Australian Journal of Crop Science AJCS

AJCS 8(1):18-26 (2014) ISSN:1835-2707

Review article

Field performance of *Bt* transgenic crops: A review

Fei Wang, Shaobing Peng, Kehui Cui, Lixiao Nie, Jianliang Huang*

National Key Laboratory of Crop Genetic Improvement, MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China

***Corresponding author: jhuang@mail.hzau.edu.cn**

Abstract

Genetically modified crops have been wildly cultivated in all over the world since the commercialization of *Bacillus thuringiensis* (*Bt*) transgenic cotton in 1996. Some review papers have been summarized the benefit from the *Bt* transgenic crops and its ecological effects, but fewer of them focused on the agronomic and physiological performance comparison between *Bt*-transgenic crops with their non-*Bt* transgenic counterparts. Here this review summarizes the researches on the field performance of *Bt* transgenic crops relative to their non-*Bt* counterparts by focusing on differences in agronomic traits and *Bt* protein content, and the effect of abiotic stresses on the performance of *Bt* transgenic crops. Furthermore, we discussed the physiological mechanisms underlying the variation of *Bt* protein content at different growth stages, or due to various environmental factors. In the end, crop management practices for maintaining stable *Bt* protein content and efficient control of pests were discussed.

Keywords: Abiotic factors, *Bacillus thuringiensis* (*Bt*), Efficacy, Transgenic crops.

Introduction

Lepidopteron pests cause severe damage to crop production in many areas of the world (Gressel et al., 2004). Besides the damage to yield by their feeding, winds easily knock over the larvae-hollowed stems, causing breakage before harvest (Gressel, 2010). For a long time, they are mainly controlled by insecticide spraying, which not only is not effective enough but also cause severe environmental pollution. Meanwhile, extensive adoption of modern varieties and improved cultivation practices very often result in an increase of insect numbers (Khush, 2001). Therefore, alternative approaches of pest control are needed. One of other choices is *Bacillus thuringiensis* (*Bt*), which has been intensively studied for the last two decades (Fujimoto et al., 1993; Datta et al., 1998; Tu et al., 2000; Chen et al., 2005c; Tang et al., 2006). China government made a landmark decision that approved the safety of two *Bt*-transgenic rice cultivars in 2009 (James, 2009). Since the first commercialization of transgenic cotton in 1996, the increase of transgenic crop cultivation has been 87 fold since 1996 to 2010 (148 million ha) (James, 2010). Although lots of review papers have concentrated on the benefit from the deployment of *Bt* transgenic crops (High et al., 2004; Marra et al., 2002; Raybould and Quemada, 2010; Rice, 2004), and ecological effects of *Bt* endotoxin protein on non-target organisms (Clark et al., 2005; Cattaneo et al., 2006; Chen et al., 2006; Liu 2009), fewer of them focused on the agronomic and physiological performance of *Bt*-transgenic crops compared with their non-*Bt* counterparts. Therefore, this review focused on the following three aspects from the perspective of an agronomist: (1) the differences in agronomic and physiological performance between *Bt*-transgenic crop cultivars and their counterparts; (2) the spatial and temporal expression of *Bt* genes and its efficacy on target insects; (3) the effect of abiotic factors on the expression of *Bt* genes and the efficacy against target pests.

Agronomic and physiological performance of *Bt*-transgenic crops

Yield performance

Agronomic traits of *Bt*-transgenic cotton and corn have been widely investigated. Generally, *Bt* corn hybrids out yielded their non-*Bt* hybrids by 13-23% when European corn borer (ECB) (*Lepidoptera: Crambidae*) infestation was severe (Mungai et al., 2005). Dillehay et al. (2004) evaluated the grain yield of *Bt* hybrids, their non-*Bt* isolines and leading non-*Bt* hybrids under natural infestation of ECB across six locations and three years. Their results indicated that *Bt* hybrids had higher grain yield by 5.5%. On the contrary, no yield advantage was observed for the *Bt* transgenic corn under low or moderate pest infestation (Graeber et al., 1999; Ma and Subedi, 2005; Laserna et al., 2013). Lauer and Wedberg (1999) compared the yield performance of *Bt*-transgenic hybrids, their non-*Bt* isolines and standard high yielding hybrids under natural infestation, inoculation and insecticide application treatments, and found that yield of isoline hybrids was 10% lower than that of *Bt*-transgenic hybrids and standard hybrids regardless of ECB treatments. They also demonstrated that yield of *Bt* hybrids was 4%-8% higher than that of standard hybrids with ECB inoculation, but 8% lower with insecticides application. In Asia, Rasco et al. (2010) found *Bt* corn hybrids were effective against Asian corn borer (ACB) (*Ostrinia furnacalis*) under natural field infestation. Meanwhile, the yield of *Bt* hybrids was slightly higher than that of their isogenic hybrids. Therefore, the incorporation of *Bt* gene into corn hybrids provides an effective protection against ECB or ACB, but yield performance is little if any changed under insect free condition (Graeber et al., 1999; Magg et al., 2001; Laserna et al., 2013).

In cotton, a number of researches have demonstrated that the incorporation *Bt* gene into cotton can significantly reduce insecticide application (Wu et al., 2003; Wan et al., 2005; Cattaneo et al., 2006). A survey of 283 farmers in Northern China showed that *Bt*-cotton substantially reduced insecticide application, but the average yield of non-*Bt* cotton was higher than that of *Bt*-transgenic cotton because of different location, varieties and low pest infestation (Pray et al., 2001). But yield gain from the deployment of *Bt* cotton in India was much higher than that in other regions, because small-scale farmers especially suffer big pest-related yield losses because of technical and economic constraints in many developing countries (Qaim and Zilberman, 2003). Farm-scale evaluation of *Bt* transgenic cotton on yield performance showed that non-*Bt* and *Bt*-transgenic cotton have the same yield overall when insecticide was applied (Cattaneo et al., 2006). Blanche et al. (2006) found that cultivars containing the Bollgard gene (the gene for the insecticidal protein from *Bacillus thuringiensis spp. kurstaki*; Monsanto Co.) yielded more than its conventional cultivars under optimal growth condition. However, Moser et al. (2001) found that six of nine Bollgard cultivars yielded significantly higher than their conventional parents indicating that not all transgenes and insertion events affect yield equally. In China, Dong et al. (2006) studied the agronomic and photosynthetic performance of three types of commercial *Bt* cotton varieties and found that the introduced *Bt* cotton differed significantly from the indigenous Chinese *Bt* cotton in plant growth and photosynthetic rate. Sun et al. (2007a) found that there were no significant differences between *Bt* transgenic cotton and their non *Bt* counterpart in stomatal conductance, transpiration rate, intercellular CO_2 concentration, and chlorophyll content, although *Bt* transgenic cotton has significantly higher Rubisco activity and lower GO activity. Therefore, *Bt* cotton may differ from its non-*Bt* counterpart in some agronomic and physiological traits, but lint yield is not changed due to the manipulation of boll numbers, boll weight and lint percentage under insect free condition (Dong et al., 2006). In rice, yield of *Bt* rice increased by 60–65% compared to non-*Bt* rice under no control of any pests, but decreased by 28–36% with pesticide application, which indicated that pesticide sprays were still required to avoid losses caused by non-target insect pests (Wang et al., 2010; Xia et al., 2010). A *Bt* transgenic line with *Cry1C** was reported having significantly lower grain yield than the control variety Minghui63, and the yield decrease was mainly caused by lower grain filling (Wang et al., 2012a).

