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REVIEW

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Effects of Bt transgenic crops on soil ecosystems: a review of a ten-year research in China

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Abstract *Bacillus thuringiensis* (Bt) transgenic cotton is the unique Bt transgenic crop planted on a large scale in China, and its commercialized varieties and hectareage had increased rapidly in China during the past decade (1997–2006) with broad geographic distribution for the economic, environmental, and health benefits. In 2004, the planting area of Bt transgenic cotton in China ranked first worldwide with up to 370×10^6 hm^2. In addition, Bt transgenic rice varieties in field tests have been close to approval for commercialization. However, ecological risks, a complex issue of Bt transgenic crops on soil ecosystem is urgently faced in China due to more than 60 varieties transferred single or bivalent Bt genes grown under diverse geographic regions. Two main pathways, biomass incorporation and root exudates, are involved in the effects of Bt transgenic crops on soil ecosystems. In this paper, the research results in recent years in China involved in the effects of Bt transgenic crops (Bt transgenic cottons and rice) on soil ecosystems were summarized with special attentions paid to the release and persistence of Bt toxins, and the toxicology to microorganisms, as well as the change of soil biochemical properties in soils where Bt transgenic crops were planted or incubated with their biomass. In addition, the complexity and current research defaults of ecological risk evaluation of Bt transgenic crops in China were highlighted.

Keywords Bt transgenic crops, soil ecosystems, enzyme activity, microorganisms, Bt toxins

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1 Introduction

Bt transgenic crops are plants that have been genetically modified to express the insecticidal proteins (e.g., Cry1Ab, Cry1Ac, and Cry13A) from subspecies of the bacterium, Bt to kill the lepidopteran pests that feed on cotton, rice, and maize and the coleopteran pests that feed on potato (Flores et al., 2005). Many excellent accounts of the economic, environmental, and health benefits for Bt transgenic crops with low pesticide input in agroecosystems have been published (Huang et al., 2002; Huang et al., 2003; Brookes and Barfoot, 2006; Ferry et al., 2006). However, the use of Bt transgenic plants asks for a well-defined risk assessment (Wolfenbarger and Phifer, 2000; Bruinsma et al., 2003). Even today, the release of Bt transgenic crops is still highly controversial in many countries due to the concern over their potential detrimental effects on ecology and human health (Liu et al., 2005). In short, ecological risk evaluation mainly focuses on three issues, i.e., (1) the transfer of genes from crops to wild relatives and related species (Snow and Palma, 1997; Hails, 2000), (2) the resistance evolution to herbicide-tolerant crops, virus-resistant crops, and insect-resistant crops (Tabashnik et al., 1994), and (3) the impacts on nontarget organisms and ecosystems (Hilbeck et al., 1998; Watrud and Seidlet, 1998; Losey et al., 1999). The increased concerns of the impacts of Bt transgenic plants on soil ecosystems are particularly over soil microorganism species, populations, and biodiversity (Angle, 1994; Jepson et al., 1994). The risk evaluation to human health of Bt transgenic products is another issue of concern. Negative results have been presented by Heritage (2004), and his data show that there was no transferred genes in the excrement and urine of 12 healthy individuals who ate food from transgenic soybean. This means that the transferred genes are degraded in the gastrointestinal

tract, which might affect the function of the gastrointestinal tract (Netherwood et al., 2004).

