Field performance of Bt transgenic crops: A review

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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 isolate 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 285 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 CO₂ 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 Minghu63, 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 38W36Br) 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 38W36Bt 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 soil (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, hially in Chrysogenic 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 can’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, 2005). 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 cases.
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 Cry1Aa 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 Cin4/Ab (protein (estimated to be 5–10 g Cin4/Ab (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 (Petitgrew 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 cry1Aa than the environment. Adamczyk and Meredith (2004b) further studied the genetic basis for variability of cry1Aa expression among commercial transgenic Bt cotton cultivars in the United States. Four cultivars, ST 4691B and PM1218BR expressing low level
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; Bomireddy 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).

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

<table>
<thead>
<tr>
<th>Crop</th>
<th>Plant tissues</th>
<th>Bt genes</th>
<th>Concentration (µg g⁻¹ FW)</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton</td>
<td>Root</td>
<td>Cry1Ac</td>
<td>0.82-1.33</td>
<td>Jiang et al. 2006</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td></td>
<td>0.56-1.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Upper leaves</td>
<td></td>
<td>0.05-5.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle leaves</td>
<td></td>
<td>0.05-3.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bottom leaves</td>
<td>Cry1Ac</td>
<td>0.10-5.49</td>
<td>Kranthi et al. 2005</td>
</tr>
<tr>
<td></td>
<td>Flowers</td>
<td></td>
<td>0.25-0.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Squares</td>
<td></td>
<td>0.06-0.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bolls</td>
<td></td>
<td>0.19-2.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td></td>
<td>0.32-11.07</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Root</td>
<td>Cry1Ab</td>
<td>0.27-4.17</td>
<td>Székács et al. 2010</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td></td>
<td>0.35-2.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grain</td>
<td></td>
<td>0.01-0.51</td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>Leaf</td>
<td>Cry1Ab</td>
<td>4.0-9.1</td>
<td>Bai et al. 2005</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td></td>
<td>3.7-8.0</td>
<td></td>
</tr>
</tbody>
</table>
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). Glutamate-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 still 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. Coviglia et al. (2002) found that C:N ratio in transgenic plants under elevated CO₂ 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 CO₂ 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 CO₂ for 1, 2 or 3 months compared with ambient CO₂ for same time in 2004 and 2005, respectively. Their results indicated that elevated CO₂ 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 CO₂, but decreased these under elevated CO₂ (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).

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