Nitrogen assimilation and metabolism

Nitrogen metabolism is essential to the formation of grain yield in crops (Kropff et al., 1993; Lawlor, 2002). It is reasonable to speculate that *Bt* endotoxin as a kind of soluble proteins will consume N in its production and may cause a redistribution of N in different plant organs. Therefore, whether the insertion of external genes would change nitrogen metabolism in transgenic crops attracted the attention of many scientists. In corn, *Bt*-transgenic cultivars had the same whole-plant N concentration with their non-*Bt* counterparts (Bruns and Abel, 2003), however, most of *Bt* hybrids had lower N accumulation in grain, but higher N accumulation in stover than that of their non-*Bt* counterpart (Ma and Subedi, 2005). Subedi and Ma (2007) further studied dry matter production, N uptake and dry matter and N partitioning pattern of *Bt*-transgenic hybrid (Pioneer 38W36*Bt*) and their conventional hybrid (Pioneer 38W36). They demonstrated that both hybrids had similar harvest index, leaf chlorophyll content, and N concentration and content at key growth stages. Pioneer 38W36*Bt* accumulated more biomass and N in kernels and on a whole-plant base than Pioneer 38W36, but they had a similar partitioning of N and NUE in different plant parts. *Bt* and non-*Bt* corn residues did not differ in their effect on N dynamics when they were incorporated into field (Mungai et al., 2005). In cotton, Pettigrew and Adamczyk (2006) studied the effect of N fertilizer and planting date on lint yield and *Cry1Ac* endotoxin production, they concluded that plant receiving the 112/56 kg N/ha (split application) treatment exhibited 14% greater leaf *Cry1Ac* concentration and a 3% greater leaf Chlorophyll concentration than the other N treatments. Meanwhile, Reddy et al. (2009) found that four split application of nitrogen fertilizer at 120kg/ha dose could increase boll number and hence seed yield. Chen et al. (2005a) studied the effect of *Bt* gene on nitrogen metabolism in cotton, and the results indicated that *Bt* cotton cultivars had more intense nitrogen metabolism than their parents during reproductive growth stage. There were higher total leaf nitrogen, soluble protein content, and free amino acid, greater Nitrate Reductase (NR) and Glutamic-Pyruvic Transaminase (GPT) activity in *Bt*-transgenic cotton during peak square and boll developing period, compared with their parents. However, Sun et al. (2007b) found that there were no significant differences in the content of free amino acid and the activity of NR between transgenic *Bt* cotton and non transgenic counterpart at seedling stage, although *Bt* transgenic cotton had a significantly higher GPT activity and lower content of soluble protein.

Potassium assimilation

Several studies suggest that *Bt* cotton cultivars are more sensitive to K deficiency than non-*Bt* cultivars (Zhang et al., 2007; Yukui et al., 2009). Zhang et al. (2007) compared the responses of two *Bt* cotton cultivars and two conventional cultivars to K deficiency, and demonstrated that *Bt* cotton cultivars had lower dry weight and K uptake than that of conventional cultivars under low K condition. Yukui et al. (2009) analyzed 12 elements distribution in leaf, stem and root, and found that content of K of *Bt*-transgenic cotton in all the three organs was lower than that of non-*Bt* cotton, especially in leaves by one time. Otherwise, *Bt* cotton cultivars respond well to K fertilizer application. Lint yield was increased by 2.3-18% when 150-375kg/ha KCL was applied, and fiber quality was also improved due to K fertilizer application (Zhou et al., 2006). However, there are no similar reports in other transgenic crops. In consideration of cotton as a K-favoring crop, it cann’t be concluded that *Bt*-transgenic crops are more sensitive to K deficiency. In fact, there are no differences in grain yield, K concentration in straw and grain at maturity between *Bt*-transgenic rice cultivars and their non-*Bt* counterparts at three K treatments in our research (unpublished data).

Optimum population density

Bt cultivars was found to require a bigger population density to fulfill its yield potential than their non-Bt counterparts (Stanger and Lauer, 2006). But the higher harvest costs related to those greater yields and the higher seed costs associated with attaining those populations resulted in no difference in the economically optimum plant population between *Bt* and non-*Bt* corn (Stanger and Lauer, 2006). Singer et al. (2003) also found that *Bt* corn cultivars are more efficient than their isolines to produce a higher yield as plant density increases. But different optimum population densities was not found for *Bt*-transgenic cultivars and their isolines because of low stalk lodging, plant density treatment that did not maximize yield in most circumstances and the absence of different optimum densities. In cotton, two field experiments were conducted by Dong et al. (2006) to find the optimum planting density for three types of commercial *Bt* cotton varieties. Their results found that the optimal plant densities in terms of lint yield for the introduced *Bt* cotton, indigenous Chinese *Bt* cotton and Chinese hybrid *Bt* cotton were 6.0, 4.5 and 3.0 plants m^2 respectively.

Other traits

There were no significant differences on the responses to drought stress between *Bt* corn and non-*Bt* corn. Drought stress decreased leaf area as much as 33% and plant height by 15% for both (Traore et al., 2000). Yan et al. (2007) studied the effect of incorporation of *Bt* gene on the low-molecular-weight components in root exudates and found that *Bt*-transgenic cotton secretes more organic acid, but less amino acid and soluble sugars than wide type cotton when supplemented with all nutrient elements. When nitrogen was removed from the solution, the secretion amount of organic acid of *Bt*-transgenic cotton was less than that of wild-type cotton. There are some other studies on the effect of incorporation of *Bt* genes on the lignin content of leaves and stems in corn. Saxena and Stotzky (2001) confirmed that all *Bt*-transgenic varieties used in their experiment had a higher content of lignin (33-97%) than their non-*Bt* counterpart. Poerschmann et al. (2005) studied the lignin pattern of leaves and stems of *Bt*-transgenic corn and their near-isogenic non-*Bt* varieties, and their results showed that the total lignin content in the stems of *Bt*-transgenic corn was higher than that in the stems of the respective isogenic lines. There was no significant difference in lignin pattern of leaves between isogenic and transgenic lines. However, contrary to some earlier reports, presence of the *cry1Ab* gene did not alter lignin concentration or other forage quality of corn stover in commercial corn hybrids (Jung and Sheaffer, 2004).