Soil ecosystem is not only the reservoir pool of exotic genes and their expression products of Bt transgenic crops but also the center of biosphere and terminal habitat of microorganisms. More and more attention has been paid worldwide to soil ecosystem effects of the Bt transgenic crops in recent years. Usually, the Bt gene-expressing products of transgenic Bt crops and Bt toxin could be introduced into soil through root exudation or decomposition of the crop residues (Palm et al., 1996; Sims and Holden, 1996; Saxena et al., 1999; Saxena and Stotzky, 2000). Once in the soil, the toxin could be adsorbed or bound on clay particles, humic components, or organic–mineral complexes and then be protected against degradation by soil microorganisms. In this way, it could accumulate to a certain concentration that might affect the composition and activity of soil microbial communities (Tapp and Stotzky, 1995; Crecchio and Stotzky, 1998; Tapp and Stotzky, 1998; Stotzky, 2000; Crecchio and Stotzky, 2001; Rui et al., 2005) and the soil biochemical properties (Rui et al., 2005; Sun et al., 2007). In addition, there are many modifications in crop–soil ecosystem being raised after replacement of conventional crops by Bt transgenic crops, such as plant physiological characteristics, biomass quantity and composition, degree of dependence on pesticides, fertilization structure, etc. For example, Flores et al. (2005) found that Bt maize had a significantly higher lignin content than near-isogenic non-Bt maize. In soil ecosystems, therefore, Bt transgenic products expose potential toxicity to sensitive microorganisms, which affect the transformation and cycle of carbon and nutrients. In the past decade, Bt transgenic cotton, rice, and maize were the main model crops for soil ecosystem risk evaluation studies of Bt transgenic crops worldwide. Although cotton is the unique Bt transgenic crop that has been commercialized in large scales in China, the ecological effects of Bt transgenic cotton and rice are extensively studied by Chinese scientists. However, Bt transgenic maize is scarcely studied in China due to its commercialization impossibility in recent years as a staple crop. More than ten reviews have been published in English (Liu et al., 2005; Liu and Du, 2008) or Chinese by Chinese authors concerning the progress of this issue worldwide. However, no overall summary of research advances in China on this issue have been reviewed. In this paper, the results in recent years in China involving the effects of Bt transgenic crops (Bt transgenic cottons and rice) on soil ecosystems were summarized, with special attentions paid on the release and persistence of Bt toxins the toxicity to microorganisms, and the change of soil biochemical properties in soils where Bt transgenic crops were planted or incubated with their biomass. In addition, the complexity and current research defaults of ecological risk evaluation of Bt transgenic crops in China were highlighted.

2 Research and commercialization status of Bt transgenic crops in China

From 1997 to 1999, four kinds of Bt transgenic plant species (cotton, potato, sweet pepper, and petunia) were licensed by the Office of Agricultural Biological Genetic Engineering Safety Administration (OABGESA) of the Ministry of Agriculture in China. However, only Bt transgenic cotton was planted on a large scale in China from 1997 to 2006 with an ever-most acreage of 370×10^6 hm^2 in 2004 (Fig. 1, data mainly come from the reports of ISAAA), which ranked first in the world. In 2006, the acreage of Bt transgenic cottons in China was 350×10^6 hm^2, the second in the world following India. Chinese scientists began the research on Bt transgenic cotton in the late 1980s (Zhang et al., 2000), and the first Bt transgenic cotton variety (Newcot 33B) was licensed in 1997 by OABGESA, China (Zhang and Wang, 2001). China has become the second country that possesses the technology of Bt transgenic cotton breeding, and more than 60 varieties have been developed in the past several years. Nowadays, Chinese scientists are working on transgenic cottons with triple genes (Bt + CpTI + GNA) resistant to insects. Currently, domestic and exotic varieties from America are coplanted in China. The acreage of domestic varieties of Bt transgenic cottons reaches 75% of the total, and the principal production provinces, e.g., Hebei, Shandong, and Henan, nearly adopt 100% domestic varieties. Cotton planting regions in China are mainly distributed in three areas, i.e., Region of Middle and Lower Reaches of Yellow River, Basin Region of Yangtze River, and Inland Region of Northwest of China, which cross a broad geographic span. Since 2005, more than 100 genetically modified (GM) rice varieties have been in field test. Most of them are insect-resistant lines. Two Bt rice varieties, one rice transgenic for the *Xa21* gene and one herbicide-resistant rice, are now close to approval for commercialization (Wang and Johnston, 2007). China also can become an exporter of biotechnology research methods and commodities. Globally, China has several advantages of many well-trained scientists, a low-cost research environment, and large collections of germplasm (Huang et al., 2002).

3 Impacts of Bt transgenic crops on soil ecosystems

Due to the expression of Bt insecticidal protein, unintentional modifications of agronomic traits changed for Bt transgenic crops compared with the isogenic non-Bt counterparts. Based on the summary of previous results, we suggest that Bt transgenic crops can affect soil ecosystem through two pathways, i.e., biomass incorporation and root exudates (Fig. 2). Contribution degree of biomass incorporation pathway depends on changes of

