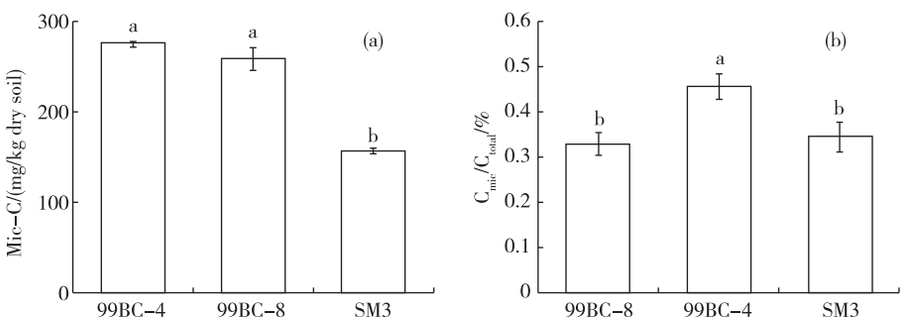

Fig. 1　Values of biological indicators in the studied soils. Different letters above bars indicated significant differences ($p < 0.05$).

3.4 Effects of 99BC-4, 99BC-8 and SM3 on the rhizospheric soil enzyme activities

The activities of alkaline phosphatase and acid phosphatase in the rhizospheric soil of 99BC-4, 99BC-8 and SM3 were shown in Fig. 3. There were no significant differences for activities of both alkaline phosphatase and acid phosphatase.

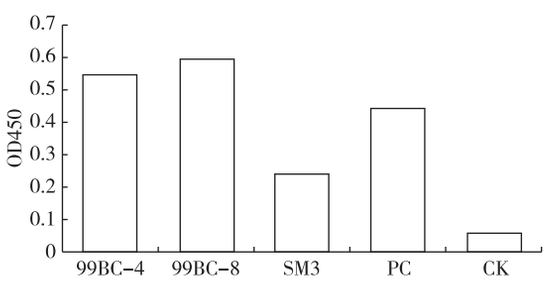

Fig. 2　Comparison of ELISA of Bt endotoxins in the studied soils. Bars represented standard deviations.

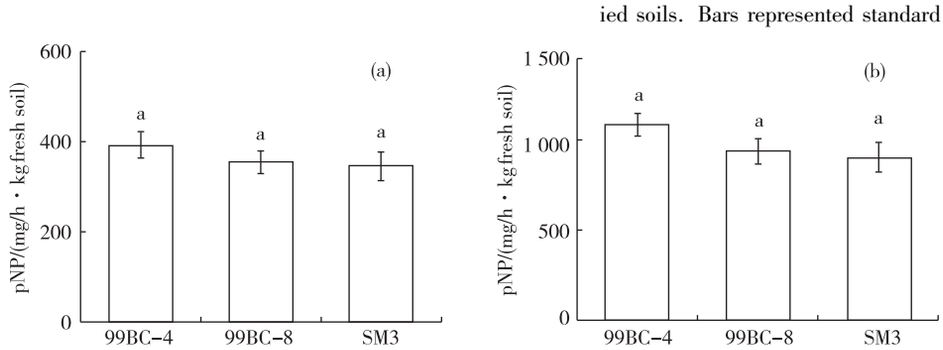

Fig. 3　Activities of alkaline phosphatase (a) and acid phosphatase (b) in the studied soils. Same letters above bars indicated no significant differences ($p > 0.05$).

The two *Bt* cotton lines performed inconsistently for their rhizospheric soil FDA hydrolase activities. FDA hydrolase activity possessed a higher value in the rhizospheric soil of Line 99BC-4 than in that of its non-*Bt* recipient. Conversely, the counterpart of Line 99BC-8 had a lower value. And there appeared significant differences, as shown in Fig. 4.