The spatial and temporal expression of *Bt* genes and its efficacy against target insects

Genes for the toxic *Bt* crystal proteins from the bacteria are transferred into crop plants to protect them from attack by economically important insect pests. Right now, *Bt* genes have been successfully incorporated into several crops, for example, in tomato (Delannay et al., 1989), in *Nicotiana tabacum* (Barton et al., 1987), and in rice (Tu et al., 2000; Chen et al., 2005c; Tang et al., 2006); and some tree species, for example, in white spruce (Lachance et al., 2007). In rice, *Bt*-transgenic cultivars expressing different *cry* genes all show high level of efficacy against lepidopteran pests (Benedict et al., 1996; Ye et al., 2003; Han et al., 2007; Wang et al., 2010). The mechanism of action of *Bt* insecticidal crystal proteins include expression of the protein as a protoxin crystal during the sporulation phase; solubilization of the crystal in the alkaline reducing environment of the insect midgut; processing of the protoxin by insect midgut trypsin-like proteases; binding of the activated toxin to specific receptors on the midgut epithelial cells; insertion of the toxin into the apical midgut membrane; and formation of an ion channel, which disrupts midgut ion (mostly K^+) flow causing paralysis and death of the insect (Dean et al., 1996).

Spatial and temporal variation and the possible mechanism

Although transgenic *Bt* crops are engineered to express δ-endotoxin proteins in almost all parts of the plant, variation in the amount of insecticidal proteins occurs according to the age of the plant (Adamczyk and Sumerford, 2001b; Dong and Li, 2007b; Llewellyn et al., 2007; Poongothai et al., 2010) and in different plant parts (Fearing et al., 1997; Adamczyk et al., 2001a; Wu et al., 2002; Badea et al., 2010; Table 1.). Meanwhile, the fluctuate of δ-endotoxin proteins may logically cause variation in efficacy of *Bt* crops against target pests (Gore et al., 2001; Wan et al., 2005; Kranthi et al., 2005; Siebert et al., 2009). Generally, insecticidal protein levels and its efficacy were high during the early stages of growth and then declined (Greenplate, 1999; Kranthi et al., 2005; Olsen et al., 2005a). But Wan et al. (2005) reported that the expression of *Cry1Ac/Cry1Ab* and single *Cry1Ac* protein in cotton both declined in middle season and rebounded in late season. Meanwhile, Llewellyn et al. (2007) stated that there was no dramatic reduction in production of the *vip3A* protein during growth and maturation of the crop in transgenic cotton with *vip3A* gene. In corn, the highest amount of *CryIA(b)* protein (estimated to be 5–10 g *CryIA(b)* protein ha^{-1}) was found to occur at anthesis, consistent with the stage at which maximum plant vegetative biomass was reached (Fearing et al., 1997). Among different plant parts, the fully expanded leaf usually contains the highest amount of *Bt* δ-endotoxin protein, larger than reproductive organs and roots (Fearing et al., 1997; Adamczyk et al., 2001a; Kranthi et al., 2005; Siebert et al., 2009). Because the content of δ-endotoxin proteins correlates with the efficacy against target insects (Adamczyk et al., 2001a; Olsen et al. 2005a), it is important to keep the stability of expression of *Bt* genes at sufficiently high levels to achieve insect control (Llewellyn et al., 1994). Either temporal or spatial variability in efficacy may increase the probability of surviving pests, thus has been and continues to be concern of *Bt*-transgenic crops growers, researchers and breeders (Oosterhuis and Brown, 2004). Although a large number of researches have been concentrated on the spatial and temporal expression of *Bt* genes and its efficacy in transgenic crops, much less attention was paid to the physiological mechanism behind it and how to overcome it by crop management practice in the field. There are some researches which have demonstrated that the concentration of δ-endotoxin in plant tissues are significantly correlates with the concentration of total soluble protein and overall nitrogen (Bruns and Abel, 2003; Oosterhuis and Brown, 2004; Dong et al., 2007a; Wang et al., 2012b). The decrease in efficacy against target pest in late season results from the enhanced remobilization of soluble protein (Pettigrew and Adamczyk, 2006). However, other research found that there was no such correlation between them and argued that other physiological mechanism for the changes of survival of target pests in *Bt*-transgenic plants existed (Gore et al., 2001; Olsen et al., 2005a). Therefore, physiologists and agronomists should pay more attention to design elaborate experiments to study the physiological mechanism on the fluctuation of *Bt* genes expression and build up a kind of crop management practice which can realize the potential of *Bt*-transgenic crops with both high grain yield and efficacy against target insects.

Genotypic variation and the inheritance of Bt genes expression

It has been demonstrated that all varieties and plant structures of Bollgard® cotton (containing *cry1Ac* gene) did not provide the same level of lepidopteran control. Adamczyk and Sumerford (2001b) demonstrated that parental background has a stronger impact on the expression of *cry1Ac* than the environment. Adamczyk and Meredith (2004b) further studied the genetic basis for variability of *cry1Ac* expression among commercial transgenic *Bt* cotton cultivars in the United States. Four cultivars, ST 4691B and PM1218BR expressing low level

Table 1. The concentration of different kinds of *Bt* proteins varies in different plant tissues in cotton, corn and rice.

Crop	Plant tissues	*Bt* genes	Concentration (μg g^{-1} FW)	Citation
	Leaf		0.97-2.29	
Cotton	Root	*Cry1Ac*	0.82-1.33	Jiang et al. 2006
	Stem		0.56-1.07	
	Upper leaves		0.05-5.51	
	Middle leaves		0.05-3.48	
Cotton	Bottom leaves	*Cry1Ac*	0.10-5.49	Kranthi et al. 2005
	Flowers		0.25-0.80	
	Squares		0.06-0.63	
	Bolls		0.19-2.02	
	Leaf		0.32-11.07	
Corn	Root	*Cry1Ab*	0.27-4.17	Székács et al. 2010
	Stem		0.35-2.06	
	Grain		0.01-0.51	
Rice	Leaf		4.0-9.1	Bai et al. 2005
	Stem	*Cry1Ab*	3.7-8.0	

of *cry1Ac* and NuCOTN 33B and DP 458BR expressing high level of *cry1Ac*, are chosen, and NuCOTN 33B and DP 458BR have the same genetic background. Reciprocal crosses were made in the greenhouse between DP 458BR x PM 1218BR plants and NuCOTN 33B x ST 4691B plants to produce F1 and F2 progenies. They studied the genetic basis of the expression of *cry1Ac* in parents, F1 progenies and F2 progenies, and the results concluded that the same number of major genes affecting the expression level of *cry1Ac* across the two breeding populations was calculated even when sampling across the two breeding populations was conducted over different times in the season and during different crop growth stages, which indicates that genetic background has a major effect on *cry1Ac* expression in Bollgard cultivars. What's more, there were also differences in the efficacy against target pests among different kinds of *Bt* genes (Magg et al., 2001; Adamczyk and Gore, 2004a), and the expression of two insecticidal proteins of *Bt* is more toxic to bollworm in cotton (Stewart et al., 2001; Bommireddy and Leonard, 2008). Strategies to improve the insecticidal activity of *cry* toxins from *Bt* were discussed by Pardo-Lopez (2009). Wu et al. (2002) studied the inheritance and expression of *cry 1Ab* gene in *Bt* transgenic rice and their results indicated that the *cry1Ab* gene was stably transmitted in an intact manner via successive sexual generations, and the concentration of the *cry1Ab* protein was kept quantitatively stable up to the R6 generation. They also demonstrated that in the F2 population, the *cry1Ab* gene in all crosses between japonica and japonica rice displayed a 3:1 one-locus Mendelian segregation ratio, while the cross between indica and japonica displayed a distorted segregation ratio. In corn, Fearing et al. (1997) found that *cry 1Ab* expression was stable over at least four successive generations. Moreover, there existed considerable heterosis in yield, yield components and δ-endotoxin expression in some *Bt* transgenic cotton hybrids (Dong et al., 2007a).