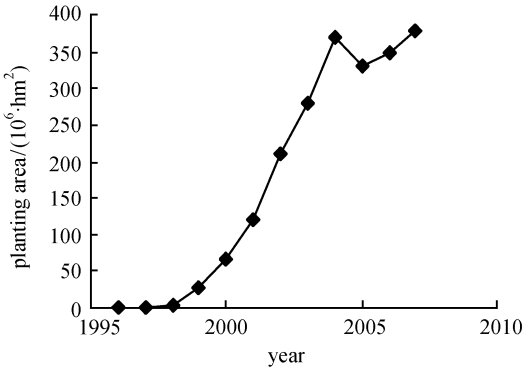

Fig. 1 Planting area of Bt transgenic cottons in China from 1996 to 2007

quantity and composition of biomass (e.g., lignin content), nutrient requirement and metabolism characteristics, decompose rate, and Bt expression level, etc., whereas the contribution degree of root exudates pathway depends on the changes of quantity and composition of exudates, quantity of Bt toxin, persistence of Bt toxin, and toxicity of Bt toxin, etc. Through the above pathways, changes of microbial flora, enzyme activity, hormone content, transformation of nutrients, organic matter, and so on may occur in soil ecosystems. Essentially, there may occur a change in the matter exchange between Bt transgenic crops and soil ecosystem during growth period caused by some modifications of crops, e.g., contents of fructose and soluble carbohydrate (Escher et al., 2000), P requirement (Liu and Du, 2007), photosynthetic parameters (Sun et al., 2004), nitrogen metabolism (Sun et al., 2007b) of Bt transgenic cotton seedlings, lignin content (Saxena and Stotsky, 2001b), decomposition rate (Flores et al., 2005), composition of root exudates (Grayston et al., 1998), etc. Moreover, there may be an exposure of biological toxicity to microorganisms for Bt toxins no matter how they are incorporated into the soil. The degree of effect of the two pathways is all related to the expression level of Bt insecticidal protein in Bt transgenic crops. Recent results indicated that Bt insecticidal protein expression in the tissues of Bt transgenic crops were impacted by climatic conditions, e.g., drought (Sachs et al., 1998; Traore et al., 2000) and agronomic measures (Bruns et al., 2003), e.g., nitrogen fertilizer.

4 Soil persistence and dynamics of Bt toxins from Bt transgenic crops

Soil persistence and dynamics of Bt toxins from transgenic crops (Bt transgenic cotton and rice) were mainly investigated in the rhizosphere and soils incubated with Bt transgenic crop tissues. As the study revealed, there was a significant accumulation (0.2–0.3 $\mu g \cdot g^{-1}$) and then a decreasing process of Bt toxin concentration in rhizosphere during entire growth period of Bt cotton SGK321 and NuCOTN99B, and finally, there was no detectable Bt toxin (Rui et al., 2005). The change of Bt toxin in the rhizosphere of Bt cotton was related to the expression of Bt toxin in leaves because the expression of Bt toxin in leaves had two peaks (Cui and Xia, 1999), which was well correlated with the content of Bt toxin in the rhizosphere of Bt cottons. Rui et al. (2007) assessed the degradation of CpTI in the rhizosphere of the transgenic Bt + CpT1 transgenic cotton cultivar (SGK321). As the plant developed, the residue of CpT1 in the rhizosphere increased and reached a peak (25 $\mu g \cdot g^{-1}$) at topping stage. After this stage, the residue began to decrease and was nil in the following year. Therefore, the authors suggested that Bt transgenic cotton could safely be grown for no significant accumulation occurrence in the rhizosphere soil (Rui et al., 2007). Another experiment indicated that there was a linear