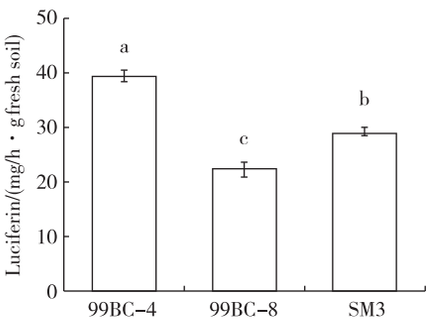

Fig. 4 Activities of FDA hydrolase in the studied soils. Different letters above bars indicated significant differences ($p < 0.05$).

4 Discussion

Bt cottons can produce crystal toxic protein for protecting themselves against the insects *Helicoverpa armigera*, but suppressing these bollworm in multiple crops in area with *Bt* cotton[3], justifying the large-scale release of these transgenic cotton varieties. Nevertheless, the ecological and environmental risks of them, such as of transgenic escape or super-weeds, and the impacts on non-target organisms, are widely concerned with[8].

Since Bt protein can be released into soil from root exudates[11], pollen[15], plant residues[16], and the feces of animals feeding on *Bt* cotton vegetative parts[17], the potential risks of *Bt* cotton on the soil ecosystem should be paid more attention, especially on the soil microbial communities which are essential for biogeochemical cycles and soil fertility.

Although not few studies have been focused on the effects of *Bt* cotton on soil microbial communities the conclusions remained inconsistent or even contradictory possibly due to the different techniques, varieties and target genes used. In this study, two *Bt* cotton lines (99BC-4, 99BC-8) and their non-*Bt* recipient (SM3) were used after cultivation in rhizoboxes for assessing the integrated effects of *Bt* cottons on soil nutrients, enzymes activities.

After cultivation in rhizoboxes, the presence of Cry1Ac *Bt* toxic protein could be determined in *Bt* cotton rhizospheric soils. This result contradicted another report that no detectable levels of Cry1Ac protein by ELISA in soils collected from field of *Bt* cotton that had been grown were incorporated with the biomass into soil for 3 to 6 yrs[18]. Albeit more than 60 Cry proteins have been identified, Cry1Ac Bt toxin was the main protein introduced into cotton for targeting against the cotton bollworm (Lepidoptera), while Cry1Ab or Cry3Bb1, Cry1F and Cry9C proteins was mainly gendered in corn and Cry3A or Cry3C protein in potato. Several laboratory studies in soil microcosms reported that the Cry proteins from *Bt* cotton, *Bt* corn, *Bt* potato were generally degraded rapidly with a half-life of 20 d or less without persistence[19-22]. However, in comparison, some reports showed that Cry2A protein from *Bt* cotton was still detectable after 120 d in the field[23], and Cry1Ab protein was detected in most soils of *Bt* corn[5]. The apparent differences of Cry protein persistence were probably attributed to the plant species, Cry protein type, pH, soil type, microbial activity as well as the binding characterization of Cry protein with the soil clays and humic acids[5].

Soil microbial communities play a critical part in soil processes, such as nutrient cycling, formation of soil structures, and transformation of pollutants in this system as the important soil components[24]. Soil nutrient cycle involves biochemical processes completed by soil enzyme catalytic reactions. Soil enzymes are indispensable for the assessment of soil 'function' and 'health'. Soil enzyme activity is also a sensitive indicator of soil microbial activity, and in turn the factors that influence soil microbial activity will be reflected on the soil enzyme activity, which finally results in the change of soil nutrition. Soil enzyme activity is the integrated reflection of soil physicochemical properties and microbial activity. So, to test the effects of *Bt* cotton on soil nutrition and microbial communities, it is imperative to measure the major related soil enzyme activ-

ities. Phosphatase is a general designation for a broad group of enzymes catalyzing the hydrolysis of both esters and anhydrides of phosphoric acid. Phosphatase activity also reflects the activation competence to phosphorus of soil. Fluorescein diacetate (FDA) hydrolase activity could be used to detect the number of soil bacteria and fungi with activity.

Commonly used indicators of the status and function of soil microbial communities include microbial community enumeration, which can be depicted by microbial biomass, and so on. Microbial biomass correlates with soil fertility and soil health and is recommended as a useful ecological indicator of stress caused by anthropogenic activities (including genetic modification).