Management practices to keep stable expression of Bt genes

In order to enhance δ-endotoxin levels and thereby improve insect mortality, Oosterhuis and Brown (2004) studied foliar applications of Chaperone on protein and δ-endotoxin levels of cotton leaves and squares, and they concluded that plant growth regulator Chaperone can increase the content of δ-endotoxin by 1-16% in relation to the amount of Chaperone application and the plant parts measured. Moreover, Wang et al. (2009) found that boll size affects the δ-endotoxin content in boll shell and cotton seed, and the correlation between the 100-seed weight and the cotton seed δ-endotoxin content is significant and negative. They also found that leaf cut and square removal could enhance and reduce the δ-endotoxin content in boll shell and cotton seed due to its effect on the boll size, respectively. Moreover, removal of early fruiting branches is proved to be effective to enhance the expression in the fully expanded young leaves (Dong et al., 2008). Therefore, it is feasible to increase the expression of *Bt* genes and keep the stability of the efficacy against target pests in transgenic crops by adopting appropriate crop management practices.

Effects of abiotic factors on the expression of *Bt* genes and the efficacy against target insects

An analysis of major U.S. crops shows that there is a large genetic potential for yield that is unrealized because of the need for a better adaptation of the plants to the environments in which they are grown (Boyer, 1982). Global climate change, like high temperature, drought, changes in the pattern of precipitation, rising seas, will have profound influences on the distribution and production of crops (Nguyen, 2002). For example, rice yield declines with higher night temperature from global warming (Peng et al., 2004). What's more, environmental factors certainly affect the efficacy of *Bt* cotton plants, for example, temperature (high or low) during all stages of plant growth influenced the survival of H. armigera larvae on *Bt* cotton (Mahon et al., 2002). Therefore, it is meaningful to evaluate the effect of climate change on the expression of *Bt* genes and its efficacy against target insects (Dong and Li, 2007b).

Effects of abiotic factors on the expression of Bt genes

Different abiotic factors may have different effects on the expression of *Bt* genes. Chen et al. (2005b) found that high temperature treatment at flowering period had no effect on the content of *Cry1A* protein, but profound effect at boll period was observed with a decrease of *Cry1A* content by 30-63% according to different cultivars. What's more, there are no consistent responses to temperature treatments for different plant tissues. The *Cry1Ab* content in different plant tissues of *Bt*-transgenic rice varied individually with temperature (Wu et al., 2002). Glutamic-pyruvic transaminase (GPT) activity, total free amino acid and soluble protein content, and the activity of protease in the leaf involved in nitrogen metabolism were regarded as the possible causes for the variation of *Bt* protein concentration in response to temperature treatments in *Bt* transgenic cotton (Chen et al., 2005b). Hallikeri et al. (2009) studied the effect of sowing date and moisture regime on the expression of *Bt* genes, and concluded that early sowing crop had higher *Cry* protein than late sowings, which was related with higher SPAD reading and leaf N concentration in early sowing crops. *Bt* protein content could also be decreased by salt stress, which might be related to the concomitant decrease of total soluble protein (Jiang et al., 2006). Luo et al. (2008) studied individual and combined effect of salinity and waterlogging on *Cry1Ac* protein production and its efficacy, and concluded that waterlogging and a combination of waterlogging and salinity reduced the production of total soluble protein and δ-endotoxin protein by 40-65% and 38-72%, respectively, relative to the control treatment. From the discussion above, it is obvious that the changes of *Bt* protein content caused by various abiotic factors are all related with the concomitant variation in nitrogen metabolism. It is found that δ-endotoxin concentration was highly correlated with nitrogen fertilizer level (Bruns and Abel, 2003; Wang et al., 2012b), but little is known about how nitrogen metabolism influences the content of *Bt* proteins in different environments.

Effects of abiotic factors on the efficacy against target insects

It has been discussed that expression of *Bt* genes in transgenic crops varies temporally and spatially, which is highly related with its efficacy against target pests. However, several studies found that the content of *Bt* δ-endotoxin protein and its efficacy against target pests were not changed congruously by abiotic factors, in other words, the response of efficacy of transgenic plants on target pests to environmental stresses is independent of *Bt* δ-endotoxin protein production (Mahon et al., 2002). It was found that *Bt* protein content was decreased by salt stress, but sill biologically effective to control neonate bollworm larvae in seedling cotton (Jiang et al., 2006). Salinity stress also did not reduce the control efficacy, although it did result into a reduction of total soluble protein and δ-endotoxin protein by 5.7-7.2% and 11-22%, respectively (Luo et al., 2008). The same with salinity, water-deficit stress also had no effect on the efficacy against first-instar cotton bollworm larvae, although δ-endotoxin protein production in leaves, flowers and bolls was reduced (Martins et al., 2008). Kranthi et al. (2005) identified a critical level of 1.9 μg g^{-1} for *Bt* transgenic cotton, and found that only after 110 days after sowing did the toxin levels fall below the critical level. On the contrary, Olsen et al. (2005b) found that the survival of *H. armigera* larvae on *Bt* plants was influenced by exposure to higher or lower temperatures, either for short periods or throughout growth, although δ-endotoxin concentration was not changed. It is speculated that increases in secondary compounds which is toxic to insects may be involved in maintaining the efficacy when *Bt* protein production was reduced under salinity (Luo et al., 2008). In fact, plants produce many chemicals and some of these appear to limit damage from insects and other herbivores under environmental stresses. The effect may be a direct one, where the secondary chemicals limit larval survival, or an indirect effect where the chemicals interact with the *Bt* toxin (Mahon et al., 2002). Lots of studies have demonstrated that the ratio of C to N in crops is influenced by environmental factors. Coviella et al. (2002) found that C-N ratio in transgenic plants under elevated CO_2 was increased and the allocation of N to δ-endotoxin decreased, but the reduction was greatly alleviated by the addition of nitrogen. Wu et al. (2007) also concluded that elevated CO_2 decreased the expression of δ-endotoxin by 3.0%, 2.9%, 3.1% and 2.4%, 2.5%, 2.9%, but increased the content of condensed tannin occurrence by 17%, 11%, 9% and 12%, 11%, 9%, in transgenic *Bt* cotton after exposed to elevated CO_2 for 1, 2 or 3 months compared with ambient CO_2 for same time in 2004 and 2005, respectively. Their results indicated that elevated CO_2 could alter the folia chemical composition in transgenic cotton, which might in turn affect the plant-herbivore interactions (Wu et al., 2007). In rapeseed, elevated temperature increased C and N contents, total chlorophyll and carotenoid concentrations under ambient CO_2, but decreased these under elevated CO_2 (Himanen et al., 2008). Therefore, changes in the background physiology of the plant may contribute to the observed larval survival (Olsen et al., 2005b).