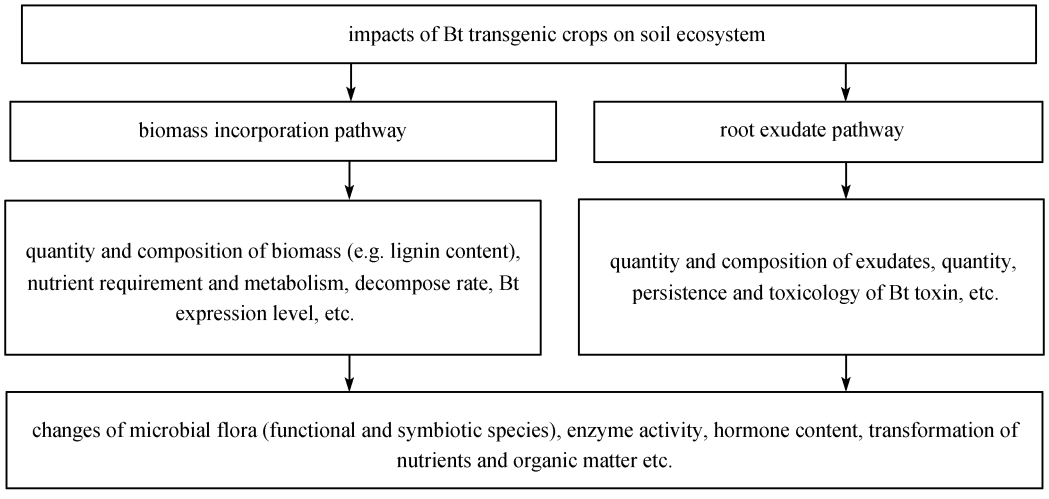

Fig. 2 Impact mechanisms of Bt transgenic crops on soil systems

degradation curve of CpTI in the rhizosphere soil samples during the first 48 h, but the degradation rates of CpTI at the 96th hour were different. In addition, the half-life ($t_{1/2}$) of CpTI toxin in the rhizosphere soil samples calculated from the degradation curves was 1.1–3.8 d. Compared with the 2–41-day-half-life ($t_{1/2}$) of Bt toxin (Sims and Ream, 1997), it seemed that the CpTI toxin degraded more quickly. Sun et al. (2007) incubated the leaves and stems of two transgenic Bt cottons (GK12 and ZK30) and nontransgenic Bt lines (ZM30) in silty loam soil. The results showed that Bt toxin in the soils treated with Bt cotton tissues rapidly decreased in the first 7 d of incubation, but then, the decrease rate lowered, and the Bt toxin content remained almost unchanged after 28 d of incubation. By the end of incubation (the 56th day), the Bt toxin content was still high (14.19–22.69 $ng \cdot g^{-1}$ soil), representing 40.79% (ZK30) and 59.98% (GK12) of its initial introduced amounts. Throughout the whole period of incubation, the Bt toxin content was higher in ZK30 than in GK12 treatment (Sun et al., 2007). This may relate to the higher Bt transgenic insecticidal protein expression in ZK30 (208.14 $ng \cdot g^{-1}$) than in GK12 (169.35 $ng \cdot g^{-1}$). The dynamic concentrations of Bt toxin released from root exudates of Bt cotton (NuCOTN99B and SGK321) were measured by an enzyme-linked immunosorbent assay (ELISA) (Rui et al., 2005). The levels of Bt toxin in the rhizosphere of NuCOTN99B were significantly higher than those of SGK321 within all sampling dates except on June 17th in the whole growth season.

Bt insecticidal protein in cotton leaves degraded rapidly in the first several days, then got slowed down, and then entered a relative stable stage, in which Cry1Ac protein content was kept at a concentration of about 50 $ng \cdot g^{-1}$. The higher temperature and lower humidity significantly increased the degradation speed of the toxin. However, there was no significant difference in the degradation under light or dark treatments. In natural environment, the insecticidal protein in Bt cotton degraded much rapidly in the initial period, it reached 85% in the first month, slowed down during winter season, then degraded quickly in the next spring until it became undetectable in late April (Li et al., 2005). Bt insecticidal protein was applied directly into the soil with the results showing that Bt toxin concentration in the soil increased gradually along with the incubation time and reached the peak on the 15th day and then decreased. On the 30th day, the concentration of Bt toxin was almost the same as at the initial stage after the application. There was a rapid decline at the initial stage of the incubations and the trend leveled off in the middle and late stages. On the 56th day of the incubation, Bt toxin in the soil was at 44.7% (ZK) and 56.1% (GK) of the initial values.

The Cry1Ab protein contents in the shoot and root of KMD were 3.23–8.22 and 0.68–0.89 $mg \cdot g^{-1}$ (fresh weight), respectively (Wang et al., 2006). The content of the Cry1Ab toxin proteins via the root exudate was 1.66–48.02 $ng \cdot individual^{-1} \cdot d^{-1}$ (Wu et al., 2004). The residue of Cry1Ab protein in KMD rhizosphere soil was undetectable (below the limit of 0.5 $ng \cdot g^{-1}$ air-dried soil). The residence time of the protein varied significantly in a Fluvio-marine yellow loamy soil amended with KMD straw at the rate of 3%, 4%, and 7%, with half-lives of 9.9, 13.8, and 18 d, respectively. It suggested that the KMD straws returned to the field might have the higher potential environmental risk than the root exudation (Wang et al., 2006).