There exist some interactions between crop root exudates and rhizospheric soil microbial communities. Some rhizospheric microbes can affect root exudates through changing the content level of the allelopathic compounds, and vice versa, the allelopathic compounds can also affect non-target soil rhizospheric microbial communities. In our study, the result of higher N, Ca, Zn, Co and Cu but lower K and Mg contents in 99BC-4/99BC-8 rhizospheric soils, could be comprehended by that the releasing of Bt endotoxin raised the content of N in the soil and also affected the physiological functions, especially assimilation of some metal cations into cotton plants from soil environment. Bt endotoxin as root exudate might induced more non-target soil microbes appearing in the rhizosphere, which significantly altered C_{mic} as measured. The rapid decomposition of Bt endotoxin might have allowed C_{mic} change to occur because soil microbes might rapidly degrade such a cotton-released allelopathic compound[25]. As for P content, no significant difference emerged among two *Bt* cotton lines and their non-*Bt* recipient, only a slightly decreased with the non-*Bt* recipient. This result also coincided with our observation of no significant differences for activities of both alkaline phosphatase and acid phosphatase since phosphatase activity was significantly positively correlated with soil total P content. A lower FDA hydrolysis activity and yet a higher C_{mic} in 99BC-8 rhizospheric soil exposed the truth of a greater quantity of microbes without activity within, suggesting that *Bt* endotoxin released severely inhibited the growth of the microbes assiduously at work in 99BC-8 rhizospheric soil[20].

5 Conclusions

The widely planting of transgenic crops has made a research hit of the risks potentially imposed to soil microbes and agro-ecosystem. In the rhizospheric soils which had been planted by 2 transgenic *Bt* cotton lines (99BC-4, 99BC-8), Cry1Ac Bt toxic protein could be determined by using the EnviroLogix QualiPlate Kit for Cry1Ab/Cry1Ac, which changed soil nutrient conditions such as increasing N, Ca, Zn, Co and Cu but decreasing K and Mg contents. Bt endotoxin likely triggered more non-target soil microbes appearing in the rhizosphere, significantly raising C_{mic}, to degrade more rapidly such a cotton-released allelopathic compound, but inhibit severely the growth of the microbes at work in 99BC-8 rhizospheric soil. As for enzyme activities, compared with their recipient, 2 *Bt* cotton lines performed more or less inconsistently since no significant difference emerged for phosphatase, a higher value in 99BC-4 rhizospheric soils but a lower value in that of 99BC-8 for FDA hydrolysis. It was alleged that Bt endotoxin selectively affected microbial composition, altered their metabolic patterns, which seemed to dominate the microbial responses in the soil although the responses were inconsistent in the two transgenic *Bt* lines.

References:

[1] James C. Global status of commercialized biotech / GM Crops: 2009. ISAAA Brief No. 41. ISAAA: Ithaca, NY. 2009.

[2] Mendelson M, Kough J, Vaituzis Z, et al. Are *Bt* crops safe [J]? Nature Biotechnology, 2003, 21(9):1003-1009.

[3] Wu K M, Lu Y H, Feng H Q, et al. Suppression of cotton bollworm in multiple crops in China in areas with *Bt* toxin-containing cotton[J]. Science, 2008,321(5896):1676-1678.

[4] Marvier M, McCreedy C, Regetz J, et al. A meta-analysis of effects of *Bt* cotton and maize on nontarget invertebrates [J]. Science, 2007, 316(5830): 1475-1477.

[5] Icoz I, Saxena D, Andow D A, et al. Microbial populations and enzyme activities in soil in situ under transgenic corn expressing Cry proteins from *Bacillus thuringiensis* [J]. Journal of Environ-

mental Quality, 2008,37(2): 647-662.

[6] Lilley A K, Mark J B, Cartwright C, et al. Life in earth: the impact of GM plants on soil ecology [J]? Trends in Biotechnology, 2006, 24(1):9-14.

[7] Cartwright C D, Lilley A K. Mechanisms for investigating changes in soil ecology due to GMO releases[R]. Defra report EPG 1/5/214: Department for Environment, Food and Rural Affairs, 2004.