Future Prospects

Generally, most of the surveys indicate that the deployment of *Bt*-transgenic crops brings benefits to farmers in both higher yield and lower costs, especially in the developing countries. Scientific research also demonstrates that there is a great yield advantage in *Bt*-transgenic crops under severe insect infestation. However, the incorporation of *Bt* genes into crops rarely change the yield performance significantly under strict pesticide control. The percentage of δ-endotoxin protein account for the total soluble protein is barely more than 1%, so it seems to hardly cause any obvious influences to the nitrogen metabolism in *Bt*-transgenic crops. But morphological and physiological variations have been demonstrated in previous studies, which will definitely influence the management practices of *Bt* transgenic crops. Therefore, it is critical for the agronomists and physiologists to design professional experiments to evaluate how the rest of plant organisms are affected by the insertion of *Bt* gene (Edmeades et al., 2004).

Full expression of *Bt* genes at a sufficiently high level is crucial to the production of *Bt*-transgenic crops, but expression levels of a gene may decrease as the age of the crop advances, vary between young and older parts, and be affected by environmental stresses, although some scientists stated that not all decreases of efficacy against target pests in *Bt*-transgenic crops are due to the reduction of δ-endotoxin production Right now, lots of researches have been concentrated on the expression of *Bt* genes in transgenic crops and its efficacy against target pests. However, relatively fewer papers focus on the physiological mechanism behind the dynamic of *Bt* protein production. Most of them attribute the dynamic of *Bt* protein production to changes in nitrogen metabolism. Abel and Adamczyk (2004) found that factors regulating photosynthesis at transcription and translation level should be studied for its effect on *Bt* protein production and insect control. Therefore, more and more endeavors are required to study the specific mechanism involved in the expression of *Bt* genes and how environmental stresses affect it. It will be beneficial to both the breeders to select elite cultivars and the agronomist to build up appropriate crop management practices for transgenic crops. Site-specific and wound-induced promoters are used to keep the expression of transgenes in specific plant parts, or under the attack (See review by Schuler et al. 1998). But genetic background profoundly influences the expression of *Bt* genes, and environmental factors also have a great effect on the content of δ-endotoxin in the transgenic plants, even when the same promoter is used. It has been demonstrated that crop management practices can alleviate the reduction of δ-endotoxin content caused by environmental factors, or by senescence. Therefore, in order to pursue great improvement of crops in the field, multidisciplinary efforts are needed. Combining physiological and genetic information can provide a more complete understanding gene-phenotype relationship and genotype-by-environment interaction (Edmeades et al. 2004).

Acknowledgements

This work is supported by National Basic Research Program of China (No. 2009CB118605), and the National Key Technology R&D Program of the Ministry of Science and Technology of China (No. 2012BAD04B12).

References

Abel CA, Adamczyk JJ (2004) Relative concentration of *Cry1A* in maize leaves and cotton bolls with diverse chlorophyll content and corresponding larval development of fall armyworm (*Lepidoptera: Noctuidae*) and southwestern corn borer (*Lepidoptera: Crambidae*) on maize whorl leaf profiles. J Econ Entomol. 97: 1737-1744.

Adamczyk JJ and Gore J (2004a) Laboratory and field performance of cotton containing *Cry1Ac*, *Cry1F*, and both *Cry1Ac* and *Cry1F* (WIDESTRIKE®) against beet armyworm and fall armyworm larvae (*Lepidoptera: NoctuIdae*). Fla Entomol. 87: 427-432.

Adamczyk JJ and Meredith WR (2004b) Genetic basis for variability of *Cry1Ac* expression among commercial transgenic *Bacillus thuringiensis* (*Bt*) cotton cultivars in the United States. J Cotton Sci. 8: 17-23.

Adamczyk JJ, Hardee DD, Adams LC, Sumerford DV (2001a) Correlating differences in larval survival and development of bollworm (*Lepidoptera: Noctuidae*) and fall armyworm (*Lepidoptera: Noctuidae*) to differential expression of *Cry1A(c)* δ-endotoxin in various plant parts among commercial cultivars of transgenic bacillus thuringiensis cotton. J Econ Entomol. 94: 284-290.

Adamczyk JJ and Sumerford DV (2001b) Potential factors impacting season-long expression of *Cry1Ac* in 13 commercial varieties of Bollgard® cotton. J Insect Sci. 1:13. Available online: http://insectscience.org/1.13.

Badea EM, Chelu F, LĂCĂTUȘU A (2010) Results regarding the levels of *Cry1Ab* protein in transgenic corn tissue (MON810) and the fate of *Bt* protein in the three soil types. Rom Biotech Lett. 15: 55-62.

Bai YY, Jiang MX, Cheng JA (2005) Temporal expression patterns of *Cry1Ab* insecticidal protein in *Bt* rice plants and its degradation in paddy soils. Acta Ecologica Sinica. 25: 1583-1590.

Barton KA, Whiteley HR, Yang N (1987) *Bacillus thuringiensis* δ-endotoxin expressed in transgenic *Nicotiana Tabacum* provides resistance to lepidopteran insects. Plant Physiol. 85: 1103-1109.

Benedict JH, Sachs ES, Altman DW, Deaton WR, Kohel RJ, Ring DR, Berberich SA (1996) Field performance of cottons expressing transgenic *CrylA* insecticidal proteins for resistance to *Heliothis virescens* and *Helicoverpa zea* (*Lepidoptera: Noctuidae*). J Econ Entomol. 89: 230-238.

Blanche SB, Myers GO, Zumba JZ, Caldwell D, Hayes J (2006) Stability comparisons between conventional and near-isogenic transgenic cotton cultivars. J Cotton Sci. 10: 17-28.

Bommireddy PL and Leonard BR (2008) Survivorship of *Helicoverpa zea* and *Heliothis virescens* on cotton plant structures expressing a *Bacillus thuringiensis* vegetative insecticidal protein. J Econ Entomol. 101: 1244-1252.

Boyer JS (1982) Plant Productivity and Environment. Science. 218: 443-448.

Bruns HA and Abel CA (2003) Nitrogen fertility effects on *Bt* -endotoxin and nitrogen concentrations of maize during early growth. Agron J. 95: 207-211.