The degradation rate of Cry1Ab of Bt rice was high at the early experimental stage but slowed down steadily at middle and later stages, which could be described by exponential equations, with the half-life period of degradation determined as 1.8 – 4.0 d. The soil water content, pH, and temperature could affect the degradation of Cry1Ab, but the effects of soil pH and temperature were relatively greater. In general, Cry1Ab degradation was slower under lower soil pH and temperature conditions, especially in the marine-fluvigenic yellow loamy paddy soil compared with that in blue clayey paddy soil and pale paddy soil on quaternary red soil (Bai et al., 2007). Cry1Ab in ground leaves of KMD2 degraded rapidly in all the soils during 36 d after treatment (DAT), particularly during the first six DAT, with the highest degradation rate in blue clayey paddy soil and the lowest in marine fluvigenic yellow loamy paddy soil. However, such difference decreased gradually as time proceeded thereafter and became fairly little at 78 DAT. As the soil was flooded, the degradation of Cry1Ab in the buried leaves of Huachi B6 was significantly accelerated and exhibited a similar pattern among all the flooded soils. Such impacts of flooding occurred only in the first 12 DAT, and during most of the subsequent periods, no significant difference could be observed in the Cry1Ab residue between the flooded and unflooded treatments (Bai et al., 2004). In comparing with the amount of Bt toxin expressed in a transgenic Bt cotton plant, which was expressed in its root exudates (less than 4 $ng \cdot g^{-1}$), was rather low (Sun et al., 2005).

To sum up, Bt toxins could be introduced into the soil ecosystem by the abovementioned two pathways. For the root exudates pathway, there was limited Bt toxin accumulation (0.2–0.3 $\mu g \cdot g^{-1}$) observed in rhizosphere of Bt transgenic cotton (Rui et al., 2005). This may relate with the low expression level in the root compared with shoot. For example, a pot culture study showed that the amounts of Bt toxin expressed in transgenic Bt cotton leaves and stems (103.5–134.1 $ng \cdot g^{-1}$) were rather higher than those expressed in transgenic Bt cotton roots (21.2–44.7 $ng \cdot g^{-1}$) (Sun et al., 2005). These results may indicate that the effect of Bt toxin released through an exudate pathway on soil ecosystems seems to be negligible at postharvest for no Bt and CpT1 toxin detectable in soil (Rui et al., 2005; Rui et al., 2007). However, there are still puzzles that what will occur when Bt transgenic crops are planted in the same field continuously. In addition, no clear conclusions have been drawn about the effects of Bt toxins in the rhizosphere

at maximal value during growth period on soil ecosystems. On the contrary, Bt toxin released from the biomass incorporated into soil could last for a long time and might accumulate at a high concentration for its large quantity and continuous planting. Sims and Ream (1997) estimated that maximum content of CryIIA protein was 1.6 $\mu g \cdot g^{-1}$ dry-soil when incorporating the Bt transgenic cotton matrix (34 μg CryIIA protein $\cdot g^{-1}$ fresh weight) uniformly into the top 7.6 cm soil. Previous studies showed that the Bt toxin could still be detected when the experiments were terminated after 28–140 d (Palm et al., 1996; Sims and Ream, 1997). In addition, Saxena and Stotzky (2001a) reported that Bt toxin from transgenic corn root exudates and from degrading Bt corn biomass could persist in the soil for up to 350 d. Sims and Ream (1997) found that the Bt toxin in root residuum of Bt transgenic cotton disappeared 1447 d after harvest. As Liu et al. (2005) concluded, Bt toxins could remain in the soil for a long time, but it was still not known whether continuous planting of Bt transgenic crops over many years could lead to further increasing of Bt toxins accumulation or what impacts this might have on soil microorganisms or soil fauna. Therefore, many authors suggested that biomass incorporation pathway might have higher potential environmental risk than root exudation. No matter what pathway of Bt toxin was released into soil ecosystems, its quantity was related with Bt insecticidal protein expression in biomass. There are large differences between species and varieties on expression, release, and dynamics of Bt toxins. Two Chinese Bt transgenic cultivars, ZK30 and GK12, express on the average 208.14 $ng \cdot g^{-1}$ and 169.35 $ng \cdot g^{-1}$, respectively. Furthermore, many factors may potentially influence the accumulation of Bt toxin in soil, including the amount in the plant tissues, the resistance of the proteins to degradation, and soil chemical, physical, and environmental factors that influence availability and persistence of proteins in the soil (Li et al., 2005; Bai et al., 2007). Apparently, agronomic measures, e.g., tillage, irrigation, etc., may directly determine the surrounding conditions pertaining to the decomposition rate of the incorporated biomass of Bt transgenic crops. Furthermore, previous results showed that Bt toxin (Cry1Ab) released to soil through root exudates of Bt corn, from the degradation of the biomass of Bt corn or as purified toxin, was not taken up by crops such as non-Bt corn, carrot, radish, and turnip (Saxena and Stotzky, 2001c, 2002). For other Bt toxins, e.g., cowpea trypsin inhibitor (CpTI) toxin, no similar results were presented.