[8] Wolfenbarger L L, Phifer R P. The ecological risks and benefits of genetically engineered plants [J]. Science, 2000, 290 (5499): 2088-2093.

[9] Chen L C (陈立昶), Yu J Z(俞敬忠), Ji S Y (吉守银), et al. Selection and breeding of new cotton variety Simian3 [J]. Acta Gossypii Sin (棉花学报), 1998,10(1):20-25.

[10] Rice C W, Moorman T B, Beare M. Role of microbial carbon and nitrogen in soil quality[A]. In: Doran J D, Jones A J. Methods for assessing soil quality[M]. Madison: Soil Science Society of America, 1996:203-215.

[11] Saxena D, Flores S, Stotzky G. Transgenic plants: insecticidal toxin in root exudates from *Bt* corn [J]. Nature, 1999, 402 (6761):480.

[12] Tabatabai M A. Soil enzyme[A]. In: Weaver R W, Angle J R, Bottomley P S. Mehods of soil anhysis: microbiological and biochemical properties[M]. Madison: Soil Science Society of America, 1994:775-833.

[13] Sarkar B, Patra A K, Purakayastha T J, et al. Assessment of biological and biochemical indicators in soil under transgenic *Bt* and non-*Bt* cotton crop in a sub-tropical environment[J]. Soil Biology & Biochemistry, 2004, 36(4):571-579.

[14] Li Q C, Allen H L, Wollum II A G. Microbial biomass and bacterial functional diversity in forest soils: effects of organic matter removal, compaction, and vegetation control [J]. Soil Biology & Biochemistry, 2004, 36(4):571-579.

[15] Losey J E, Rayor L S, Carter M E. Transgenic pollen harms monarch larvae [J]. Nature, 1999, 399(6733): 214.

[16] Zwahlen C, Hilbeck A, Gugerli P, et al. Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field[J]. Molecular Ecology, 2003a, 12(3):765-775.

[17] Wandeler H, Bahylova J, Nentwig W. Consumption of two *Bt* and six non-*Bt* corn varieties by the woodlouse *Porcellio scaber* [J]. Basic and Applied Ecology, 2002, 3(4):357-365.

[18] Head G, Surber J B, Watson J A, et al. No detection of Cry1Ac protein in soil after multiple years of transgenic *Bt* cotton (Bollgard) use [J]. Environmental Entomology, 2002, 31 (1): 30-36.

[19] Ream J E, Sims S R, and Leach J N. Aerobic soil degradation of *Bacillus thuringiensis* var *kurstaki* HD-73 protein bioactivity [R]. Monsanto: Monsanto Company Laboratory Project MSL 13267 (11), 1994.

[20] Palm C J, Schaller D L, Donegan K K, et al. Persistence in soil of transgenic plant-produced *Bacillus thuringiensis* var *kurstaki* d-endotoxin [J]. Canadian Journal of Microbiology, 1996 (42): 1258-1262.

[21] Sims S R, Holden L R. Insect bioassay for determining soil degradation of *Bacillus thuringiensis* subsp. *kurstaki* Cry1A(b) protein in corn tissues[J]. Environmental Entomology, 1996, 25(3): 659-664.

[22] Icoz I, Stotzky G. Cry3Bb1 protein from *Bacillus thuringiensis* in root exudates and biomass of transgenic corn does not persist in soil [J]. Transgenic Research, 2007, 17:609-620.

[23] Sims S R, Ream J E. Soil inactivation of the *Bacillus thuringiensis* subsp. *kurstaki* Cry IIA insecticidal protein within transgenic cotton tissue: laboratory microcosm and field studies [J]. Journal of Agricultural and Food Chemistry, 1997(45):1502-1505.

[24] Liu B, Zeng Q, Yan F M, et al. Effects of transgenic plants on soil micro-organisms [J]. Plant Soil, 2005, 271(1/2): 1-13.

[25] Weidenhamer J D, Romeo J T. Allelochemicals of *Polygonella myriophylla*: chemistry and soil degradation [J]. Journal of Chemical Ecology, 2004, 30(5):1067-1082.