Cattaneo MG, Yafuso C, Schmidt C, Huang C, Rahman M, Olson C, Kirk CE, Orr BJ, Marsh SE, Antilla L, Dutilleul P, Carriere Y (2006) Farm-scale evaluation of the impacts of transgenic cotton on biodiversity, pesticide use, and yield. Proc Natl Acad Sci USA. 103: 7571-7576.

Chen D, Ye G, Yang C, Chen Y, Wu Y (2005a) The effect of high temperature on theinsecticidal properties of *Bt* Cotton. Environ Exp Bot. 53: 333-342.

Chen D, Ye G, Yang C, Chen Y, Wu Y (2005b) Effect after introducing *Bacillus thuringiensis* gene on nitrogen metabolism in cotton. Field Crop Res. 87: 235-244.

Chen H, Tang W, Xu C, Li X, Lin Y, Zhang Q (2005c) Transgenic indica rice plants harboring a synthetic *cry2A** gene of *Bacillus thuringiensis* exhibit enhanced resistance against lepidopteran rice pests. Theor Appl Genet. 111: 1330-1337.

Chen M, Zhao J, Ye G, Fu Q, Shelton AM (2006) Impact of insect-resistant transgenic rice on target insect pests and non-target arthropods in China. Insect Sci. 13: 409-420.

Clark BW, Phillips TA, Coats JR (2005) Environmental fate and effects of *Bacillus thuringiensis* (*Bt*) proteins from transgenic crops: a review. J Agric Food Chem. 53: 4643-4653.

Coviella CE, Stipanovic RD, Trumble JT (2002) Plant allocation to defensive compounds: interactions between elevated CO_2 and nitrogen in transgenic cotton plants. J Exp Bot. 53: 323-331.

Datta K, Vasquez A, Tu J, Torrizo L M, Alam F, Oliva N, Abrigo E, Khush GS, Datta SK (1998) Constitutive and tissue-specific differential expression of the *cryIA(b)* gene in transgenic rice plants conferring resistance to rice insect pest. Theor Appl Genet. 97: 20-30.

Dean DH, Rajamohan F, Lee MK, Wu SJ, Chen XJ, Alcantara E, Hussain SR (1996) Probing the mechanism of action of *Bacillus thuringiensis* insecticidal proteins by site-directed mutagenesis - a minireview. Gene. 179: 111-117.

Delannay X, LaVallee BJ, Proksch RK, Fuchs RL, Sims SR, Greenplate JT, Marrone PG, Dodson RB, Augustine JJ, Layton JG, Fischhoff DA (1989) Field Performance of transgenic tomato plants expressing the *Bacillus Thuringiensis Var. Kurstaki* insect control protein. Nat Biotechnol. 7: 1265-1269.

Dillehay BL, Roth GW, Calvin DD, Kratochvil RJ, Kuldau GA, Hyde JA (2004) Performance of *Bt* hybrids, their near isolines and leading corn hybrids in Pennsylvania and Maryland. Agron J. 96: 818-824.

Dong H, Li WJ, Tang W, Li ZH, Zhang DM (2006) Effects of genotypes and plant density on yield, yield components and photosynthesis in *Bt* transgenic cotton. J Agron Crop Sci. 192: 132-139.

Dong HZ, Li WJ (2007b) Variability of endotoxin expression in *Bt* transgenic cotton. J Agron Crop Sci 193: 21-29.

Dong HZ, Li WJ, Tang W, Li ZH, Zhang DM (2007a) Heterosis in yield, endotoxin expression and some physiological parameters in *Bt* transgenic cotton. Plant Breed. 126: 169-175.

Dong H, Tang W, Li W, LI Z, Niu Y, Zhang D (2008) Yield, leaf senescence, and *Cry1Ac* expression in response to removal of early fruiting branches in transgenic *Bt* cotton. Agr Sci China. 7: 101-105. (In Chinese with English abstract)

Edmeades GO, McMaster GS, White JW, Campos H (2004) Genomics and physiologists: bridging the gap between genes and crop response. Field Crop Res. 90: 5-18.

Fearing P L, Brown D, Vlachos D, Meghji M, Privalle L (1997) Quantitative analysis of *CryIA(b)* expression in *Bt* maize plants, tissues, and silage and stability of expression over successive generations. Mol Breed. 3: 169-176.

Fujimoto H, Itoh K, Yamamoto M, Kyozuka J, Shimamoto K (1993) Insect resistant rice generated by introduction of a modified -endotoxin gene of *Bacillus thuringiensis*. Nat Biotechnol. 11: 1151-1155.

Gore J, Leonard BR, Adamczyk JJ (2001) Bollworm (Lepidoptera: Noctuidae) survival on ‘Bollgard’ and ‘Bollgard II’ cotton flower bud and flower components. J Econ Entomol. 94: 1445-1451.

Graeber JV, Nafziger ED, Mies EW (1999) Evaluation of transgenic, *Bt*-containing corn hybrids. J Prod Agric. 12: 659-663.

Greenplate JT (1999) Quantification of *Bacillus thuringiensis* insect control protein *Cry1Ac* over time in Bollgard cotton fruit and Tterminals. J Econ Entomol. 92: 1377-1383.

Gressel J (2010) Needs for and environmental risks from transgenic crops in the developing world. New Biotechnol. 27: 522-527.

Gressel J, Hanafi A, Head G, Marasas W, Obilana AB, Ochanda J, Souissi T, Tzotzos G (2004) Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions. Crop Prot. 23: 661-689.

Hallikeri SS, Halemani HL, Katageri IS, Patil BC, Patil VC, Palled YB (2009) Influence of sowing time and moisture regimes on cry protein concentration and related parameters of *Bt*-cotton. Karnataka J Agric Sci. 22: 995-1000.

Han L, Wu K, Peng Y, Wang F, Guo Y (2007) Efficacy of transgenic rice expressing *Cry1Ac* and *CpTI* against the rice leaffolder, *Cnaphalocrocis medinalis* (Guenee). J Invert Pathol. 96: 71-79.

High SM, Cohen MB, Shu Q, Altosaar I (2004) Achieving successful deployment of *Bt* rice. Trends Plant Sci. 9: 286-292.

Himanen A, Nisinen A, Dong W, Nerg AM, Stewart CN, Poppy GM, Holopainen JK (2008) Interactions of elevated carbon dioxide and temperature with aphid feeding on transgenic oilseed rape: Are *Bacillus thuringiensis* (*Bt*) plants more susceptible to nontarget herbivores in future climate? Global Change Biol. 14: 1-18.

James C (2009) Executive Summary of Global Status of Commercialized Biotech/GM Crops: 2009. ISAAA Briefs No. 34, Ithaca, New York, USA.

James C (2010) Global status of commercialized Biotech/GM crops: 2010. ISAAA Briefs No.42, Ithaca, New York, USA.