5 Effects of Bt transgenic crops on soil microorganisms

Soil microorganisms are closely related with soil biochemical properties, e.g., enzyme activities, which determine the turnover cycle of many soil components and persistence of Bt toxins. Some results suggested that there was no evidence to indicate any adverse effects of Bt cotton on the soil ecosystem, while some gave the adverse results. Shen et al. (2006) showed that the richness of the microbial communities in rhizosphere soil did not differ between Bt (Sukang-103) and the non-Bt cotton (Sumian-12). The functional diversity of microbial communities was not different in rhizosphere soils between Bt and non-Bt cotton by a Biological system after a complete cotton growth cycle. In addition, no significant differences were found in the number of functional bacteria (potassium-dissolving bacteria, inorganic phosphate-dissolving bacteria, and nitrogen-fixing bacteria) among three cultivars after growth season. Fortification of pure Bt toxin into rhizospheric soil did not result in significant changes in the number of culturable functional bacteria, except the nitrogen-fixing bacteria when the concentration of Bt toxin was higher than 500 $ng \cdot g^{-1}$ (Rui et al., 2005).

The influences of transgenic Bt rice straw on anaerobic microbial populations in paddy soil were investigated. The results showed that the transgenic Bt rice straw could not only increase the number of hydrolytic fermentative bacteria and decrease obviously that of denitrifying and methanogenic bacteria but also increase the population of anaerobic nitrogen-fixing bacteria significantly (Xu et al., 2004). Wu et al. (2004) studied the influence of the transgenic rice (KMD) straw on the culturable microbiota in a flooded paddy soil under laboratory conditions. The results showed that there were only some occasional significant differences in the number of colony forming units of aerobic bacteria, actinomycetes, and fungi and in the number of anaerobic fermentative bacteria, denitrifying bacteria, hydrogen-producing acetogenic bacteria, and methanogenic bacteria between the paddy soils amended with Bt-transgenic rice straw and with the non-Bt parental rice straw during the early stages of incubation.

In short, no consistent conclusions have been obtained in China on this aspect, which is the same situation as the international progress. Previous studies provided valuable insight into the possible mechanisms by which Bt transgenic crops might affect soil microorganisms. Some authors suggested that Bt toxin was not the direct factor causing the decrease of the number of bacteria in the rhizosphere, and other factors may be involved (Rui et al., 2005). Liu et al. (2005) suggested that direct impacts were the spectrum of activity of the transgenic proteins (Oger et al., 1997) and the quantity of the protein that accumulated in the soil, and indirect impacts were changes of plant protein and root exudates composition. As mentioned above, Bt expression and release variations between varieties are the basic factors that influence experimental data. For example, Bt toxin concentration of BT transgenic cottons in rhizosphere soil was 0.2 $\mu g \cdot g^{-1}$ (SGK321) and 0.3 $g \cdot g^{-1}$ (NuCOTN99B), respectively (Rui et al., 2005), with an average expression of 208.14 $ng \cdot g^{-1}$ (ZK30) and 169.35 $ng \cdot g^{-1}$ (GK12) (Sun et al., 2007). Thus, before the

studies on this issue, the situation of Bt expression in Bt transgenic crops should be investigated to select model plants in terms of Bt level as experimental materials. Two of the three transgenic Bt cottons (Line 247 and Line 249) caused a transient increase in total bacterial and fungal population levels but neither the third Bt (Line 81) cotton nor the purified Bt toxins had any significant effect on the total number of bacteria and fungi (Donegan et al., 1995). In addition, symbiotic and sensitive microorganisms should be the focus for research in the future (Liu et al., 2005; Liu and Du, 2008). Liu and Du (2008) suggested the interaction between Bt transgenic crops and arbuscular mycorrhizal fungi (AMF) is a new urgent issue of soil ecology in agroecosystems, because many evidences has proved the detrimental effects of Bt transgenic maize on AMF (Castaldini et al., 2005; Turrini et al., 2005). Further studies are urgently needed for sufficient information to interpret what causes that. AMF has more opportunities to contact Bt toxin intraradical and rhizosphere than other microorganisms because Bt toxin is distributed in the cytoplasm and intercellular space of Bt transgenic cotton (GK12) by immunocyte localization (Dong et al., 2006).