Jiang L, Duan L, Tian X, Wang B, Zhang H, Zhang M, Li Z (2006) NaCl salinity stress decreased *Bacillus thuringiensis* (*Bt*) protein content of transgenic *Bt* cotton (*Gossypium hirsutum L.*) seedlings. Environ Exp Bot. 55: 315-320.

Jung HG and Sheaffer CC (2004) Influence of *Bt* transgenes on cell wall lignification and digestibility of maize stover for silage. Crop Sci. 44: 1781–1789.

Khush GS (2001) Green revolution: the way forward. Nat Rev Genet. 2: 815-822.

Kranthi KR, Naidu S, Dhawad CS, Tatwawadi A, Mate K, Patil E, Bharose AA, Behere GT, Wadaskar RM, Kranthi S (2005) Temporal and intra-plant variability of *Cry1Ac* expression in *Bt*-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Noctuidae: Lepidoptera). Current Sci. India. 89: 291-298.

Kropff MJ, Cassman KG, Van Laar HH, Peng S (1993) Nitrogen and yield potential of irrigated rice. Plant Soil. 155/156: 391-394.

Lachance D, Hamel LP, Pelletier F, Valéro J, Cardou MB, Chapman K, Frankenhuyzen K, Séguin A (2007) Expression of a *Bacillus thuringiensis cry1Ab* gene in transgenic white spruce and its efficacy against the spruce budworm (*Choristoneura fumiferana*). Tree Genet Genomes. 3: 153-167.

Laserna MP, Maddonni GA, Lopez CG (2013) Phenotypic variations between non-transgenic and transgenic maize hybrids. Field Crop Res. 134: 175-184.

Lauer J and Wedberg J (1999) Grain yield of initial *Bt* corn hybrid introductions to farmers in the Northern Corn Belt. J Product Agric. 12: 373-376.

Lawlor DW (2002) Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. J Exp Bot. 53: 773-787.

Liu W (2009) Effects of *Bt* transgenic crops on soil ecosystems: a review of a ten-year research in China. Front Agric China. 3: 190-198.

Llewellyn D, Cousins Y, Mathews A, Hartweck L, Lyon B (1994) Expression of *Bacillus thuringiensis* insecticidal protein genes in transgenic crop plants. Agric Ecosyst Environ. 49: 85-93.

Llewellyn D, Mares C L, Fitt GP (2007) Field performance and seasonal changes in the efficacy against *Helicoverpa armigera* (Hübner) of transgenic cotton expressing the insecticidal protein vip3A. Agric Forest Entomol. 9: 93-101.

Luo Z, Dong H, Li W, Zhao M, Zhu Y (2008) Individual and combined effects of salinity and waterlogging on *Cry1Ac* expression and insecticidal efficacy of *Bt* cotton. Crop Protect. 27: 1485-1490.

Ma BL and Subedi KD (2005) Development, yield, grain moisture and nitrogen uptake of *Bt* corn hybrids and their conventional near-isolines. Field Crop Res. 93: 199-211.

Magg T, Melchinger AE, Klein D, Bohn M (2001) Comparison of *Bt* maize hybrids with their non-transgenic counterparts and commercial varieties for resistance to European corn borer and for agronomic traits. Plant Breed. 120: 397-403.

Mahon R, Finnegan J, Olsen K, Lawrence L (2002) Environmental stress and the efficacy of *Bt* cotton. Aust Cotton Grower. 23: 18-22.

Marra MC, Pardey PG, Alston JM (2002) The payoffs of transgenic field crops: An assessment of the envidence. AgBioForum 5: 43-50. Available on the World Wide Web: http://www.agbioforum.org.

Martins CM, Beyene G, Hofs JL, Kruger K, Vyver CV, Schlter U, Kunert KJ (2008) Effect of water-deficit stress on cotton plants expressing the *Bacillus thuringiensis* toxin. Ann Appl Biol. 152: 255-262.

Moser HS, McCloskey WB, Silvertooth JC (2001) Performance of transgenic cotton varieties in Arizona. p. 420-423. In Proc. Beltwide Cotton Conf., Anaheim, CA. 9-13 Jan. 2001. Natl. Cotton Counc. Am., Memphis, TN.

Mungai NW, Motavalli PP, Nelson KA, Kremer RJ (2005) Differences in yields, residue composition and N mineralization dynamics of *Bt* and non-*Bt* maize. Nutr Cycl Agroecos. 73: 101-109.

Nguyen N (2002) Global climate changes and rice food security. Rome, Italy: FAO.

Olsen KM, Daly JC, Fennegan EJ, Mathon RJ (2005b) Changes in *Cry1Ac Bt* transgenic cotton in response to two environmental factors: temperature and insect damage. J Econ Entomol. 98: 1382-1390.

Olsen KM, Daly JC, Holt HE, Finnegan EJ (2005a) Season-long variation in expression of *Cry1Ac* gene and efficacy of *Bacillus thuringiensis* toxin in transgenic cotton against *Helicoverpa armigera* (Lepidoptera: Noctuidae). J Econ Entomol. 98: 1007-1017.

Oosterhuis DM and Brown RS (2004) Effect of foliar Chaperone™ applications on endotoxin and protein concentration, insect mortality, and yield response of cotton. Ark Agr Exp Sta Res Ser. 533: 51-56.

Pardo-Lopez L, Munoz-Garay C, Porta H, Rodriguez-Almazan C, Soberon M, Bravo A (2009) Strategies to improve the insecticidal activity of *Cry* toxins from *Bacillus thuringiensis*. Peptides. 30 (3): 589-595.

Peng S, Huang J, Sheeh JE, Laza RC, Visperas RM, Zhong X, Centeno GS, Khush GS, Cassman KG (2004) Rice yields decline with higher night temperature from global warming, Proc Natl Acad Sci USA. 101: 9971-9975.

Pettigrew WT and Adamczyk JJ (2006) Nitrogen fertility and planting date effects on lint yield and *Cry1Ac* (*Bt*) endotoxin production. Agron J. 98: 691-697.

Poerschmann J, Gathmann A, Augustin J, Langer U, Gorecki T (2005) Molecular composition of leaves and stems of genetically modified *Bt* and near-isogenic non-*Bt* maize-characterization of lignin patterns. J Environl Qual. 34: 1508-1518.

Poongothai S, Ilavarasan R, Karrunakaran CM (2010) *Cry 1Ac* levels and biochemical variations in *Bt* cotton as influenced by tissue maturity and senescence. J Plant Breed Crop Sci. 2: 96-103.

Pray CE, Huang J, Ma D, Qiao F (2001) Impact of *Bt* cotton in China. World Develop. 29: 813-825.

Qaim M and Zilberman D (2003) Yield effects of genetically modified crops in developing countries. Science. 299: 900-902.

Rasco ET, Mangubat JR, Burgonio AB, Logrono ML, Villegas VN, Fernandez EC (2010) Agronomic performance and Asiatic Corn Borer resistance of tropical converted transgenic corn hybrids containing the truncated *Cry1A(b)* Gene (*Bt*-11) in Davao City, Philippines. Philipp J Crop Sci. 35: 1-15.

Raybould A and Quemada H (2010) *Bt* crops and food security in developing countries: released benefits, sustainable use and lower barriers to adoption. Food Secur. 2: 247-259.

Reddy P, Kumar B, Gopinath M, Rao L (2009) Nitrogen levels and scheduling studies in *Bt* cotton hybrids. Crop Res. 38: 61-63.

Rice ME (2004) Transgenic rootworm corn: Assessing potential agronomic, economic, and environmental benefits. Plant Health Progress. Available at: http://www.plantmanagementnetwork.org/pub/php/review/2004/rootworm/

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

Schuler TH, Poppy GM, Kerry BR, Denholm I (1998) Insect-resistant transgenic plants. Trends Biotechnol. 16: 168-175.

Siebert MW, Patterson TG, Gilles GJ, Nolting SP, Braxton LB, Leonard BR, Van Duyn JW, Lassiter RB (2009) Quantification of *Cry1Ac* and *Cry1F Bacillus thuringiensis* insecticidal proteins in selected transgenic cotton plant tissue types. J Econ Entomol. 102: 1301-1308.

Singer JW, Taylor RW, Bamka WJ (2003) Corn yield response of *Bt* and near-isolines to plant density, Available at www.plantmanagementnetwork.org/cm/ Crop Manage. doi:10.1094/CM-2003-0829-01-RS.

Stanger TF and Lauer JG (2006) Optimum plant population of *Bt* and non-*Bt* corn in Wisconsin. Agron J. 98: 914-921.

Stewart SD, Adamczyk JJ, Knighten KS, Davis FM (2001) Impact of *Bt* cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis Berliner* on growth and survival of *Noctuid* (Lepidoptera) larvae. J Econ Entomol. 94: 752-760.

Subedi KD and Ma BL (2007) Dry matter and nitrogen partitioning patterns in *Bt* and non-*Bt* near-Isoline maize hybrids. Crop Sci. 47, 1186-1192.

Sun C, Qi H, Sun J, Zhang L, Miao L (2007a) Photosynthetic characteristics of *Bt* or *CpTⅠ-Bt* transgenic cotton at seedling stage. Acta Agronomica Sinica. 33: 469-475. (In Chinese with English abstract)

Sun C, Zhang L, Wu Q, Miao L, Wang G, Li S (2007b) Nitrogen metabolism of transgenic *Bt* cotton and transgenic *Bt+CpTⅠ* cotton at seedling stage. Chin J Ecol. 26: 187-191. (In Chinese with English abstract)

Székács A, LauberÉ, Takács E, Darvas B (2010) Detection of *Cry1Ab* toxin in the leaves of MON 810 transgenic maize. Anal Bioanal Chem 396: 2203-2211.

Tang W, Chen H, Xu C, Li X, Lin Y, Zhang Q (2006) Development of insect-resistant transgenic indica rice with a synthetic *cry1C** gene. Mol Breed. 18: 1-10.

Traore SB, Carlson RE, Pilcher CD, Rice ME (2000) *Bt* and non-*Bt* maize growth and development as affected by temperature and drought stress. Agron J. 92: 1027-1035.

Tu J, Zhang G, Datta K, Xu C, He Y, Zhang Q, Khush GS, Datta SK (2000) Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* δ-endotoxin. Nat Biotechnol. 18: 1101-1104.

Wan P, Zhang Y, Wu K, Huang M (2005) Seasonal expression profiles of insecticidal protein and control efficacy against *Helicoverpa armigera* for *Bt* cotton in the Yangtze River Valley of China. J Econ Entomol. 98: 195-201.

Wang F, Ye C, Zhu L, Nie L, Cui K, Peng S, Lin Y, Huang J (2012a) Yield differences between *Bt* transgenic rice lines and their non-*Bt* courterparts and its possible mechanism. Field Crop Res. 126: 8-15.

Wang F, Jian Z, Nie L, Cui K, Peng S, Lin Y, Huang J (2012b) Effects of N treatments on the yield advantage of *Bt*-SY63 over SY63 (*Oryza sativa*) and the concentration of Bt protein. Field Crop Res. 129: 39-45.

Wang Y, Ye G, Luan N, Xiao J, Chen Y, Chen D (2009) Boll size affects the insecticidal protein content in *Bacillus thuringiensis* (*Bt*) cotton. Field Crop Res. 110: 106-110.

Wang Y, Zhang G, Du J, Liu B, Wang M (2010) Influence of transgenic hybrid rice expressing a fused gene derived from *cry1Ab* and *cry1Ac* on primary insect pests and rice yield. Crop Prot. 29: 128-133.

Wu G, Chen F, Ge F, Sun Y (2007) Effects of elevated carbon dioxide on the growth and foliar chemistry of transgenic *Bt* cotton. J Integrat Plant Biol. 49: 1361-1369.

Wu G, Cui H, Ye G, Xia Y, Sardana R, Cheng X, Li Y, Altosaar I, Shu Q (2002) Inheritance and expression of the cry1Ab gene in *Bt* (*Bacillus thuringiensis*) transgenic rice. Theor Appl Genet. 104: 727-734.

Wu K, Guo Y, Lv N, Greenplate JT, Deaton R (2003) Efficacy of transgenic cotton containing a *cry1Ac* gene from *Bacillus thuringiensis* against *Helicoverpa armigera* (*Lepidoptera: Noctuidae*) in Northern China. J Econ Entomol. 96: 1322-1328.

Xia H, Chen L, Wang F, Lu B (2010) Yield benefit and underlying cost of insect-resistance transgenic rice: Implication in breeding and deployment of transgenic crops. Field Crop Res. 118: 215-220.

Yan W, Shi W, Li B, Zhang M (2007) Overexpression of a foreign *Bt* gene in cotton affects the low-molecular-weight components in root exudates. Pedosphere. 17: 324-330.

Ye GY, Yao HW, Shu QY, Cheng X, Hu C, Xia YW, Gao MW, Altosaar I (2003) High levels of stable resistance in transgenic rice with a *cry1Ab* gene from *Bacillus thuringiensis* Berliner to rice leaffolder, *Cnaphalocrocis medinalis* (Guenee) under field conditions. Crop Protect. 22: 171-178.

Yukui R, Wenya W, Pinghui L, Fusuo Z (2009) Mineral element distribution in organs of dual-toxin transgenic (*Bt+CpTI*) cotton seedling, Plant Biosystems. 143: 137-139.

Zhang Z, Tian X, Duan L, Wang B, He Z, Li Z (2007) Differential Responses of Conventional and *Bt*-Transgenic Cotton to Potassium Deficiency. J Plant Nutr. 30: 659-670.

Zhou T H, Zhang H P, Liu L (2006) Studies on effect of potassium fertilizer applied on yield of *Bt* cotton. Chin Agric Sci Bull. 22: 292-296. (In Chinese with English abstract).