6 Effects of Bt transgenic crops on soil biochemical properties

The biochemical properties of soil have often been described as early and sensitive indicators of ecological changes in both natural soil and agroecosystem. Activities of soil enzymes indicate the direction and strength of all kinds of biochemical processes in soil andact as key biological indicators of soil. The significant effect of transgenic Bt rice straw was observed on the activities of phosphatase and cellulase; however, the activity of dehydrogenase was seriously inhibited in short time after returning transgenic rice straw into flooded soil (Xu et al., 2004). Sun et al. (2007) suggested that activities of soil urease, acid phosphomonoesterase, invertase, and cellulase were stimulated by the addition of Bt cotton tissues (GK12 and ZK30), whereas activity of soil arylsulfatase was inhibited. In addition, the activities of urease, phosphatase, dehydrogenase, phenol oxidase, and protease in cotton rhizosphere (Bt cotton, Sukang-103, and its non-Bt cotton counterpart, Sumian-12) were assayed during the vegetative, reproductive, and senescing stages of cotton growth and after harvest. There were few significant differences in enzyme activities between Bt and non-Bt cottons at any of the growth stages and after harvest; amendment with cotton biomass to soil enhanced soil enzyme activities, but there were no significant difference between Bt and non-Bt cotton (Shen et al., 2006)

Based on a pot experiment and a soil incubation experiment with cotton biomass (Bt cotton ZM30 and Bt + CpTI cottons ZM 41 and SGK321), it was found that Bt-transgenic cotton had no apparent effects on soil biochemical properties. After CpTI toxin was introduced into the soil 30 d after seedling growth, the introduced amount of CpTI toxin varied with the cultivars. Compared with the relevant nontransgenic cottons, the planting of ZM 30 and ZM 41 had no significant effects on the activities of soil urease, proteinase, and phosphomonoesterase, while that of SGK321 decreased soil phosphomonoesterse activity significantly. From the viewpoints of toxin release and its effects on soil hydrolase activity, the planting of ZM41 caused fewer disturbances on soil biological activities (Zhang et al., 2006).

Wu et al. (2004) investigated the impacts of the amendment of Bt-transgenic rice (KMD) straw on biological activities in water-flooded soil under laboratory conditions and compared it with nontransgenic rice (Xiushui 11) straw. The results showed that there were some differences in protease, neutral phosphatase, and cellulase activities between soil amended with Bt-transgenic rice straw and nontransgenic rice straw at the early stage of incubation, and none of these differences were persistent. However, differences in dehydrogenase activity, methanogenesis, hydrogen production, and anaerobic respiration between the soils supplemented with Bt-transgenic rice straw and nontransgenic rice straw were persistent over the course of incubation (63 d). The results demonstrated that the amendment of the Bt-transgenic rice straw altered some important biological properties in water-flooded soil, indicating a shift in microbial populations or a change in the metabolic abilities of the microbial community as a result of substrate availability in soil. From 14 d to 84 d, there was a significant increase in soil dehydrogenase and soil neutral phosphatase activity in the soil amended with rice straw, compared to the soil without the straw. The dehydrogenase activity was significantly great (almost 1.95-fold) in the soil amended with Bt transgenic straw from 7 to 14 d, but from 21 to 49 d, there was significantly greater activity (about 1.47-fold) in the soil amended with non-Bt-straw. There were no apparent differences between the activity of soil neutral phosphatase in the soils with non-Bt-straw and Bt-straw added. The above results indicate that the Bt-straw from KMD transgenic rice is not toxic to a variety of culturable microorganisms in the flooded paddy soil (Wu et al., 2004). Rui (2005) found that the content of plant hormone in Bt transgenic cottons (99B and SGK321) rhizosphere was different from that in general cotton (Shiyuan 321). Specifically, ABA and IAA in the rhizosphere of the transgenic cotton was much narrower than that of the regular cotton, but the dynamics was the same during all the growth phase. During the former growth stage, the content of GA_3 (gibberellin acid) in the transgenic cotton was much higher than that of the regular cotton however the content of Z + ZR (zeation) changed little.

In short, negative effects on soil enzyme activities of Bt transgenic cotton biomass have been observed. However, the explanation for the consequence is not convincing.

Currently, no results about the effects of Bt transgenic crops on soil enzyme activities have been published except those from China. For non-Bt transgenic plants, Donegan et al. (1999) examined the engineered alfalfa with three genotypes of alfalfa plants (parental, transgenic amylase producing, and transgenic lignin peroxidase producing). They found lower activity of the soil enzymes dehydrogenase and alkaline phosphatase, and higher soil pH levels were associated with the lignin peroxidase transgenic plants. Biochemical properties of soil are associated with activities of soil microorganisms and root system, which are more sensitive than soil microorganisms to environmental changes. More extensive experiments should be conducted to examine the effects of Bt transgenic crops on soil chemical properties since they might help to understand the interaction mechanisms between Bt transgenic crops, soil microorganisms, and soil ecosystem.

7 Research prospects

Commercialization of Bt transgenic crops was conducted in 22 countries in 2006, but the release of Bt transgenic plants was still highly prohibited in the majority of the countries due to concern over the potential detrimental effects of genetically modified plants on ecology and human health (Liu et al., 2005). Although a survey of agricultural producers in China demonstrated that Bt cotton adoption increased production efficiency and improved farmer health (Huang et al., 2002), no consistent conclusions were obtained in the research data from China, even the entire world, and the issues of Bt transgenic crops' effects on soil ecosystems involving three key issues, i.e., soil persistence and dynamics of Bt toxins, effects of Bt transgenic cottons on soil microorganisms, and effects of Bt transgenic cottons on soil biochemical properties. What caused that fact? Basically, the effect of commercialized Bt transgenic crops on soil ecosystem is very complex in China, even in the world, after the following two points are taken into consideration. First, there is a combination complexity of ecological regions, crop species, varieties, and Bt genes transferred. In China, three regions where more than 60 varieties of Bt transgenic cottons with various Bt genes (single, bivalent, and trivalent in the future) were planted across a large geographic span. Second, there is an inherent complexity of soil ecosystems, where components are complex and dynamic, including physical, chemical, and biochemical factors and processes. Many factors in the soil ecosystems are interlocked and have interaction. For example, dynamics of soil organic matter, nutrients, hormones, and soil enzyme largely depend on the status of plants and soil microorganisms, and the soil microorganisms interact closely with plants especially in rhizosphere. Therefore, more multisite experiments should be conducted in order to confirm the effects of Bt transgenic crops on soil ecosystems and their interaction mechanisms. Furthermore, current results almost come from the experiments conducted at a special site during a short period (one growth season), due to lacking of long-term monitoring of experiments in different sites covering all the cotton planting areas. Risk assessment of Bt transgenic crops to soil ecosystems is also an urgent issue worldwide. Today, the knowledge about the three mentioned issues is surprisingly limited. As to soil microorganisms, sensitive, functional, and symbiotic microorganisms (Liu and Du, 2008) should be highlighted prior to others for their greater roles in soil ecosystems or close relationships with crops. More experiments are needed to systematically examine the relationship among Bt toxin, soil microorganisms, and soil biochemical properties. Factually, except Bt toxins, transgenic crops, as well as transgenic plants with herbicide-resistant traits, may produce lectins and proteinase inhibitors, T4 lysozyme, cecropin B, and so on for the resistance to biological diseases (Liu et al., 2005). In further investigations, particular attention should be paid to the effects of other genetically modified plants on soil ecosystem.

Anyway, although some studies to date have suggested that Bt transgenic crops may cause minor usually transient changes in microbial community structure, some data indicate that Bt transgenic crops really have significant effects on soil population of non-target microorganisms (Donegan et al., 1995; Wu et al., 2004), soil enzyme activities (Wu et al., 2004; Sun et al., 2007), hormone content in rhizosphere (Rui, 2005), and AMF colonization (Castaldini et al., 2005; Turrini et al., 2005). Indeed, we surprisingly have little evidence of environmental risks to nontarget organisms arising directly from the use of current GM crops. There is a strong need to reassess environmental risks using a number of large-scale field studies on a trait-by-trait basis (Firbank et al., 2005). In order to accelerate the research progress, three measures should be taken into considerations. First, a series of proper model Bt transgenic crops should be selected in terms of Bt toxin expression level. Second, more multisite field trials should be designed and conducted. Finally, except plating method, more determination methods, such as substrate utilization assay with Biological GN microtiter plates, DNA fingerprint analysis, and microbial lipid analysis (FAME and PLFA) should be adopted and compared.

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